THE COUGH REFLEX - PROPERTIES OF RAPIDLY ADAPTING STRETCH RECEPTORS IN RELATION TO INHALED TUSSIGENIC STIMULI AND SOME POTENTIAL ANTI-TUSSIVE/ANTI-ASTHMATIC AGENTS

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To my Mum
with love

Abstract

Activation of airway afferent sensory nerve endings evokes numerous airway reflexes including cough. However, the distribution and type of nerve endings involved in evoking individual reflexes are unclear. The primary purpose of the present study was to examine the distribution of airway afferent sensory nerve endings in man responsive to "selective" C-fibre stimulants used to evoke cough and to establish the degree of selectivity of these agents on airway afferent sensory nerve endings in anaesthetized cats. A secondary purpose was to investigate if a group of these sensory nerve endings, i.e. rapidly adapting stretch receptors (RARs) are involved in the anti-tussive/anti-bronchoconstrictor action of the loop diuretic, frusemide. A third purpose was to ascertain if neurotransmitters, which cause hyperalgesia, are important in the development of airway hyperreactivity through a sensory neuronal mechanism.

Inhaled capsaicin and prostaglandin E_2 evoked coughing in human subjects; the central airways more responsive to prostaglandin E_2 and the peripheral airways more responsive to capsaicin. In anaesthetized cats, inhaled capsaicin was not selective for C-fibres, since a proportion of RARs were activated independently of changes in pulmonary mechanics. Furthermore, PGE_2 caused a dose-dependent increase in RAR discharge and a dose-related bronchoconstriction, possibly through activation of EP_2 receptors. Frusemide had no effect on the spontaneous activity of RARs in anaesthetized cats whereas 443C81, the peripherally-acting μ-opioid agonist, significantly inhibited the spontaneous activity of RARs. The neurotransmitter glutamate which causes hyperalgesia had no effect on airway tone or the spontaneous discharge of RARs in cats. Although lamotrigine, a glutamate release inhibitor, was anti-bronchoconstrictor at high doses, it is unlikely to have any therapeutic potential in asthma.

Capsaicin and PGE_2 are **not**, as previously thought, selective for either C-fibre endings or RARs. Furthermore, RARs may be the sensory nerve endings primarily responsible for evoking the cough reflex, and represent a potential therapeutic target for inhibiting this reflex response.

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Abbreviations

A	AH13205
(A)	aerosol
AA	arachidonic acid
ABP	arterial blood pressure
ACE	angiotensin converting enzyme
AI	adaptation index
ANOVA	analysis of variance
AP	action potential
Arg	arginine
^{o}C	degree centigrade
C/CAP	capsaicin
C_{Dyn}	dynamic lung compliance
CGRP	calcitonin gene related peptide
Cl^-	chloride ions
CNS	central nervous system
cmH_2O	centimetres of water, pressure
CSF	cerebral spinal fluid
d-	dextro
DMT	dimethyl tubocurarine
DTPA	diethyletriaminepentaacetic acid
EC_{50}	effective concentration of a drug producing an effect in 50% of the sample group.
ED_{50}	effective dose of a drug producing an effect in 50% of the sample group.
F	flow
FEV_1	forced expired volume in one second
FVC	forced vital capacity
Fig.	figure
G	glutamate
Gly	glycine
GSD	geometric standard deviation
h	hour
H	histamine
HETE	hydroxyeicosatetraenoic acid
HLT	heart-lung transplant

HPETE	hydroperoxyeicosatetraenoic acid
HR	heart rate
5-HT	5-hydroxytryptamine
i.a.	intra-arterial
imp/IMP	impulses
inh	inhalation
IP_3	inositol 1-4-5 trisphosphate
i.v.	intravenous
JET	jet nebuliser
K^+	potassium ions
kg	kilogram
l-	laevo
l	litre
L	Lamotrigine
Leu	leucine
LTB_4	leukotriene B_4
m	metre
M	molar
Met	methionine
MBq	megabecquerel
mg	milligram
min	minute
ml	millilitre
mm	millimetre
MMAD	mass median aerodynamic diameter
mmHg	millimetres of mercury, pressure
mSv	millisievert
n	number
Na^+	sodium ions
NaCl	sodium chloride
NANC	nonadrenergic noncholinergic
ng	nanogram
NMDA	N-methyl-D-aspartate
NTS	nucleus tractus solitarii
O_2	oxygen
P/PGE_2	prostaglandin E_2
Pr	pressure

PAF	platelet activating factor
P	probability
PF	peak frequency
PG's	prostaglandins
$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$
Phe	phenylalanine
p.o.	*per os*
ppm	parts per million
Pro	proline
P_{Tp}	transpulmonary pressure
P_{TB}	tracheal balloon pressure
R-	*rectus*
RAR	rapidly adapting stretch receptor
R_L	total lung resistance
RT	respiratory tract
S	salbutamol
S-	*sinister*
s/sec	second
SAR	slowly adapting stretch receptor
sem	standard error of the mean
SO_2	sulphur dioxide
SPECT	single photon emission computed tomography
Tc	technetium
Tyr	tyrosine
µg	microgram
UNDW	ultrasonically nebulised distilled water
USN	ultrasonic nebuliser
VIP	vasoactive intestinal peptide
V/Veh	vehicle
Vo	volume
V_T	tidal volume
<	less than
>	greater than
%	percentage

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Chapter 1

Introduction

1.1 Cough and asthma, neural involvement

The principal role of the lungs is to maintain an adequate oxygenation of the body's cells, to excrete waste products (for example, carbon dioxide) and to act as a metabolic and endocrine organ. To ensure the proper function and integrity of the air tissue barrier, the respiratory system has several mechanisms to minimise the impact and spread of noxious agents possibly present in the environment. These mechanisms consist of reflexes, mucociliary clearance, and cellular events, involving phagocytic and immunocompetent cells. In addition, a well developed sensory innervation ensures that harm to the lung can also be sensed, for example, as rawness, irritation, tearing, tightness of the chest and dyspnoea (Widdicombe, 1986a).

Airway reflexes can be described as being protective or defensive (Korpás & Tomori, 1979). Protective reflexes (for example, apnoea and laryngeal narrowing) do not actively remove noxious agents from the respiratory tract, but prevent penetration of the noxious agents into the distal airways and alveoli. In contrast, defensive reflexes, such as cough, sneezing and the expiration reflex, actively remove irritants from the respiratory tract. Reflex bronchoconstriction can be viewed as being both defensive and protective. Bronchoconstriction may extend the turbulent flow to the peripheral airways and increase linear velocities in cough. In a protective role, bronchoconstriction would stabilise the tracheobronchial walls and reduce the chance of compression of the airways (Widdicombe, 1977a).

Cough is rare in complete health. This suggests that normally, clearance of the respiratory tract is satisfactorily managed by other mechanisms (for example, mucociliary clearance and alveolar macrophages). However, when mucociliary clearance is ineffective, or when the respiratory tract is overloaded with mucus, the cough reflex provides an effective means for the removal of harmful endogenous and

exogenous materials from the lungs and airways. Cough is also a simple symptom that can be traced in over 100 diseases. Until this century, the physiological and pathological nature of cough, because of its familiarity in every day life, has been overlooked. However, analysis of airway reflexes, such as cough, was greatly facilitated by Adrian's development of the techniques for recording activity in single nerve fibres from the lungs and airways. The systematic exploration of afferent pathways, begun by Adrian (1933), has been continued by a number of investigators.

Unequivocal evidence for a neural involvement in the cough reflex has come from findings in both experimental animals and man. Deafferentation studies in cats have shown the importance of the vagus nerve in evoking airway reflexes: bilateral cervical vagotomy abolishes airway reflexes, such as cough and reflex bronchoconstriction, from the tracheobronchial tree (Korpás & Tomori, 1979). In man, ipsilateral vagotomy has been used to abolish persistent cough in patients with inoperable bronchial carcinoma (Klassen *et al.*, 1951). Further and more recent information has come from studies conducted in patients who have undergone heart-lung transplantation (HLT). After HLT, there is complete denervation of the heart and lungs below the tracheal anastomosis. From immunohistochemical studies there is a clear loss of afferent nerves to airway epithelium, with only the post-ganglionic efferent nerves remaining in any number in the transplanted lung (Springall *et al.*, 1990). Furthermore, there is no evidence to suggest that re-innervation of the heart and lungs occurs in man (Stinson *et al.*, 1972; Rowan & Billingham, 1988). Inhalation of ultrasonically nebulised solutions, low in permeant anion concentration (for example, distilled water), can evoke coughing in normal and asthmatic subjects, the cough response being mediated by afferent sensory nerve endings in the airways (Boggs & Bartlett, 1982; Eschenbacher *et al.*, 1984; Godden *et al.*, 1986; Pisarri *et al.*, 1992). However, in heart-lung transplant recipients the cough response to

ultrasonically nebulised distilled water is abolished, with no evidence of re-occurrence up to three years after surgery (Higenbottam *et al.*, 1989b). Furthermore, Hathaway *et al.*, (1993) have demonstrated that the cough response to inhaled capsaicin (an extract of red peppers), a "selective" stimulant of airway C-fibre endings, is also absent in HLT patients.

Asthma is a major chronic disease in many industrialised countries in which a number of separate mechanisms contribute to its characteristic presentation (Snapper, 1990). Asthma is characterised by wide variations over short periods of time in resistance to airflow in intrapulmonary airways and is associated with variable degrees of bronchoconstriction and inflammation of the bronchial mucosa. The introduction of the concept of increased sensitivity (hyperreactivity) of the airways to a variety of specific (allergic) and non-specific stimuli (American Thoracic Society, 1987) has added a further dimension to the physiological definition of asthma, as it enables inclusion of individuals with apparently normal airway calibre (Higenbottam *et al.*, 1983). Clinically, asthma is characterised by the triad of dyspnoea, wheezing and cough, which may vary from mild and almost undetectable to severe and unremitting (status asthmaticus). However, it is important to recognise that, in some cases, cough may be the sole presenting symptom of asthma (Glauser, 1972; McFadden, Jr., 1975).

That neural mechanisms may be important in asthma has for a long time been suspected (Salter, 1868). For many centuries, it has been recognised that the airways are innervated. Bartholinus (1663) clearly described nerve branches supplying human bronchi. Later, Roy and Brown (1885), following experiments in dogs, suggested that the vagus nerve contained both bronchoconstrictor and bronchodilator nerves. This was confirmed by Dixon and Brodie (1903), who also found that bronchoconstriction in dogs could also occur as the result of a reflex mechanism.

Despite the popularity of the neurogenic theories, that asthma could be explained by excessive irritability of the airways, much of this century has been concerned with immunological theories of pathogenesis. The phenomenon of anaphylaxis was first described in 1902 by Portier and Richet. Later, Meltzer (1910) demonstrated anaphylaxis in guinea-pigs and likened it to human asthma. Although the importance of allergy in asthma cannot be denied, it must be conceded that attempts at hypo-sensitisation and the use of mediator antagonists have not been very successful in the treatment of the disease. More recently, the study of neuronal regulation of the airways has become yet again a progressive field of investigation. This is primarily because of further advances in the knowledge of the sensory and motor innervation of the lungs and airways. Indeed, autonomic regulation of the airways, that includes the classical cholinergic and adrenergic pathways (Nadel & Barnes, 1984), has been added to with the recognition of the nonadrenergic and noncholinergic pathways (NANC) and the identification of several neuropeptides, as possible transmitters of such pathways, present in the respiratory tract (Richardson, 1981; Barnes, 1984).

Although neurogenic activity may contribute to the development of asthma, there is evidence to suggest that airway afferent sensory nerves are not necessarily required for the development of airway hyperreactivity. Some HLT patients, who had no history of asthma prior to transplantation and had received heart-lung blocks from donors with no history of asthma, show clinical features suggestive of asthma (Morrison *et al.*, 1992). Furthermore, hyperreactivity to methacholine and ultrasonically nebulised distilled water challenge has been demonstrated in some HLT patients (Higenbottam *et al.*, 1989a; Hathaway *et al.*, 1993). However, the mechanism by which this bronchial hyperresponsiveness occurs in HLT patients is unclear.

1.2 Afferent Innervation of the Respiratory Tract

The respiratory tract, from the larynx to the smallest airways and alveoli, is supplied by the vagus nerves. Sensory and/or afferent information is conducted from sensory nerve endings in the walls of the different airways. The afferent networks also feed locally into other parts of the respiratory tract, where they have motor actions and may affect airway calibre and function. The vagus also has direct motor control of zones throughout the respiratory tract. There are, therefore, vago-vagal reflexes to the larynx and tracheobronchial tree, vagal afferent reflexes from these sites back to the same areas and to other parts of the respiratory tract. In addition, there are reflexes that arise from the whole respiratory tract and other areas that influence the vagal motor supply of the larynx and the lower respiratory tract. Furthermore, the anatomical structures in the respiratory tract vary considerably along its length and therefore the supply will differ in each area. Thus it is a complex system which may be complicated further by the sympathetic nervous system, although evidence suggests that sympathetic innervation of airway smooth muscle is sparse, if at all (Barnes, 1988). It is complicated even further in that the vagal supply to many of the relevant tissues may not be a single cholinergic motor pathway, but may involve NANC pathways either together with, or separate from, cholinergic nerves.

Several types of afferent sensory nerve endings have been described in the airways, their function being to send sensory/afferent information up the vagus nerve so that appropriate changes in breathing pattern and bronchomotor tone may occur. Most of the information about afferent nerves is derived from animal studies (Sant'Ambrogio, 1982; Coleridge & Coleridge, 1986), but there are considerable differences in afferent nerve supply and function between species, so that it may be misleading to extrapolate to humans.

By far the most direct evidence of the afferent properties and distribution of the nerve endings in the airways comes from action potential studies of the impulse traffic in "single fibres" of the vagus nerves in laboratory animals. When considering the afferent innervation of the respiratory tract, it is easier to subdivide it into its obvious anatomical areas, for example nose, pharynx, larynx and tracheobronchial tree. Although the nose, pharynx and larynx are very important structures, they are not necessarily relevant to the present study and, therefore, are not discussed here.

Tracheobronchial Tree and Lungs

Afferents from the tracheobronchial tree and lungs have probably been studied more than those from elsewhere in the respiratory tract because of the ease of access to the vagus nerves. In addition, the most studied of airway diseases, asthma and chronic bronchitis, are generally associated with changes in calibre of the tracheobronchial tree and lungs rather than elsewhere in the respiratory tract.

The afferent innervation of the lower respiratory tract resembles that of skin. It is made up of mechanoreceptors supplied by myelinated fibres that are mostly in the Aδ range, having conduction velocities of 5 m s^{-1} and above. In addition, there are large numbers of non-myelinated C-fibres with endings that are loosely classed as "polymodal". The conduction velocities of the C-fibres in the lungs and airways range from 0.8 - 2.4 m s^{-1} (Coleridge & Coleridge, 1984). C-fibres are in the majority. Estimates of the ratio of C- to A-fibres in the pulmonary and bronchial branches of the vagus nerve of various species range from 4:1 to 9:1 (Coleridge & Coleridge, 1984).

There are 3 main groups of sensory nerve endings in the tracheobronchial tree and lungs, rapidly adapting (irritant) stretch receptors (RARs), slowly adapting stretch

receptors (SARs), and C-fibres. Each is distributed throughout the tracheobronchial tree and the last is also in the alveolar wall. A fourth type of sensory receptor, less well defined, is also present, and is associated with neuro-epithelial bodies (Pack & Widdicombe, 1984). Table 1.1. summarises the 3 main groups of airway afferent sensory nerve endings in the lower airways, together with the properties of the fibre types and the airway responses elicted from each group of sensory nerve ending.

Table 1.1 Airway afferent sensory nerve endings, fibre types and airway reflexes.

sensory nerve ending	vagal fibre type	reflex
RAR	Aδ fibre, myelinated, fast conducting (> 5 m s^{-1})	cough, bronchoconstriction, mucus secretion.
SAR	Aδ/β fibre, myelinated, fast conducting (> 5 m s^{-1})	Hering Breuer, bronchodilatation, cough facilitation
C-fibres (pulmonary and bronchial)	C-fibres, non-myelinated, slow conducting (0.8 - 2.4 m s^{-1})	cough? bronchoconstriction, tracheal dilatation, mucus secretion, oedema, cough inhibition
Note: the sensory nerve endings of SARs, RARs and C-fibre endings are probably non-myelinated.		

Rapidly Adapting "Irritant" Stretch Receptors (RARs)

RARs were first described by Knowlton and Larrabee (1946). They identified nerve endings that had myelinated fibres and were stimulated by inflation and deflation but differed from Adrian's SARs (1933) in having a more rapid rate of adaptation with a prominent off response, a higher volume threshold and a more irregular pattern of discharge.

Since the first description of RARs by Knowlton and Larrabee (1946), there has been considerable confusion in the literature regarding the nomenclature of these nerve endings. The term "rapidly adapting stretch receptors" describes their behaviour to inflation, deflation and local probing of the airways and properly contrasts them with SARs. Widdicombe and colleagues (Fillenz & Widdicombe, 1971) introduced the term "irritant receptors" to acknowledge the response of the nerve endings to respiratory irritants such as ammonia, dust and cigarette smoke. Although this term describes some of the properties of nerve endings, it does not describe the original observations made by Knowlton and Larrabee (1946); both terms *irritant receptor* and *rapidly adapting stretch receptor* are widely used. In the present thesis, these airway sensory nerve endings will be referred to as rapidly adapting stretch receptors (RARs).

Other terms such as "cough receptors" (Fillenz & Widdicombe, 1971), "deflation receptors" (Koller & Ferrer, 1973; Roumy & Leitner, 1980) or Kollapsafferenzen (Homberger, 1968) have also been used to describe these afferent sensory nerve endings. The term "cough receptors" is only applicable to those sensory nerve endings located in the trachea and the major bronchi, since stimulation of these sensory nerve endings located deeper in the airways causes hyperpnoea rather than cough (Mills *et al.*, 1970; Widdicombe, 1981). In addition, stimulation of other areas outside the

respiratory tract can also elicit cough. The terms "deflation receptor" and Kollapsafferenzen have been used to describe nerve endings that are stimulated by forced deflation. However, they do not describe the responses of the nerve endings to hyperinflation and local probing of the airways. Moreover, the term "deflation receptors", albeit wrongly, has also been used to describe C-fibre endings (Paintal, 1955).

RARs are believed (Fillenz & Widdicombe, 1971; Widdicombe, 1981) to correspond to the epithelial nerve endings in the airways of several mammalian species (Larsell, 1921; Larsell & Dow, 1933; Elftman, 1943; Fisher, 1964; Fillenz & Woods, 1970). Although the vagal fibres are myelinated, the epithelial nerve endings are non-myelinated. The nerve endings branch extensively in the tracheobronchial submucosa, particularly at points of bronchial branching. The fine endings pass towards the mucosal surface between the columnar cells of the epithelium (Larsell, 1921; Elftman, 1943; Fisher, 1964a; Fillenz & Woods, 1970). The most superficial endings lie less than 1 μm from the airway lumen, where they are well sited to intraluminal irritation (Das *et al.*, 1978). The parent axons of the sensory nerve endings branch extensively to supply end formations to a wide area of the mucosa (Elftman, 1943; Fillenz & Woods, 1970). Superficial lesions of the receptor field abolishes the response to local probing but leaves the response to inflation and deflation (Sant'Ambrogio *et al.*, 1978). This confirms the histological evidence in suggesting a multi-branching structure for these endings. Degeneration experiments confirm that these intraepithelial terminals are vagal afferents (Das *et al.*, 1979; Hoyes & Barber, 1981).

RARs are located throughout the trachea and larger bronchi, with concentrations at the carina and the points of bronchial bifurcation (Widdicombe, 1964; Sant'Ambrogio, 1982; Coleridge & Coleridge, 1986). Although, findings in the cat would suggest that

there are few RARs deep in the lung (Widdicombe, 1954a), it has been shown that endings of myelinated fibres are present in the mucosa of the more peripheral airways, where columnar cells are replaced by progressively flatter cells (Coleridge & Coleridge, 1986). In the trachea and bronchi, RARs are distributed around the circumference, unlike SARs, which are found only in the posterior wall (Sant'Ambrogio *et al.*, 1978). In addition to their sensitivity to mechanical stimuli, RARs can be activated by inhalation of various gases such as ammonia, sulphur dioxide and cigarette smoke, and by a wide range of inflammatory mediators (Sant'Ambrogio, 1982; Coleridge & Coleridge, 1986).

A major difficulty in investigating the mechanism by which inflammatory mediators stimulate RARs is that all the mediators known to stimulate these nerve endings are themselves potent bronchoconstrictors, the inflammatory mediators acting directly on the airway smooth muscle to mediate the bronchoconstriction. Histamine, whether administered intravenously or as an inhaled aerosol, provokes bronchoconstriction and an increase in RAR activity (Mills *et al.*, 1969; Armstrong & Luck, 1974; Sampson & Vidruk, 1975; Adcock, 1989). Evidence from studies in guinea-pigs (Bergren & Sampson, 1982) and rabbits (Mills *et al.*, 1969, 1970) would suggest that stimulation of RARs by histamine is secondary to contraction of the airway smooth muscle, with RAR activation being blocked by the administration of bronchodilators. Coleridge *et al.*, (1978) also favour an indirect mechanism of action for histamine. They found an effect with histamine only when lung mechanics changed. Injection of histamine into the right heart caused bronchoconstriction and an increase in RAR activity, whereas injection of histamine into the left heart had little or no effect on RAR activity or pulmonary mechanics. In addition, stopping the respiratory movements, in artificially ventilated dogs, completely abolished the activating effect of histamine on RARs. However, a direct effect of histamine on RARs ending cannot be

ignored. Vidruk *et al.*, (1977) demonstrated in dogs that histamine, applied locally via a bronchoscope to the receptive field of RARs, still evoked an increase in RAR activity after the administration of a bronchodilator agent. In dogs, a comparison of the effects of histamine and acetylcholine on RARs would also indicate that, in this species, the afferent response to histamine cannot be accounted for by mechanical changes alone (Dixon *et al.*, 1979a). Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), administered intravenously or as an inhaled aerosol, also caused bronchoconstriction and an increase in RAR activity in dogs (Coleridge *et al.*, 1976). The investigators suggested from their findings that the effect of $PGF_{2\alpha}$ was secondary to changes in lung mechanics. However, in the same study, intravenous $PGF_{2\alpha}$ still activated RARs, even when the bronchoconstrictor effects of $PGF_{2\alpha}$ had been attenuated by inhaled isoprenaline, a β-adrenoceptor agonist.

In dog lungs, autocoids such as bradykinin and prostaglandin E_2 (PGE_2), which do not contract airway smooth muscle directly, have a weak effect on RARs (Coleridge *et al.*, 1976; Kaufman *et al.*, 1980; Roberts *et al.*, 1985). Even so, both bradykinin and PGE_2 evoke airway reflexes in humans and experimental animals.

An important function of RARs may be to signal the onset of pathophysiological changes in the airways. Support for the concept of a nociceptive function for RARs in the airways comes from the observations that the discharge and sensitivity of the RARs is increased in a number of pathological conditions, including acute pulmonary congestion and oedema, microembolism, anaphylaxis, pneumothorax and to various bronchoconstrictor drugs (Sellick & Widdicombe, 1969, 1970; Mills *et al.*, 1970). The increased discharge is related to a decrease in lung compliance which can be reversed by large lung inflations that restore the lung compliance to its baseline level (Coleridge & Coleridge, 1986).

There are species differences with regards to epithelial sensory nerves and the cough reflex from the tracheobronchial tree. Mice cannot cough and lack intraepithelial nerves (Korpás & Kalocsayová, 1975; Pack *et al.*, 1981). Ferrets also lack these nerve endings (Robinson *et al.*, 1986) and have no cough reflex from the trachea or carina, although coughing can be induced weakly from the bronchi (Korpás & Widdicombe, 1983). Rats have no cough reflex from the trachea or bronchi in response to mechanical or chemical stimulation, but have intraepithelial nerves (Jeffrey & Reid, 1973). However, rats do show a hyperpnoeic response to tracheal irritation, presumably mediated by these receptors (Korpás & Tomori, 1979). In the cat, the concentration of the intraepithelial nerves, highest at the carina, corresponds very closely with the mechanical stimuli for coughing (Das *et al.*, 1978). The lower incidence of coughing by tracheobronchial stimulation in new-born infant children (Miller *et al.*, 1952; Fleming *et al.*, 1978) and in new-born cats (Korpás & Kalocsayová, 1973) is consistent with sparse RAR activity in the early stages of development (Fisher & Sant'Ambrogio, 1982). In ageing dogs, there is both a decrease in the number of RARs and a loss of the cough reflex (Pontoppidan & Beecher, 1960).

The site of RARs and their responses to intraluminal stimuli indicate that the reflex action of these sensory nerve endings is to cause coughing from the larger airways and hyperpnoea from the smaller bronchi which can be abolished by vagotomy (Mills *et al.*, 1970; Widdicombe, 1981). The evidence is based on the correlation between reflexes from the airways and impulse activity from sensory nerve endings recorded from the vagus. Stimuli that excite RARs can induce tachypnoea by shortening expiratory time (Davies & Roumy, 1982). The same stimuli can cause occasional deep augmented breaths, that may reverse the tendency to collapse the lungs. The stimuli that excite RARs also cause laryngeal constriction, bronchoconstriction and tracheal

mucus secretion (Widdicombe, 1963, 1964; Coleridge & Coleridge, 1986). RARs have been described in human airways (Laitinen, 1985) and it seems likely that they play a role in physiological control of breathing, as well as in pathological conditions.

Slowly Adapting Stretch Receptors (SARs)

Slowly adapting stretch receptors with myelinated fibres are localised mainly to the airway smooth muscle of the trachea and larger bronchi and have the ultrastructural appearance of mechanoreceptors. They discharge during inflation and adapt slowly to maintained stretch of the airways. Collapse of the airways may either inhibit or stimulate them. They have been delineated by three-dimensional electron microscopy (Krauhs, 1984). Although their attachments to the smooth muscle are not certain, they are sensitised by smooth muscle contraction. They can also be inhibited by high carbon dioxide tension, but the physiological significance of this is uncertain. In general, their role is to signal the degree of stretch of the lungs, but their activity may be affected by various mechanical and chemical factors, including contraction of smooth muscle (Sant'Ambrogio, 1982; Coleridge & Coleridge, 1986).

These nerve endings are responsible for the Hering-Breuer reflex, which inhibits inspiratory activity; tonic discharge at high lung volumes prolongs expiration (Knox, 1973; Bradley, 1977; Coleridge & Coleridge, 1986). Thus they play a major reflex role in modifying the pattern of breathing, which is primarily set by the respiratory rhythm generator in the brainstem. They also evoke a reflex bronchodilatation, although the importance of this reflex in pathophysiology has not been studied (Widdicombe, 1963, 1987; Nadel, 1980; Coleridge & Coleridge, 1986). Despite extensive studies in animals there is little direct information about their role in humans. The bronchodilator reflex, however, may be correlated with the fact that

asthmatics tend to find that increasing their functional residual capacity is beneficial in relieving airway obstruction.

C-Fibres

It was Paintal (1955) who first investigated nerve fibre endings at the alveolar level, and subsequently called them J-receptors (Paintal, 1969). Nowadays, they are classified into two groups of similar nerve fibre endings, namely pulmonary and bronchial C-fibre endings, based on the branch of the blood circulation that supplies them. The fibres from these nerve endings are sometimes silent or have irregular, sparse discharges under normal conditions. Moreover, their action potentials are usually small and inconspicuous compared to those of myelinated fibres. It is not surprising, therefore, that action potential studies have tended to emphasise the function of nerve endings with myelinated fibres. Thus, C-fibres have, until recently, been somewhat neglected. They are found throughout the tracheobronchial tree and in the alveolar walls and probably in the laryngeal mucosa (Sant'Ambrogio, 1982; Coleridge & Coleridge, 1986). The nerve endings have not been unequivocally identified histologically, although non-myelinated nerves have been identified in the alveolar wall in rats (Meyrick & Reid, 1971) and in human airway epithelium (Laitinen, 1985). However, it cannot be established that these are not fibres passing through to other sites.

The nerve fibre endings are activated by almost the same group of stimuli as those that affect RARs, although in general they are less sensitive to mechanical stimuli such as lung volume changes (Coleridge & Coleridge, 1984, 1986). They are excited by some inhaled irritants such as sulphur dioxide, and many mediators associated with inflammatory conditions such as prostaglandins, histamine and bradykinin. In addition, they are also stimulated by foreign chemicals such as capsaicin (the extract of red

pepper) and phenylbiguanide, which have been used frequently to determine the reflex responses of C-fibre ending stimulation. The reflex responses to stimulation of C-fibre endings include apnoea, followed by rapid shallow breathing (Coleridge & Coleridge, 1984). The same pattern of breathing can be seen in the pathological conditions that stimulate C-fibre endings (and often RARs) for example, microembolism, lung congestion, oedema and pneumonia (Coleridge & Coleridge, 1984). C-fibre activation also causes reflex bronchoconstriction, laryngeal constriction, tracheal mucus secretion, hypotension, bradycardia and inhibition of spinal reflexes (Coleridge & Coleridge, 1984, 1986).

Local Axon Reflexes

Some of the non-myelinated nerves, connected higher up to myelinated nerves, contain neuropeptides such as substance P, calcitonin gene-related peptide (CGRP) and neurokinins A and B (Wharton *et al.*, 1979; Lundberg *et al.*, 1988; Springall *et al.*, 1988). These nerves are found in the airway epithelium around glands and airway smooth muscle and in the submucosa. They provide the potential for local axon reflexes, as have been studied in other tissues, such as skin and alimentary tract. The fibres can be activated by electrical antidromic stimulation down the vagus nerve. In addition, axon reflexes can be induced by capsaicin, which releases neuropeptides such as substance P, leading to local responses in the vasculature and airway smooth muscle, including bronchoconstriction (Lundberg & Saria, 1983). These studies are important, since they point to local pathological effects in diseases such as asthma mediated by sensory nerve endings, rather than by a direct action of mediators released from cells such as mast cells. The local responses can be prevented by substance P antagonists and depleting drugs, but not by atropine (Lundberg & Saria, 1983). Sensory nerve activation can also induce plasma protein extravasation and this, together with vasodilatation, is often referred to as "neurogenic inflammation" (Jansco

et al., 1967). Which group of nerve fibre endings is involved in axon reflexes is not entirely clear. Certain researchers believe that C-fibre endings seem to be the most likely candidates (Barnes, 1986; Lundberg *et al.*, 1988), whereas others favour the RARs (McDonald, 1987; Widdicombe, 1988). Nevertheless, it is possible that the impulse initiated at a sensory nerve ending not only reaches the central nervous system (CNS), where it may elicit a variety of reflexes, but it may also be transmitted into peripheral axonal ramifications with subsequent local release of neuropeptides. Whether local axon reflexes can occur without the CNS reflex is not yet clear.

Role of airway sensory nerves in airway reflexes

Assigning a specific role to the individual groups of respiratory tract afferents is becoming increasingly more difficult, primarily because many of the reflexes from the lungs such as laryngeal constriction, bronchoconstriction and mucus secretion, are thought to be common features of stimulation of both C-fibres and RARs in the airways. Single fibre recordings show that both RARs and C-fibre endings are stimulated in a variety of diseases (allergic reactions, microembolism, pulmonary oedema) and by mediators involved in tissue reactions in lung inflammation. They may also be stimulated by inhaled substances including mediators, foreign chemicals etc. Neuropeptides, involved in local axon reflexes and inflammatory reactions, may well be present in both RAR and C-fibre endings. There are some differences between the two groups of nerve fibre endings and between the pulmonary and bronchial C-fibres, but they may be quantitative rather than qualitative. Some foreign chemicals, for example, capsaicin and phenylbiguanide in small doses, are thought to affect C-fibre endings and not RARs (Coleridge & Coleridge, 1986). However, it is likely that larger doses stimulate both groups. Nevertheless, experiments with these agents support the role, along with the already established role of RARs, of C-fibres in reflex bronchoconstriction. There appears to be no selective chemical stimuli for RARs,

although histamine and $PGF_{2\alpha}$ were once thought of as such. A method commonly used to distinguish the two groups of afferent fibres is to differentially block conduction in the vagus nerves using cooling techniques. However, there is some inconsistency in the literature concerning these methods.

The reflex evoked from stimulation of sensory endings in the respiratory tract surrounded by the most controversy is that of cough. Cough is probably the most powerful and commonest reflex in airway diseases. Its function, to expel secretions and inhaled irritants from the airways, is immediately obvious. It is also accompanied by a reflex increase in airway secretion, by reflex bronchoconstriction (Widdicombe, 1963, 1977b) and by irritant sensations transmitted by the vagus nerve (Noble *et al.*, 1970; Guz, 1977). The sensations can be crudely localised to the region from which the cough arises and may be described as having a tickling, scratching or burning quality depending on the intensity of the stimulus. Coughing often gives temporary relief to such irritant sensations even when no secretion or inhaled material appear to be expelled, similar to relief of an itch of the skin by scratching. Cough and reflex bronchoconstriction often occur simultaneously in airway diseases and have been considered closely related. However, accumulating evidence, that demonstrates inhibition of cough reflex but not reflex bronchoconstriction by local anaesthetics, indicates that the reflexes may not have a common afferent pathway (Karlsson *et al.*, 1988). Many reviewers believe that cough is mediated by RARs in the airway walls (Sant'Ambrogio, 1982, 1987; Coleridge & Coleridge, 1986; Widdicombe, 1986b). Recently a number of authors have suggested that cough can be induced by C-fibre stimulation in the lungs and larynx (Collier & Fuller, 1984; Forsberg & Karlsson, 1986, 1987; Midgren *et al.*, 1986; Paintal, 1986; Fuller *et al.*, 1987a, b). It is generally accepted that RARs are involved in the cough reflex, based on evidence from reflex and nerve recording experiments. In contrast, most of the evidence that C-

fibres are involved comes from the fact that capsaicin, bradykinin and similar agents cause cough from administration of these agents by aerosol to conscious animals and man. Unfortunately, few studies, in particular not in man, have been undertaken to record action potentials in single fibres from the larynx and lower airways when agents used to stimulate C-fibre endings via the vasculature are administered as aerosols. Recent studies by Tatar *et al.* (1988) and Jackson *et al.*, (1989) have suggested that stimulation of C-fibre endings may suppress the cough response. Nedocromil sodium has been shown to inhibit citric acid aerosol-induced cough in conscious dogs (Jackson, 1988). Single nerve fibre recording studies, in dogs, have demonstrated that nedocromil sodium, administered intravenously or as an inhaled aerosol, does not stimulate or inhibit the pattern of discharge of SARs, RARs or pulmonary C-fibre endings but causes a stimulation of bronchial C-fibre endings (Jackson *et al.*, 1989). Indeed, the duration of the stimulation of bronchial C-fibre endings by nedocromil sodium given by aerosol, is similar to the duration of cough suppression when the drug is given by the same route (Jackson, 1988). Tatar *et al.*, (1988) demonstrated that the pulmonary C-fibre reflex, induced by i.v. capsaicin, inhibited mechanically-induced coughing and sneezing in cats. These researchers suggested that cough due to capsaicin administration in man and experimental animals (i.v. or as an inhaled aerosol) might be explained by activation of RARs in the larynx or lower airways.

Considering the evidence available, it is thus sometimes difficult to assign a specific reflex to one group of sensory nerve ending. It seems reasonable to suggest that the different groups of afferents may be involved, to a greater or lesser extent, in the reflex responses of the airways in disease and those evoked by inhalation of irritants into the respiratory tract. Even slowly adapting stretch receptors are now thought to play an integral part in the pattern of cough reflex (Sant'Ambrogio *et al.*, 1984).

1.3 The Cough Reflex

Coughing, a response which can be evoked voluntarily and involuntarily, is one of the body's defence mechanisms which prevents the entry of noxious materials into the respiratory tract, and clears potentially harmful debris from the lungs and airways. The act of coughing itself is simply a perturbation of the normal breathing pattern. The sequence of a cough (Fig. 1.1), whether it is voluntarily or involuntarily, usually involves a rapid deep inspiration. This is followed by glottic closure, relaxation of the diaphragm and muscle contraction against a closed glottis which raises thoracoabdominal pressures to 100 cmH_2O or more above ambient pressure. These positive pressures result in a narrowing of the central intrathoracic airways. The narrowed cross section is associated with high gas linear velocities and therefore with high shearing forces at airway walls and high kinetic energies. These conditions are probably important in suspending and clearing materials adhering to the airway walls. Once the glottis is opened, the combination of a large pressure differential between the airways and the atmosphere, coupled with airway narrowing, produces flow rates close to the speed of sound. There are wide variations in the sequence of a cough (for example, glottis remains open during cough, variable volumes in the initial inspiration of air) to the above description. Readers are referred to Leith *et al.*, (1986) for a more descriptive review.

The principal role of coughing, as mentioned earlier, is the clearance of harmful endogenous and exogenous materials from the lungs and airways. Coughing is also elicited by application of high distending pressures to the lungs. Such coughs set to limit maximal inspiration, for example in the presence of atelectasis (Burger & Mead, 1969), presumably protecting the lungs from over-distension and mechanical disruption. Coughing also has psycho-social functions. It may be used voluntarily as a discreet sign, or unconsciously with symbolic meaning (Gortjahn, 1972), or as a tic

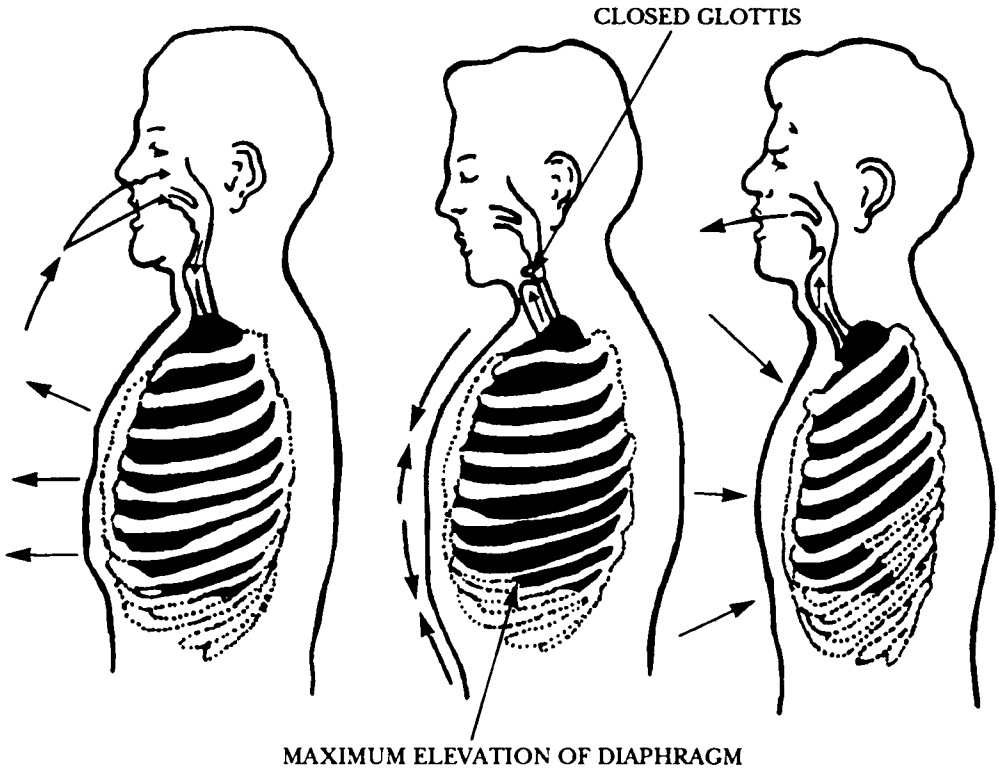


Fig. 1.1 Sequence of a cough. From left to right, inspiratory phase of coughing: a deep inspiration with maximum dilation of the tracheobronchial tree; compression phase (middle) compression of intrathoracic air against a closed glottis and the expiratory phase (right) forced expiration with open glottis.

(Berman, 1966; Huizinga, 1967). From a clinical viewpoint, cough has an important diagnostic value in both general and respiratory medicine. Cough is a simple symptom that can be traced in well over a 100 known diseases. In some cases, it is often the only symptom, for example in bronchial carcinoma. In other cases the duration of the cough is the main criterion for diagnosis, for example in chronic bronchitis.

Physiology of the cough reflex.

Reflex coughing, like any other reflex process, is made up of 5 components: afferent sensory nerve endings, an afferent pathway, a "cough centre" located in the medulla

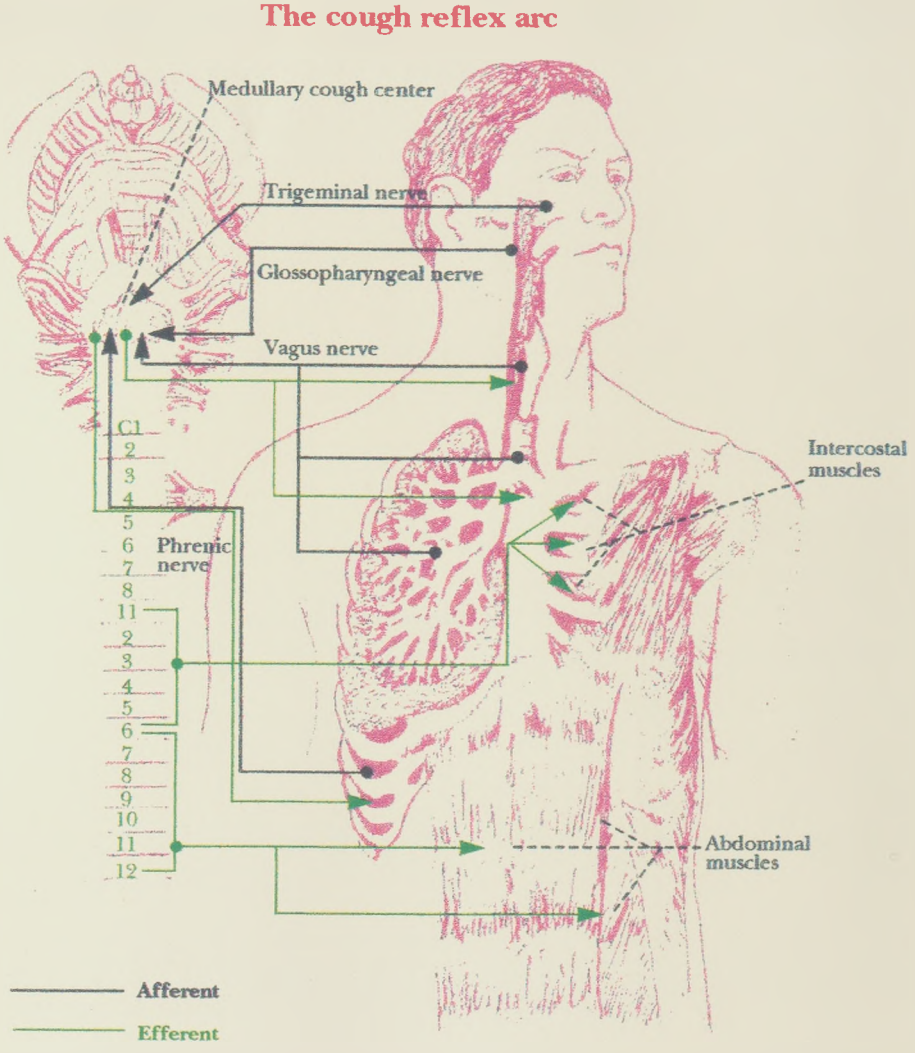


Fig. 1.2 Reflex arc of the cough reflex, showing the location of the main afferent sensory nerve endings, the main afferent pathways, the "cough centre" in the medulla oblongata, the efferent pathways and end-effector organs.

(Korpás & Tomori, 1979). Electrical stimulation of some areas of the brainstem causes coughing in the cat (Kase *et al.*, 1970), but, because the region is so close to the nucleus of the tractus solitarius, it is possible that the afferent input of the cough reflex is being stimulated. However, a dissociation of the central control of coughing from the respiratory rhythm generator can be established; some anti-tussive drugs such as codeine will stimulate breathing at the same time as the cough reflex is depressed (Bucher, 1958). Furthermore, the descending pathway to the abdominal

oblongata, an efferent pathway and end-effector organs (Fig. 1.2). In both man and experimental animals coughing is easily provoked by stimulation of "cough receptors" located predominantly, but not solely, within the respiratory tract but below the level of the oropharynx.

The larynx, trachea, large bronchi and, in particular, the carina and points of large bronchial branching are sensitive to both mechanical and chemical stimuli (Widdicombe, 1954b; Korpás & Tomori, 1979). However, it is very difficult to induce cough from the smaller airways and alveoli (Widdicombe, 1954b; Berglund. 1980). This is understandable, since in the smaller airways even a vigorous cough would not move gas fast enough to cause turbulence and shearing forces at the airway wall. Therefore, cough at this level would be ineffective.

The tracheobronchial tree takes its innervation from the vagus nerve and the larynx from the superior laryngeal nerve (although fibres innervating the most caudual part may also pass through the inferior laryngeal nerve). Cough can also be elicited from areas other than the larynx and tracheobronchial tree. Other afferent pathways from "cough receptors" include the trigeminal nerve from the nose and paranasal sinuses; the vagus from nerve endings in the stomach, pleura and ear passages; the phrenic nerve from nerve endings in the stomach and pericardium and the glossopharyngeal nerve from nerve endings in the larynx (Irwin *et al.*, 1977).

The afferent pathways for the cough reflex pass to the medulla oblongata, where they relay in or around the nucleus of the tractus solitarius. However, little is known about their further connections or how they link with the brainstem respiratory complex (Korpás & Tomori, 1979). Animal studies suggest that there is a "cough centre" independent of those areas responsible for the generation of respiratory pattern

muscles participating in coughing can be separated from spinal pathways driving rhythmic inputs to the diaphragm (Newsom Davis & Plum, 1972).

A number of interactions between coughing and other nervous inputs can be demonstrated. One of the best established is that from slowly adapting stretch receptors (which are responsible for the Hering Breuer Inflation reflex). Augmentation of this reflex enhances the strength of the expiratory efforts of cough (Bucher, 1958; Hanacek *et al.*, 1984; Sant'Ambrogio *et al.*, 1984); abolition of slowly adapting stretch receptor activity weakens the strength of the expiratory efforts of coughing (Davies *et al.*, 1978). Other interactions include stretching of the respiratory muscles (myotatic reflexes) and the influence of higher brain centres (especially of the cerebral cortex and hypothalamus).

The efferent pathways include the vagus nerve, which transmits impulses to the larynx, trachea and bronchi, and the spinal and phrenic nerves which transmit impulses to the diaphragm and other respiratory skeletal muscles.

Etiology of cough.

Coughing can be provoked by a multiplicity of diseases and stimuli (Leith., 1977; Lowry & Higenbottam; 1991) (Table 1.2). The causes may be obvious (for example, respiratory tract infection, asthma) or as rare and obscure as neurilemomas of the vagus (Leichtling *et al.*, 1963), hairs touching the tympanic membrane (Wolff & May, 1973) and transvenous pacemakers (Kang *et al.*, 1971). In essence, an increase in the activity of the "cough receptor" or afferent pathway above threshold level will provoke cough.

Table 1.2 Some Causes of Cough

Chemical irritants
tobacco smoke, smog, noxious gases in the workplace
high levels of atmospheric SO_2
capsaicin, histamine, prostaglandins, acetylcholine, citric acid

Mechanical irritants
retained bronchopulmonary secretions
foreign bodies within the airways
postnasal drip; fine dusts

Infectious diseases
Viral: most common, rhinovirus, parainfluenza and adenovirus
Bacterial:
Atypical pneumonia organisms, for example, Chlamydia, Bordetella pertussis, pneumonias, tuberculosis

Opportunistic organisms in immunocompromised patients: pneumocystis carinii, cytomegalovirus, herpes simplex virus
acute bronchitis
pericarditis, pleuritis

Airways disease
asthma and allergic rhinitis
chronic bronchitis, bronchiectasis, emphysema
cystic fibrosis

Interstitial lung disease
sarcoidosis, pulmonary fibrosis (e.g., cryptogenic fibrosing alveolitis)
pneumoconiosis, collagen vascular disease, granulomatous disease

Pulmonary vascular disease
pulmonary emboli, pulmonary hypertension

Neoplastic
laryngeal tumour, endobronchial tumour
extrinsic compression of the airway

Drug-induced
ACE-inhibitors

Psychogenic

The precise mechanism by which "cough receptors" are activated is unclear. Findings, from both experimental animals and man, suggest that there are three possible mechanisms by which "cough receptors" can be activated: either indirectly, or by a direct chemical action on the sensory nerve ending or a combination of the two.

Salem & Aviado (1970) suggested that the proximate cough stimulus is invariably mechanical. They called attention to the fact that most tussive agents provoke bronchoconstriction and that bronchodilators have anti-tussive properties. Hence, contraction of the airway smooth muscle and/or a reduction in lung compliance may cause distortion of the airway tissue surrounding the sensory nerve endings, thus activating the sensory nerve endings (Mills *et al.*, 1969; Coleridge *et al.*, 1978). In addition to bronchoconstriction, other mechanical stimuli may be involved in spontaneous cough. Respiratory manoeuvres, such as deep inspiration and forced expiration, can also provoke cough in susceptible individuals. The capacity of respiratory manoeuvres to discharge airway sensory nerve endings is well established, and is the basis for classifying vagal fibres (Section 1.2). The presence of excess airway secretions, probably by distorting the airway tissue and/or altering the sensory nerve ending environment, is another well known mechanical stimulus for cough.

It is doubtful that a mechanical effect is the sole mechanism by which "cough receptors" are activated. Bradykinin, capsaicin, and some prostaglandins (Coleridge & Coleridge, 1984) are believed to provoke cough and bronchoconstriction by direct stimulation of non-myelinated C-fibres endings. Vidruk *et al.*, (1977) have shown that histamine can increase the activity of RARs in the absence of changes in lung mechanics. In normal human volunteers, Eschenbacher *et al.*, (1984) demonstrated that inhalation of hypotonic aerosols (low anion concentration) provoked cough that was not dependent on bronchoconstriction.

It is more likely that "cough receptors" are, in most cases, activated by a combination of a direct and indirect effect. Inert carbon particles are thought to elicit cough by mechanical distortion of nerve endings. However, the carbon particles may also damage the airway mucosa, causing release of chemical mediators that stimulate afferent nerves. Smoking is by far the most commonly implicated etiological factor causing chronic persistent cough (Wyander *et al.*, 1965) and may do so by two mechanisms. It may act as a bronchial irritant and directly stimulate the sensory nerve endings, or more importantly, it may induce inflammatory changes and large amounts of secretions that stimulate the sensory nerve endings by a mechanical action. One way to reduce the frequency of cough in this population is to discontinue smoking. Indeed, within four weeks, 54% of smokers with a bronchial cough are freed of their cough (Wyander *et al.*, 1967).

Experimental cough in man.

Coughing can be induced by inhalation or intravenous administration of a number of irritants and inflammatory mediators: this gives some insight into the mechanism of the human cough reflex and a means of assessing the effectiveness of anti-tussives. These tussive agents can be classified into a number of different groups (Table 1.3).

Table 1.3 Tussive agents in humans

Inflammatory mediators	Chemical Irritants	Osmotic/low Cl^- solutions	Mechanical
Histamine	Capsaicin	Distilled water	Bronchoconstriction
Bradykinin	Nicotine	Hypertonic saline	Instrumentation
PGE_2	Metabisulphite	Urea solutions	Aerosols
$PGF_{2\alpha}$	Sulphur dioxide	Sugar solutions	Dust
Acetylcholine	Cs gas	Lactose	Carbon particles

Tachykinins	Lobeline
	Citric acid
	Acetic acid
	Ammonia

Earlier cough studies incorporated the use of ammonia fumes, citric acid, acetylcholine and less frequently, sulphuric acid and cigarette smoke as tussive agents (Korpás & Tomori, 1979; Braga & Allegra, 1989). Citric acid, first used by Bickerman and Barach (1954), has been the most frequently used tussive agent for experimental investigation and in the evaluation of anti-tussive drugs in man (Braga & Allegra, 1989). However, the use of citric acid has been limited by tachyphylaxis of the cough response to repeated exposure of this agent (Bickerman *et al.*, 1956).

More recent studies to investigate the cough reflex in man, have incorporated the use of stimuli which are believed to be selective for different groups of afferent sensory nerve endings. Inhalation of solutions of low chloride ion concentration (for example, distilled water) are thought to elicit cough in normal and asthmatic subjects by stimulation of "irritant receptors" (Boushey *et al.*, 1974; Boggs & Bartlett, 1982). Cough can also be elicited by inhalation of capsaicin and PGE_2 (a product of arachidonic acid metabolism) (Collier & Fuller, 1984; Costello *et al.*, 1985). Both of these agents are thought to evoke cough through their "selective" action on non-myelinated C-fibre endings (Coleridge & Coleridge, 1984). The use of PGE_2 in evaluating possible anti-tussive therapies is limited by the finding that the cough response to PGE_2 shows both adaptation and tachyphylaxis (Lowry & Higenbottam, 1989; Higenbottam & Lowry, 1990). Capsaicin, on the other hand, causes coughing which is dose-dependent and more reproducible (Collier & Fuller, 1984; Fuller *et al.*, 1988) hence, the increasing use of this agent, by a number of investigators, to assess

the effectiveness of anti-tussives. However, to date; only local anaesthetics (lignocaine) and systemic opiates (for example, morphine and codeine) have been successful in attenuating capsaicin aerosol-induced cough (Fuller, 1991).

The findings that inhaled capsaicin and can evoke cough in laryngectomised (Morice & Harris, 1990) and that the cough response to distilled water is abolished in heart-lung transplant recipient patients (Higenbottam *et al.*, 1989b) would suggest the involvement of lower respiratory tract afferent nerve endings in the cough response to these two cough stimuli. Hence, evaluation of the cough response to a particular irritant requires not only information on the afferent susceptibilities of the various sensory nerve endings, but also knowledge of the anatomical distribution, along the airways, of the sensory nerve endings responsive to that particular irritant.

Clinical Implications of Coughing

When cough does not fulfil its clearance role, or if the cough is excessive, or if it persists, the cough discredits the patient both physically and socially (especially if the cough is incessant and in other cases when it causes urinary incontinence). Although, in most cases it is not life threatening, coughing demands increased oxygen consumption and can impair respiration. In addition, it stresses the patient, robs the patient of sleep, disturbs food intake and may cause giddiness, vomiting and general exhaustion.

Although coughing does not over distend the lungs, it can cause considerable lung disruption. Coughing can cause rupturing of pulmonary alveoli (Munsell, 1967), infiltration of air into the interstitial tissues of the lungs (Macklin & Macklin, 1944), mediastinum (Munsell, 1967; Roe & Kulkami, 1967), body wall and body cavities (pleural, pericardial and peritoneal) but not into the circulation (James, 1968). The

violent and intense muscular contractions during coughing can result in substantial stresses on the body wall, causing laryngeal and/or airway injuries (Fisher, 1964b; Von Leden & Isshiki, 1965), fractured ribs (Warmington, 1966; Kopcsanyi *et al.*, 1969), vertebrae fractures (Bickerman, 1960) and barotraumas (Munsell, 1967). In addition, injuries may arise in other areas of the body, for example torn recto abdominal muscles, hernias or vaginal prolapses. In asthmatic patients, coughing can precipitate wheezing, reduced expiratory flow-rates and asthma attacks (Simonsson *et al.*, 1967; Stanescu & Teculescu, 1970).

The high thoracoabdominal pressures, transient or sustained, generated during cough are transmitted to both the circulatory and cerebral spinal fluid (CSF) systems, producing a range of important but yet incompletely understood effects (Leith *et al.*, 1986). Venous return, left and right filling of the heart and afterloads, systemic arterial flow distribution and circulatory reflexes are also profoundly influenced during cough. Coughing can result in elevation of CSF pressures. If the increase in CSF pressure is equal to and simultaneous with the increase in thoracoabdominal pressure, cerebral perfusion pressure can fall. Persistent falls in cerebral perfusion pressure can result in syncope (Leith *et al.*, 1986).

Cough, when it fulfils its physiological role, should be preserved and supported by treatments that make expectoration easier and more efficient. However, when cough becomes pathological, the cough reflex should be suppressed. Suppression of cough is the basis for a whole field of pharmacology. However, in spite of the extensive study of the cough reflex in the last three decades, the physiological and pathological mechanisms of cough have not been classified. Although much is known about the processes that elicit cough, our knowledge concerning which group/groups of afferent

sensory nerve endings and the mechanisms that initiate their stimulation is still incomplete.

The absence of cough, or its failure to be effective, can also be life threatening, particularly in the new-born or in patients who are comatose or paralysed or in individuals who, after surgery, are limited by expiratory effort. Thus the developmental aspects of cough, the mechanisms of its failure, the methods to stimulate it, to evaluate and enhance its effectiveness and to replace it with various therapeutic manoeuvres and devices, are also of considerable interest (Leith *et al.*, 1986).

1.4 Asthma, bronchial hyperreactivity and hyperalgesia

Evidence from clinical studies emphasises that inflammation of airways is associated with even mild forms of asthma (Alexander *et al.*, 1991). In other tissues such as the skin and joints, the cardinal signs of inflammation are erythema, oedema, leucocyte infiltration, pain/hyperalgesia and tissue damage with loss of function. Most of these signs of inflammation are also apparent in the asthmatic airway. Mucosal oedema, along with smooth muscle contraction and mucus secretion, contributes to narrowing of the airways. Sensitisation of airway afferent sensory nerve endings involved in evoking cough (for example by inflammatory mediators) may result in a reduction in the threshold level required to evoke coughing. Infiltration of leukocytes, particularly eosinophils, is evident, loss of pulmonary epithelium occurs and the laying down of sub-mucosal collagen may be evidence of other aspects of tissue damage. "Loss of function" in the asthmatic airway is the impairment of airflow and diminished gas exchange.

Exaggerated reactivity of the trachea and bronchi to various physical, chemical and pharmacological stimuli is probably the most characteristic feature of patients with asthma or respiratory tract infection. The mechanisms of this non-specific bronchial hyperreactivity are still not known. Many theories have been put forward to explain bronchial hyperreactivity, including autonomic control of the smooth muscle, the smooth muscle cell itself, mucosal permeability, and epithelial damage and inflammation (Boushey *et al.*, 1980; Nadel, 1983; Nadel & Holtzman, 1984; Barnes, 1985; Boushey, 1985).

Bronchial hyperreactivity was first described in 1921 by Alexander and Paddock when it was observed that asthmatics wheezed when given pilocarpine, which was relieved by an injection of epinephrine. This led to the speculation that the basic defect in asthma was an imbalance between the cholinergic (excitatory) and sympathetic (inhibitory) nervous systems so that the balance favours excitation, resulting in excessively sensitive airways. Several different autonomic abnormalities have been proposed, including enhanced cholinergic (Simonsson *et al.*, 1967; Reed, 1974), α-adrenergic (Reed, 1974), or non-cholinergic excitatory (substance P) mechanisms (Lundberg *et al.*, 1983a, b, c), or reduced β-adrenergic (Szentivanyi, 1968; Reed, 1974) or non-adrenergic inhibitory (vasoactive intestinal peptide, VIP) mechanisms (Richardson, 1981; Barnes, 1984).

The non-specific hyperreactivity is probably not due to an increase in the sensitivity of the airway smooth muscle to spasmogens as *in vitro* smooth muscle from asthmatic lungs are not more sensitive than normal to contractile agonists (Schellenberg & Foster, 1984; deJonste *et al.*, 1987; Whicker *et al.*, 1988). Hence, the expression of hyperreactivity appears to be due to the increased responsiveness of cells within the lung apart from smooth muscle.

One alternative cause of non-specific hyperreactivity may be that lung sensory nerve endings have a decreased threshold to stimulation due to local inflammation in the lung, analogous to hyperalgesia in other inflamed tissues (Adcock & Garland, 1993). Thus, subsequent stimulation of the airway receptors may lead to exaggerated vagal cholinergic reflexes as well as NANC bronchoconstriction and proliferation of neurogenic inflammation (Adcock & Garland, 1993).

The proposal that hyperalgesia may be analogous to the airway hyperreactivity seen in asthma leads to an interesting line of investigation, that is, whether inflammatory mediators that evoke hyperalgesia are capable of inducing airway hyperreactivity. Clearly, the most appropriate organ in which to study hyperalgesia in the context of airway hyperreactivity is the lung. Although it is possible to demonstrate sensitisation of nerve endings in the airways (Adcock, 1989), it is difficult to assess pain in the airways. However, data from experiments on hyperalgesia using simpler techniques in, for example, the rat hind paw (Randall & Selitto, 1957; Ferreira & Nakamura, 1979) provide preliminary evidence that may point the way for future studies in the lung.

Many studies of hyperalgesia have made use of a technique in rats where a mildly painful stimulus is provided by applying a metal wedge at increasing pressure to a hind paw and recording the time taken for the rat to withdraw the paw (Randall & Selitto, 1957). In control rats, the time taken for such a response to occur is very reproducible and becomes shorter after injection of a hyperalgesic stimulus. Measuring the reaction time before and after injection of a hyperalgesic stimulus into the plantar surface of a paw provides an assessment of the degree of hyperalgesia.

Prostaglandins (for example, PGE_2 and prostacyclin) are capable of producing both acute and sustained hyperalgesia in the rat paw model (Ferreira, 1972; Willis & Cornelsen, 1973; Ferreira *et al.*, 1990). In acute hyperalgesia, the effect of prostacyclin is greater but of shorter duration than that of PGE_2. Acute hyperalgesia can also be induced by 5-lipoxygenase products (LTB_4, Levine *et al.*, 1986) and 15-lipoxygenase products (8R, 15S-diHETE, Levine *et al.*, 1986; 15-HPETE, Follenfant *et al.*, 1990). In addition, repeated injection of 15-HPETE (1 ng), into the rat paw, induces sustained hyperalgesia (Follenfant *et al.*, 1990). Platelet activating factor and neuropeptides (for example, substance P, neurokinin and CGRP) can also provoke acute and sustained hyperalgesia (Adcock & Garland, 1993). Recent interest, in stimuli that evoke hyperalgesia, has focused upon glutamate. Glutamate, a major excitatory neurotransmitter in the CNS, has been shown to coexist with substance P in some primary neurones (Battaglia & Rustioni, 1988). In the rat paw model, glutamate administered by sub-plantar injection, is a potent hyperalgesic agent (Follenfant & Nakamura-Craig, 1992).

These observations with inflammatory mediators (for example, 15-HPETE), neuropeptides (for example, substance P) and neurotransmitters (for example, glutamate) may be relevant to airway hyperreactivity in asthma as the majority of these agents are already implicated in bronchospasm and other airway responses. It is interesting that PAF provokes sustained hyperalgesia in the rat paw (Nakamura-Craig, unpublished observations) since recently it has been found that PAF-induced airway hyperreactivity in rabbits is prevented by pre-treatment with capsaicin, implying the involvement of sensory neurones in this response (Campbell *et al.*, 1989). Sustained hyperalgesia with 15-HPETE is also very relevant since 15-lipoxygenase is the major route of metabolism for arachidonic acid in lung epithelial cells. Thus, 15-HPETE, produced by lung epithelial cells, may decrease stimulation

threshold of sensory nerves that lie between or beneath the cells. Thus it becomes important to discover whether inhalation of hyperalgesic agents, as determined for example, in the rat paw model, increase airway hyperreactivity *in vivo*, and whether these agents lower the stimulation threshold of sensory nerve endings in the lung.

1.5 New approaches to the attenuation of afferent sensory nerve activity

In a number of airway diseases (for example, asthma) airway reflexes, such as cough and bronchoconstriction, are often exaggerated. Cough and reflex bronchoconstriction, like any other reflex processes, are made up of 5 basic components: afferent sensory nerve endings, an afferent pathway, a "regulating centre" in the CNS, an efferent pathway and end-effector organs. Therefore, pharmacological intervention is possible at a number of sites (for example, attenuation of afferent sensory nerve activity, inhibition of neurotransmitter release from efferent nerve endings, inhibition of end-effector organs, decrease in the activity of the "regulating centres" in the CNS).

Opiates (for example, codeine, hydrocodone, dextromethorphan) are the most effective and commonly used agents in the treatment of cough. It is generally assumed that the anti-tussive effects of these agents are mediated through an action on the "cough centre" in the medulla (Eddy *et al.*, 1969; Salem & Aviado, 1970). However, use of these agents is often associated with unpleasant side effects, such as respiratory depression, constipation and, sometimes, addiction.

Atropine and related anti-cholinergic agents are non-selective muscarinic receptor antagonists, which antagonise the effects of acetylcholine on post-junctional cholinoceptors. These drugs, therefore, should attenuate the vagal cholinergic bronchoconstriction that may contribute to airway obstruction. The variable effect of

these agents, possibly as a result of their non-selectivity for muscarinic receptor binding sites, has limited their use in the clinical management of asthma. However, ipratropium bromide, which is available in aerosol form, is widely used in asthma for its topical action; although minimal systemic absorption may contribute to certain side-effects such as urinary retention, particularly in elderly patients.

β_2 sympathomimetics, (for example, salbutamol and salmeterol) are highly potent bronchodilators and antagonists of bronchoconstriction (Choo-Kang *et al.*, 1969; Kennedy & Simpson, 1969; Ahrens *et al.*, 1987). These agents can, therefore, attenuate the vagal cholinergic bronchoconstriction that may contribute to airway obstruction, albeit indirectly. Since stimulation of β_2 adrenoceptors can inhibit the release of acetylcholine from cholinergic nerves of human bronchi, *in vitro* (Rhoden *et al.*, 1989), it is possible that inhaled β_2 agonists may also be able to attenuate vagal tone. The role of β_2 agonists in the treatment of asthma, however, has come under scrutiny following the recent controversy surrounding the regular use of β_2 agonists and asthma deaths (Sears *et al.*, 1990) and fears that new longer acting β_2-adrenoceptor agonists, such as salmeterol, may be used inappropriately by asthmatic patients and thereby mask the underlying pathology of the disease (Abbot, 1988). However, the Committee on Safety of Medicine has since advised doctors in the UK that there is no evidence to link the use of β_2 agonist aerosols with asthma mortality seen in the UK between 1980 and 1990 (CSM working party, 1992). Indeed, β_2 agonists, if used appropriately, are likely to continue to have a major beneficial role in the treatment of asthma (Twentyman & Higenbottam, 1992).

In addition to their bronchodilator properties, β_2 agonists and muscarinic antagonists are also effective in inhibiting experimentally-induced cough in man (Lowry *et al.*, 1987, 1988). However, their use in treating pathological cough may be limited; the

muscarinic antagonist oxitropium bromide has been shown to be ineffective in inhibiting cough associated with an upper respiratory tract viral infection (Lowry *et al.*, 1990).

Considering the side effects and/or the lack of direct effect of treatments in attenuating vagally-mediated airway reflexes, an alternative approach is required to examine the hypothesis that airway reflexes are involved in asthma. One such approach has been based upon a local anaesthetic action on afferent sensory nerve endings (Dain *et al.*, 1975, Cross *et al.*, 1976; Karlsson, 1987). Topically applied local anaesthetics (for example, lignocaine) are used during bronchoscopies to inhibit the sensory nerve endings responsible for evoking cough. However, inhalation of local anaesthetics is not without hazard (Efthimiou *et al.*, 1982, Karlsson, 1987) and is often associated with bronchoconstriction (Thomson, 1978). Despite the limited uses of local anaesthetics in attenuating afferent sensory nerve activity, there has been continued interest in the development of pharmacological agents which may act on the "afferent limb" of vagally-mediated airway reflexes. Indeed, a number of compounds have recently been described (Adcock & Garland, 1993) which may attenuate afferent sensory nerve activity, however, only those which are relevant to the present study are discussed.

443C81

H-Tyr-D-Arg-Gly-Phe(4-NO_2)-Pro-NH_2

443C81, a compound originally developed as an analgesic agent, is an opioid peptide which activates μ-opioid receptors on peripheral sensory nerve endings (Adcock, 1989). Structurally, 443C81 is a highly polar pentapeptide and because of its low lipid solubility, crosses the blood-brain barrier and penetrates the CNS very poorly, if at all (Follenfant *et al.*, 1988). The compound has anti-tussive properties in guinea-pigs and

cats, an effect which is blocked by the peripheral opioid antagonist N-methyl nalorphine (Adcock, 1987, 1988). Evidence for an effect of 443C81 on sensory nerves comes from single vagal nerve fibre recording studies in anaesthetized, paralysed and artificially ventilated cats (Adcock, 1989). In these experiments, 443C81 (intravenously or as an inhaled aerosol) attenuated spontaneous and capsaicin-evoked discharges in C-fibres. 443C81 was equally effective in inhibiting spontaneous and histamine-evoked discharges in Aδ fibres arising from intrathoracic RARs. The effects of 443C81 on sensory nerves have been corroborated by the ability of this compound to inhibit the cough reflex in cats (Adcock, 1987) and reflex responses to either inhaled capsaicin in normal airways (Adcock & Smith, 1989) or to inhaled histamine in hyperreactive airways (Adcock, & Beesley, 1989). In addition, 443C81 also inhibits capsaicin aerosol-induced cholinergic and non-adrenergic non-cholinergic bronchoconstriction in anaesthetized guinea-pigs (Buchan *et al.*, 1989). Therefore, in addition to its ability to prevent sensory nerve activation, 443C81 has the potential to modulate local axon reflexes by acting pre-junctionally to inhibit the release of sensory neuropeptides (Adcock, & Smith, 1989; Shankley *et al.*, 1989).

Frusemide

Inhalation of the loop diuretic frusemide attenuates the cough response to distilled water (low ion concentration and low osmolarity) in both normal and asthmatic subjects (Ventresca *et al.*, 1990; Wood *et al.*, 1990b). The cough response to distilled water is thought to be mediated by myelinated laryngeal "irritant receptors" (Boggs & Bartlett, 1982). It is possible, therefore, that frusemide may act on afferent sensory nerves in the respiratory tract. Indeed, Sant'Ambrogio and colleagues (1993) demonstrated that frusemide, inhaled or instilled into the larynx, can attenuate dextrose solution (a solution with normal osmolarity but which lacks ions) induced

stimulation of myelinated laryngeal "irritant receptors" in spontaneously breathing dogs.

Lamotrigine

Lamotrigine, a glutamate-release inhibitor (Leach *et al.*, 1986), is currently used as an anti-convulsant agent in the treatment of epilepsy. However, the finding that lamotrigine attenuates both PGE_2-induced acute and sustained hyperalgesia (Nakamura-Craig & Follenfant, 1992), has raised the possibility that glutamate-release inhibitors, such as lamotrigine, may also represent a new class of analgesic agents. The mechanism by which lamotrigine exerts its anti-nociceptor properties, is unclear, but may involve an inhibitory effect on afferent sensory nerve endings and/or dorsal horn transmitter release (for example, glutamate and neuropeptides).

1.6 Aims of the thesis

The aims of the present research are as follows:

a) To investigate the distribution of airway sensory receptors that are responsive to inhaled capsaicin and prostaglandin E_2 in normal human subjects.

b) To examine the effects of inhaled capsaicin and prostaglandin E_2 on rapidly adapting stretch receptors in cats, using the technique of single nerve fibre recording.

c) To determine if any observed effect of inhaled capsaicin and prostaglandin E_2 on rapidly adapting stretch receptor nerves is mediated by direct stimulation of the nerve ending or through a secondary mechanism.

d) To examine if the neurotransmitter glutamate, when administered to the lungs and airways by inhalation, contributes to airway hyperreactivity by an action on airway sensory nerves.

e) To investigate the actions of two agents, marketed for use in completely unrelated conditions, frusemide (diuretic) and lamotrigine (anti-epileptic), on the activity of vagal afferents and airway reflexes, for the following reasons:

i) To determine if frusemide exerts its anti-tussive and anti-bronchoconstrictor actions in the airways, by modifying the spontaneous impulse activity of vagal afferents from rapidly adapting stretch receptors.

ii) To determine if the glutamate release inhibitor lamotrigine represents a new class of anti-asthma agents by an action on airway sensory nerve endings or at a point more centrally in the reflex, .

g) To contribute to the understanding of the physiological roles of rapidly adapting stretch receptors in vagal reflexes in the airways.

Chapter 2

General Methods and Materials

2.1 Introduction

This chapter describes the methodology which applies to nearly all of the forthcoming chapters. Methods and protocols which are unique to a chapter are not included here, but in the chapter to which they relate.

All of the animal experiments were carried out in the Department of Pharmacology, Wellcome Research Laboratories (W.R.L.). For Home Office purposes, the animal experiments were carried out under the supervision of Dr. J.J. Adcock and Mr. Peter Buchan.

2.2 Animals

Animals used throughout were male mongrel cats which were supplied by Hillgrove Family Farm (Oxfordshire). The cats were in the weight range 2.5-5.0 kg. They were housed in communal pens in the Department of Animal Health Control, W.R.L., and fed on a normal diet of "Whiskers" or "Kit E Kat", milk and water. Each cat was fasted overnight prior to experiment.

2.3 Anaesthesia and neuromuscular blockade

Anaesthesia was induced with 5% halothane (carrier gas, O_2 8 l min^{-1}) in a perspex box. The animals were removed from the box and maintained on halothane through a face mask. The right femoral vein was cannulated (Portex 4fg) for administration of α -chloralose (dissolved in saline) at 60 - 80 mg kg^{-1}. Depending on the depth of anaesthesia, this was supplemented occasionally during the experiment with i.v. α-chloralose, 3 mg kg^{-1}. The depth of anaesthesia was frequently assessed by monitoring the response of heart rate and blood pressure to noxious stimuli.

In all of the experiments, the animals were paralysed with dimethyl tubocurarine (DMT). DMT (dissolved in saline) was administered intravenously, initially at a dose of 0.125 mg kg^{-1}, and was then followed 90 mins later and every hour thereafter with 0.0625 mg kg^{-1} to maintain paralysis. Each dose of DMT was accompanied by 3 mg kg^{-1} α-chloralose, to ensure maintenance of anaesthesia. All of the animals were artificially ventilated using the parameters described in the next section.

2.4 General Surgical Techniques and Recording of Pulmonary Mechanics and Cardiovascular Parameters

Figure 2.1 shows a schematic representation of the recording arrangement for measuring pulmonary mechanics. The cervical trachea was cannulated with a short length of endotracheal tubing (Magill's tube 4.5 mm). Airflow was recorded with a heated Fleisch pneumotachograph (size 00) and an Ether UP1 or Grass differential pressure transducer. Depending on the type of the experiment, transpulmonary pressure (P_{Tp}) was measured with either an Ether UP1 or Statham differential pressure transducer in two ways. In single nerve fibre recording experiments, the difference in pressure between the endotracheal cannula and atmosphere was measured, mid-line thoracotomies having been performed in all of the animals. The chest was held open with retractors and covered with cling film to preserve moisture and heat. Opening of the chest, as well as providing an accurate measurement of pulmonary mechanics, was necessary for determining the location of airway sensory nerve endings at the end of this particular type of experiment (Section 2.6). In preliminary reflex bronchoconstriction experiments, cats with a closed chest, an oesophageal balloon was inserted via the throat and positioned in the region of the thorax (Chapter 7). The difference between the pressure in the oesophageal balloon and that in a side arm of the endotracheal cannula was measured. However, this method produced artefacts, especially when histamine was administered

intravenously; histamine as well as causing contraction of airway smooth muscle, would also cause contraction of the oesophageal tissue surrounding the balloon. In later reflex bronchoconstriction experiments, transpulmonary pressure was measured in animals with an open chest. This method was found to be a more accurate means of measuring transpulmonary pressure and was less likely to produce artefacts. In both experimental set-ups, the lungs were prevented from collapse by maintaining a positive pressure of 2 cmH_2O and artificially ventilated (Palmer ventilator) at 27 strokes min^{-1} of laboratory air with a tidal volume (V_T) of 15 ml kg^{-1}. The electronic signals from the differential pressure transducers were input into an on-line analogue pulmonary mechanics analyser (Buxco Electronics, Model 4 or 6) for derivation of the peak values of airflow, tidal volume, transpulmonary pressure, total lung resistance (R_L) and dynamic lung compliance (C_{Dyn}). The parameters were recorded continuously, on a breath-by-breath basis, on a Beckman R611 or Grass 78B recorder. Internal calibration signals within the analyser allowed simple calibration of all the parameters.

The left femoral artery was cannulated (Portex 4fg) for the measurement of arterial blood pressure (Statham P23 pressure transducer). Heart rate was derived from the arterial pulse with a cardiotachometer. Systemic arterial blood pressure and heart rate were recorded continuously on a Beckman R611 recorder or Grass 78B recorder.

The left femoral vein, right jugular vein and right carotid artery (passed to the ascending aorta/aortic arch) were also cannulated (Portex 4fg) for the administration of drugs. Body temperature was monitored with a rectal thermometer and maintained at 37-39^{o}C with a heated blanket and control unit (Harvard). Arterial blood gases were measured (Radiometer ABL-3) occasionally during the experiments, to ensure that ventilation of the animals was sufficient.

2.5 Single Nerve Fibre Recording

All animals were paralysed and artificially ventilated. The left cervical vagus nerve was located, via a cervical incision, and dissected free from the carotid artery, sympathetic and aortic nerves (Fig. 2.2). The vagus was cleared of its surrounding fascia and cut at the central end. The skin and muscle in the neck at either side of the incision was lifted and tied to a metal ring to form a well, which was filled with light mineral oil. Bipolar plastic coated silver electrodes (exposed at the tips) were used for recording purposes, using fascia positioned on one electrode for a reference. The vagus nerve was placed on a small black perspex plate to aid subsequent dissection (Fig. 2.3). Thin filaments were teased from the vagus nerve, under a binocular microscope (Nikon SMZ 1-B), and placed on the second electrode until a single active unit, or one of not more than two or three units, was obtained. Action potentials were recorded in a conventional manner. The pick-up electrodes were connected to a pre-amp headstage (Digitimer NL100K), and the signal was amplified (Digitimer NL104), and filtered (Digitimer NL125) before being input into a spike processor (Digitimer 130). The spike processor allowed pulse train counting, with a digital display, over selectable time periods. In addition, a digital to analogue converter circuit enabled chart recorder display (Grass 78B) of the "firing rate" of the nerve fibre. Upper and lower discriminator outputs plus a "window" detector facility allowed, when more than one active unit was present, selection of an action potential if the spike height was sufficiently different from the others. Monitoring of the input signal to the spike processor was carried out on an oscilloscope (Tektronix 5113 Dual Beam Storage) and relevant information photographed with an oscilloscope camera (Tektronix C-5). The input signal to and from the spike processor was also fed through an audio amplifier to a loudspeaker. Finally, the data were recorded on a Racal Thermionic 4 channel tape recorder for playback through a Medelec fibre optic

recording oscilloscope (For-4) onto light sensitive photographic paper (Kodak Linagraph Type 1801).

In later experiments, Chapter 7, action potentials from single nerve fibre recordings were stored directly onto an Compaq 386 computer using an R.C. Electronics ISC-16 data acquisition system. After completion of the experiment, the raw data were analysed using R.C. Electronics Enhanced Graphics Acquisition and Analysis software.

2.6 Criteria for Identification of Sensory Nerve Endings

In the present study only vagal fibres arising from RARs were investigated. To identify these sensory nerve endings from other groups of sensory nerve endings in the respiratory tract, a modified criterion from that used by Adcock (1989) was adopted.

Identifying A-fibres from C-fibres is much more clear cut than identifying RARs from mediastinal, oesophageal and even some cardiac sensory nerve endings. The following sections detail the criteria used to identify vagal fibres arising from RARs rather than those from C-fibres. For the criteria used to identify C-fibres readers are referred to Adcock (1989).

Spontaneous Activity

The spontaneous activities in vagal nerve fibres arising from lower respiratory tract afferent sensory nerve endings were quite different and often indicative of the sensory nerve ending groups from which they originated. SARs fired either during inspiration or continuously throughout the respiratory cycle, increasing during inspiration (Fig. 2.4). Alternatively, some fired during expiration and were presumably extrathoracic.

RARs tended to fire occasionally with 1 - 3 impulses during inspiration (intrathoracic) or expiration (extrathoracic) (Fig. 2.5). Some were completely silent until stimulated.

Hyperinflation

This was the primary manoeuvre carried out to identify sensory nerve endings in the respiratory tract. The lungs were inflated in a cumulative manner up to 5 x tidal volume by clamping the expiratory line from the animal. SARs increased their firing rate during this manoeuvre, with virtually no adaptation, until they fired continuously, or faster if already firing continuously (Fig. 2.6). The majority had a low threshold to the inflation stimulus. Sparsely firing, or silent, RARs increased their firing rate with short bursts of activity coinciding with the stepwise hyperinflation (Fig. 2.7). This was usually at a higher threshold than the SARs. The discharges were rapidly adapting, often with a prominent "off" response when the expiratory line was re-opened. Although hyperinflation indicated that the fibre under test had a respiratory origin, it was by no means definitive. For instance, some mediastinal, oesophageal and cardiac nerve endings were also stimulated during hyperinflation, presumably due to movement or touching of these tissues by the lungs.

Deflation

The lungs were deflated by turning off the ventilator, clamping the inspiratory line to the lungs and withdrawing air (100 ml) rapidly from the expiratory line with a syringe. Intrathoracic SARs ceased firing during this manoeuvre, whereas extrathoracic nerve endings increased their firing rate. Deflation provoked rapidly adapting bursts of activity in fibres from RARs with a prominent "off" response when the inspiratory line was re-opened.

Adaptation Index

This was used to identify and separate the SARs and RARs. The respiratory pump was turned off and the expiratory line clamped. A known volume of air, i.e. 100ml, was injected into the lungs in 1s and maintained. A sensory nerve ending was assigned to one or other group on the basis of its "adaptation index" (AI), which showed the decline in frequency of discharge from the point of peak frequency (PF) to the 2nd second of inflation. The AI was expressed as a percentage calculated as follows :

$$AI = \frac{PF - \text{average frequency during 2nd s inflation}}{PF} \times 100$$

Widdicombe, 1954a

Sensory nerve endings with an AI of 70% or less were considered SARs (Fig. 2.8a). Although those above 70% are generally considered as RARs, only those sensory nerve endings that had AIs of 90% (usually 100%) and above were classed as possible RARs (Fig. 2.8b). In some cases mediastinal and oesophageal nerve endings were encountered with very similar characteristics. These were distinguished at a later stage.

Response to Chemical Stimuli

Although the difference between an SAR and RAR was established at this stage, it was not certain whether the RAR was irritant. Histamine, until recently, was considered to be a selective stimulant of RAR receptors. However, it also stimulates C-fibre endings, albeit weakly, when compared to capsaicin or phenylbiguanide. In contrast, capsaicin and phenylbiguanide are generally considered as selective C-fibre endings stimulants, but these chemicals may also stimulate RARs at high doses. Therefore, the response of the different groups of sensory nerve endings to histamine,

capsaicin or phenylbiguanide, although indicative, is not definitive proof of the sensory nerve ending group.

In general, it was found that RARs responded to i.v. histamine (10 μg kg^{-1}) with increased discharges that adopted a characteristic respiratory or cardiac rhythm. The discharges tended to correspond to the decrease in compliance produced by histamine. To distinguish an RAR from one that was mediastinal or oesophageal, histamine was also administered as an aerosol (6 breaths of 1 mg ml^{-1} solution). RARs responded with a respiratory or cardiac rhythm. Sensory nerve endings that did not respond to histamine aerosol were considered to be from either the oesophagus, mediastinum or elsewhere and were discarded.

Location of sensory nerve endings.

At the end of the experiment the animal was killed with an overdose of pentobarbitone (Euthesate). The lungs were probed with a cotton bud in an attempt to locate the sensory nerve ending and confirm its origin in the lungs or respiratory tract. When a sensory nerve ending was located, gentle prodding evoked bursts of activity in the fibre. Occasionally the sensory nerve ending was not located because either the fibre was lost during the procedure or activity in the fibre had declined at this stage of the experiment. In these cases, the previous criteria were relied upon as sufficient evidence to justify the nature of the sensory nerve ending.

2.7 Generation of Aerosols

Aerosols of drugs were generated, using a modified DeVilbiss ultrasonic nebuliser (Lees & Payne, 1986). The adapted nebuliser assembly (Fig. 2.9) was connected directly to the afferent limb of the ventilator circuit and arranged so that the inspired air passed through the medication chamber before entering the lungs of anaesthetized

animals via the tracheal cannula. Continuous quantitative measurements of changes in pulmonary mechanics were possible using this technique.

2.8 Data Presentation and Statistical Analysis

Data are presented as either absolute values, Δ, or expressed as a percentage change from a pre-treatment value. In all cases the mean ± standard error of mean (sem) is shown. In most cases, these data were subjected to either a paired analysis using Student's "t" test or to a one-way or two-way analysis of variance test (ANOVA). The presentation and statistical analysis of the data are described, where necessary, in more detail in each chapter.

2.9 Materials

The following materials were used during these studies. Preparation and administration of drugs and chemicals are discussed in the relevant chapters.

Atropine sulphate (BDH Laboratories), α-chloralose (Koch Light Ltd), capsaicin (Fluka Chemicals AG), condoms (Durex elite), dimethyl tubocurarine (Wellcome Research Laboratories), absolute ethanol (BDH Laboratories), frusemide (Antigen Pharmaceuticals Ltd), L-glutamic acid (Sigma Chemical Company Ltd), halothane (May and Baker), heparin (Evans Medical Ltd), histamine acid phosphate (Sigma Chemical Company Ltd), indomethacin (Sigma Chemical Company Ltd), lamotrigine isothionate (Wellcome), mineral oil (Sigma Chemical Company Ltd), naloxone hydrochloride (Sigma Chemical Company Ltd), oxygen medical grade (BOC), pentobarbitone sodium (Euthesate, Willows Francis Veterinary), photographic film (Polaroid type 667), prostaglandin E_2 (Prostin E_2, Upjohn), salbutamol (Ventolin, Allen & Hanbury's), saline (0.85% sodium chloride w/v; Wellcome Research Laboratories), serotonin (Sigma Chemical Company Ltd), silver wire (Clark

Electromedical Instruments), anhydrous di-sodium hydrogen orthophosphate (BDH Laboratories), sodium di-hydrogen orthophosphate (BDH Laboratories), Tween 80 (Sigma Chemical Company Ltd).

Compound 443C81 was synthesised by the Medicinal Chemistry Department, Wellcome Research Laboratories.

The prostaglandin A analogue, AH13205, was synthesised and supplied by Glaxo Group Research.

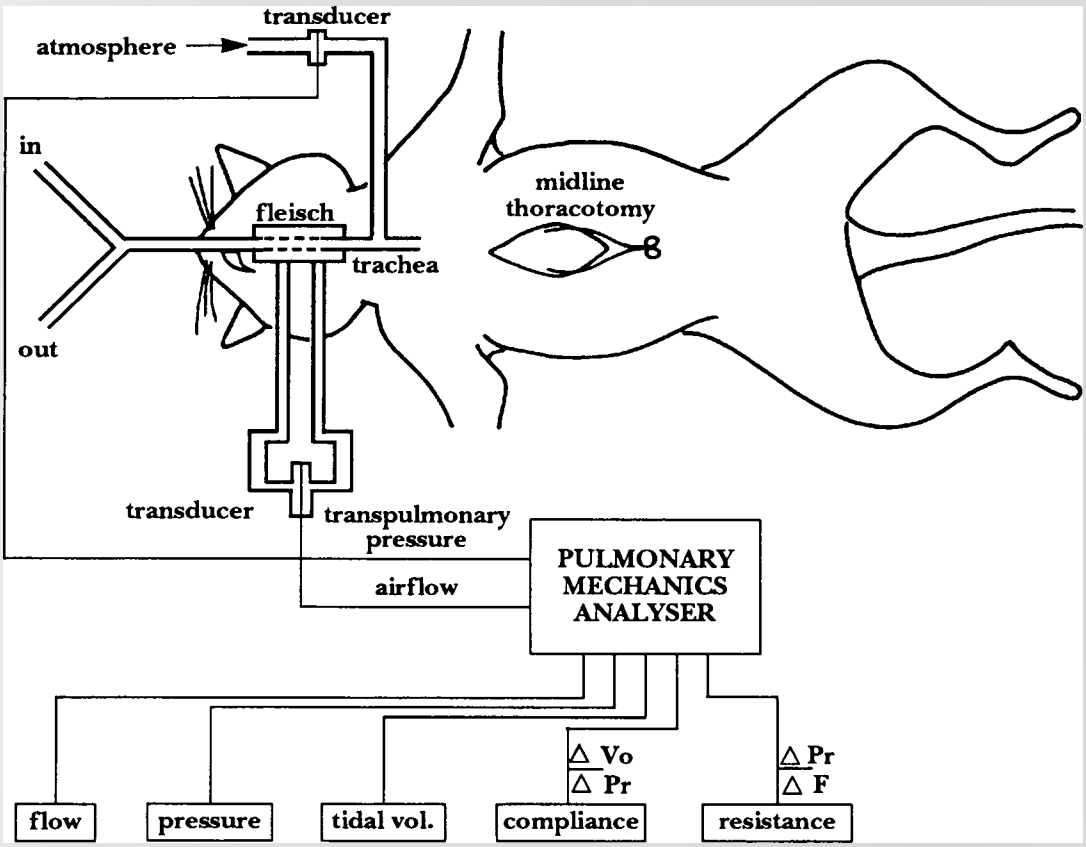


Fig. 2.1 Schematic representation of the experimental set-up for recording pulmonary mechanics in anaesthetized, paralysed, artificially ventilated cats. The cervical trachea is cannulated with a short length of endotracheal tubing and attached to a Fleisch pneumotachograph, which is connected to a differential pressure transducer. A side arm of the endotracheal cannula is connected to a differential pressure transducer; the other port of the transducer is open to atmosphere. A mid-line thoracotomy was performed. The signals from the differential pressure transducers are input to a Buxco pulmonary mechanics analyser for determination on a breath-by-breath basis of airflow, transpulmonary pressure, tidal volume, dynamic compliance and lung resistance. Vo=volume, Pr=pressure, F=flow.

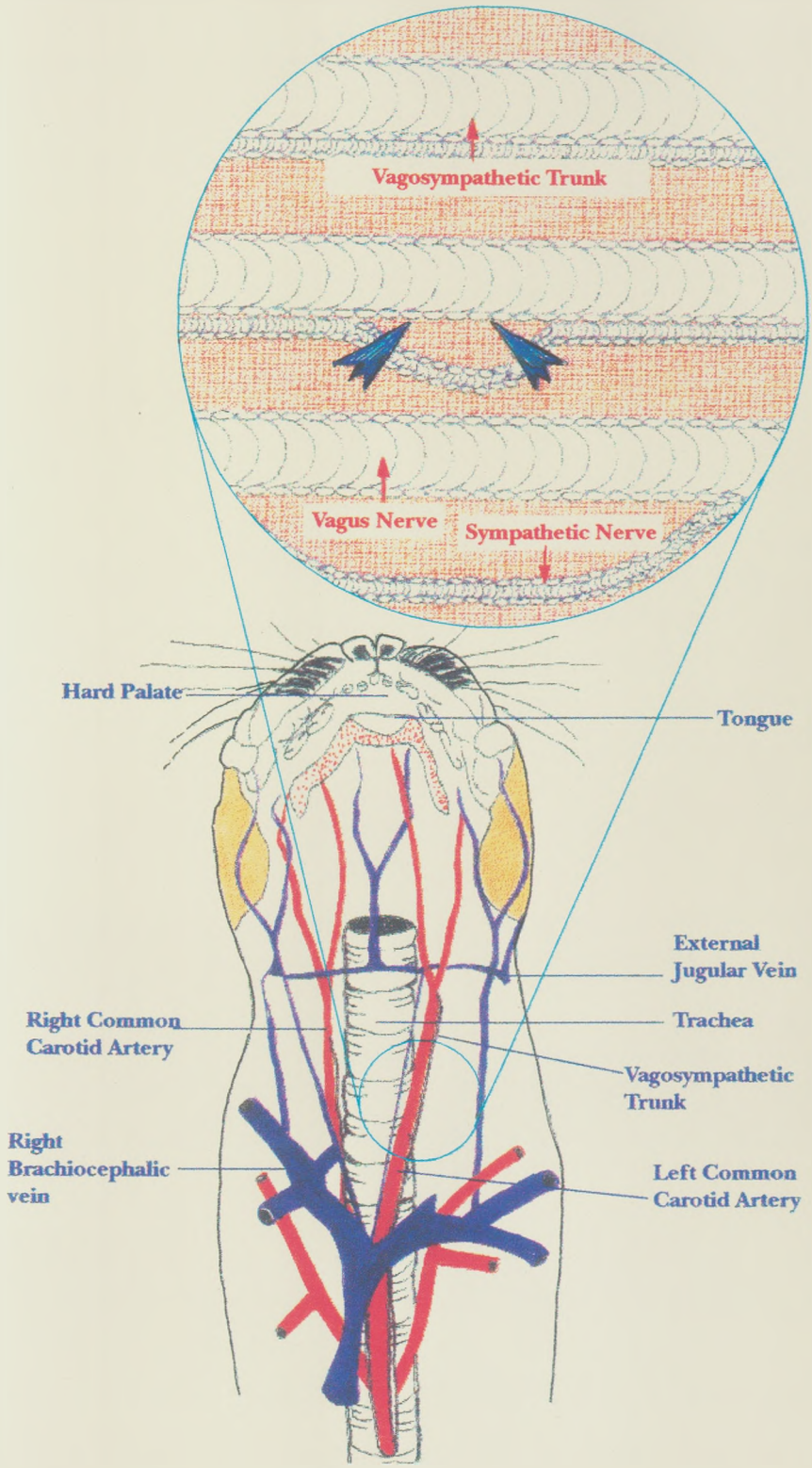


Fig. 2.2 *Main:* Diagram showing the location of the vago-sympathetic trunk, which was used in single nerve fibre recording experiments, in relation to the carotid artery and trachea in the cat. *Insert:* The vagus nerve, in single nerve fibre recording experiments, was dissected free from the sympathetic nerve, carotid artery and aortic nerve (latter two not shown) with fine dissecting forceps and with the aid of a binocular microscope.

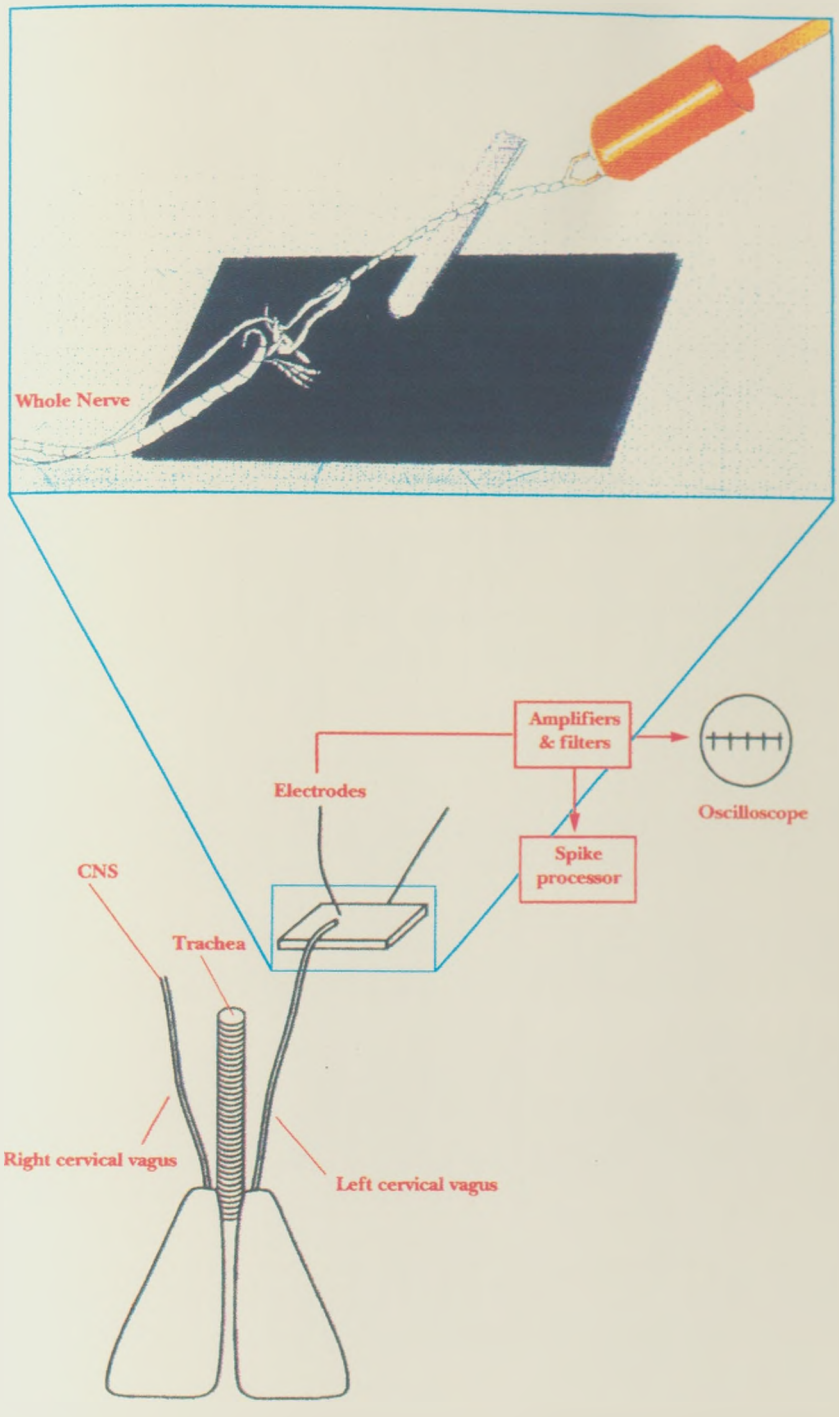


Fig. 2.3 Diagram showing the general arrangement for recording action potentials in single nerve fibres of the vagus nerve in anaesthetized, paralysed and artificially ventilated cats. *Insert:* Single nerve fibres are dissected and placed over a silver recording electrode (the fascia from the vagus nerve is placed over another electrode for reference). *Main:* Action potentials are pre-amplified, filtered and monitored visually on an oscilloscope and counted with a spike processor

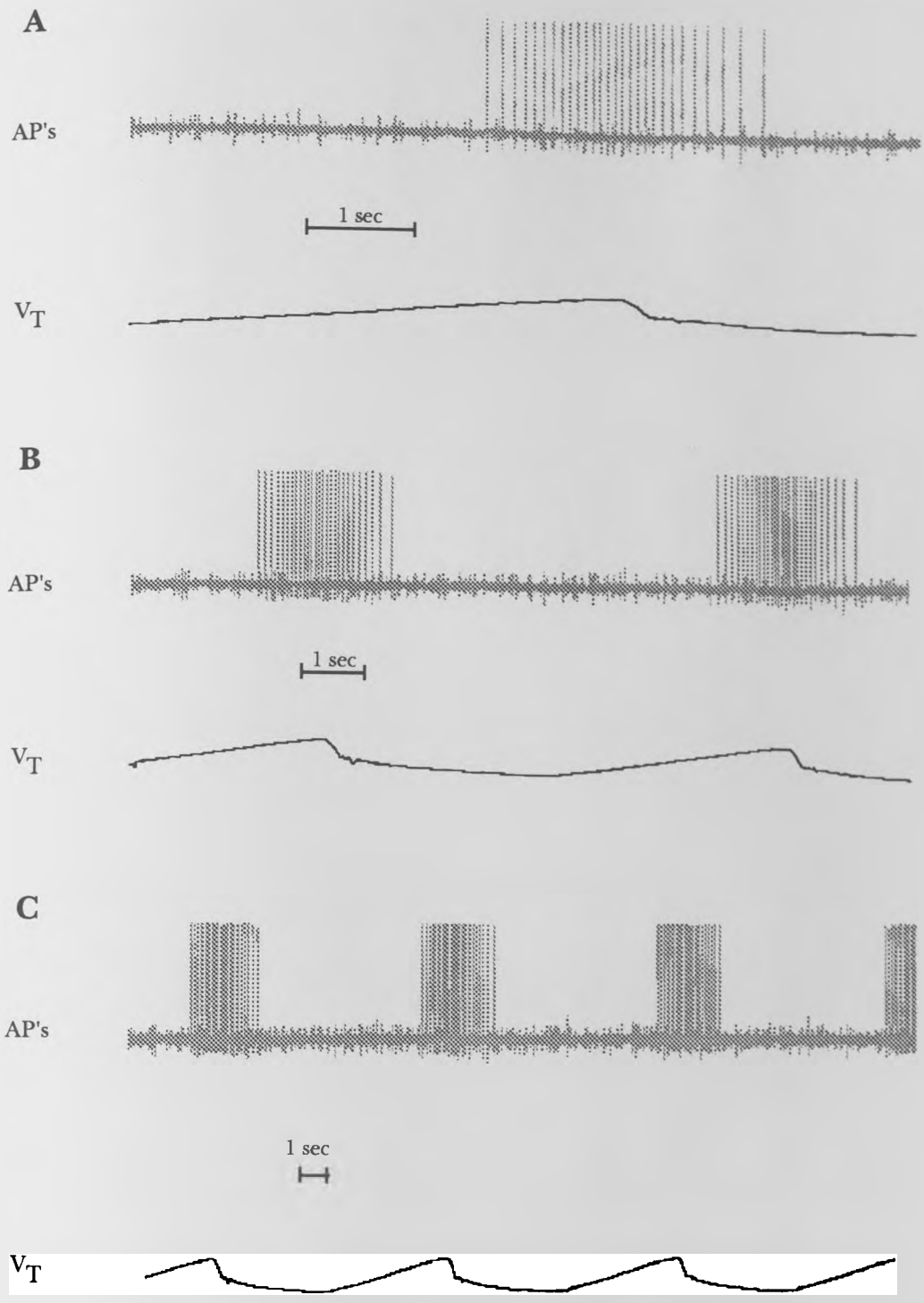


Fig. 2.4 Spontaneous impulse activity in a single nerve fibre from the left cervical vagus in an anaesthetized, paralysed and artificially ventilated cat. Tidal volume (V_T) is also shown. **A-C**) show the recorded impulse activity of a vagal fibre arising from a slowly adapting stretch receptor in the left lung at various time sweeps; note the frequent discharges with respiratory rhythm. AP = action potentials.

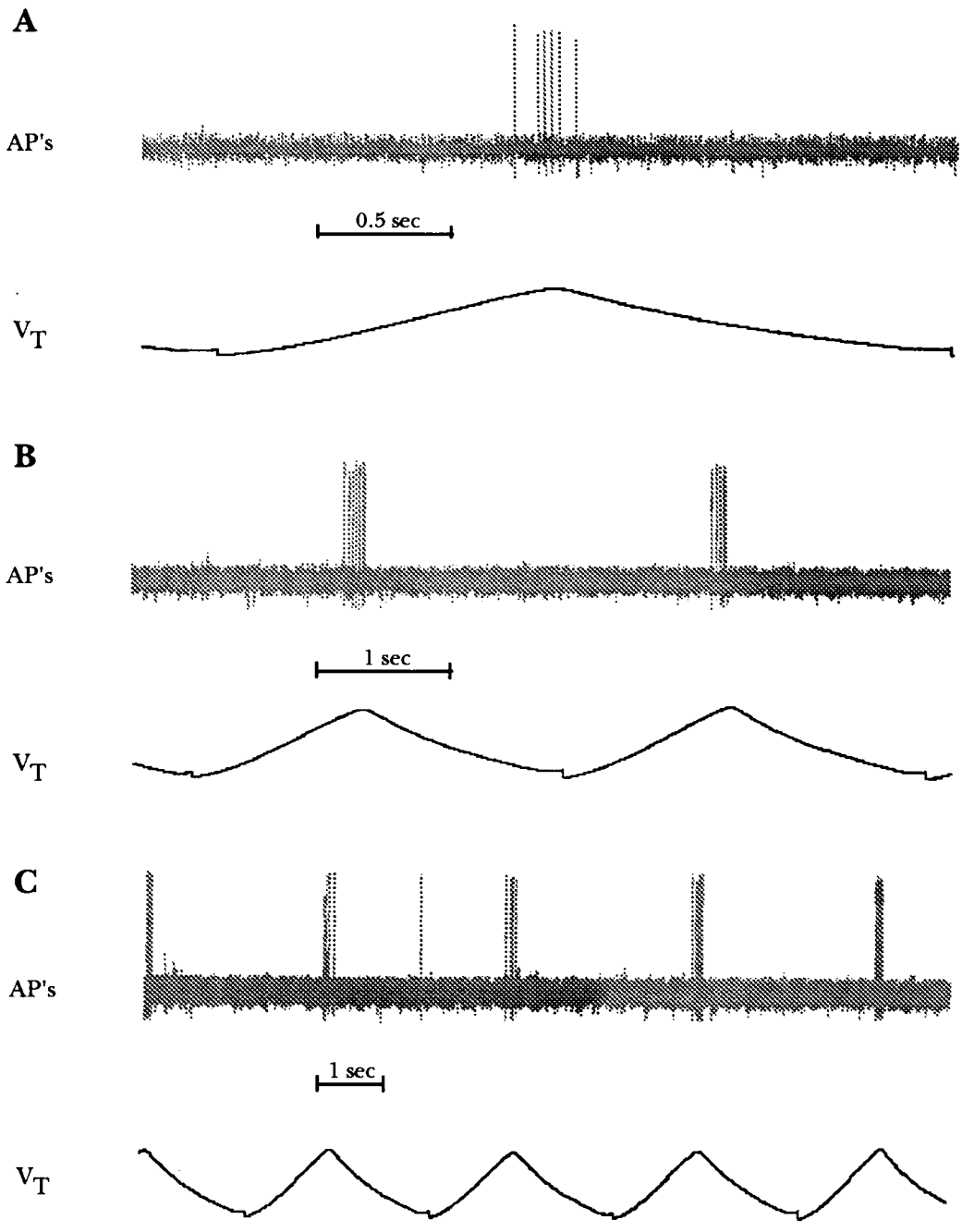


Fig. 2.5 Spontaneous impulse activity in a single nerve fibre from the left cervical vagus in an anaesthetized, paralysed and artificially ventilated cat. Tidal volume (V_T) is also shown. **A-C**) show the recorded impulse activity of a vagal fibre arising from a rapidly adapting stretch receptor in the left lung at various time sweeps; note the occasional discharges with respiratory rhythm. AP = action potentials.

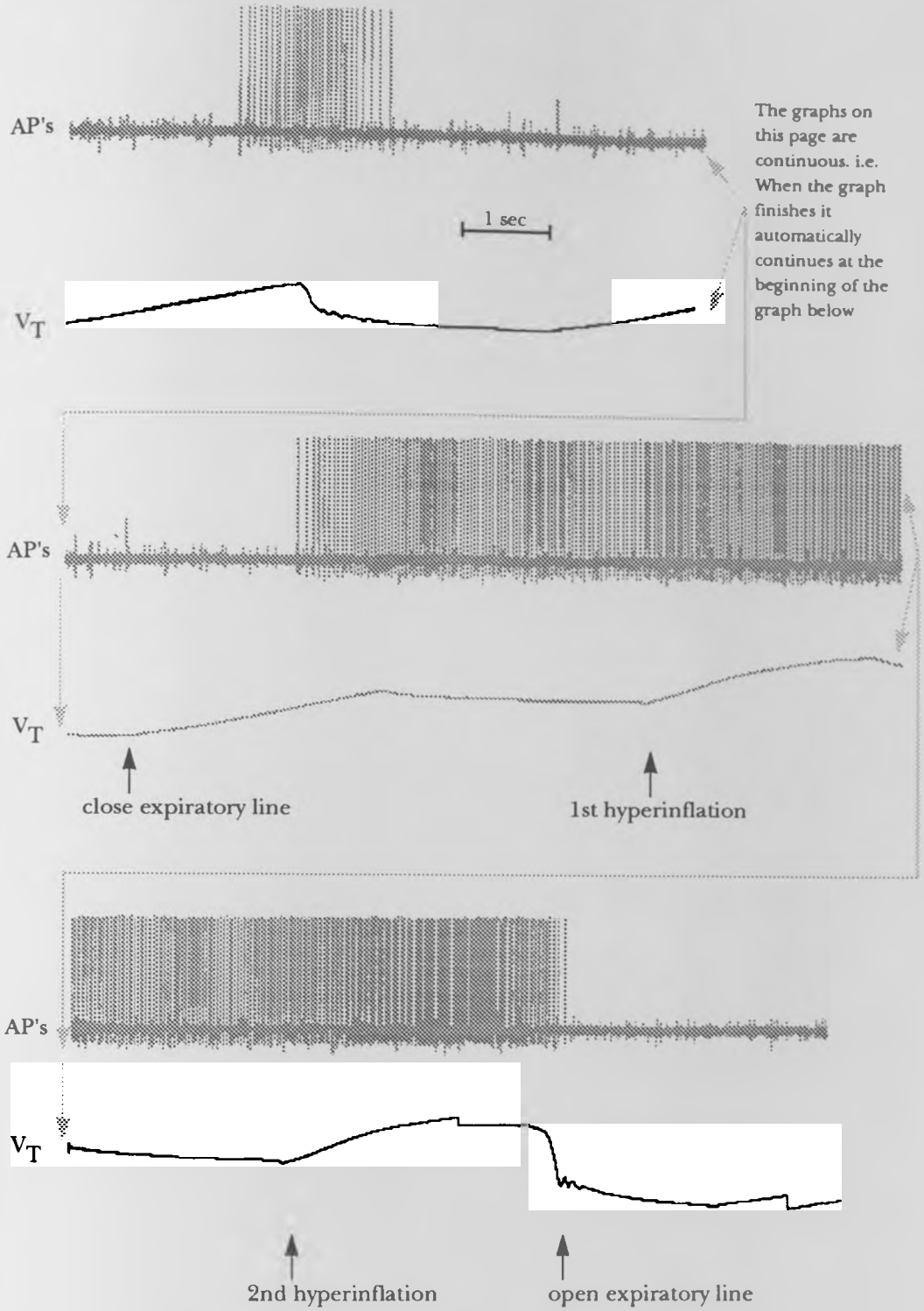


Fig. 2.6 Diagram showing the effect of consecutive hyperinflations of the lungs on the impulse activity in a vagal fibre arising from a slowly adapting stretch receptor in an anaesthetized, paralysed and artificially ventilated cat. Tidal volume (V_T) is also shown. This particular slowly adapting stretch receptor increases its firing rate with each hyperinflation, with virtually no adaptation. AP = action potentials.

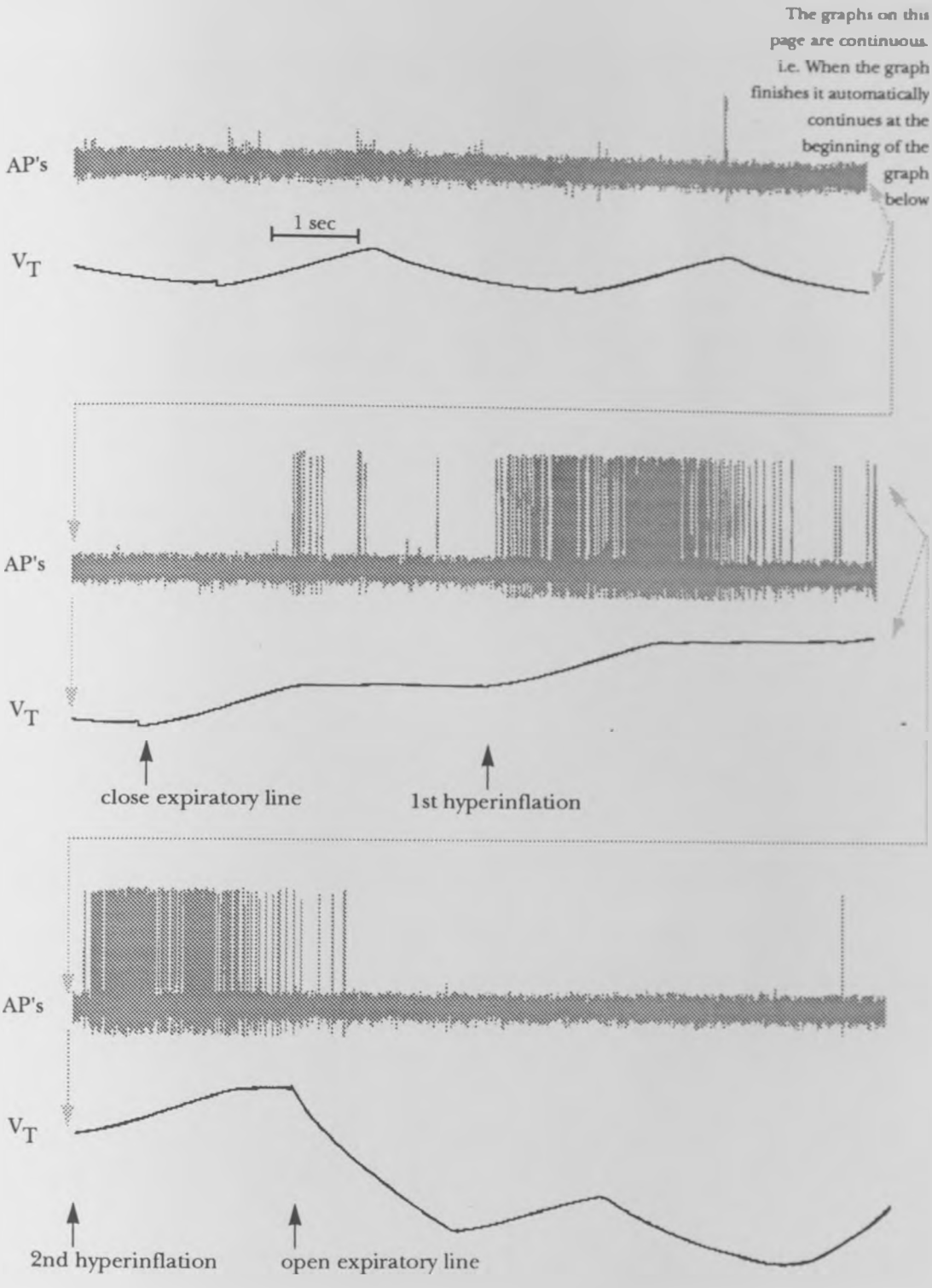


Fig. 2.7 Diagram showing the effect of consecutive hyperinflations of the lungs on the impulse activity in a vagal fibre arising from a rapidly adapting stretch receptor in an anaesthetized, paralysed and artificially ventilated cat. Tidal volume (V_T) is also shown. This particular rapidly adapting stretch receptor increases its firing rate with short bursts of activity coinciding with each stepwise hyperinflation, with rapid adaptation. AP = action potentials.

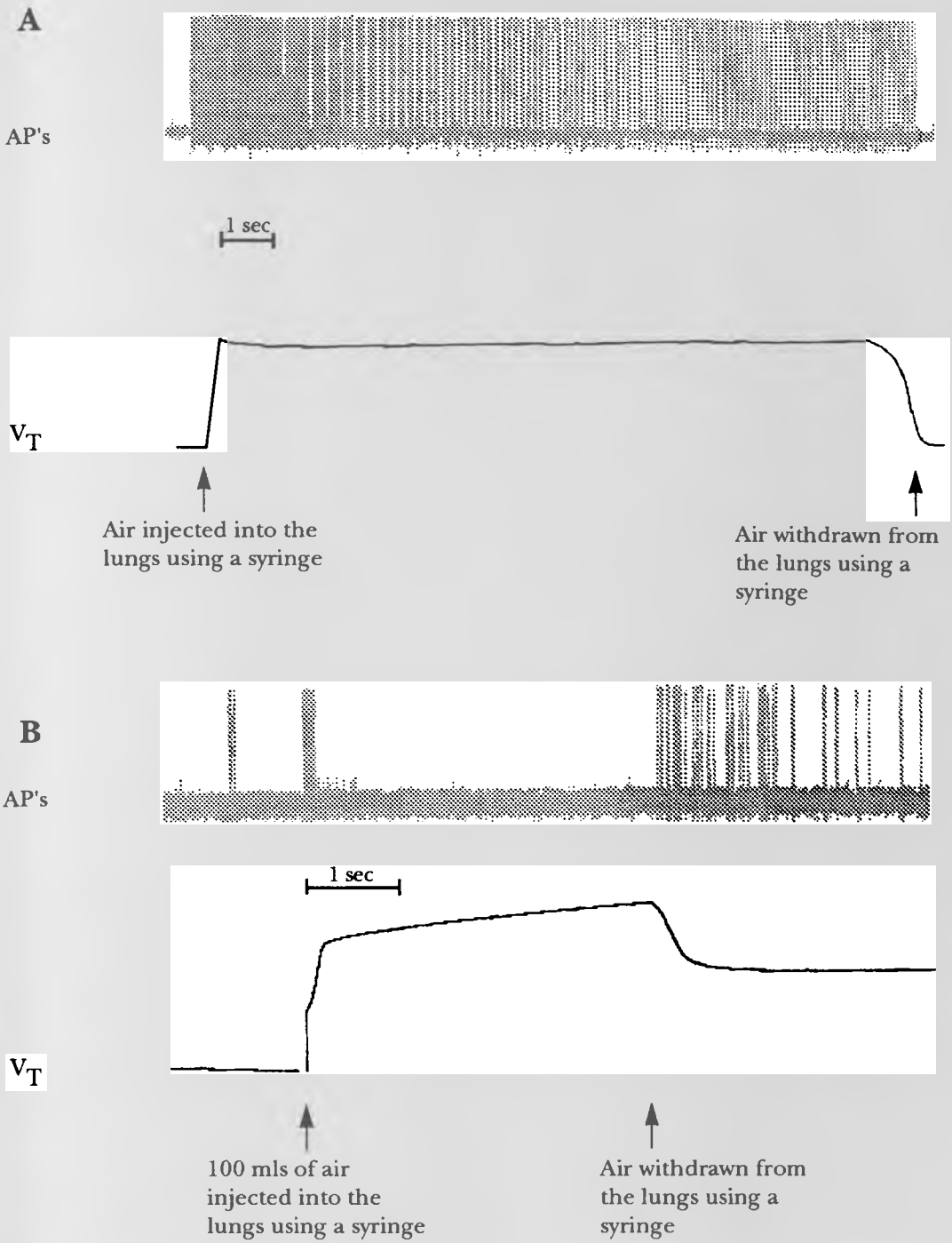


Fig. 2.8 Adaptation indices of single nerve fibres from the left cervical vagus in anaesthetized, paralysed and artificially ventilated cats. Tidal volume (V_T) is also shown. **A)** adaptation index of a vagal fibre arising from a slowly adapting stretch receptor; note, a rapid injection of 100 mls of air into the lungs causes an increase in discharge, but the response is very slow in adapting. **B)** adaptation index of a vagal fibre arising from a rapidly adapting stretch receptor; note, a rapid injection of 100 mls of air into the lungs causes an increased discharge which adapts rapidly, within one second of injection of the air. AP = action potentials.

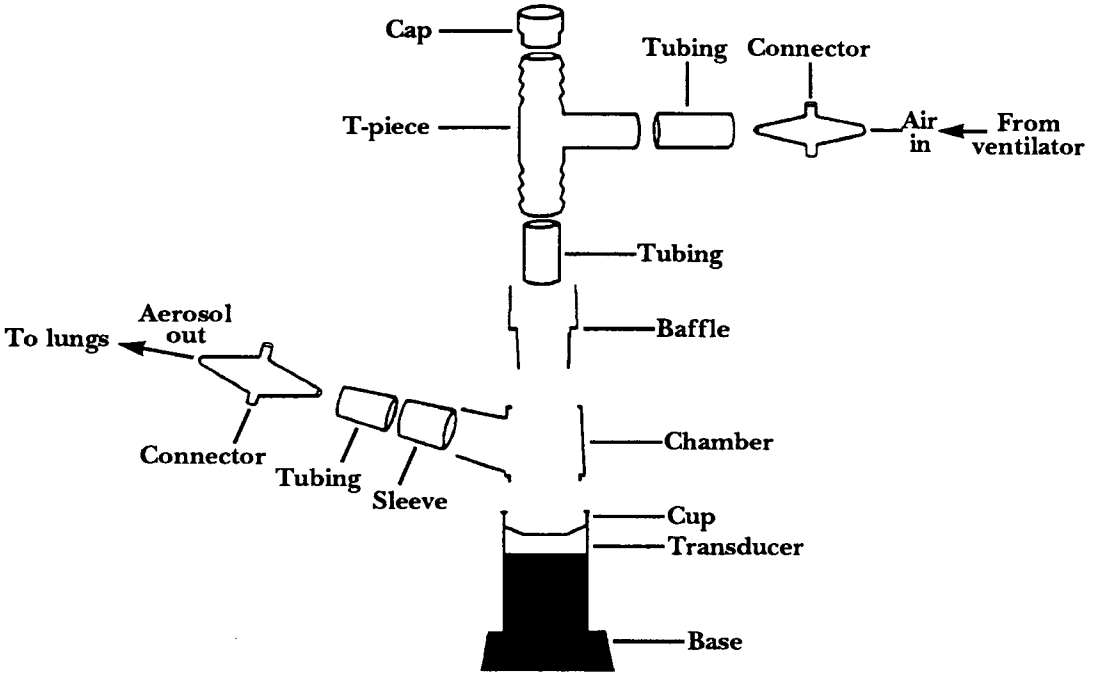


Fig. 2.9 Diagram of a modified DeVilbiss ultrasonic nebuliser used to generate aerosols for inhalation. The solution is placed in the cup above the transducer. The nebuliser is positioned in the air inflow line from the ventilator to the lungs, allowing continuous monitoring of pulmonary mechanics during the generation of aerosols. (from Lees & Payne, 1986).

Chapter 3

Distribution of airway sensory nerve endings responsive to inhaled capsaicin and prostaglandin E_2 in man

3.1 Introduction

Irritation of airway sensory nerve endings can evoke respiratory reflexes such as cough and reflex bronchoconstriction. Traditionally, the oropharyngeal and laryngeal airways have been considered the most sensitive areas of the human respiratory tract to mechanical and chemical irritation. However, studies conducted in experimental animals and man, have demonstrated regional differences in the sensitivity of the respiratory tract to tussive stimuli (Widdicombe, 1954b; Higenbottam, 1989b; Forsberg *et al.*, 1990). A comparison of the effectiveness of dusts and sulphur dioxide gas in producing cough in cats led Widdicombe (1954b) to conclude that nerve endings, stimulated by particulate irritants, were likely to be concentrated in the larger airways, whereas those whose primary function was chemosensitivity were probably more plentiful within the lung. Thus, the reflex response to an inhaled irritant will be influenced not only by the afferent susceptibilities of sensory nerve endings but also by their anatomical distribution along the respiratory tract.

Coughing can be induced experimentally in man by a variety of inhaled mediators and irritants (for example, citric acid, cigarette smoke). More recent studies to investigate the cough reflex in man, have incorporated the use of selective stimuli for different airway afferent sensory nerve endings (for example, distilled water, capsaicin and PGE_2) (Karlsson *et al.*, 1988). Isolated nerve studies in animals suggest that distilled water selectively stimulates RARs (Boushey *et al.*, 1974; Boggs & Bartlett, 1982). By contrast both capsaicin and PGE_2 are believed to preferentially excite C-fibres endings (Armstrong & Luck, 1974; Coleridge *et al.*, 1976; Coleridge & Coleridge, 1984; Roberts *et al.*, 1985).

Aerosol deposition within the respiratory tract can be influenced by a number of factors - droplet size, mode of inhalation and the subject inhaling the particles.

Properties of the particles themselves are important in determining the site of deposition. Large aerosol droplets (5 - 10 μm in diameter) deposit predominantly in the large airways, particles 1 - 5 μm in diameter are distributed evenly throughout the airways, whilst particles less than 1 μm in diameter are deposited primarily in the alveoli (Lippman *et al.*, 1980; Emmet *et al.*, 1982). The quantity of aerosol deposited in the respiratory tract and its distribution are both dependent on the mode of inhalation of the aerosol. As the volume of inhalation is increased, aerosol particles are likely to penetrate further into the lung and may be deposited on more peripheral airways (Pavia *et al.*, 1977). Conversely, as the flow rate of inspired air increases, the probability of inertial impaction also increases, and droplets are more likely to deposit in large central airways (Lippman & Albert, 1969; Goldberg & Lourenço, 1973). A period of breath holding after inhalation increases the probability of droplets settling by sedimentation (Palmes *et al.*, 1967, Palmes, 1973). The physical characteristics of the subject inhaling the aerosol are also very important in determining the subsequent site of deposition. In healthy subjects there is considerable subject to subject variability in deposition which probably reflects random anatomical differences in airway dimensions (Palmes & Lippman, 1977; Yu *et al.*, 1979). In subjects with obstructive airways, the degree of aerosol penetration to the lung periphery is related to the degree of airway obstruction (Pavia *et al.*, 1977), deposition in the central airways being much more likely if the airways are narrowed.

The cough reflex is a suitable physiological response which allows the study of airway afferent sensory nerve endings involved in evoking airway reflexes, since it is not dependent on the conditioning of the vagal efferent system; a factor that has caused problems in the study of bronchoconstriction. By altering factors which influence aerosol deposition, such as droplet size, it is possible to modify the

deposition of inhaled aerosols deliberately (Brain *et al.*, 1985). It is possible, therefore, by altering the deposition pattern of inhaled aerosols and using inhaled irritants, which are "selective" for different airway afferent sensory nerve endings, to map out the distribution of airway sensory nerve endings involved in evoking respiratory reflexes.

Despite what is known about the morphology and the chemical specificity of different afferent sensory endings from isolated nerve studies in animals, there have been few attempts to map out the distribution of vagal afferent sensory nerve endings which are involved in evoking airway reflexes, such as cough and reflex bronchoconstriction, in man (Woolman *et al.*, 1988). The aim of the present study was to examine the sensitivity of different regions of the respiratory tract to inhaled aerosols of the "selective" C-fibre ending stimulants, capsaicin and PGE_2. Selective deposition of aerosols was achieved by varying aerosol droplet size. In a separate study, the deposited dose and the distribution of the aerosol, for each of the nebuliser systems, was assessed by inhalation of radiolabeled aerosol and gamma camera imaging.

3.2 Method

Aerosol Generation and Inhalation

A large droplet aerosol was produced from a DeVilbiss ultrasonic nebuliser (USN; DeVilbiss Healthcare, Feltham, UK) with a power setting of 5 and vent fully open. The aerosol passed through corrugated tubing, 1 metre in length, into a one litre cone-shaped dead space, to ensure a continuous supply of "similar" aged aerosol. Each subject breathed from the cone through a two-way low resistance valve (Godden *et al.*, 1986). A small droplet aerosol was generated from four acorn jet nebulisers (JET; Medic Aid, Pagham, UK) connected in parallel with two Y pieces

and short lengths of plastic tubing. The output from all four acorn nebulisers passed through the same system as described above for the USN. The JET system was run from a pressurised gas cylinder. Airflow was controlled by two needle valves and measured by two floating ball flow meters. The airflow through each needle valve was kept at 15 l min^{-1}.

Aerosol Output

A corrugated anaesthetic filter (Pall Biomedical, Portsmouth, UK) was placed on the inspiratory end of the two-way valve. The USN and JET nebuliser reservoirs (50 mls and 4x5 mls of 0.9% saline, respectively) were loaded with 50 MBq of the radiopharmaceutical ^{99m}Technetium Diethylene Triamine Penta Acetic Acid (^{99m}Tc DTPA). The radioactivity in 1 ml of solution, removed from the nebuliser reservoir, was measured prior to nebuliser operation. The quantity of radioactivity trapped on the anaesthetic filter was measured after running the nebuliser for 1 min. The volume of liquid output from the nebuliser was calculated as:

$$\frac{\text{Quantity of radioactivity trapped on the filter}}{\text{Quantity of radioactivity in 1 ml of solution from the nebuliser reservoir}}$$

and expressed as the volume of liquid output (in mls) per minute of nebuliser operation.

Particle Size

A May cascade impactor (Biral, Bristol, UK) was used to determine the droplet size characteristics of the aerosols produced by the two nebuliser systems. The USN and JET nebuliser reservoirs (50 mls and 4x5 mls of 0.9% saline, respectively) were loaded with approximately 50 MBq of ^{99m}Tc DTPA. Each nebuliser system was run

for 1 min. The aerosols were sampled from the inspiratory end of the cone-shaped dead space. A calibrated scintillation counter was used to measure the quantity of radioactivity deposited on each plate of the cascade impactor. It was assumed that each droplet contained radioactivity proportional to its mass. The radioactivity distribution was then treated as if it were a mass distribution. The mass distribution was fitted to a log normal distribution, from which the mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD), of the two aerosols, were calculated.

Protocols

Distribution studies using Radioaerosols

Four healthy, non-smoking male volunteers participated in this study (Table 3.1). District Ethical Committee approval was obtained for the study. Each subject gave informed consent. The USN and JET nebuliser reservoirs contained approximately 7000 MBq of ^{99m}Tc DTPA (in 50 mls of 0.9% saline) and 5000 MBq of ^{99m}Tc DTPA (in 4 x 5 mls of 0.9% saline), respectively. The radioaerosols from the two nebuliser systems were inhaled, for 1 min, on two occasions, at least 1 week apart, by each subject. Each subject received approximately 20 MBq of ^{99m}Tc DTPA per visit, giving a combined radiation dose of 0.2 mSv effective whole body dose equivalent for the two studies. Immediately after radioaerosol inhalation, water was gargled and expectorated and the subjects placed supine on a tomographic imaging couch. Subjects were imaged using a large field of view gamma camera with a low energy general purpose collimator (IGE400AT, International General Electric Medical Systems, Slough, UK). The gamma camera was connected to an on-line computer (Nodecrest Ltd., Byfleet, UK). For 20 s, 64 x 64 word images were acquired at each of 64 angles around the subject. Since ^{99m}Tc DTPA deposited in the lungs and airways is lost by a number of absorptive and non-absorptive processes

(Lippman *et al.*, 1980; Bennet & Ilowite, 1989), by looking at the number of counts in the first and last frames, a correction factor was determined and applied to each frame of the study assuming exponential removal of the ^{99m}Tc DTPA Transaxial slices were reconstructed using standard Nodecrest filtered back-projection software, attenuation correction was applied using a mean attenuation coefficient obtained from transmission studies. Transverse and coronal mid-lung images were formed from the reconstructed transaxial dataset as described by Phipps *et al* (1989) (Fig 3.1). Right and left lungs were both assessed. The boundary of each lung was defined as the contour drawn at 20% of the maximum count in that lung. A separate region was created to incorporate extrapulmonary and extrathoracic airways (excluding mouth). A central region was defined which included intrapulmonary airways down to segmental bronchi, as well as extrapulmonary and extrathoracic airways (excluding mouth). A peripheral region was defined which included intrapulmonary airways from the segmental bronchi to the alveoli. In each subject, the peripheral and central regions defined for the JET were used for the USN images. From the anterior lung view, the quantity of radioactivity deposited in the lungs and airways was calculated using a gamma camera counter efficiency factor. The volume of liquid output deposited in the lungs and airways was calculated as:

$$\frac{\text{MBq of radioactivity in the lungs and airways}}{\text{MBq of radioactivity in 1 ml solution from the nebuliser reservoir}}$$

and expressed as the volume of liquid output (in mls) deposited in the lungs and airways during one minute of nebuliser operation.

Cough Studies

Each subject inhaled irritant aerosols from both nebuliser systems for 1 min. The number of coughs were recorded on a hot pen chart recorder (Model 220, Gould Electronics Ltd., UK) as described by Godden *et al*., (1986).

Capsaicin study

Ten volunteers (5 male and 5 female, 2 current smokers and 2 ex-smokers), mean age 28 yrs (range 19 - 44), were recruited for this study. All of the subjects had normal lung functions (mean forced expired volume in one second, FEV_1, as a % of predicted, was 113%, range 96 - 129%) and all were without respiratory tract infections. Since, capsaicin-induced cough does not exhibit tachyphylaxis (Collier & Fuller, 1984) all of the subjects inhaled increasing concentrations of capsaicin (0.1 - 2 μmoles l^{-1}) until greater than or equal to 4 coughs were obtained. Dose response curves, using the two different nebuliser systems, were obtained on separate days. FEV_1 measurements were taken 30 seconds before and after completion of each dose response curve.

PGE_2 study

Seven subjects (4 male and 3 female, 2 current smokers and 2 ex-smokers) from the capsaicin study also participated in this study. Each subject inhaled increasing doses of PGE_2 (0.014 - 14 millimoles l^{-1}) until greater than or equal to 4 coughs were obtained. Each dose was inhaled on separate days to avoid tachyphylaxis. FEV_1 measurements were taken 30 seconds before and after each inhalation challenge.

Control study

A vehicle control (50 µl of absolute alcohol) was inhaled from the ultrasonic nebuliser system by all subjects. FEV_1 was measured before and after the inhalation challenge.

Data Analysis

Distribution studies using Radioaerosols

All results are expressed as actual values or %'s of actual values ± standard error of the mean (sem). These data were subjected to either a paired or unpaired Student's "t" test. Statistical values of $P < 0.05$ were considered to be significant

Cough studies

The Sign Test (Siegel & Castellan, 1988) was used to compare the frequency of coughing, using USN and JET nebulisers, for each of the inhaled irritants Actuarial survival techniques (Armitage & Berry, 1971) were used to analyse the concentration at which each subject coughed at least four times to each of the inhaled irritants, for both USN and JET nebulisers. FEV_1 measurements, before and after inhalation of irritant agents, were analysed using analysis of variance tests (ANOVA). Statistical values of $P < 0.05$ were considered to be significant.

3.3 Results

3.3.1 Distribution studies using Radioaerosols

The USN and JET nebuliser systems were matched for volume of liquid output in 1 min (USN = 0.85 ± 0.01 mls, n=18; JET = 0.86 ± 0.01 mls, n=16). Although both nebuliser systems produced heterodispersal aerosols (USN, GSD = 2.12; JET, GSD = 2.91) the nebuliser systems produced significantly ($P < 0.001$) different droplet

sized aerosols (USN, MMAD of 7.9 ± 0.7 μm, n=8; JET, MMAD of 3.83 ± 0.53 μm n=7).

The mean volume of liquid output deposited in the lungs and airways was significantly ($P < 0.05$) higher with the USN than with the JET nebuliser system (0.14 ± 0.01 mls and 0.09 ± 0.01 mls, respectively). Individual values for each subject are shown in Fig. 3.2.

There was no significant difference between peripheral and central deposition, with the USN, for coronal or transverse lung sections (coronal: peripheral 45.5 ± 7.1%, central 54.4 ± 7.1%; transverse: peripheral 54.5 ± 5.5%, central 45.5 ± 5.5%). However, peripheral deposition was significantly ($P < 0.05$) higher than central deposition, with the JET, for coronal and transverse lung slices (coronal: peripheral 57.5 ± 3.0%, central 42.5 ± 3.0%; transverse: peripheral 63.8 ± 3.2%, central 36.2 ± 3.2%). Individual values for each subject are shown in Fig. 3.3.

3.3.2 Cough Studies

In all subjects, a cough concentration-response curve to capsaicin and PGE_2 could be constructed (examples of capsaicin and PGE_2 response curves are shown in Figs 3.4 & 3.5). The cough threshold for each subject could be accurately defined since no subject coughed during exposure to the vehicle or to the lowest concentrations of capsaicin or PGE_2. In all of the subjects, the frequency of coughing, to inhaled capsaicin and PGE_2, was always higher when using the USN than when using the JET nebuliser (examples are shown in Figs 3.4 & 3.5). Sign test for capsaicin, $P < 0.001$ and PGE_2, $P < 0.008$.

From Fig. 3.6, the effective concentration of capsaicin necessary to cause 50% of subjects to cough at least four times (EC_{50}) using the USN was 0.4 μmoles l^{-1}, compared to 0.7 μmoles l^{-1} with the JET. For PGE_2 (Fig. 3.7) the EC_{50} using the USN was 0.059 millimoles l^{-1} compared to 0.44 millimoles l^{-1} with the JET. Thus, in comparison with the USN, a 1.75-fold and a 7.5-fold higher concentration of capsaicin and PGE_2, respectively, is required when using the JET to reach the cough threshold of four or more coughs. However, in two subjects, both of whom were current smokers, no difference was observed in the concentration of PGE_2 required to induce four or more coughs using either USN or JET nebulisers.

None of the subjects coughed during inhalation of the vehicle. FEV_1 was not affected by more than 10%, by the inhalation solutions, nebuliser systems or by diurnal variation.

Using the mean liquid volume output deposited in the lungs and airways, mean regional deposition (transverse data used in this example) and the EC_{50} to cause cough at least four times, it is possible to calculate the effective dose of capsaicin and PGE_2 deposited in peripheral and central airways causing at least four coughs using USN and JET nebulisers (Table 3.2). For capsaicin, the effective dose deposited in central and peripheral airways is essentially similar for both USN and JET nebulisers. In contrast, for PGE_2, the effective dose deposited in central and peripheral airways using the JET was ten fold greater than that deposited using the USN.

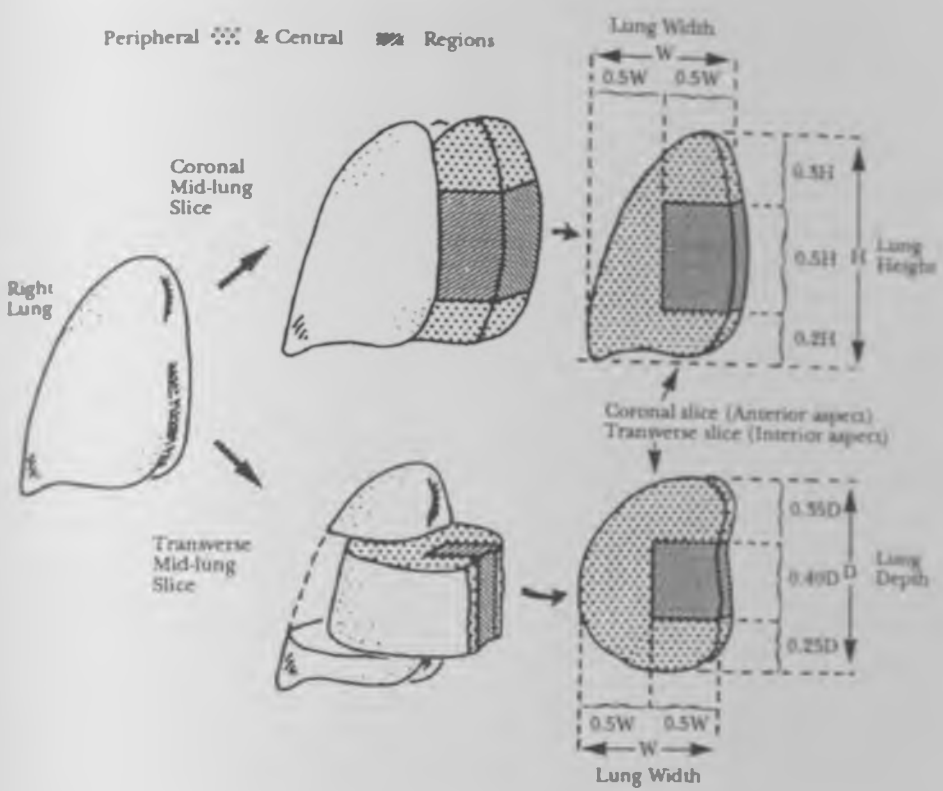


Fig. 3.1 Diagram showing peripheral and central regions and mid-slicing of the lung

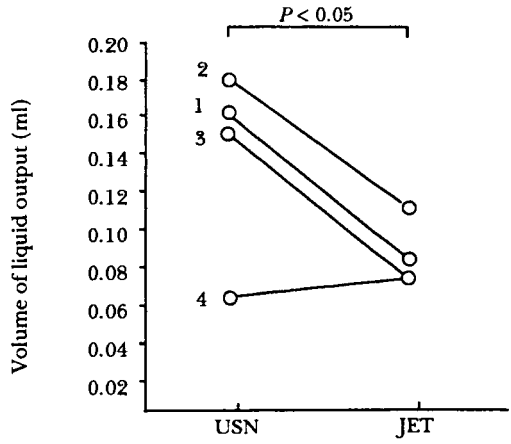


Fig 3.2 Individual values of the volume of liquid output (mls) deposited in the lungs and airways using USN and JET nebulisers.

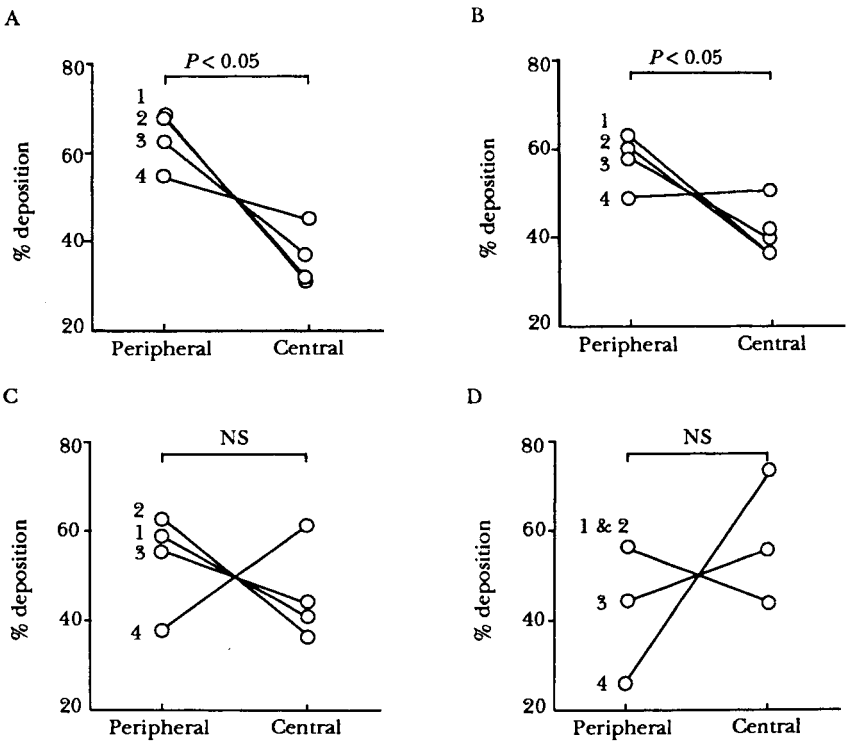


Fig. 3.3 Percentage of peripheral and central deposition for individual subjects using USN and JET nebulisers. **A, B** Transverse and coronal lung sections for the JET. **C, D** Transverse and coronal lung sections for the USN.

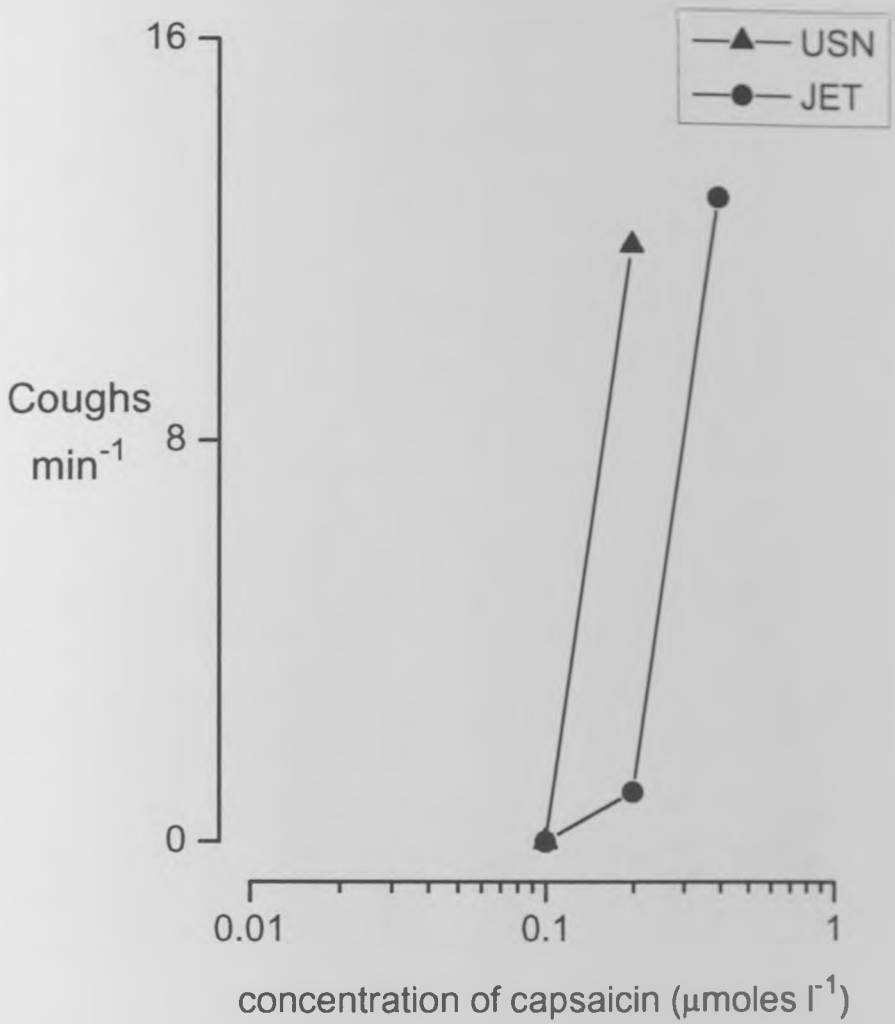


Fig. 3.4 A typical cough concentration response curve to inhaled capsaicin, in a normal volunteer, using USN and JET nebulisers.

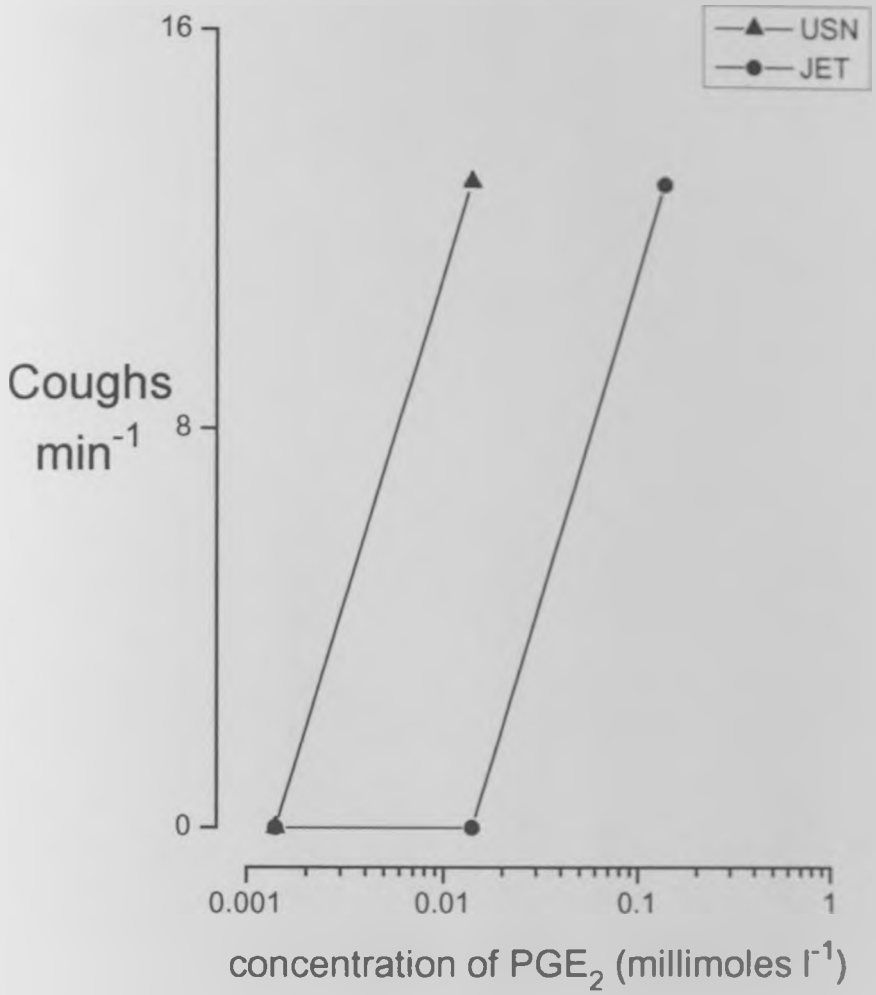


Fig. 3.5 A typical cough concentration response curve to inhaled prostaglandin E_2, in a normal volunteer, using USN and JET nebulisers

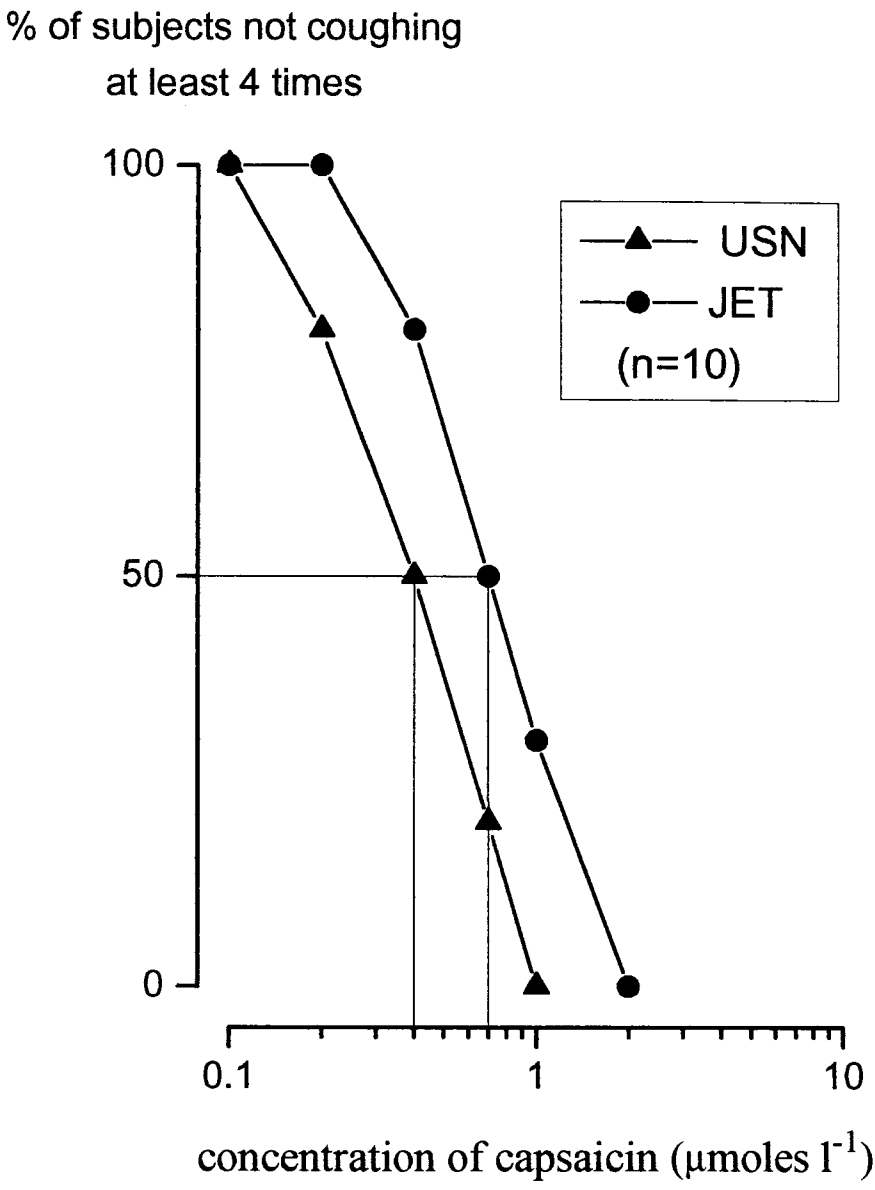


Fig. 3.6 Actuarial survival graph, showing the % of subjects not coughing at least four times, for USN and JET nebulisers, against increasing concentrations of capsaicin.

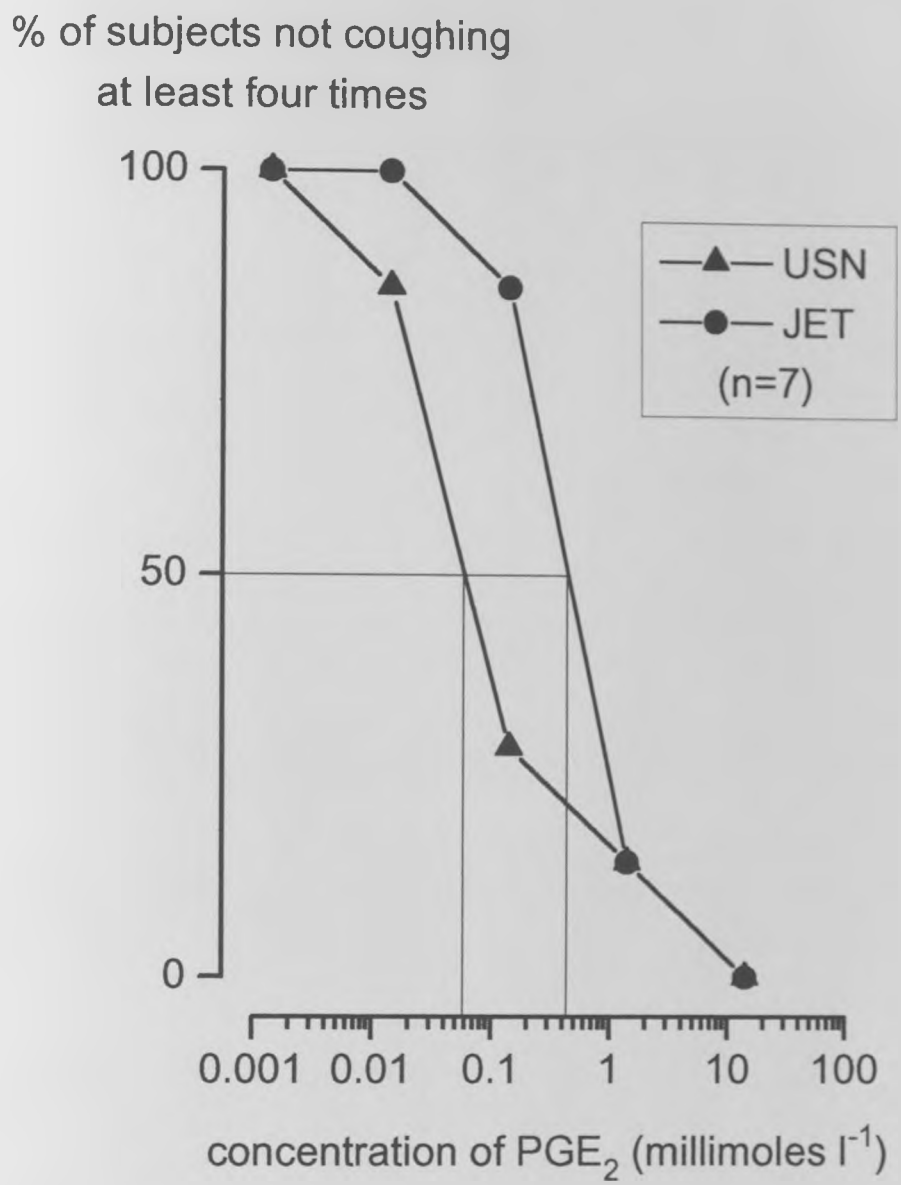


Fig. 3.7 Actuarial survival graph, showing the % of subjects not coughing at least four times, for USN and JET nebulisers, against increasing concentrations of prostaglandin E_2.

Table 3.1 Details of subjects participating in the radioaerosol distribution study.

Subject	Age (years)	Height (cm)	FEV_1 (l)	FVC (l)	FEV_1 (% of predicted)	FVC (% of predicted)
1	40	175	4.3	5.7	117	123
2	31	178	4.4	5.8	106	114
3	27	178	4.8	5.9	105	113
4	22	168	3.9	4.8	104	107

FEV_1, Forced expired volume in one second; FVC, Forced vital capacity.

Table 3.2 Deposited dose (moles) of capsaicin and PGE_2 in central and peripheral airways using both USN and JET nebulisers.

	Capsaicin		PGE_2	
	USN	JET	USN	JET
Central	2.6×10^{-11}	2.3×10^{-11}	2.4×10^{-9}	1.4×10^{-8}
Peripheral	3×10^{-11}	4×10^{-11}	2.9×10^{-9}	2.5×10^{-8}

3.4 Discussion

The purpose of the present study was to examine the cough responses, evoked by inhaled capsaicin and PGE_2, when delivered preferentially to the central or peripheral airways of normal subjects. The pattern of aerosol deposition was varied using two nebuliser systems which generated different droplet-sized aerosols. The USN produced a large droplet-sized aerosol which was deposited almost equally in central and peripheral airways. In comparison, the JET produced a small droplet-sized aerosol which had a deposition pattern which favoured peripheral deposition. However, there was no significant difference in the deposition patterns between the large and small aerosols, because of the relatively high peripheral deposition attained with the large aerosol. This finding, however, is not surprising, since the USN produces a heterodispersal aerosol and, therefore, it is probable that the smaller droplets, generated by the USN, are likely to deposit in the periphery of the lung. Furthermore, Agnew *et al.*, (1985) have shown that particles with a diameter 5 μm can penetrate down to the alveolar level. Although both nebuliser systems were matched for liquid volume output, there was a significantly ($P < 0.05$) higher volume of aerosol deposited in the lungs and airways with the USN than with the JET. Large aerosol droplets will deposit in the respiratory tract primarily by the process of inertial impaction (Brain *et al.*, 1985). In comparison, small aerosol droplets will deposit mainly by the mechanisms of sedimentation and diffusion (Brain *et al.*, 1985). It is possible, therefore, that a greater number of droplets of aerosol, principally because of their size, are being exhaled with the JET than with the USN.

Radioaerosol imaging, in the present study, was performed using the technique of 3-dimensional single photon emission computed tomography (SPECT). Although a longer time is required for SPECT (20 mins) and the patient receives a higher dose of radioactivity (but is within safety levels) than with two-dimensional gamma

scintigraphy (4 mins), the technique of SPECT is more sensitive in discriminating between aerosol deposition in large and small airways than conventional two-dimensional scintigraphy (Phipps *et al.*, 1989).

Ideally, the evaluation of deposition should have been done by adding radioactive isotope to the active drug. In the present study, however, this was not possible with either tussive agent since the deposition would be greatly affected by the cough and not reflect the site of the initiation for the cough reflex (Smaldone & Messina, 1985; Swift, 1987). Moreover, it would have been impractical to try to contain radioactivity during episodes of coughing. It was probable, however, that in the present study the same pattern of deposition was produced by both tussive agents and DTPA aerosols since both droplet size and inhalation technique were controlled appropriately.

There have been few studies examining the distribution of the airway sensory nerve endings in the respiratory tract in man which evoke airway reflexes such as cough (Woolman *et al.*, 1988). Using the same nebuliser systems, as described in the present study, Woolman *et al.*, (1988) demonstrated that a small droplet aerosol of distilled water, significantly, produced less coughing than a large droplet aerosol in normal subjects. From this study, the authors suggested that the "cough receptors" responsive to distilled water were located in the central airways. Further information regarding the location of the "cough receptors" responsive to distilled water has come from studies conducted in HLT patients. In these patients the afferent innervation is absent below the tracheal anastomosis. Although distilled water applied to the larynx caused coughing, there was a much reduced response to ultrasonically nebulised distilled water (Higenbottam *et al.*, 1989b). Taken together

these findings indicate that the "cough receptors" responsive to distilled water are located in the central airways, probably in the hilar region.

In the present study there is also evidence to suggest differences in the regional sensitivity in the respiratory tract of "cough receptors" responsive to capsaicin and PGE_2. Concentration-dependent cough responses were obtained by inhalation of both large and small droplet aerosols of capsaicin and PGE_2. Almost all of the subjects were more sensitive to the large droplet aerosol, both the cough threshold concentration and the concentration causing four coughs or more were lower with the large droplet aerosol than with the small droplet aerosol.

With the small droplet aerosol, a 7.5-fold higher concentration of PGE_2 was required, in comparison with the large droplet aerosol, to cause 50% of the subjects to cough at least four times. Deposition of an equivalent dose of PGE_2, which would compensate for the differences in the volume of liquid output deposited and the deposition pattern of the aerosols in the respiratory tract between the two aerosols, could explain a doubling in the concentration of PGE_2 when using the small droplet aerosol. However, since the increase in concentration was 7.5-fold, these results would indicate that the "cough receptors" in human central airways may be more responsive to inhaled PGE_2 than those in peripheral airways.

A 1.7-fold higher concentration of capsaicin was required when using a small droplet aerosol, in comparison with a large droplet aerosol, to cause 50% of the subjects to cough at least four times. However, when differences in the volume of liquid output deposited in the respiratory tract and the deposition pattern of the two aerosols are taken into account, the dose of capsaicin deposited by both aerosols in central and peripheral airways are almost equal. If "cough receptors" responsive to capsaicin

were located in the central airways, differences in the concentration of capsaicin used with both aerosols would have been expected, similar to PGE_2. However, this was not the case. The observation that HLT patients do not cough to inhaled capsaicin (Hathaway *et al.*, 1993), taken together with the findings in the present study, would suggest that the "cough receptors" responsive to inhaled capsaicin are located in the peripheral airways. This suggestion has gained support from a similar study of the selective deposition of inhaled capsaicin in normal subjects by Hansson *et al.*, (1992). From their results, the authors also concluded that capsaicin aerosol-induced coughing is mediated by "cough receptors" located in the peripheral airways.

Considering that capsaicin and PGE_2 are both believed to be "selective" C-fibre ending stimulants, it is quite surprising to find differences in the regional sensitivity of the respiratory tract to these agents. Coughing was also observed to continue after inhalation of PGE_2 but not after inhalation of capsaicin. In addition, some subjects after inhaling PGE_2 complained of retrosternal soreness and chest tightness. All of these observations raise the possibility that coughing evoked by capsaicin and PGE_2 aerosols may involve the activation of other airway afferent sensory nerve endings (for example, RARs). Indeed, capsaicin, when administered via the vasculature and in slightly higher concentrations than those used to stimulate C-fibre endings, can activate a proportion of RARs in cat airways (Armstrong & Luck, 1974). However, to date, there have been very few investigations examining the effects of inhaled capsaicin and PGE_2 on airway afferent sensory nerve endings using the technique of single nerve fibre recording (Roberts *et al.*, 1985; Adcock *et al.*, 1989).

In the PGE_2 study, it was interesting to note that, although the cough frequency was higher with the large droplet aerosol compared to the small droplet aerosol, the concentration of PGE_2 required to evoke coughing in the two current smokers was

the same. These results could be explained by an increased sensitivity of peripheral "cough receptors" to inhaled PGE_2. Smoking may, either directly or through airway inflammation, sensitise airway sensory nerve endings. Indeed, Niewoehner and colleagues (1974) demonstrated definite pathological changes in the peripheral airways of young cigarette smokers which were characteristic of respiratory bronchiolitis.

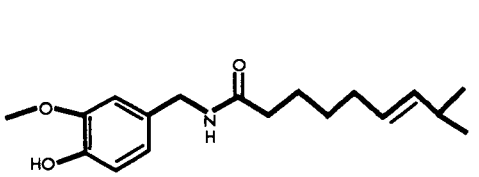
In the present study, it appears that in normal subjects, the "cough receptors" in the peripheral airways are particularly sensitive to inhaled capsaicin, whereas the "cough receptors" in the central airways appear to be more sensitive to inhaled PGE_2. The use of "selective" stimulants, such as capsaicin and PGE_2, is important in determining the topography and pharmacological nature of the various airway sensory nerve endings involved in mediating airway reflexes in man. However, to be in a position to draw positive conclusions, a comprehensive understanding of the pharmacological characteristics of the various airway afferent sensory nerve endings to each "selective" stimulant is required. From the original studies of Boushey *et al.*, (1974) and Boggs and Bartlett (1982), distilled water is thought to evoke cough through stimulation of myelinated laryngeal "irritant receptors". In these studies, however, there was little or no examination of the effect of distilled water on C-fibre endings. More recently, Pisarri and colleagues (1992) demonstrated that distilled water, instilled in the diaphragmatic lobe of dogs, is also an effective stimulus of both pulmonary and bronchial C-fibres. Thus it appears that distilled water is not selective for either myelinated "irritant receptors" or non-myelinated C-fibre endings. Indeed, there is no direct evidence to suggest that either capsaicin or PGE_2 are selective for one particular group of sensory nerve endings when administered by inhalation.

Chapter 4

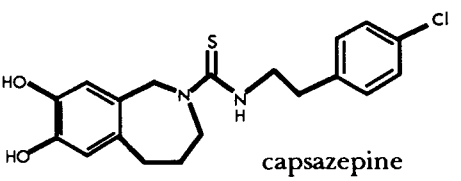
Effect of inhaled capsaicin and histamine on the spontaneous discharge of RARs

4.1 Introduction

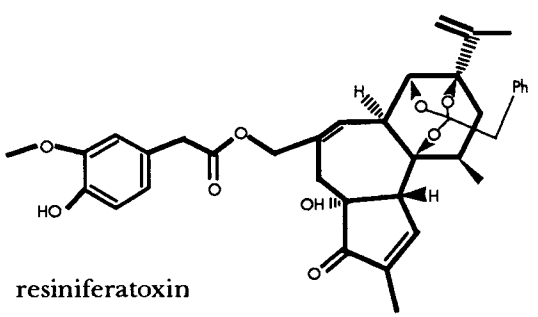
Capsaicin, 8-methyl-N-vanillyl-6-nonenamide (Fig. 4.1), is the main pungent ingredient in a wide variety of red peppers of the genus *Capsicum*. Peppers have been eaten by humans since pre-historical times (Lembeck, 1987). Yet, it was only first suggested by Höyges in 1878, that the pungent and irritant action of capsicol, an extract of *Capsicum*, was mediated mainly by an action on sensory nerves. Moreover, it was not until the middle of the 20th century that N. Jancso, realised that topical application of capsaicin, the pure extract, produced an initial inflammatory response which was followed by a specific and long-lasting insensitivity to chemical irritants; an effect mediated by a long-term sensory receptor blocking-action and which could be useful in the functional investigation of sensory neurons and the treatment of pain (Jansco, 1960, 1968; Szolcsanyi, 1982, 1984). A large number of studies have since corroborated this discovery and established capsaicin as an important probe for sensory neuron mechanisms and have been reviewed extensively by several research groups (Buck & Burks, 1986; Maggi & Meli, 1988; Holzer, 1988, 1991; Dray, 1992a, b; Maggi, 1992).



capsaicin



capsazepine



resiniferatoxin

Fig. 4.1 Structures of capsaicin, resiniferatoxin and capsazepine.

Primary afferent sensory neurons can be classified according to morphological, functional and neurochemical criteria (Holzer, 1991). Three groups of afferent nerve fibres can be separated (a) thick myelinated (b) thin myelinated and (c) thin non-myelinated. Physiologically, the thick myelinated fibres have the highest conduction velocities (Aαβ- fibres) and carry non-nociceptive mechanical information from the muscle and the skin. The thin non-myelinated C-fibres are primarily nociceptors (polymodal nociceptors, chemo-nociceptors) which respond to noxious mechanical, thermal and/or chemical stimuli and have the slowest conduction velocities. In addition, they also comprise some specific thermo-nociceptors as well as some non-nociceptive mechanical, cold and warmth receptors. The thin myelinated fibres have intermediate conduction velocities (Aδ-fibres) and carry both non-nociceptive (mechanoreceptors, cold receptors) and nociceptive (polymodal nociceptors and mechano-nociceptors) information.

Numerous studies have suggested that capsaicin preferentially excites thin primary afferent sensory nerves that are connected to distinct sensory receptors (Holzer, 1991). In particular, capsaicin seems to excite C-fibre chemo-nociceptors and polymodal nociceptors and some Aδ-fibre polymodal nociceptors (Table 4.1). On the basis of its "selectivity" for non-myelinated C-fibres, capsaicin has been used extensively to investigate the physiological and pharmacological profile of C-fibres in the respiratory tract (Coleridge & Coleridge, 1984, 1986). In the airways, afferent single nerve fibre recording studies have shown that minimal doses of capsaicin "preferentially" excite lung C-fibres, although larger doses of capsaicin may activate a proportion of myelinated Aδ-fibres (Armstrong & Luck, 1974; Kaufman *et al.*, 1980; Coleridge *et al.*, 1983).

Table 4.1 Selectivity of capsaicin's action on neuronal cells in mammals*.

1. Excitation of primary afferent nerves:

A) Application of capsaicin to the peripheral endings of sensory nerves.

i) Excitation of most, if not all, non-myelinated afferent nerves (C-fibres) connected to chemo-nociceptors.

ii) Excitation of many, but not all, non-myelinated afferent nerves (C-fibres. group IV afferents) connected to polymodal nociceptors.

iii) Excitation of some afferent C-fibres connected to some warmth receptors.

iv) Excitation of a minority of thinly myelinated afferent axons (Aδ-fibres. group III afferents) connected to polymodal nociceptors.

B) Application of capsaicin perineurally or to cell bodies in sensory ganglia.

i) Excitation of C-fibres and some Aδ-fibres when applied perineurally.

ii) Excitation of somata connected to only C-fibres.

2. Excitation of thermosensitive neurons in the pre-optic region of the hypothalamus

Table taken from Holzer, (1991). * Potency and effectiveness of capsaicin depend on the route of administration, age, strain and species of mammal being studied.

In spontaneously breathing animals, capsaicin administered via the vasculature, evokes a complex pulmonary chemoreflex response that includes bradycardia, hypotension and apnoea, followed by rapid shallow breathing; a response that can be abolished by bilateral vagotomy (Coleridge & Coleridge, 1984). It was first suggested, by the observations of Barer and Nusser (1953) and later clearly demonstrated by Karczewski and Widdicombe (1969a), that bronchoconstriction is also a component of the pulmonary chemoreflex. In anaesthetized, artificially ventilated animals, capsaicin administered via the vasculature, evokes bronchoconstrictor responses which can be abolished by either vagotomy or by vagal cooling (Russell & Lai-Fook, 1979; Coleridge *et al.*, 1982). Moreover, capsaicin, administered as an inhaled aerosol to the lower respiratory tract of anaesthetized, paralysed and artificially ventilated cats, has also been shown to evoke a vagally-mediated bronchoconstriction, a response which can be abolished by either bilateral vagotomy or pre-treatment with atropine (Adcock *et al.*, 1989).

In man, inhalation studies have shown that capsaicin produces cough (Collier & Fuller, 1984) and a transient (<1 min) bronchoconstriction, which is partly inhibited by ipratropium bromide, a muscarinic receptor antagonist (Fuller at al., 1985). The cough response to capsaicin is dose-dependent and reproducible, with little evidence of tachyphylaxis (Collier & Fuller, 1984; Fuller *et al.*, 1988). Although intravenous administration of capsaicin in man does not produce either bronchoconstriction or a feeling of breathlessness, coughing was reported in one of three subjects studied (Winning *et al.*, 1986). Inhaled capsaicin, has also been reported to cause coughing and bronchoconstriction in conscious guinea-pigs (Forsberg & Karlsson, 1986; Forsberg *et al.*, 1986).

Inhalation of histamine in man has been shown to cause both coughing and bronchoconstriction (Empey *et al.*, 1976). It is well established that histamine, administered either via the vasculature or as an inhaled aerosol, is a potent stimulus of RARs in a number of species (Mills *et al.*, 1969; Armstrong & Luck, 1974; Sampson & Vidruk, 1975; Adcock, 1989). Histamine also stimulates C-fibre endings, although this effect is relatively weak when compared with capsaicin (Coleridge & Coleridge, 1984).

From the single nerve fibre studies, it has been suggested that the airway reflexes, cough and reflex bronchoconstriction, evoked by inhaled capsaicin in both man and experimental animals are mediated mainly by stimulation of C-fibres (Collier & Fuller, 1984; Fuller *et al.*, 1985; Forsberg *et al.*, 1986; Forsberg & Karlsson, 1986, 1987; Midgren *et al.*, 1986). However, there have been few, if any, studies examining the effects of inhaled capsaicin on RARs (Adcock *et al.*, 1989). Therefore, the aim of the present series of experiments was to compare the effect of inhaled solutions of

capsaicin with those of histamine on vagal fibres arising from intrathoracic RARs in anaesthetized, paralysed and artificially ventilated cats.

4.2 Methods

Male cats (3.1 - 4.6 kg) were anaesthetized and prepared for surgery as described in Chapter 2. Airflow (ml s^{-1}) and transpulmonary pressure (cmH_2O) were measured. Tidal volume (ml), C_{Dyn} (ml cmH_2O^{-1}) and R_L ($cmH_2O\ l^{-1}$ s) were computed from these measurements, on a breath-by-breath basis, with an on-line Buxco pulmonary mechanics analyser. Identification and single afferent nerve fibre recording of the RARs was carried out as described in Chapter 2. Arterial blood pressure and heart rate were also recorded. Additional cannulae (Portex 4fg) were inserted in the right jugular vein and right carotid artery (pushed into the aortic arch) for administration of drugs. The animals were paralysed with DMT and artificially ventilated according to the protocol in Chapter 2. Drugs were administered by two routes, i.v. and inhalation (DeVilbiss Ultrasonic nebuliser, Chapter 2).

Materials

Histamine diphosphate and atropine sulphate were dissolved in saline (0.85% wt/vol); capsaicin in an absolute ethanol, Tween 80 and saline mixture in the ratio 1:1:23. A stock solution of capsaicin (1 mg ml^{-1}) was prepared and further dilutions were made in saline. Capsaicin vehicle consisted of an ethanol, Tween 80 and saline mixture in the ratio of 1:1:23. All of the drugs were expressed as the free base.

Protocols

After surgery the animals were allowed to stabilise for at least 30 min. Single nerve fibres were dissected from the left vagus nerve and action potentials recorded. Each

animal, and, therefore each nerve fibre, was subjected to only one of the following protocols.

Unilateral vagotomy

Upon identification of a vagal fibre originating from an intrathoracic RAR, capsaicin vehicle aerosol was administered by inhalation (6 breaths). This was followed by administration of capsaicin aerosols, in each case 6 breaths from solutions of 0.1 and 1 mg ml^{-1}, at 8 min intervals. Inhalation of histamine aerosol, 6 breaths of a 1 mg ml^{-1} solution, was repeated at the end of the protocol to ensure that the RAR was still active.

Bilateral vagotomy

Bilateral vagotomy was performed prior to the task of dissecting single nerve fibres from the left vagus nerve. After identification of a vagal fibre originating from an intrathoracic RAR, capsaicin vehicle aerosol was administered by inhalation (6 breaths). This was followed eight minutes later by administration of capsaicin aerosol, 6 breaths of a 1 mg ml^{-1} solution. Inhalation of histamine aerosol, 6 breaths of a 1 mg ml^{-1} solution, was repeated at the end of the protocol to ensure that the RAR was still active.

Atropine pre-treatment

When a vagal fibre originating from an intrathoracic RAR was found, atropine was administered intravenously (0.1 mg kg^{-1}). After a 10 min interval, capsaicin aerosol was administered by inhalation (6 breaths generated from a 1 mg ml^{-1} solution). Inhalation of histamine aerosol, 6 breaths of a 1 mg ml^{-1} solution, was repeated at the end of the protocol to ensure that the RAR was still active.

Data Analysis

Nerve impulses were counted in 5 second period "bins". Control periods were counted for 1 min before administration of the irritant agent and expressed as the average impulses s^{-1} (imp s^{-1}). Impulse discharges, evoked by the vehicle and irritant agents, were averaged (imp s^{-1}) during the period of the response (in the case of the vehicle 1 min) and expressed as the Δ (imp s^{-1}) from the control period. In all cases the mean ± standard error of the mean (sem) is shown.

The effect of vehicle and irritant agents on arterial blood pressure and heart rate are expressed as the peak changes in absolute values, whereas C_{Dyn} and R_L responses are expressed as the maximum percentage change from pre-treatment values.

Histamine aerosol and i.v. effects: The effect of histamine (aerosol and i.v.) on airway sensory nerve discharge, C_{Dyn}, R_L, arterial blood pressure and heart rate were compared with baseline using Student's "t" test for paired data. In addition, the responses to histamine aerosol, before and after the capsaicin protocols, were analysed using Student's "t" test for paired data. Statistical values of $P < 0.05$ were considered to be significant.

Capsaicin effects in unilaterally vagotomised cats: The effect of capsaicin aerosols (0.1 and 1 mg ml^{-1} solutions) on airway sensory nerve discharge were compared with the vehicle aerosol using Student's "t" test for paired data. Data comparing the effects of the capsaicin aerosols (0.1 and 1 mg ml^{-1} solutions) with the vehicle aerosol on arterial blood pressure, heart rate, C_{Dyn} and R_L were analysed using one-way analysis of variance (ANOVA). Statistical values of $P < 0.05$ were considered to be significant.

Capsaicin effects in bilaterally vagotomised cats: The effect of capsaicin aerosol (1 mg ml^{-1} solution) on airway sensory nerve discharge, arterial blood pressure, heart rate, C_{Dyn} and R_L were compared with the vehicle aerosol using Student's "t" test for paired data. Statistical values of $P < 0.05$ were considered to be significant.

Capsaicin effects in atropine-treated cats: The effect of capsaicin aerosol (1 mg ml^{-1} solution) on airway sensory nerve discharge, arterial blood pressure, heart rate, C_{Dyn} and R_L were compared with baseline using Student's "t" test for paired data. Statistical values of $P < 0.05$ were considered to be significant.

4.3 Results

4.3.1 Baseline Values

20 male cats were used. The mean ± sem of the resting values of pulmonary mechanics and cardiovascular parameters for 20 open chest animals are shown in Table 4.2.

Table 4.2 Baseline values for C_{Dyn}, R_L, systolic and diastolic blood pressure and heart rate in anaesthetized, paralysed and artificially ventilated cats. Values are shown as mean ± sem, (n=20, unless otherwise stated).

parameters	baseline values
C_{Dyn}, ml cmH_2O^{-1}	5.8 ± 0.6 (n=19)
R_L, $cmH_2O\ l^{-1}\ s$	19.4 ± 1.4
systolic blood pressure, mmHg	130 ± 6.6 (n=19)
diastolic blood pressure, mmHg	89 ± 4.7 (n=19)
Heart rate, beats min^{-1}	154 ± 7.5 (n=19)

4.3.2 Effect of Capsaicin and Histamine aerosols on RARs in cats with the right vagus intact

Nine intrathoracic RARs were examined in nine cats. The spontaneous discharge rate in the fibres was 2.17 ± 0.6 imp s^{-1} (range 0.08 - 6.12 imp s^{-1}). The adaptation index of each receptor was 100%. Administration of histamine intravenously (10 μg kg^{-1}) and by aerosol (6 breaths, 1 mg ml^{-1} solution) evoked a significant increase in the rate of discharge of RARs (5.13 ± 1.58 imp s^{-1}, $P < 0.01$; 2.62 ± 0.83 imp s^{-1}, $P < 0.01$, respectively) and caused significant bronchoconstriction as shown by a fall in C_{Dyn} (-33 ± 3%, $P < 0.001$; -30 ± 4%, $P < 0.001$, respectively) and increase in R_L (+70 ± 11%, $P < 0.01$; +67 ± 25%, $P < 0.01$, respectively) (Fig. 4.2). The effect of histamine aerosol (6 breaths generated from a 1 mg ml^{-1} solution) on the discharge of an RAR, together with the effect on C_{Dyn}, is shown in Fig. 4.3A. At the end of the protocol, histamine aerosol still evoked significant ($P < 0.001$) increases in RAR discharge (1.9 ± 0.3 imp s^{-1}) and caused significant bronchoconstriction (C_{Dyn} -24 ± 4%, $P < 0.001$; R_L +53 ± 13%, $P < 0.01$) (Fig. 4.2). There was no significant difference between histamine aerosol-evoked responses before or after capsaicin aerosols on RARs or R_L, but the response on C_{Dyn} was significantly ($P < 0.01$) different (Fig. 4.2).

In the same animals, capsaicin vehicle (6 breaths) had no significant ($P > 0.05$) effect on either pulmonary mechanics (C_{Dyn} -0.1 ± 2%, R_L -6 ± 2%) or rate of RAR discharge (0.17 ± 0.19 imp s^{-1}). However, capsaicin aerosol (0.1 or 1 mg ml^{-1} solution) stimulated 6 of the 9 RARs examined. An example of the response of a single RAR to capsaicin (6 breaths, 0.1 mg ml^{-1} solution), together with the effects of capsaicin on either C_{Dyn} or C_{Dyn}, R_L and cardiovascular variables are shown in Figs 4.3B and 4.4. The responses of all the RARs studied to capsaicin aerosols are shown in Table 4.3.

Table 4.3 Varying response of RARs to inhalation of capsaicin aerosols.

Number of RARs	Capsaicin Vehicle	Capsaicin (0.1 mg ml^{-1})	Capsaicin (1 mg ml^{-1})
3	-	-	-
3	-	+	+
1	-	-	+
2	-	+	-

+, stimulation **-, no stimulation**

When data from all 9 RARs (including the non-responders) were pooled, capsaicin (6 breaths, 0.1 mg ml^{-1} solution) significantly ($P < 0.05$) increased RAR discharge from 0.17 ± 0.19 imp s^{-1} (vehicle) to 2.06 ± 0.74 imp s^{-1}. Subsequent inhalation of a higher concentration of capsaicin (6 breaths, 1 mg ml^{-1} solution) also apparently increased RAR discharge rate by 1.29 ± 0.67 imp s^{-1}, but this was not significantly different from the control (Fig. 4.2). The mean duration of capsaicin aerosol evoked discharges was 43.3 ± 9.57 s, range being 15 - 95 s. Inhalation of capsaicin aerosols (0.1 & 1 mg ml^{-1} solutions) caused significant bronchoconstriction as shown by a fall in C_{Dyn} (-9 ± 3%, $P < 0.01$; -6 ± 2%, $P < 0.05$, respectively) and rise in R_L (+96 ± 37%, $P < 0.01$; +54 ± 15%, $P > 0.05$, respectively) (Fig. 4.2).

Administration of capsaicin aerosol (1 mg ml^{-1} solution) and i.v. histamine (10 μg kg^{-1}) evoked significant decreases in arterial blood pressure and heart rate (Table 4.4). Histamine aerosol, before and after capsaicin administration, also evoked significant decreases in arterial blood pressure, but had no significant effect on heart rate (Table 4.4). Inhalation of capsaicin vehicle had no significant effect on either arterial blood pressure or heart rate (Table 4.4). Although capsaicin aerosol (0.1 mg ml^{-1}) reduced arterial blood pressure and heart rate, only diastolic blood pressure was significantly affected (Table 4.4).

4.3.3 Effect of Capsaicin and Histamine aerosols on RARs in cats which were either bilaterally vagotomised or treated with atropine

Bilateral vagotomy

Seven intrathoracic RARs were examined in seven cats. The spontaneous discharge rate in the fibres was 2.2 ± 0.6 imp s^{-1} (range 0.2 - 4.3 imp s^{-1}). The adaptation index of each receptor was 100%. Administration of histamine by aerosol (6 breaths, 1 mg ml^{-1} solution) before and after capsaicin administration, evoked significant increases in the rate of discharge of RARs (2.3 ± 0.5 imp s^{-1}, $P < 0.01$; 2.51 ± 0.6 imp s^{-1}, $P < 0.01$ respectively) and caused significant bronchoconstriction as shown by a fall in C_{Dyn} (-22 ± 3 %, n=7, $P < 0.001$; -19 ± 2%, n=6, $P < 0.01$, respectively) and increase in R_L (+32 ± 7%, n=7, $P < 0.01$; +31 ± 8%, n=4, $P < 0.05$, respectively) (Fig. 4.5). Histamine aerosol also evoked significant falls in arterial blood pressure before and after administration of capsaicin aerosols but had no effect on heart rate (Table 4.5).

In comparison with histamine aerosol, capsaicin aerosol (6 breaths, 1 mg ml^{-1} solution) failed to evoke bronchoconstriction but stimulated one of the seven RARs tested (Fig. 4.6). When these data were pooled, neither inhalation of capsaicin vehicle (6 breaths) nor capsaicin aerosol (6 breaths, 1 mg ml^{-1} solution) had any significant ($P > 0.05$) effect on pulmonary mechanics (C_{Dyn} -0.3 ± 1%, n=5 & 0.4 ± 1%, n=5; R_L -3 ± 4%, n=4 & -8 ± 2%, n=4 respectively) or rate of RAR discharge (0.2 ± 0.4 imp s^{-1}, n=7; 0.5 ± 0.1 imp s^{-1}, n=7 respectively) (Fig. 4.5). Inhalation of capsaicin aerosol (1 mg ml^{-1} solution) significantly increased arterial blood pressure when compared with the capsaicin vehicle response; neither aerosol affected heart rate (Table 4.5). These effects with capsaicin aerosol on cardiovascular parameters, in

bilaterally vagotomised animals, are in direct contrast to those with capsaicin aerosol in animals with one vagus nerve intact.

Atropine pre-treatment

Four intrathoracic RARs were examined in four cats. The spontaneous discharge rate in the fibres was 0.81 ± 0.52 imp s^{-1} (range 0.5 - 2.27 imp s^{-1}). The adaptation index of each receptor was 100%. Histamine aerosol (6 breaths, 1 mg ml^{-1} solution), before capsaicin aerosol administration, evoked a significant increase in the rate of RAR discharge (3.4 ± 0.8 imp s^{-1}, $P < 0.05$) and caused bronchoconstriction as shown by a fall in C_{Dyn} (-24 ± 4%, $P < 0.01$) and increase in R_L (+48 ± 10%, $P < 0.05$). Although, histamine aerosol apparently increased the rate of RAR (2.54 ± 1.2 imp s^{-1}) discharge after capsaicin aerosol administration, this was found not to be significant. In addition, the effects of histamine aerosol on pulmonary mechanics were variable (C_{Dyn} -17 ± 3%, $P > 0.05$; R_L +41 ± 11%, $P < 0.05$) (Fig. 4.7). Similarly, histamine aerosol, before but not after capsaicin administration, significantly decreased arterial blood pressure. Histamine aerosol had no significant effect on heart rate (Table 4.6).

Capsaicin aerosol (6 breaths, 1 mg ml^{-1}) stimulated three out of the four RARs tested, although when these data were pooled, inhaled capsaicin did not significantly ($P > 0.05$) affect the rate of RAR discharge (1.26 ± 0.55 imp s^{-1}) (Fig. 4.7). In addition, capsaicin aerosol did not affect pulmonary mechanics (C_{Dyn} +2 ± 2%, R_L - 12 ± 10%), arterial blood pressure or heart rate (Fig. 4.7; see Table 4.6 for the effects on cardiovascular parameters).

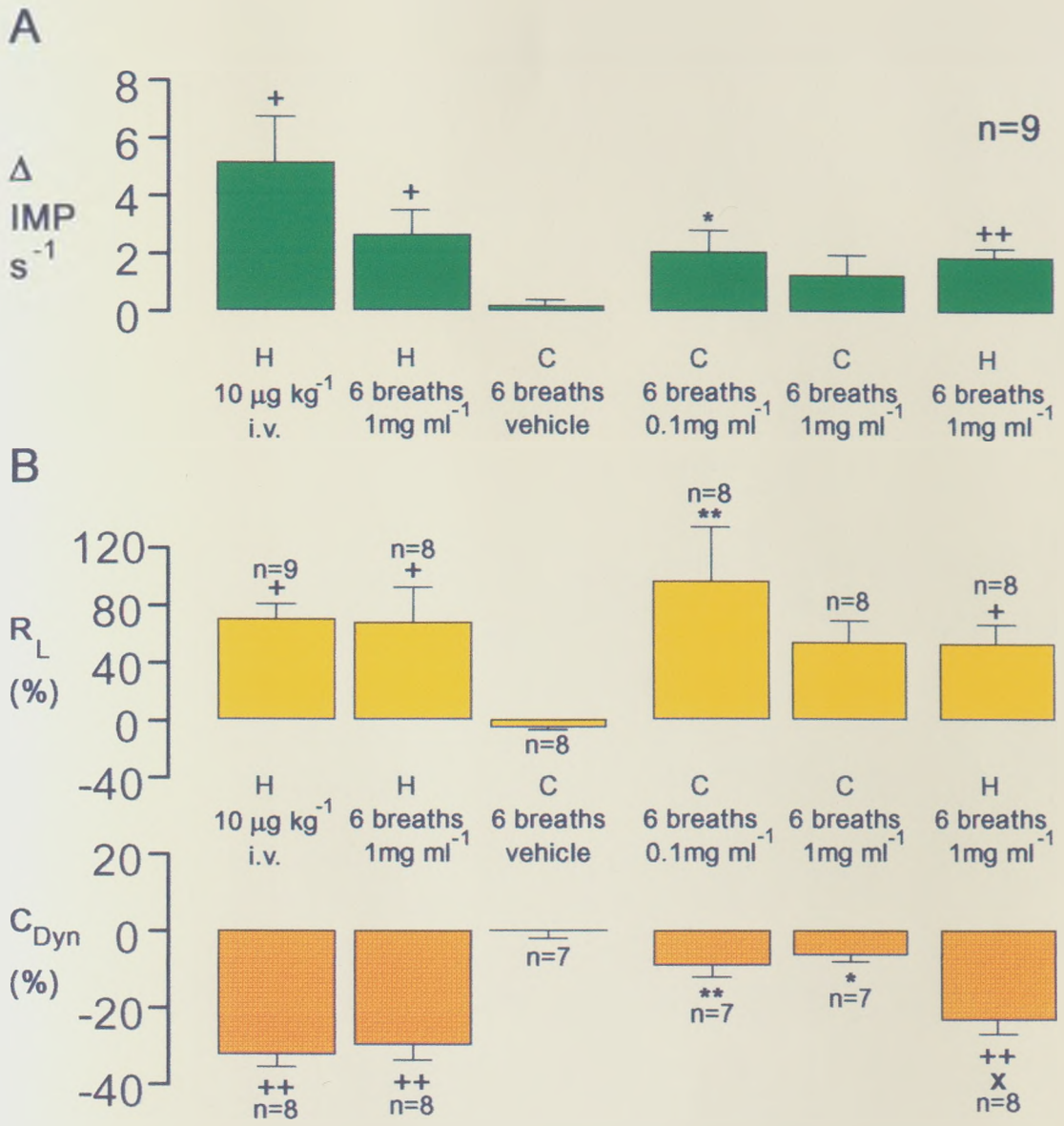


Fig. 4.2 **A**, discharges in vagal fibres arising from intrathoracic RARs (n=9), expressed as the change in impulses s^{-1} (Δ IMP s^{-1}) from spontaneous levels and **B**, percentage changes in dynamic lung compliance (C_{Dyn}) and total lung resistance (R_L) evoked by sequential administration of histamine (H) and capsaicin (C) in anaesthetized, paralysed and artificially ventilated cats with the right vagus intact. Histamine was administered intravenously (10 µg kg^{-1}) and by aerosol (6 breaths, 1 mg ml^{-1} solution). Capsaicin was administered by inhalation in increasing doses (6 breaths, vehicle, 0.1 mg ml^{-1} and 1 mg ml^{-1} solutions). Vertical bars represent sem. Significantly different histamine-evoked responses from control baselines are shown by + $P < 0.01$, ++ $P < 0.001$. Significantly different histamine aerosol-evoked responses are shown by X $P < 0.01$. Significantly different capsaicin-evoked responses (0.1 and 1 mg ml^{-1}) compared with capsaicin vehicle responses are shown by * $P < 0.05$, ** $P < 0.01$.

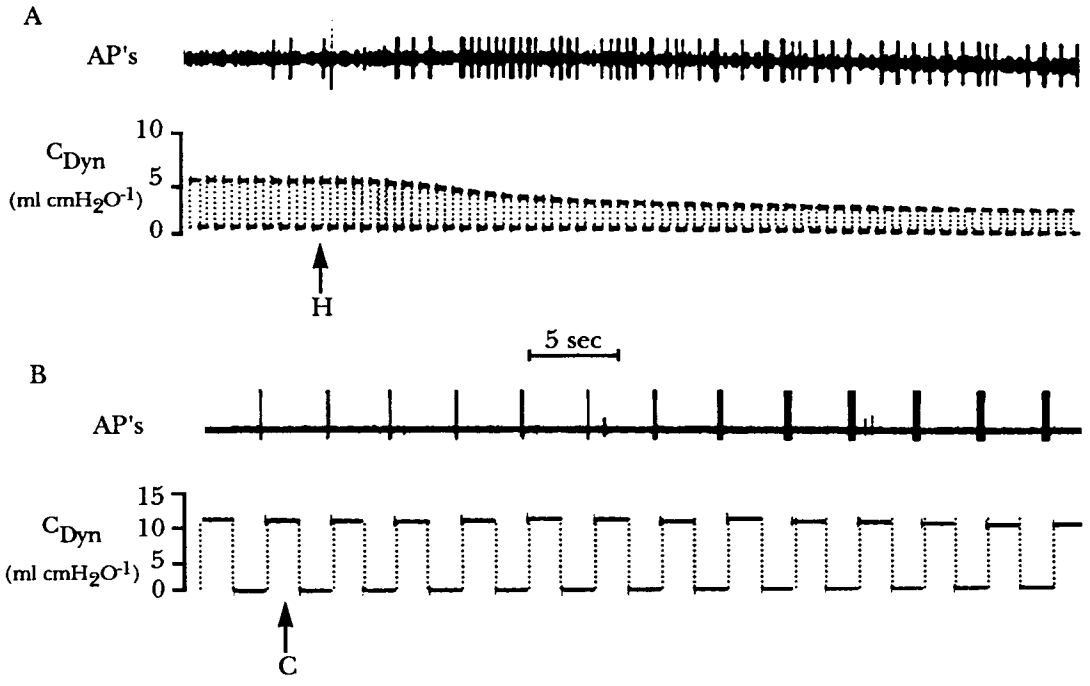


Fig. 4.3 Discharges in vagal fibres arising from RARs, evoked by inhalation of histamine (H in **A**) and capsaicin (C in **B**) in anaesthetized, paralysed and artificially ventilated cats with the right vagus intact. The RARs were stimulated by inhalation of aerosols of histamine (6 breaths, 1 mg ml^{-1} solution) and capsaicin (6 breaths, 0.1 mg ml^{-1} solution). Concomitant recordings of dynamic lung compliance (C_{Dyn}) are also shown. AP, action potentials.

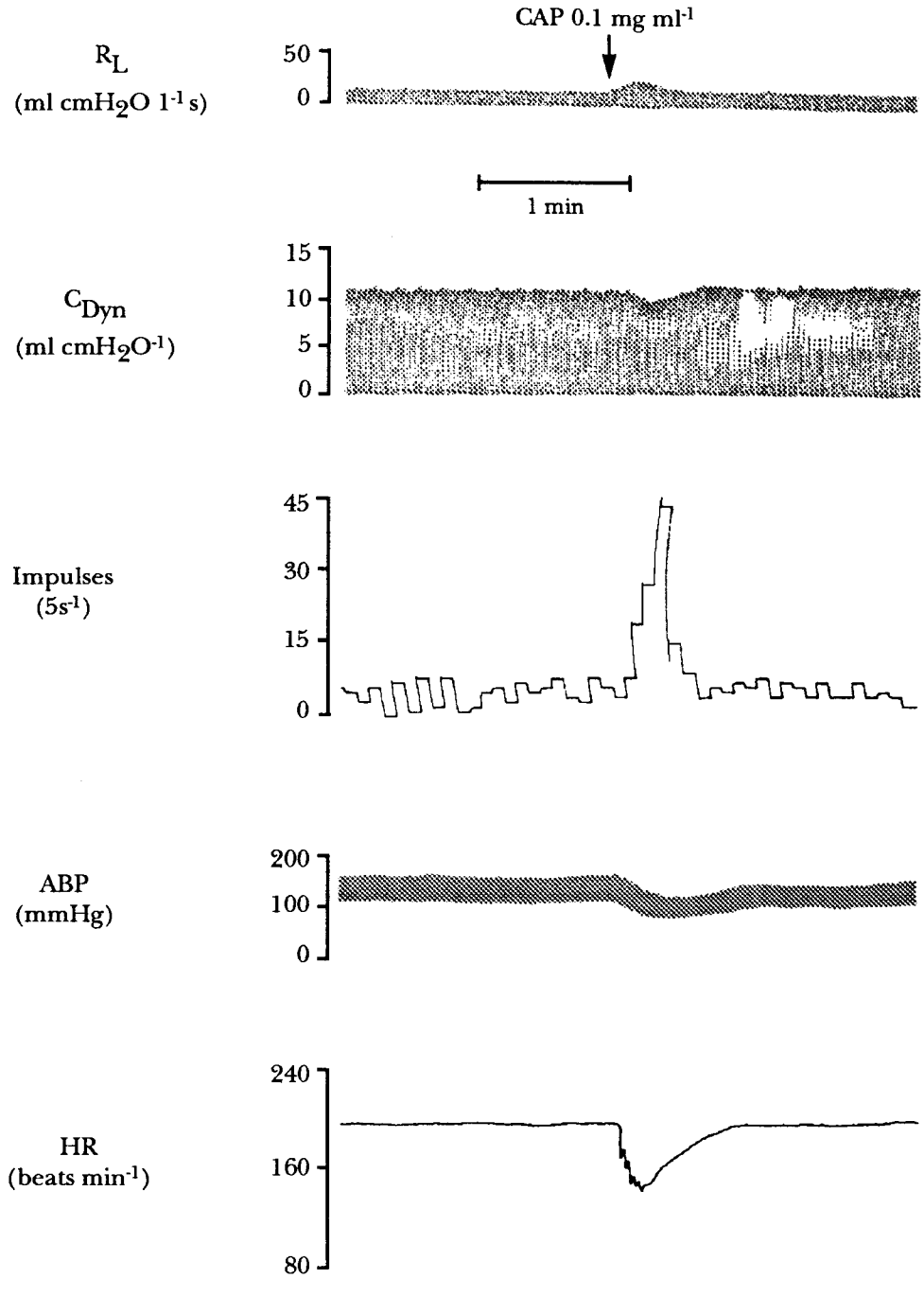


Fig. 4.4 Changes in dynamic lung compliance (C_{Dyn}), lung resistance (R_L), impulse discharges (measured in 5 s bins) arising from an intrathoracic RAR, arterial blood pressure (ABP) and heart rate (HR) evoked by inhalation of capsaicin aerosol (CAP; 6 breaths, 0.1 mg ml^{-1} solution) in an anaesthetized, paralysed and artificially ventilated cat with the right vagus intact.

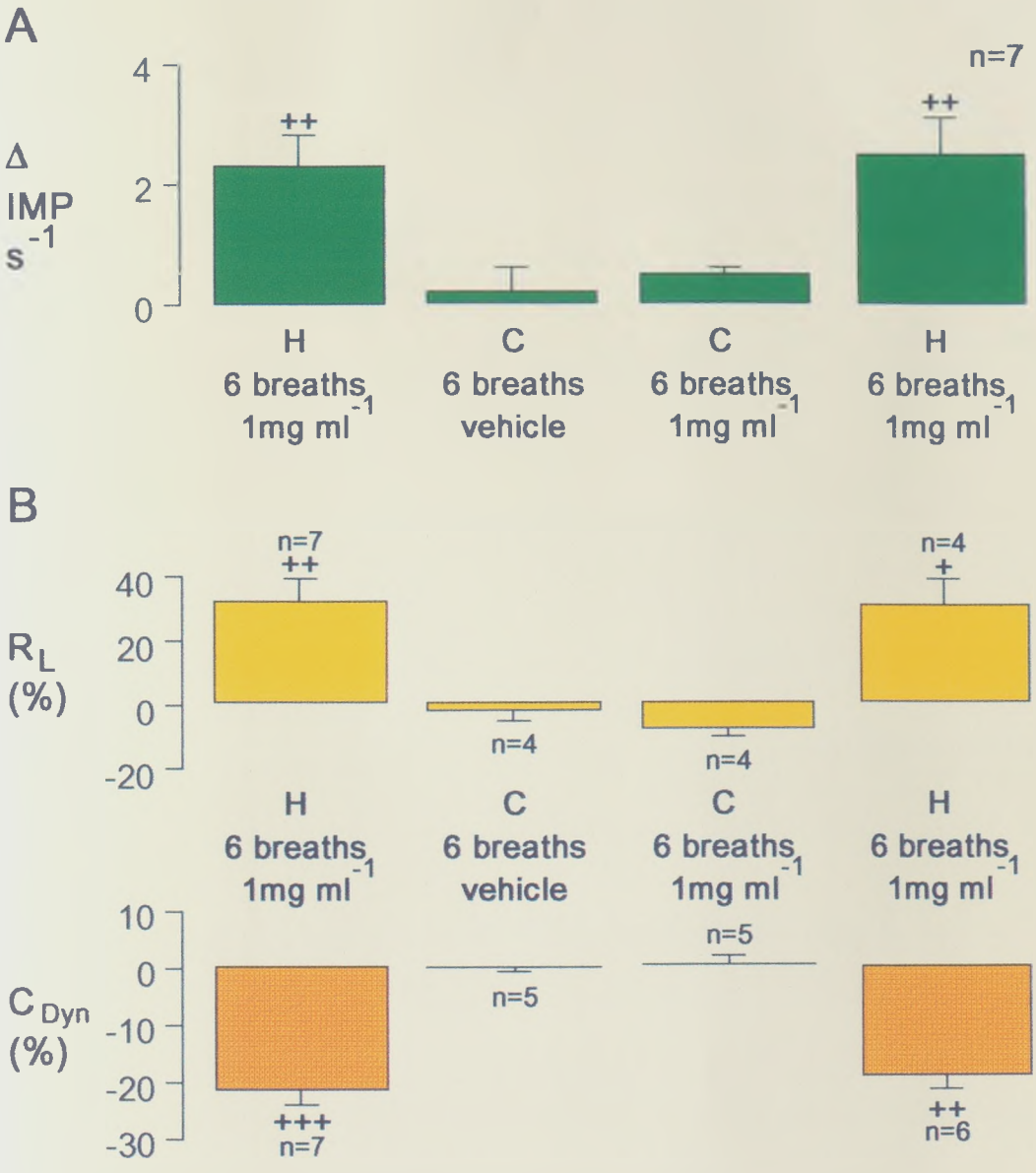


Fig. 4.5 **A**, discharges in vagal fibres arising from intrathoracic RARs (n=7), expressed as the change in impulses s^{-1} (Δ IMP s^{-1}) from spontaneous levels and **B**, percentage changes in dynamic lung compliance (C_{Dyn}) and total lung resistance (R_L) evoked by sequential administration of histamine (H) and capsaicin (C) in bilaterally vagotomised, anaesthetized, paralysed and artificially ventilated cats. Histamine was administered by aerosol (6 breaths, 1 mg ml^{-1} solution). Capsaicin was administered by inhalation in increasing doses (6 breaths, vehicle and 1 mg ml^{-1} solutions). Vertical bars represent sem. Significantly different histamine-evoked responses from control baselines are shown by + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$.

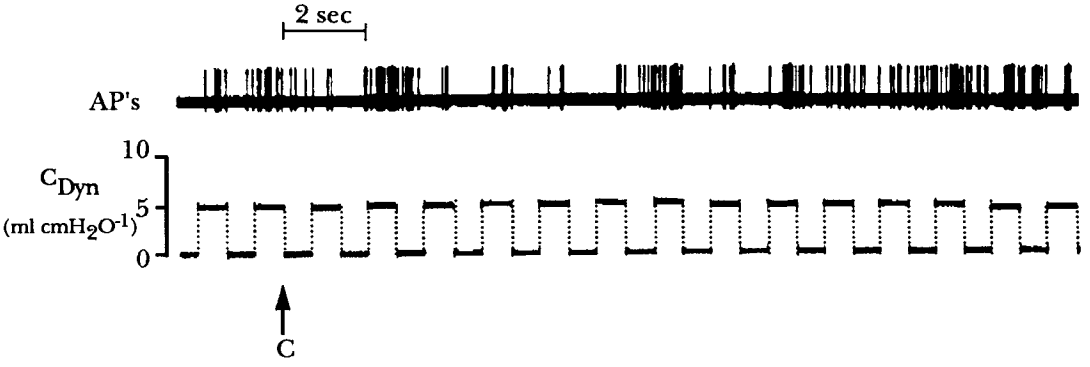


Fig. 4.6 Discharges in a vagal fibre arising from an RAR, evoked by inhalation of capsaicin (C) in a bilaterally vagotomised, anaesthetized, paralysed and artificially ventilated cat. The RAR was stimulated by inhalation of capsaicin aerosol (6 breaths, 1 mg ml^{-1} solution). Concomitant recording of dynamic lung compliance (C_{Dyn}) is also shown. AP, action potentials.

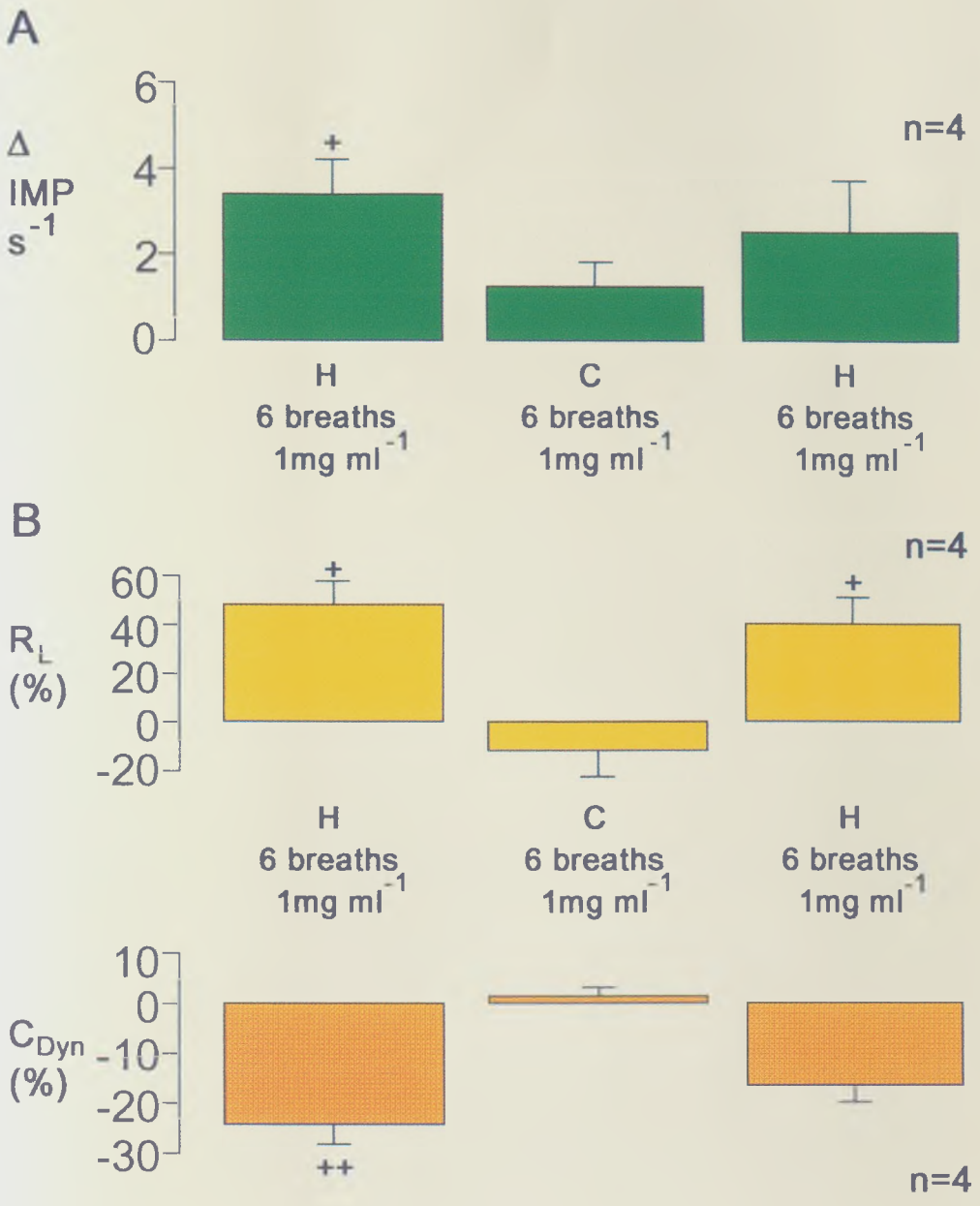


Fig. 4.7 **A**, discharges in vagal fibres arising from intrathoracic RARs (n=4), expressed as the change in impulses s^{-1} (Δ IMP s^{-1}) from spontaneous levels and **B**, percentage changes in dynamic lung compliance (C_{Dyn}) and total lung resistance (R_L) evoked by sequential administration of histamine (H) and capsaicin (C) in, anaesthetized, paralysed and artificially ventilated cats pre-treated with atropine (i.v., 0.1 mg kg^{-1}). Histamine was administered by aerosol (6 breaths, 1 mg ml^{-1} solution). Capsaicin was administered by inhalation (6 breaths, 1 mg ml^{-1} solution). Vertical bars represent sem. Significantly different histamine-evoked responses from control baselines are shown by + $P < 0.05$, ++ $P < 0.01$.

Table 4.4 Effect of histamine (i.v. and aerosol) and capsaicin aerosols on arterial blood pressure and heart rate in anaesthetized, paralysed and artificially ventilated cats with the right vagus intact.

	Δ systolic blood pressure (mmHg)	Δ diastolic blood pressure (mmHg)	Δ heart rate (beats min^{-1})
Histamine 10 μg kg^{-1}	-42 ± 5†††† (n=8)	-50 ± 5†††† (n=8)	+25 ± 8† (n=8)
Histamine aerosol (6 breaths, 1 mg ml^{-1})	-18 ± 8†	-20 ± 7†	+10 ± 3††
Capsaicin aerosol (6 breaths, vehicle)	+1 ± 3	+1 ± 1	-1 ± 1
Capsaicin aerosol (6 breaths, 0.1 mg ml^{-1})	-21 ± 9	-17 ± 6*	-26 ± 7
Capsaicin aerosol (6 breaths, 1 mg ml^{-1})	-25 ± 6*	-22 ± 5*	-34 ± 11*
Histamine aerosol (6 breaths, 1 mg ml^{-1})	-29 ± 7††	-22 ± 5††††	+13 ± 4††

+, increase -, reduction

All agents were administered sequentially. Values shown are mean ± sem absolute changes. n=9 unless otherwise stated. Significantly different histamine-evoked responses from the control baselines are shown by † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$. Significantly different capsaicin-evoked responses (0.1 and 1 mg ml^{-1} solutions) compared with capsaicin vehicle responses are shown by * $P < 0.05$, ** $P < 0.01$.

Table 4.5 Effect of histamine and capsaicin aerosols on arterial blood pressure and heart rate in bilaterally vagotomised, anaesthetized, paralysed and artificially ventilated cats.

	Δ systolic blood pressure (mmHg)	Δ diastolic blood pressure (mmHg)	Δ heart rate (beats min^{-1})
Histamine aerosol (6 breaths, 1 mg ml^{-1})	-31 ± 7††	-24 ± 5††	+8 ± 6
Capsaicin aerosol (6 breaths vehicle)	-8 ± 4	-4 ± 2	-1 ± 2
Capsaicin aerosol (6 breaths, 1 mg ml^{-1})	+11 ± 5*	+12 ± 4**	+5 ± 2
Histamine aerosol (6 breaths, 1 mg ml^{-1})	-25 ± 7†	-21 ± 6††	+8 ± 4

+, increase -, reduction

All agents were administered sequentially. Values shown are mean ± sem absolute changes, (n=7). Significantly different histamine-evoked responses from control baselines are shown by † $P < 0.05$, †† $P < 0.01$. Significantly different capsaicin-evoked responses (1 mg ml^{-1} solution) compared with capsaicin vehicle responses are shown by * $P < 0.05$, ** $P < 0.01$.

Table 4.6 Effect of histamine and capsaicin aerosols on arterial blood pressure and heart rate in anaesthetized, paralysed and artificially ventilated cats pre-treated with atropine (0.1 mg kg^{-1}, i.v.).

	Δ systolic blood pressure (mmHg)	Δ diastolic blood pressure (mmHg)	Δ heart rate (beats min^{-1})
Histamine aerosol (6 breaths, 1 mg ml^{-1})	-26 ± 7†	-18 ± 4†	0 ± 1
Capsaicin aerosol (6 breaths, 1 mg ml^{-1})	-4 ± 5	-5 ± 5	-1 ± 1
Histamine aerosol (6 breaths, 1 mg ml^{-1})	-20 ± 7	-13 ± 5	+6 ± 4

+, increase -, reduction

All agents were administered sequentially. Values shown are mean ± sem absolute changes, (n=4). Significantly different histamine-evoked responses from control baselines are shown by † $P < 0.05$.

4.4 Discussion

In initial experiments, inhaled aerosols of capsaicin, at concentrations known to cause bronchoconstriction and C-fibre stimulation in cats (Adcock & Smith, 1989), evoked bronchoconstrictor responses and stimulated a proportion of the RARs tested. Inhaled capsaicin also stimulated a proportion of the RARs tested in later experiments in which the bronchoconstrictor responses to capsaicin had been abolished by either bilateral vagotomy or pre-treatment with atropine. In both studies, inhaled histamine evoked bronchoconstriction and stimulated all of the RARs examined.

Capsaicin perhaps should not be regarded as a selective stimulant of C-fibre endings in the respiratory tract. At approximately twice the dose needed to stimulate C-fibre endings (Adcock *et al.*, 1989), 6 breaths of a 0.1 mg ml^{-1} solution, capsaicin stimulated 5 out of 9 RARs tested in animals with the right vagus intact. Armstrong and Luck (1974) had similarly noted that 37% of the RARs they examined responded to 20 $\mu g\ kg^{-1}$ of intravenous capsaicin. Other studies have shown that capsaicin can activate Aδ- polymodal nociceptors in the cat cornea (Belmonte *et al.*, 1991) and also a proportion of endings of Aδ- fibres from abdominal visceral organs in the cat (Longhurst *et al.*, 1984). Contrary to the current findings, Fox *et al.*, (1993) have recently shown that capsaicin is a potent stimulator of non-myelinated C-fibres but not of Aδ-fibres in guinea-pig airways *in vitro*. In their study, extracellular recordings were made from single vagal afferents with receptive fields located on the epithelial surface of the trachea and main bronchi, opened longitudinally on their ventral surface. Capsaicin and other inflammatory mediators were applied directly onto the receptive field and the effects on vagal afferent nerve activity were recorded using conventional electrophysiological techniques. It should be noted, however, that RARs with Aδ- fibres in the extrathoracic airways are more mechanosensitive, whereas those in the intrathoracic airways, not examined by Fox and colleagues, are more

chemosensitive. It seems likely, therefore, that Fox *et al.*, (1993) overlooked the capsaicin-sensitive RARs discussed in the present study. Alternatively, the contrasting findings may be the result of species variation in the response of Aδ- fibres to capsaicin. To date, there have been no published single nerve fibre studies describing the effects of capsaicin on airway afferent nerve fibres in guinea-pigs *in vivo*.

The findings from the present study and earlier studies (Armstrong & Luck, 1974), clearly suggest that sub-populations of RARs may exist which are capable of responding to capsaicin. It is possible that the RARs stimulated by capsaicin are similar to the more chemosensitive receptors described by Widdicombe (1954b). He classified RARs into two groups on the basis of their adaptation indices (AI). The more deeply located RARs (RARs located in the distal airways and which have AI's of between 70-100%) were thought to be more chemosensitive than the more proximal RARs (RARs located in and around the hilum and which have AI's of 100%) which were more mechanosensitive. This explanation would correlate with the findings in human studies (See Chapter 3; Hansson *et al.*, 1992), that the distal afferent sensory nerve endings are more responsive to inhaled capsaicin. However, subdivision of RARs, based on AI's, seems less likely as the RARs capable of responding to capsaicin aerosol, in the present study, were all highly rapidly adapting with AI's of 100%. Of practical importance, it may be necessary to sample large numbers of RARs to avoid overlooking the effect (Adcock *et al.*, 1989).

There has been considerable debate as to whether RARs are activated directly or indirectly by inflammatory mediators and foreign materials. Investigations into this have been complicated by the finding that most agents known to stimulate RARs, are themselves powerful bronchoconstrictors. Although inhaled capsaicin evokes reflex bronchoconstriction, this response can be abolished by either bilateral vagotomy or

pre-treatment with atropine (Adcock *et al.*, 1989). Thus by examining the effects of inhaled capsaicin on RARs, in cats which are either bilaterally vagotomised or pre-treated with atropine, experimental conditions make it possible to determine if RARs can be activated by a direct chemical stimulation of the nerve endings in the lungs and airways. In the present series of experiments, inhaled capsaicin (6 breaths, 1 mg ml^{-1} solution) activated 4 out of 11 RARs tested, in cats which were either bilaterally vagotomised or pre-treated with atropine. The highest dose of capsaicin, used in initial experiments, was used in these experiments so as not to overlook an effect on high threshold, capsaicin-sensitive RARs (Table 4.3).

Since the nerve terminal endings of the RARs in the airways are non-myelinated, like the C-fibre endings it is probable that the same mechanism of excitation by capsaicin may also pertain to those responsive RARs. Voltage clamp studies have shown that capsaicin-evoked depolarisation is carried by an inward current and which is sometimes followed by an outward current (Ault & Evans, 1980; Yanagisawa *et al.*, 1980; Williams & Zieglgansberger, 1982; Baccaglini & Hogan., 1983; Hayes *et al.*, 1984, Heyman & Rang, 1985, Bevan *et al.*, 1987; Marsh *et al.*, 1987; Winter *et al.*, 1988; Bevan & Szolcsanyi, 1990; Bleakman *et al.*, 1990; Dray *et al.*, 1990a; Docherty *et al.*, 1991). Because the inward current is associated with an inward conductance it would follow that ion channels are opened by capsaicin. However, the mechanism whereby nerve terminal depolarisation occurs is not amenable to direct study. An alternative line of approach, adopted by some investigators, has been to examine the electrophysiological effects of capsaicin, in rat dorsal root neurons in culture (Bevan & Szolcsanyi, 1990; Holzer, 1991; O'Neill, 1991). Membrane patch clamping and ion flux studies have indicated that capsaicin opens a membrane ion channel that is relatively non-selective for cations and thus allows both sodium and calcium (and possibly potassium) ions to flow down their concentration gradients,

causing initial depolarisation (Bevan & Forbes, 1988; Forbes & Bevan, 1988; Winter *et al.*, 1988; Wood *et al.*, 1988, 1989b; Bevan & Szolcsanyi, 1990; Bleakman *et al.*, 1990; Dray *et al.*, 1990a). Studies on isolated membrane patches have shown that capsaicin acts directly on the plasma membrane, without any involvement of second-messenger systems, a conclusion which is supported by biochemical findings (Wood *et al.*, 1989b; Bevan & Szolcsanyi, 1990; Dray *et al.*, 1990b, c; Winter *et al.*, 1990). There is also some evidence to suggest that the capsaicin-operated cation channel is distinct from voltage activated channels (Bevan & Szolcsanyi, 1990; Holzer, 1991).

Resiniferatoxin is a substance which occurs naturally in the latex of certain species of *Euphorbia* and like capsaicin is a potent irritant. Structurally it combines characteristics of capsaicin and phorbol esters (Fig. 4.1). Although, in keeping with the structural similarities with capsaicin and sharing most, if not all, of the effects of capsaicin on sensory neurons, resiniferatoxin is active at concentrations 100-10,000 times lower than capsaicin, both *in vivo* and *in vitro* (Bevan & Szolcsanyi, 1990; Holzer, 1991; O'Neill, 1991). A high affinity ligand, like resiniferatoxin, has proved useful in the biochemical characterisation of the cellular binding site for capsaicin. Binding studies with labelled resiniferatoxin have indicated the presence of specific saturable binding sites on membranes from dorsal root and trigeminal ganglia (Szallasi & Blumberg, 1990). Ruthenium Red, an inorganic dye with calcium entry blocking properties, inhibits the effects of capsaicin on sensory neurons and thus has been used as a capsaicin antagonist both *in vivo* and *in vitro* (Maggi *et al.*, 1988a, b, 1989; Amann & Lembeck, 1989; Chahl, 1989; Franco-Cereceda *et al.*, 1989; Dray *et al.*, 1990a). Unfortunately, Ruthenium Red can also inhibit the responses to other agents (Maggi, 1992) and this non-specificity limits its usefulness as a capsaicin antagonist. However, the development of capsazepine, a competitive capsaicin antagonist, has proved to be an important step in proving the existence of a specific recognition site

for capsaicin. Capsazepine, which is also structurally related to capsaicin (Fig. 4.1), competitively antagonises the effects of capsaicin *in vivo* and *in vitro* without any agonist activities or affecting responses to other stimuli (Bevan *et al.*, 1991, 1992; Dray *et al.*, 1991; Perkins & Campbell, 1992). The evidence to date clearly suggests that capsaicin's actions on sensory neurons is mediated by a specific recognition site with the pharmacological properties of a receptor.

Inhaled histamine stimulates both RARs and C-fibre nerve endings (Mills *et al.*, 1969; Armstrong & Luck, 1974; Sampson & Vidruk, 1975; Coleridge & Coleridge, 1977; Coleridge *et al.*, 1978; Adcock, 1989). Histamine, by inhalation and intravenously, was shown in the present study to stimulate RARs and to cause bronchoconstriction. The bronchoconstriction, under the present experimental conditions, is evoked by a direct action on the airway smooth muscle rather than through a reflex mechanism (Adcock *et al.*, 1989). In spontaneously breathing animals (Karczewski & Widdicombe, 1969b, c; Mills, *et al.*, 1970) some of the RAR stimulation by histamine may be indirect, resulting from the influence of changes in breathing pattern on lung compliance. Likewise, in paralysed animals, activation of RARs may be due to the contraction of smooth muscle of the airways through an indirect action, by distortion of the tissue surrounding the receptor (Coleridge *et al.*, 1978; Mills *et al.*, 1969). A reduction in lung compliance by histamine, which may lead to a greater pull on the airway and distortion of the airway wall, may also stimulate RARs indirectly (Mills *et al.*, 1969; Coleridge *et al.*, 1978). The exact contribution of this indirect effect is not fully understood since histamine is far more potent than acetylcholine at stimulating RARs, yet both produce similar changes in lung compliance (Dixon *et al.*, 1979a). Indeed, when isoprenaline (a β-adrenoceptor agonist) is used to block smooth muscle contraction due to histamine, RARs are still stimulated (Vidruk *et al.*, 1977) suggesting that histamine directly stimulates RARs. Histamine, therefore, appears to

have direct and indirect actions on RARs. The effects of histamine on RARs, total lung resistance and dynamic lung compliance can be blocked by chloropheniramine, a H_1 receptor antagonist, but not by cimetidine, a H_2 receptor antagonist (Dixon *et al.*, 1979b). Thus it is possible that histamine could activate RARs via H_1-receptors, which are linked to the inositol 1-4-5 trisphosphate (IP_3)/1,2-diacylglycerol messenger system (Hill, 1990). IP_3, which is released into the cytosol, has been shown to mobilise calcium from intracellular stores (Berridge, 1987). In addition, although the studies were carried out in human endothelial cells, increased intracellular calcium levels have been shown to be responsible for generating histamine-inward currents, possibly as a result of the activation of cation channels (Bregestovski *et al.*, 1988). The same may be true for histamine-induced stimulation of RARs and C-fibres. The latency to stimulation of C-fibres by histamine, following intravenous administration, is similar to that of capsaicin (Coleridge & Coleridge, 1977; Coleridge *et al.*, 1978; Adcock *et al.*, 1989). Regardless of the mechanism by which inhaled histamine stimulates RARs and C-fibres, it is surprising that inhalation of histamine, unlike capsaicin, does not induce reflex bronchoconstriction, but only bronchoconstriction caused as a result of direct smooth muscle contraction (Adcock *et al.*, 1989). A number of possible explanations are available, including histamine-induced release of catecholamines from the adrenal medulla (Staszewska-Barczak & Vane, 1965), which reduces the effect of histamine in cats (Colebatch & Engel, 1974). Hypotension resulting from i.v. injection of histamine could lead to a baroreceptor-mediated increase in sympathetic efferent activity (Diamond, 1972). However, an alternative explanation could be that histamine evokes a bronchodilator reflex, which could mask any reflex excitatory effects.

Inhalation of capsaicin aerosol (6 breaths, 1 mg ml^{-1} solution) evoked hypotension and bradycardia in unilaterally vagotomised cats. In contrast, capsaicin aerosol (6

breaths, 1 mg ml^{-1} solution) had no effect on heart rate and evoked a significant increase in arterial blood pressure in bilaterally vagotomised cats. Capsaicin aerosol (6 breaths, 1 mg ml^{-1}) had no significant effect on cardiovascular parameters in atropine-treated animals. In all three treatment groups, histamine aerosol evoked hypotension before and after capsaicin aerosol administration. Since hypotension and bradycardia are also part of the pulmonary chemoreflex evoked by inhaled capsaicin, the observation that the cardiovascular effects of inhaled capsaicin, but not those of inhaled histamine, are either absent or reduced following either bilateral vagotomy or atropine-treatment, provides further support that the cardiopulmonary reflex effects of capsaicin aerosol are removed in bilaterally vagotomised/atropine-treated animals.

Based upon the view that intravenous capsaicin is a relatively "selective" stimulant of C-fibre endings, the observation that inhalation of capsaicin aerosols in a number of animal species, including man, leads to coughing and reflex bronchoconstriction, promoted the view that C-fibre stimulation was primarily responsible for evoking these airway reflexes (Collier & Fuller, 1984; Fuller *et al.*, 1985; Forsberg *et al.*, 1986; Forsberg & Karlsson, 1986, 1987; Midgren *et al.*, 1986). From the results in the present study, it is reasonable to suggest that reflex coughing and bronchoconstriction, following the inhalation of aerosols of capsaicin, are not entirely dependent on C-fibre stimulation but may result from stimulation of sub-populations of responsive airway RARs. Indeed, detailed studies of C-fibre nerve ending stimulation, using phenylbiguanide and capsaicin, have shown that C-fibre stimulation inhibits rather than evokes coughing and sneezing in cats (Tatar *et al.*, 1988).

The present study clearly demonstrates that capsaicin, when delivered by aerosol to the airway epithelial surface, is not a "selective" stimulant of C-fibre endings. Activation of some but not all RARs tested, by inhaled capsaicin, suggests that there

are sub-populations of capsaicin sensitive and insensitive RARs. RARs can be activated by inhaled capsaicin in the absence of bronchoconstriction, which suggests that it may activate the sensory nerve endings directly and is not, therefore, dependent upon secondary stimulation brought about by bronchoconstriction, as suggested by other investigators. Direct activation of some RARs by inhaled capsaicin raises the possibility of the existence of other pharmacological receptors on RAR nerve endings for inflammatory mediators such as histamine, prostaglandins and bradykinin. Consequently, stimulation of airway RARs by a range of pharmacologically active agents released during airway inflammation may contribute to reflex coughing and bronchoconstriction in both experimental animals and man.

Chapter 5

Effects of inhaled prostaglandin E_2 and AH13205 on the spontaneous discharge of RARs

5.1 Introduction

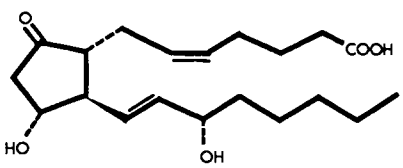
The lungs and bronchi are important sites for the synthesis and inactivation of prostanoids (for example, prostaglandin E_2, PGE_2); products of arachidonic acid metabolism. In both guinea-pigs and man, PGE_2 (Fig. 5.1) is found in relatively higher concentrations in bronchial tissue than in lung parenchymal tissue (Karim *et al.*, 1967; Spannhake *et al.*, 1981). *In vitro,* PGE_2 causes relaxation of animal (Main, 1964) and human airway smooth muscle (Sweatman & Collier, 1968). However, the degree of effect with PGE_2 is variable in comparison with that of isoprenaline, a β-adrenoceptor agonist, principally depending on the tissue and species in question (Gardiner, 1989). In addition, PGE_2 also exhibits a number of anti-inflammatory properties (Lewis 1983; Raud, 1990). Thus, in theory, PGE_2/or a related compound, which possesses both bronchodilator activity and anti-inflammatory properties, could provide a novel and advantageous approach to the treatment of bronchial asthma over established bronchodilator drugs. Indeed, in conscious guinea-pigs, inhaled PGE_2 is more potent than salbutamol in inhibiting histamine-induced bronchoconstriction (Gardiner, 1989). PGE_2, administered intravenously or as an inhaled aerosol, causes bronchodilatation in both normal and asthmatic subjects (Smith & Cuthbert, 1976). However, despite this bronchodilator activity, PGE_2, administered by either route, causes coughing and retrosternal soreness in both normal and asthmatic subjects and occasionally a paradoxical bronchoconstriction in asthmatic subjects (Smith & Cuthbert, 1976; Costello *et al.*, 1985). Consequently, these side-effects have limited the use of PGE_2, as well as other prostanoids and prostanoid analogues, in the treatment of asthma.

Prostanoids, like local hormones, produce their effects by interacting with specific receptors on cell membranes. In recent years, considerable progress has been made towards establishing the outlines of a workable classification of prostanoid receptors

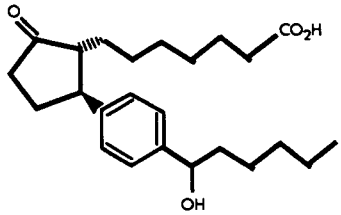
Coleman *et al.*, 1990; TIPS Supplement, 1994). According to this classification, distinct receptors exist for each of the five naturally occurring prostanoids; prostaglandin D_2 (DP receptors), $PGF_{2\alpha}$ (FP receptors), prostacyclin (IP receptors), thromboxane A_2 (TP receptors) and PGE_2 (EP receptors). With the identification of selective agonists and specific prostanoid receptor blocking drugs it has also been possible to subdivide the classification of EP receptors into EP_1, EP_2 & EP_3 receptors (Coleman *et al.*, 1990). Furthermore, there is some recent evidence in the literature to suggest the existence of an EP_4 receptor (Coleman *et al.*, 1994). It is also possible that subdivisions may exist for DP and TP receptors. Readers are referred to Coleman *et al.*, (1990) for a more detailed review of prostanoid receptor classification and their pharmacological effects.

Evidence published to date, suggests that EP_2 receptors are principally responsible for mediating PGE_2-induced airway smooth muscle relaxation and anti-inflammatory activities (Coleman, 1985; Coleman *et al.*, 1987, 1990), however, the receptor mediating airways irritancy has not been characterised. While many prostanoid analogues have been evaluated for bronchodilator activity (Large *et al.*, 1969; Rosenthale *et al.*, 1970; Kawakami *et al.*, 1973; Murao *et al.*, 1980; Nizankowska *et al.*, 1985), many are non-selective or their selectivity has not been established. AH13205 (Fig. 5.1), an analogue of prostaglandin A (a derivative of the prostaglandin E series) with a marked degree of EP_2-receptor agonist selectivity (Nials *et al.*, 1991), was developed as a tool for determining whether selective stimulation of EP_2 receptors can induce bronchodilatation and/or anti-inflammatory activity without causing concomitant airways irritancy and cough. In preliminary studies, AH13205 demonstrated both bronchodilator and anti-inflammatory activity, both *in vitro* and *in vivo* (Nials *et al.*, 1993). However, inhaled AH13205, like other

prostanoids/analogues, evokes airways irritancy in normal subjects and airways irritancy/cough in asthmatic subjects (Nials *et al.*, 1993).



prostaglandin E_2



AH13205

Fig. 5.1 Structures of prostaglandin E_2 and AH13205.

Isolated nerve fibre recordings, in dogs, have suggested that PGE_2, when administered either intravenously or as an inhaled aerosol, is a potent stimulant of C-fibre endings with little or no effect on RARs (Coleridge *et al.*, 1976; Coleridge *et al.*, 1978; Roberts *et al.*, 1985). PGE_2, intravenously or as an inhaled aerosol, also evokes reflex bronchoconstriction in the dog (Roberts *et al.*, 1985). On the basis of these findings, it has been suggested that the irritant properties and the paradoxical bronchoconstriction evoked by PGE_2 are mediated by C-fibre endings (Roberts *et al.*, 1985).

The irritant properties of PGE_2, other prostanoids and prostanoid analogues in experimental animals and man, are probably mediated by airway sensory nerve endings. However, to date, there have been few studies examining the effects of aerosols of PGE_2 on airway RARs (Roberts *et al.*, 1985). The aim of the present series of experiments was to examine the effects of aerosolised PGE_2 and the EP_2 receptor agonist, AH13205, on intrathoracic RARs in anaesthetized, paralysed and artificially ventilated cats.

5.2 Methods

Male cats (3.0-4.6 kg) were anaesthetized and prepared for surgery as described in Chapter 2. Airflow (ml s^{-1}) and transpulmonary pressure (cmH_2O) were measured. Tidal volume (ml), C_{Dyn} (ml cmH_2O^{-1}) and R_L ($cmH_2O\ l^{-1}$ s) were computed from these measurements, on a breath-by-breath basis, with an on-line Buxco pulmonary mechanics analyser. Identification and subsequent recording of single nerve fibres arising from intrathoracic RARs were carried out as described in Chapter 2. Arterial blood pressure and heart rate were also recorded. Additional cannulae (Portex 4fg) were inserted in the right jugular vein and right carotid artery (pushed into the aortic arch) for administration of drugs. The animals were paralysed with DMT and artificially ventilated according to the protocol in Chapter 2. Drugs were administered by two routes, i.v. and inhalation (DeVilbiss Ultrasonic nebuliser, Chapter 2).

Materials

Histamine diphosphate was dissolved in saline (0.85% wt/vol). PGE_2 was dissolved in saline. PGE_2 vehicle consisted of a 0.1% ethanol and saline mixture. AH13205 was initially dissolved in a 10% sodium bicarbonate solution and then diluted to give a 1% sodium bicarbonate solution. AH13205 vehicle consisted of a 1% sodium bicarbonate solution.

Protocols

After surgery, the animals were allowed to stabilise for at least 30 min. Single nerve fibres were dissected from the left vagus nerve and action potentials recorded. Each animal and, therefore, each nerve fibre was subjected to only one of the following protocols.

Prostaglandin E_2 study

After identification of a vagal fibre originating from an intrathoracic RAR, PGE_2 was inhaled using 6 breaths of aerosol, at 6 min intervals, from solutions of increasing concentrations: vehicle, 0.001, 0.01, 0.1 and 1 mg ml^{-1}. Inhalation of histamine aerosol, 6 breaths of a 1 mg ml^{-1} solution, was repeated at the end of the protocol to ensure that the RAR was still active.

If the vagal fibre was still viable at the end of the above protocol, the effects of either intravenous PGE_2 (10 μg kg^{-1}) or repeated inhalation of PGE_2 (6 breaths of a 0.1 mg ml^{-1} solution, administered at 6 min intervals) on RAR activity were examined.

AH13205 study

In initial experiments, and after identification of a vagal fibre arising from an intrathoracic RAR, AH13205 was administered as an inhaled aerosol (6 breaths, vehicle & 1 mg ml^{-1} solutions) at 6 min intervals. This was then followed 6 mins later by inhalation of PGE_2 aerosol (6 breaths, 1 mg ml^{-1} solution).

In other experiments, a higher concentration of AH13205 (6 breaths, 3 mg ml^{-1} solution) was used. In addition, PGE_2 was administered as an inhaled aerosol (6 breaths) at 6 min intervals, from solutions of increasing concentrations: 0.01, 0.1 & 1 mg ml^{-1}.

Data Analysis

Nerve impulses were counted in 5 second period "bins". Control periods were counted for 1 min before administration of the irritant agent and expressed as the average impulses s^{-1} (imp s^{-1}). Impulse discharges, evoked by the vehicle and irritant agents, were averaged (imp s^{-1}) during the period of the response (in the case of the vehicle 1 min) and expressed as the Δ (imp s^{-1}) from the control period. In all cases the mean ± standard error of the mean (sem) are shown.

The effect of the vehicle and irritant agents on arterial blood pressure and heart rate are expressed as the peak changes in absolute values, whereas C_{Dyn} and R_L responses are expressed as the maximum percentage change from pre-treatment values. In some experiments the effects on transpulmonary pressure are given and these are expressed as the maximum percentage change from pre-treatment values.

Prostaglandin E_2 study

Histamine aerosol and i.v. effects: The effect of histamine (aerosol and i.v.) on airway sensory nerve discharge, C_{Dyn}, R_L, arterial blood pressure and heart rate were compared with baseline values using Student's "t" test for paired data. In addition, the responses to histamine aerosol, before and after the PGE_2 protocols, were analysed using Student's "t" test for paired data. Statistical values of $P < 0.05$ were considered to be significant.

Prostaglandin E_2 effects: The effect of PGE_2 aerosols (0.001 - 1 mg ml^{-1} solutions) on airway sensory nerve discharge were compared with the vehicle aerosol using Student's "t" test for paired data. Data comparing the effects of PGE_2 aerosols (0.001 - 1 mg ml^{-1} solutions) with the vehicle aerosol on arterial blood pressure, heart rate,

C_{Dyn} and R_L were analysed using one-way analysis of variance (ANOVA). Statistical values of $P < 0.05$ were considered to be significant.

AH13205 study

Data for the effects of each of the treatments, histamine (i.v. and aerosol), AH13205 vehicle (6 breaths) and PGE_2 (6 breaths from a 1 mg ml^{-1} solution) on the discharge of the RARs, pulmonary mechanics and cardiovascular parameters were pooled together for both of the AH13205 protocols. Considering the sample size of the AH13205 protocols no statistical analysis was performed.

5.3 Results

5.3.1 Baseline Values

13 male cats were used. The mean ± sem of the resting values of pulmonary mechanics and cardiovascular parameters for 13 open chest animals (unless otherwise stated) are shown in Table 5.1.

Table 5.1 Baseline values for C_{Dyn}, R_L, P_{Tp}, systolic and diastolic blood pressure and heart rate in anaesthetized, paralysed and artificially ventilated cats. Values are shown as mean ± sem.

parameters	baseline values
C_{Dyn}, ml cmH_2O^{-1}	7.7 ± 0.9 (n=14)
R_L, cmH_2O l^{-1} s	17.1 ± 2.4 (n=7)
P_{Tp}, cmH_2O	4.1 ± 0.3 (n=5)
systolic blood pressure, mmHg	129 ± 4.6 (n=14)
diastolic blood pressure, mmHg	86 ± 2.8 (n=14)
Heart rate, beats min^{-1}	157 ± 7.9 (n=14)

5.3.2 Effect of PGE_2 and Histamine aerosols on RARs

Eight intrathoracic RARs were examined in six cats. The spontaneous impulse activity of the fibres was 1.6 ± 0.35 imp s^{-1} (0.1 - 2.6 imp s^{-1}). The adaptation index of each receptor was 100%. Administration of histamine intravenously (10 μg kg^{-1}) and by aerosol (6 breaths, 1 mg ml^{-1} solution) evoked a significant increase in rate of discharge of RARs (4.87 ± 1.24 imp s^{-1}, $P < 0.01$; 2.2 ± 0.44 imp s^{-1}, $P < 0.001$; respectively) and caused significant bronchoconstriction as shown by a fall in C_{Dyn} (-34 ± 2%, $P < 0.001$; -29 ± 3%, $P < 0.001$, respectively) and increase in R_L (+99 ± 12%, $P < 0.01$; +86 ± 6% $P < 0.01$, respectively) (Fig. 5.2). At the end of the protocol, histamine aerosol still evoked a significant ($P < 0.001$) increase in RAR discharge (1.1 ± 0.2 imp s^{-1}) and caused significant bronchoconstriction (C_{Dyn} -16 ± 2%, $P < 0.001$; R_L +37 ± 7%, $P < 0.001$) (Fig. 5.2). However, the effects of histamine aerosol on RAR discharge and pulmonary mechanics, at the end of the experiment, were significantly ($P < 0.01$) reduced compared to histamine aerosol-evoked responses at the start of the experiment.

In the same animals, the vehicle used for PGE_2 had no significant ($P > 0.05$) effect on either RAR rate of discharge (0.02 ± 0.07 imp s^{-1}) or on pulmonary mechanics (C_{Dyn} -0.3 ± 1%; R_L +2 ± 5%). Sequential administration of PGE_2 aerosol, with increasing concentrations 0.001 - 1 mg ml^{-1}, caused a significant dose-related decrease in C_{Dyn} (from -0.3 ± 1% to -14 ± 2%) and increase in R_L (from -2 ± 3% to +41 ± 9%). Moreover, there was a significant ($P < 0.01$) dose-related increase in rate of RAR discharge from 0.18 ± 0.18 imp s^{-1} (6 breaths, 0.001 mg ml^{-1} solution) to 2.1 ± 0.62 imp s^{-1} (6 breaths, 1 mg ml^{-1} solution) (Fig. 5.2). The effect of PGE_2 aerosol (6 breaths, 1 mg ml^{-1} solution) on rate of discharge of RARs together with the effect on either C_{Dyn} or R_L and cardiovascular variables are shown in Figs 5.3 & 5.4. The

mean duration of PGE_2 aerosol (6 breaths, 1 mg ml^{-1} solution) evoked impulse discharges was 176 ± 26.5 s, range being 50 - 285 s.

The mean effects of histamine and PGE_2 on cardiovascular parameters are shown in Table 5.2.

5.3.3 Effect of AH13205 and PGE_2 aerosols on RARs

Six intrathoracic RARs were examined in six cats. The spontaneous impulse activity of the fibres was 0.32 ± 0.1 imp s^{-1} (0.07 - 0.32 imp s^{-1}). The adaptation index of each receptor was 100%. Administration of histamine intravenously (10 μg kg^{-1}) and by aerosol (6 breaths, 1 mg ml^{-1} solution) stimulated all six RARs examined (2.5 ± 0.99 imp s^{-1} & 2.1 ± 0.73 imp s^{-1}; respectively) (Fig. 5.5). The effects of histamine (i.v. and aerosol) on pulmonary mechanics and cardiovascular parameters are shown in Tables 5.3 & 5.4 respectively.

Inhalation of AH13205 vehicle (6 breaths) did not stimulate any of the six RARs tested (0.6 ± 0.27 imp s^{-1}, Fig. 5.5). Likewise, in preliminary experiments, inhalation of AH13205 aerosol (6 breaths, 1 mg ml^{-1} solution) did not stimulate any of the three RARs tested (0.52 ± 0.27 imp s^{-1}). In contrast, inhalation of AH13205 aerosol (6 breaths, 3 mg ml^{-1} solution) stimulated two of the three RARs examined (1.1 ± 0.69 imp s^{-1}); an example of which is shown in Fig. 5.6. Inhalation of AH13205 aerosols (6 breaths, vehicle, 1 & 3 mg ml^{-1} solutions) had no effect on pulmonary mechanics or cardiovascular parameters (see Tables 5.3 & 5.4 respectively).

In contrast to AH13205, inhaled PGE_2 (6 breaths generated from 0.01, 0.1 & 1 mg ml^{-1} solutions) increased the rate of discharge of all of the RARs examined (1.5 ± 0.61, n=3; 2.7 ± 0.92, n=3 & 3.15 ± 0.72, n=6, imp s^{-1}, respectively) (Fig. 5.4). The

effects of PGE_2 aerosols on pulmonary mechanics and cardiovascular parameters are shown in Tables 5.3 & 5.4 respectively.

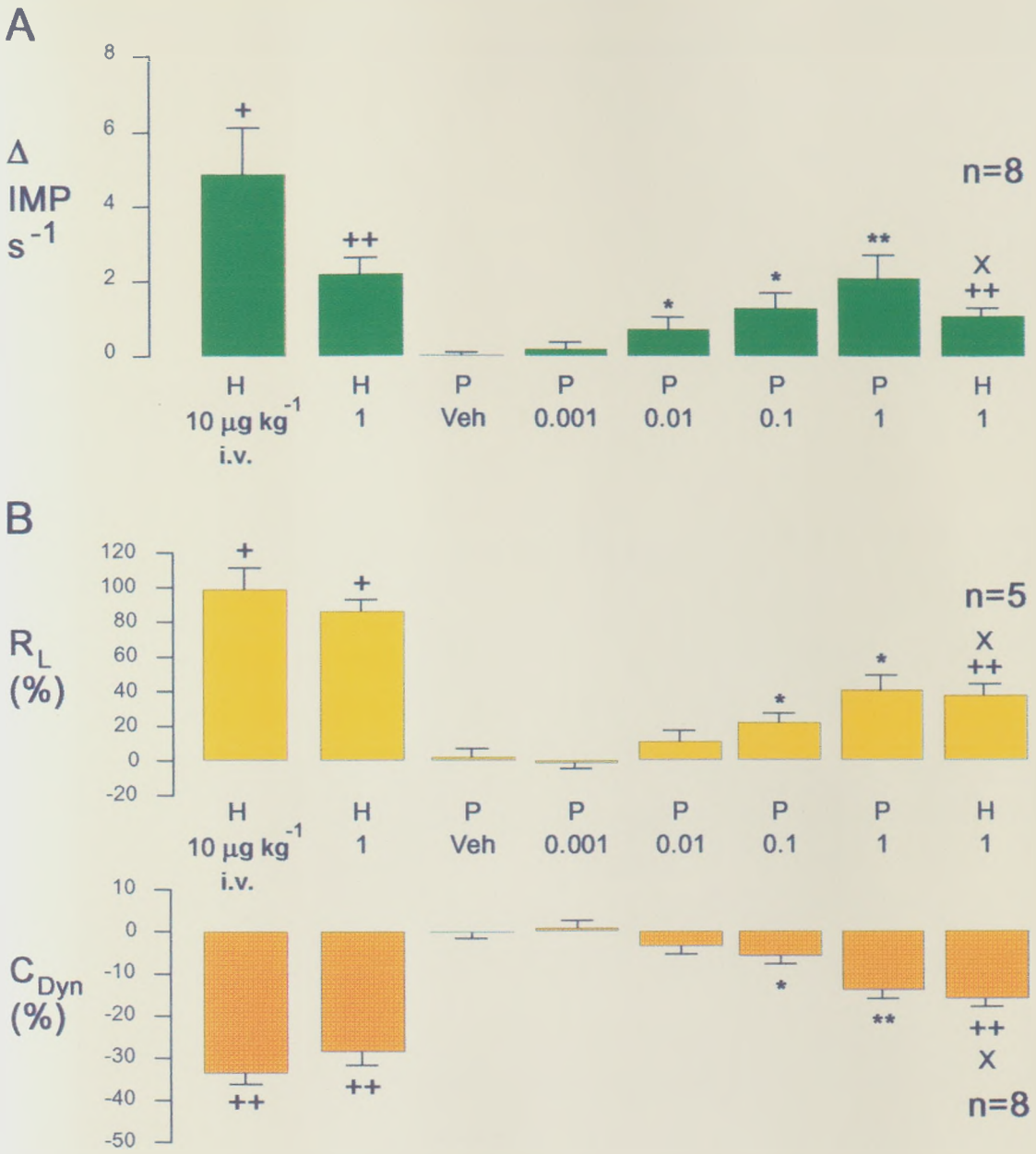


Fig. 5.2 **A**, discharges in vagal fibres arising from intrathoracic RARs (n=8), expressed as the change in impulses s^{-1} (Δ IMP s^{-1}) from spontaneous levels and **B**, percentage changes in dynamic lung compliance (C_{Dyn}) and total lung resistance (R_L) evoked by sequential administration of histamine (H) and PGE_2 (P) in anaesthetized, paralysed and artificially ventilated cats. Histamine was administered intravenously (10 µg kg^{-1}) and by aerosol (6 breaths, 1 mg ml^{-1} solution). PGE_2 was administered by inhalation in increasing doses (6 breaths, vehicle, 0.001 - 1 mg ml^{-1} solutions). Vertical bars represent sem. Significantly different histamine-evoked responses from control baselines are shown by + P <0.01, ++ P <0.001. Significantly different histamine aerosol-evoked responses are shown by X P <0.01. Significantly different PGE_2-evoked responses (0.001 - 1 mg ml^{-1}) compared with PGE_2 vehicle responses are shown by * P <0.05, ** P <0.01. Veh, Vehicle.

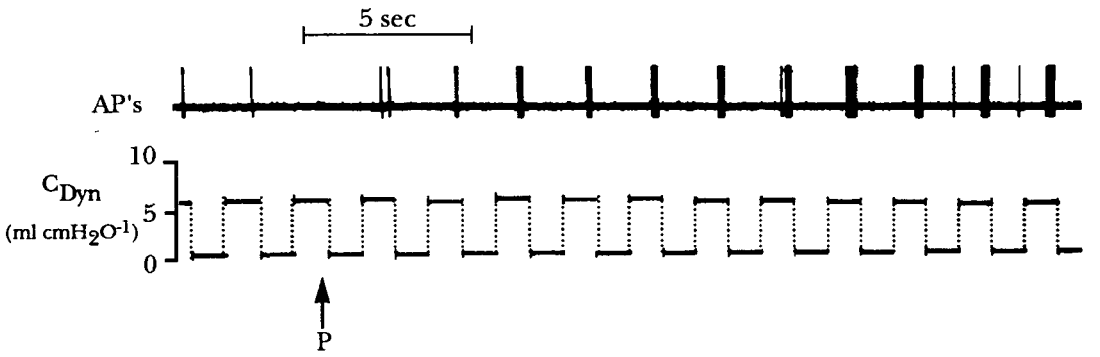


Fig. 5.3 Discharges in a vagal fibre arising from an intrathoracic RAR, evoked by inhalation of PGE_2 (P) in an anaesthetized, paralysed and artificially ventilated cat. The RAR was stimulated by inhalation of PGE_2 aerosol (6 breaths, 1 mg ml^{-1} solution). Concomitant recording of dynamic lung compliance (C_{Dyn}) is also shown. AP, action potentials.

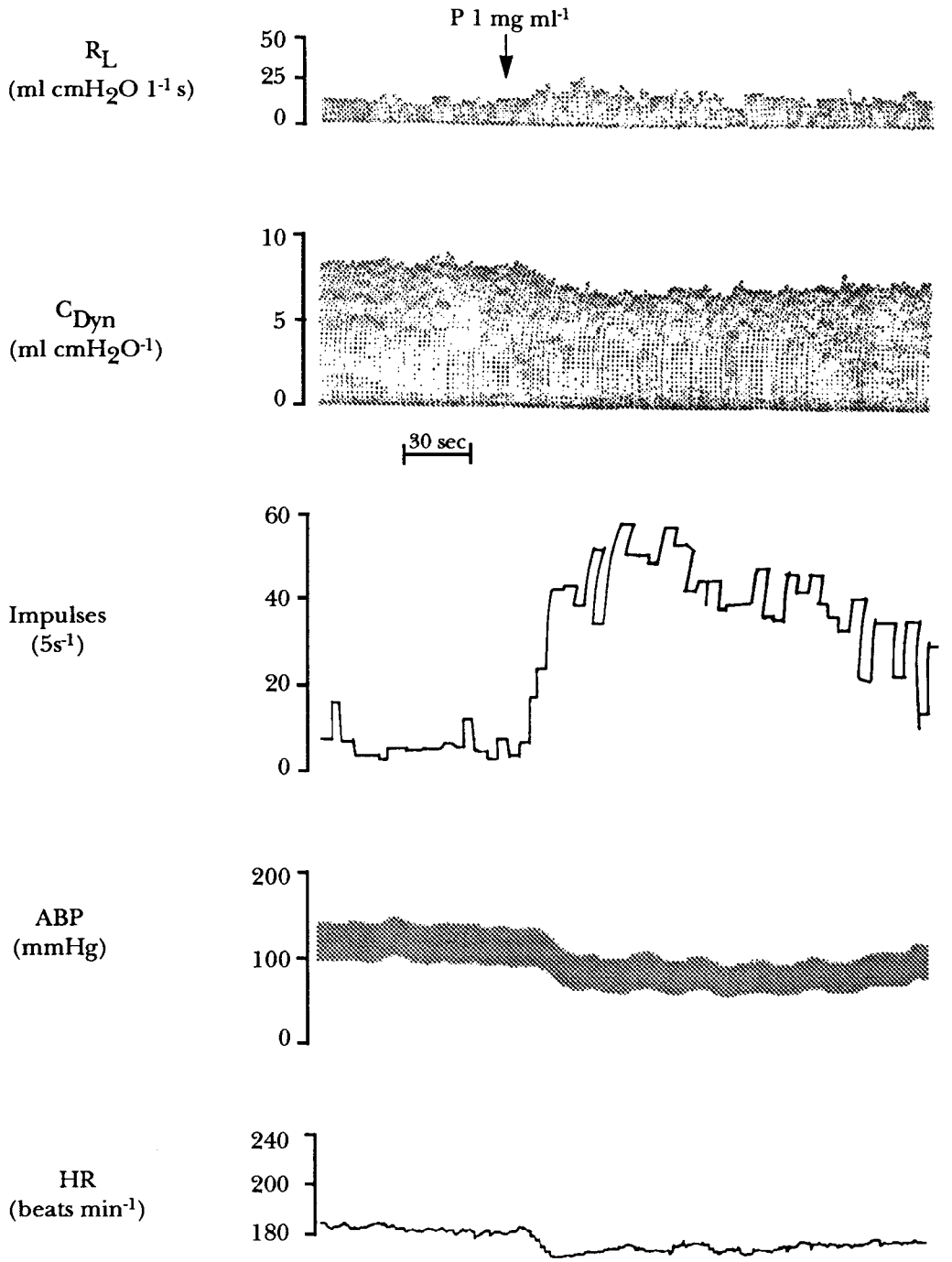


Fig. 5.4 Changes in dynamic lung compliance (C_{Dyn}), lung resistance (R_L), impulse discharges (measured in 5 s bins) arising from an intrathoracic RAR, arterial blood pressure (ABP) and heart rate (HR) evoked by inhalation of PGE_2 aerosol (P; 6 breaths, 1 mg ml^{-1} solution) in an anaesthetized, paralysed and artificially ventilated cat.

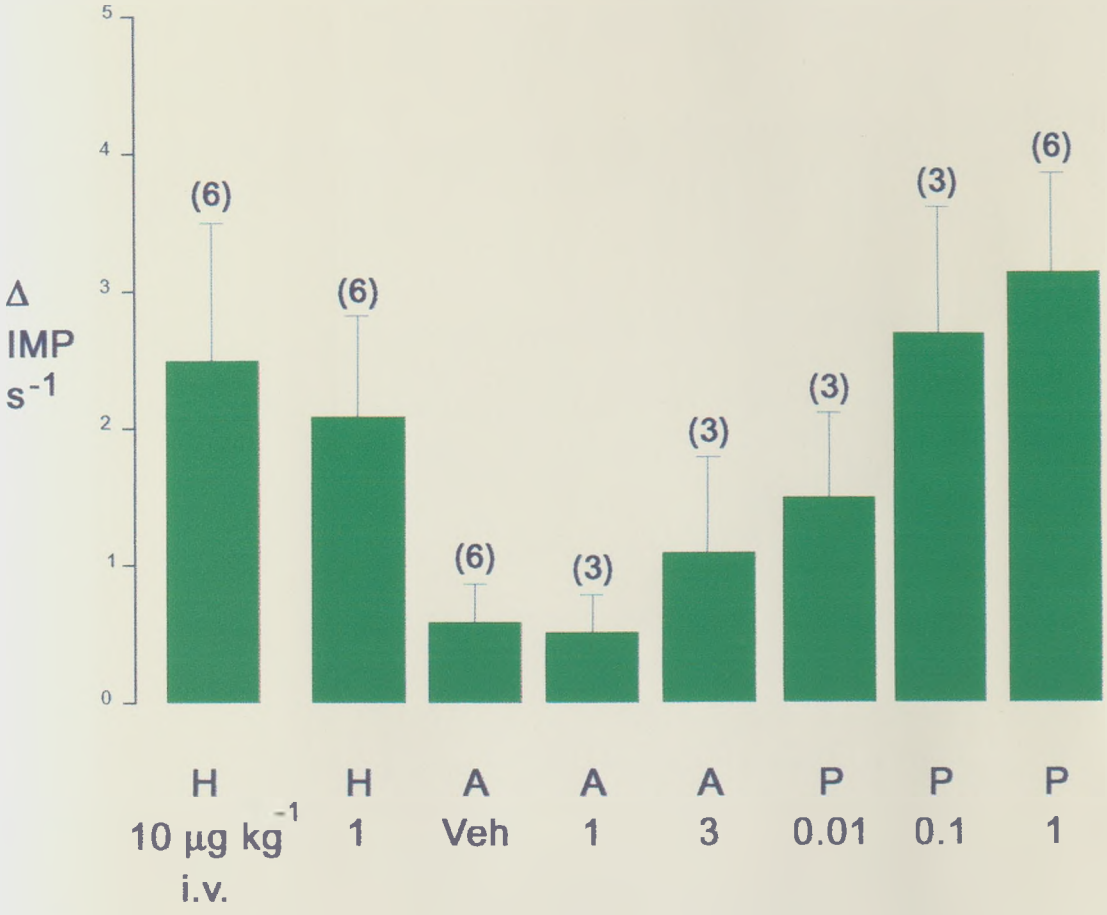


Fig. 5.5 Discharges in vagal fibres arising from intrathoracic RARs (n=6 unless otherwise stated), expressed as the change in impulses s^{-1} (Δ IMP s^{-1}) from spontaneous levels evoked by sequential administration of histamine (H), AH13205 (A) and PGE_2 (P) in anaesthetized, paralysed and artificially ventilated cats (n=6). Histamine was administered intravenously (10 $\mu g\ kg^{-1}$) and by aerosol (6 breaths, 1 mg ml^{-1} solution). AH13205 was administered by inhalation (6 breaths of vehicle, 1 & 3 mg ml^{-1} solutions). PGE_2 was also administered by inhalation (6 breaths, vehicle, 0.01, 0.1 & 1 mg ml^{-1} solutions). Data for histamine (i.v. and aerosol), AH13205 vehicle and PGE_2 (1 mg ml^{-1} solution) were pooled together from both experimental protocols. Vertical bars represent sem. Veh, Vehicle.

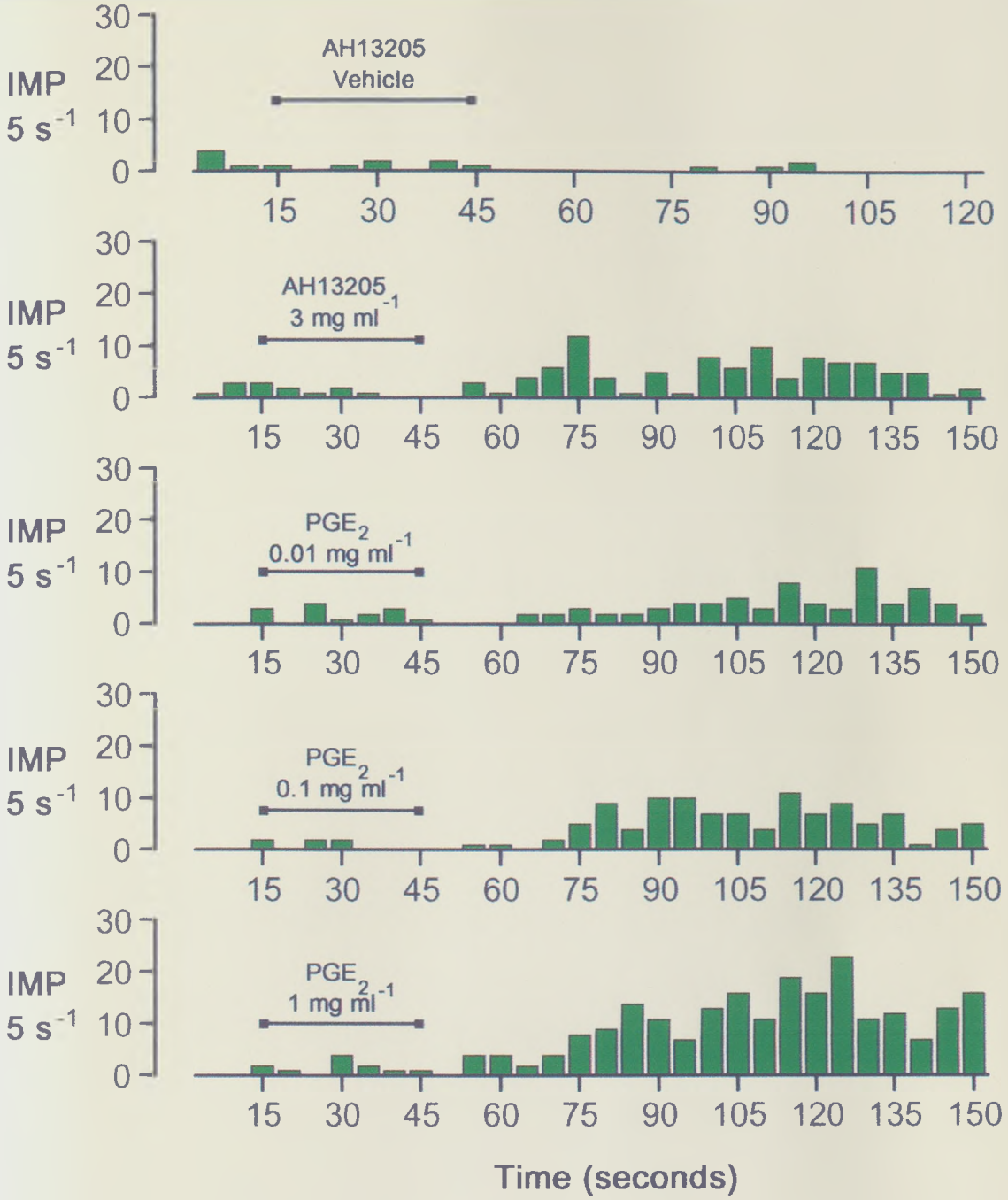


Fig. 5.6 Discharges in a vagal fibre arising from an intrathoracic RAR, expressed as impulses 5 s^{-1}, evoked by inhalation of AH13205 and PGE_2 in an anaesthetized, paralysed and artificially ventilated cat. Aerosols were administered sequentially at six minute intervals (6 breaths, AH13205 vehicle & 3 mg ml^{-1} solutions, PGE_2 0.01 - 1 mg ml^{-1} solutions).

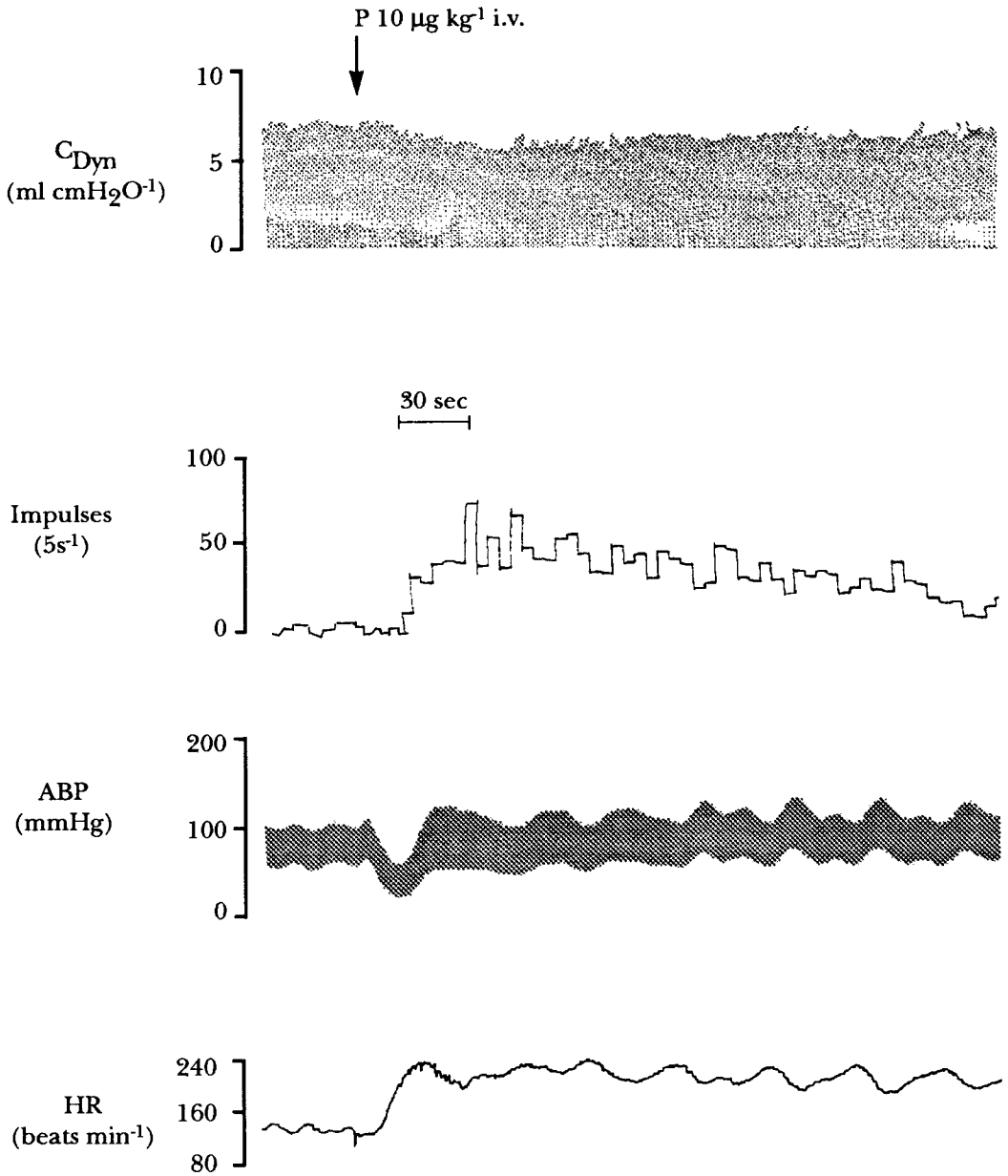


Fig. 5.7 Changes in dynamic lung compliance (C_{Dyn}), impulse discharges (measured in 5 s bins) arising from an intrathoracic RAR, arterial blood pressure (ABP) and heart rate (HR) evoked by intravenous PGE_2 (P; 10 μg kg^{-1}) in an anaesthetized, paralysed and artificially ventilated cat.

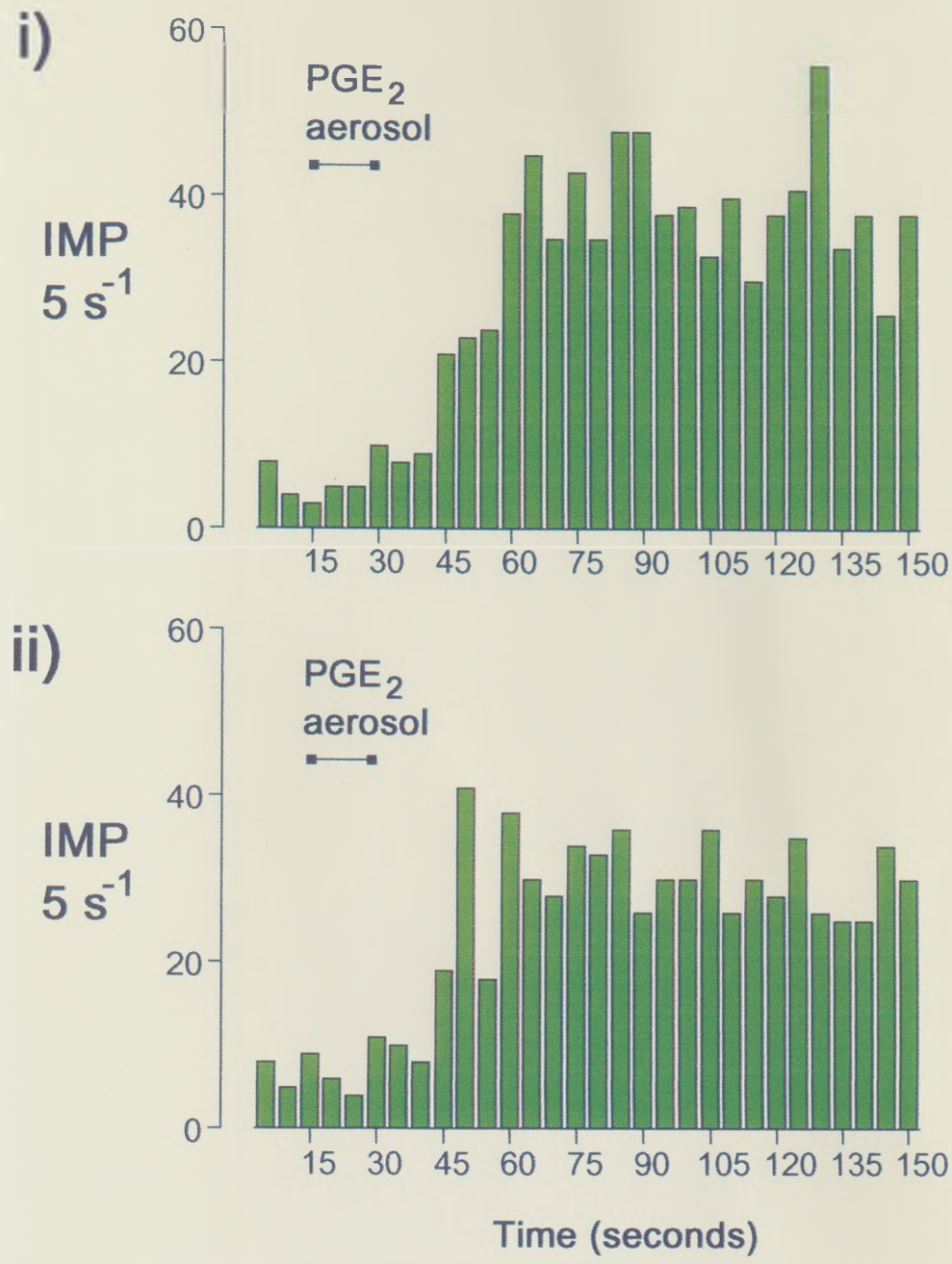


Fig. 5.8 Discharges in a vagal fibre arising from an intrathoracic RAR, expressed as impulses 5 s^{-1}, evoked by inhalation of PGE_2 in an anaesthetized, paralysed and artificially ventilated cat. **i)** Administration of PGE_2 aerosol (6 breaths from a 1 mg ml^{-1} solution) which was repeated **ii)** six minute intervals. Aerosols were administered as shown.

Table 5.2 Effect of histamine (i.v. and aerosol) and PGE_2 aerosols on arterial blood pressure and heart rate in anaesthetized, paralysed and artificially ventilated cats.

	Δ systolic blood pressure (mmHg)	Δ diastolic blood pressure (mmHg)	Δ heart rate (beats min^{-1})
Histamine 10 μg kg^{-1}	-42 ± 5†	-42 ± 2†	+47 ± 5†
Histamine aerosol (6 breaths, 1 mg ml^{-1})	-29 ± 3†	-23 ± 2†	+7 ± 6
PGE_2 aerosol (6 breaths, vehicle)	-1 ± 3	-3 ± 3	+1 ± 1
PGE_2 aerosol (6 breaths, 0.001 mg ml^{-1})	-4 ± 2	-5 ± 2	0.2 ± 1
PGE_2 aerosol (6 breaths, 0.01 mg ml^{-1})	-9 ± 3	-4 ± 1	0.3 ± 1
PGE_2 aerosol (6 breaths. 0.1 mg ml^{-1})	-14 ± 6	-14 ± 5	+6 ± 4
PGE_2 aerosol (6 breaths, 1 mg ml^{-1})	-36 ± 6*	-28 ± 5*	-14 ± 8
Histamine aerosol (6 breaths, 1 mg ml^{-1})	-9 ± 5	-8 ± 4	+1 ± 3

All agents were administered sequentially. Values shown are mean ± sem absolute changes. n=8. Significantly different histamine-evoked responses from the control baselines are shown by † $P < 0.001$. Significantly different PGE_2-evoked responses (1 mg ml^{-1} solution) compared with PGE_2 vehicle responses are shown by * $P < 0.05$.

Table 5.3 Effect of histamine (i.v. and aerosol), PGE_2 and AH13205 aerosols on transpulmonary pressure and dynamic lung compliance in anaesthetized, paralysed and artificially ventilated cats.

	Change in transpulmonary pressure (%)	Change in C_{Dyn} (%)
Histamine 10 μg kg^{-1}	+40 ± 6 (n=5)	-32 ± 2
Histamine aerosol (6 breaths, 1 mg ml^{-1})	+28 ± 8 (n=5)	-24 ± 5
AH13205 aerosol (6 breaths, vehicle)	-1 ± 2 (n=5)	+3 ± 3
AH13205 aerosol (6 breaths, 1 mg ml^{-1})	0 ± 2 (n=3)	+2 ± 2 (n=3)
AH13205 aerosol (6 breaths, 3 mg ml^{-1})	0 (n=2)	+12 ± 13 (n=3)
PGE_2 aerosol (6 breaths, 0.01 mg ml^{-1})	0 (n=2)	-1 ± 1 (n=3)
PGE_2 aerosol (6 breaths. 0.1 mg ml^{-1})	+10 (n=2)	-12 ± 1 (n=3)
PGE_2 aerosol (6 breaths, 1 mg ml^{-1})	+19 ± 3 (n=5)	-20 ± 3

Values shown are mean ± sem % changes from control baselines; (n=6 unless otherwise stated).

Table 5.4 Effect of histamine (i.v. and aerosol), PGE_2 and AH13205 aerosols on arterial blood pressure and heart rate in anaesthetized, paralysed and artificially ventilated cats.

	Δ systolic blood pressure (mmHg)	Δ diastolic blood pressure (mmHg)	Δ heart rate (beats min^{-1})
Histamine 10 μg kg^{-1}	-53 ± 4	-54 ± 2	+42 ± 6
Histamine aerosol (6 breaths, 1 mg ml^{-1})	-12 ± 7	-11 ± 5 (n=5)	+2 ± 5
AH13205 aerosol (6 breaths, vehicle)	0 ± 2	-3 ± 3	-2 ± 1
AH13205 aerosol (6 breaths, 1 mg ml^{-1})	0 (n=3)	-3 ± 3 (n=3)	0 (n=3)
AH13205 aerosol (6 breaths, 3 mg ml^{-1})	+2 ± 7 (n=3)	0 ± 6 (n=3)	+2 ± 2 (n=3)
PGE_2 aerosol (6 breaths, 0.01 mg ml^{-1})	-7 ± 3 (n=3)	-7 ± 3 (n=3)	-2 ± 2 (n=3)
PGE_2 aerosol (6 breaths. 0.1 mg ml^{-1})	-10 ± 3 (n=3)	-7 ± 2 (n=3)	+1 ± 1 (n=3)
PGE_2 aerosol (6 breaths, 1 mg ml^{-1})	-39 ± 11	-31 ± 8	+6 ± 3 (n=3)

Values shown are mean ± sem absolute changes; (n=6 unless otherwise stated).

5.4 Discussion

In the present study, inhalation of solutions of PGE_2 aerosols evoked a dose-related bronchoconstriction and an increase in RAR discharge. In a separate series of experiments, inhaled AH13205 stimulated a proportion of the RARs tested but had no effect on pulmonary mechanics. Inhaled histamine, in both experiments, evoked bronchoconstriction and stimulated all of the RARs tested.

Inhalation of PGE_2 aerosols, in the present study, evoked a dose-related stimulation of intrathoracic RARs. In contrast, PGE_2, when administered intravenously or by aerosol, has been reported by other investigators to have had either minor or no effect on RAR discharge in dogs (Coleridge *et al.*, 1976; Roberts *et al.*, 1985). From their original findings (Coleridge *et al.*, 1976) the authors later reported that PGE_2 had, in some cases, reduced RAR discharge (Coleridge *et al.*, 1978). These contrasting findings could simply reflect differences in the species used or differences in the route of administration of PGE_2, although the latter is unlikely, since in cats i.v. administration of PGE_2 also increases RAR discharge (Fig. 5.7). It has been suggested, principally from the findings of Coleridge *et al.*, (1976) and Roberts *et al.*, (1985), that PGE_2 is a "selective" stimulant of C-fibre endings. However, the doses of PGE_2 used by investigators to stimulate C-fibre endings, either intravenously or by aerosol, in the dog, are identical to those used in the present study which stimulate RARs in the cat.

The effects of inhaled agents (for example, PGE_2) on pulmonary mechanics in spontaneously breathing animals are difficult to interpret because compliance is directly affected by the pattern of breathing. This difficulty can be overcome by the use of artificially ventilated animals in which the pattern of breathing is constant. PGE_2 is generally believed to cause relaxation of airway smooth muscle, both *in vitro*

and *in vivo* (Gardiner, 1989). Most of the evidence, as one would expect, comes from preparations in which airway smooth muscle tone has been raised by a constricting agent (for example, serotonin, acetylcholine). There is evidence, however, to suggest that PGE_2 also exerts a bronchoconstrictor effect in preparations in which airway smooth muscle tone has not been raised, both *in vitro* and *in vivo* (Gardiner, 1989). Indeed, in the present study, inhalation of PGE_2 aerosols evoked a dose-related bronchoconstriction, as measured by an increase in lung resistance and fall in dynamic lung compliance. PGE_2, administered intravenously, also caused bronchoconstriction (Fig. 5.7). In contrast, PGE_2 has also been shown to be effective in relaxing airway smooth muscle in cat preparations, both *in vivo* and *in vitro*, in which tone had been increased (Gardiner, 1989). Thus, depending on airway smooth muscle tone, it appears that PGE_2 can induce either bronchoconstriction or bronchodilatation. This "dual action" of PGE_2 may reflect the presence of at least two types of prostanoid receptor, with opposing actions, in the feline respiratory tract. There is some evidence to suggest that EP_2 receptors are prominent in the cat trachea, whereas in cat lung strips both EP_2 and TP receptors may be present; EP_2 receptors mediating relaxation and TP receptors mediating contraction of the airway smooth muscle (Gardiner, 1989).

Inhalation of AH13205, the EP_2 receptor agonist (6 breaths from a 3 mg ml^{-1} solution), stimulated a proportion of the RARs tested. The effect of AH13205 on the RARs stimulated was similar to that induced by PGE_2 aerosol (6 breaths, 0.1 mg ml^{-1} solution). Thus, AH13205 appears to be at least 30 times less potent than PGE_2 in stimulating RARs, which is similar to its potency as an EP_2 receptor agonist (Nials *et al.*, 1991). AH13205 aerosols did not, however, have any effect on pulmonary mechanics or cardiovascular parameters. The apparent lack of bronchodilator activity seen with AH13205, in the current experimental set-up, is not entirely surprising since

bronchodilator activity is more commonly observed in preparations in which airway smooth muscle tone has been raised.

The mechanism by which PGE_2 stimulates RARs, in the cat, is unclear. It is possible that PGE_2 may be acting directly on the nerve endings themselves; an effect which may involve the activation of a prostanoid receptor, possibly the EP_2 receptor. Indeed, in the present study, PGE_2 at low doses, stimulated some but not all of the RARs tested without causing bronchoconstriction. Moreover, AH13205, the EP_2 receptor agonist, stimulated some of the RARs examined, without affecting pulmonary mechanics. Alternatively, it may be that the RARs are activated indirectly, as a result of the bronchoconstriction induced by PGE_2. Although, the exact contribution of each of these mechanisms is unknown, it is more than likely that both direct and indirect mechanisms are involved in PGE_2-induced stimulation of RARs.

When administered via the vasculature or as an inhaled aerosol, PGE_2 is a potent vasodilator. In the present study, inhaled PGE_2 caused significant hypotension at the highest dose (6 breaths, 1 mg ml^{-1} solution), the effect on heart rate was variable. At the lower concentrations, however, there were no significant changes in arterial blood pressure or heart rate. The absence of a decrease in arterial blood pressure, when using lower concentrations, would suggest that little or no PGE_2 reached the systemic circulation. Therefore, it would appear that PGE_2-induced bronchoconstriction and stimulation of RARs, in the present study, are independent of changes in vascular parameters.

Administration of PGE_2, intravenously or as an inhaled aerosol, causes bronchodilatation in most normal and asthmatic subjects (Smith & Cuthbert, 1976). This effect may be mediated by EP_2 receptors which are believed to be present in

human airways (Gardiner, 1989). However, when administered i.v. or as an inhaled aerosol, PGE_2 can also evoke a paradoxical bronchoconstriction in some asthmatic subjects (Smith & Cuthbert, 1976). It may be that PGE_2, in such cases, is constricting airway smooth muscle directly; possibly through stimulation of the prostanoid TP receptors (Gardiner, 1989). Alternatively, PGE_2 may be evoking bronchoconstriction through a vagal reflex process. Indeed, in dogs, Roberts *et al.*, (1985), reported that PGE_2, when administered via the bronchial artery or as an inhaled aerosol into the lower airways, evoked a vagally-mediated increase in tension in an isolated segment of trachea. Although it should also be noted that in some preparations, PGE_2 reduced tracheal tension.

The use of inhaled PGE_2 to evoke cough in the evaluation of anti-tussive therapies has been limited, principally because the cough response to PGE_2 adapts during challenge and develops tachyphylaxis to repeated challenges (Lowry & Higenbottam, 1989; Higenbottam & Lowry, 1990). The mechanisms by which adaptation and tachyphylaxis of the PGE_2-induced cough responses occur are unclear. It is possible that adaptation of the RAR response to inhaled PGE_2 may explain the adaptation of the cough response during PGE_2 challenge. However, this possibility was not explored in the current study. It is unlikely that tachyphylaxis of the cough response to repeated inhalation of PGE_2 aerosols is due to tachyphylaxis of the RAR response, since, in the present study, reproducible RAR responses were obtained, at six minute intervals, to inhaled PGE_2 (6 breaths generated from a 1 mg ml^{-1} solution) (Fig. 5.8). Alternatively, the apparent adaptation and tachyphylaxis may be the result of the involvement of another group of airway sensory nerve endings. Indeed, PGE_2, when administered via the vasculature or as an inhaled aerosol, is a potent stimulus of C-fibre endings (Coleridge *et al.*, 1976; Roberts *et al.*, 1985). Furthermore, there is

evidence to suggest that stimulation of C-fibre endings may inhibit coughing in experimental animals (Tatar *et al.*, 1988; Jackson, 1989).

PGE_2 also possesses a number of pro-inflammatory properties which include the ability to induce hyperalgesia and potentiate protein plasma exudation and pain (associated with inflammation) (Vane, 1971; Weissman, 1987). An example of this is seen in the rat, where injections of PGE_2 into the rat hind paw induces both acute and sustained hyperalgesia (Adcock & Garland, 1993). A similar effect can also be demonstrated in man. Intradermal injection of PGE_2 causes a local flare at the site of the injection but no pain or itching other than what would be expected from the intradermal injection itself (Crunkhorn & Willis, 1971). However, over the site of the local flare there is persistent hyperalgesia (Ferriera *et al.*, 1978). Thus in these two models, PGE_2 appears to sensitise nerve endings. It is possible, therefore, that PGE_2 acts in a similar manner in the airways. Indirect evidence to support this suggestion comes from human cough studies, in which, inhaled PGE_2 (at sub-cough threshold levels) has been shown to potentiate capsaicin aerosol-induced cough in normal subjects. (Choudry *et al.*, 1989; Stone *et al.*, 1992).

The conscious cat cough/irritancy model has been used to evaluate prostaglandins and their analogues for bronchodilator activity but which lack the side-effects of airway irritancy and cough (Gardiner *et al.*, 1978; Gardiner & Collier, 1980; Gardiner & Browne, 1984). However, there is evidence to suggest that man is more sensitive to prostaglandins, including PGE_2, than the cat (Costello *et al.*, 1985; Nizankowska *et al.*, 1985). Although, it has been suggested that the cat cough test is a poor predictive index of PGE_2 irritancy in man (Costello *et al.*, 1985), the present study demonstrates that evaluation of the effects of potential bronchodilators, especially those structurally related to prostaglandins, on airway sensory nerve endings using the technique of

single nerve fibre recording (Chapter 2), could identify those bronchodilators that have the potential to evoke airway irritancy.

In the cat, inhaled PGE_2 can stimulate RARs (present study) and precipitate coughing (Gardiner & Browne, 1984). In the dog, however, inhaled PGE_2 has little or no effect on the discharge of RARs but is a potent stimulant of C-fibre endings (Roberts *et al.*, 1985). Furthermore, although cough/irritancy can be demonstrated in the conscious dog, the effects are highly variable (P.J. Gardiner, personal communication). From these findings it could be argued that stimulation of RARs but not C-fibre endings is essential in evoking cough in experimental animals and possibly man.

The present study clearly demonstrates that PGE_2, when delivered by aerosol to the airway epithelial surface, is not a "selective" stimulant of C-fibre endings. Stimulation of RARs by PGE_2, released endogenously during airway inflammation or when administered as an inhaled aerosol, may contribute to the reflex coughing and the paradoxical bronchoconstriction evoked by PGE_2 in both experimental animals and man.

Chapter 6

Effects of frusemide (i.v. and aerosol) and inhaled indomethacin on the spontaneous discharge of RARs

6.1 Introduction

Cough and reflex bronchoconstriction often occur simultaneously and have been closely related mechanistically. However, there is evidence to indicate that cough and bronchoconstriction are separate airway reflexes, which can be induced individually and inhibited differentially by drugs (Karlsson *et al.*, 1988). Indeed inhalation of ultrasonically nebulised distilled water (UNDW, a solution of low osmolarity and low ion concentration) can evoke coughing and an atropine-sensitive bronchoconstriction in asthmatic subjects (Sheppard *et al.*, 1983; Eschenbacher *et al.*, 1984; Fuller & Collier, 1984). In contrast, inhalation of UNDW evokes only coughing in normal subjects (Higenbottam, 1984; Godden *et al.*, 1986). In asthmatic subjects, the bronchoconstrictor responses only occur when the tonicity of the solution is above or below iso-osmolarity (Eschenbacher *et al.*, 1984), whereas the cough response, in asthmatic and normal subjects, is related to the absence or reduced concentration of chloride ions, Cl^-, (or small anions that can functionally replace Cl^-). In asthmatic subjects, inhalation of lignocaine inhibits the cough response to UNDW, but fails to have any effect on the increased airway resistance (bronchoconstriction) whereas, the anti-asthmatic drug, sodium cromoglycate, has the reverse effect inhibiting the increase in airways resistance but having no effect on the cough response (Sheppard *et al.*, 1983; Fuller & Collier., 1984).

The cough response, to UNDW, is thought to be mediated through stimulation of myelinated airway "irritant receptors", although Pisarri and colleagues (1992) have demonstrated that UNDW injected into the lobar bronchus of anaesthetized and artificially ventilated dogs, stimulates both lung C-fibre endings and RARs. Boggs and Bartlett (1982) demonstrated that solutions, in which there was an absence or reduced concentration of Cl^- (or small anions that can functionally replace Cl^-) for example distilled water, elicited apnoea when instilled into the larynx of new-born

puppies. The principal stimulus for the apnoeic reflex was the absence or reduced concentration of Cl^- (or small anions that can functionally replace Cl^-) in the laryngeal fluid. In addition, they demonstrated that all of the solutions that evoked the apnoeic reflex could also stimulate myelinated laryngeal nerve endings. These findings confirmed the earlier findings of Boushey and colleagues (1974). However, the studies conducted by Boggs and Bartlett (1982) and Boushey *et al.*, (1974) did not examine the effects of these solutions on non-myelinated C-fibre endings and, therefore, an involvement of these nerve fibre endings in evoking airway reflexes, induced by such solutions, cannot be ruled out. The findings of Pisarri *et al.*, (1992) that suggest stimulation of RARs and lung C-fibre endings is a result of changes of tonicity rather than ion content, are in direct contrast to findings from other nerve fibre recording studies and human cough studies (Boggs & Bartlett, 1982; Godden *et al.*, 1986). These opposing findings may simply reflect differences in the regional chemosensitivity of airway nerve endings in the respiratory tract. In contrast to the cough response induced by UNDW, the mechanism by which UNDW evokes bronchoconstriction is much more uncertain, but may involve the release of inflammatory mediators from airway mast cells and other cell types (Findlay *et al.*, 1981; Eggleston *et al.*, 1983).

Conventional treatment of asthma involves the use of β-agonists, corticosteroids, and sodium cromoglycate. It is surprising, therefore, to find that the loop diuretic frusemide inhibits the bronchoconstrictor responses evoked by a variety of "indirect" airway challenges, which include adenosine, allergen, exercise, sodium metabisulphite and UNDW, in asthmatic subjects (Bianco *et al.*, 1988, 1989; Nichol *et al.*, 1990; Polosa *et al.*, 1990; Robuschi *et al.*, 1989, 1990; O'Connor *et al.*, 1991a). Inhalation of nebulised frusemide also significantly attenuates the cough response to UNDW in both normal and asthmatic subjects (Ventresca *et al.*, 1990;

Wood *et al.*, 1990b). Although frusemide inhibits the re-absorption of NaCl from the ascending loop of Henlé by competitive inhibition of the $Na^+/K^+/2Cl^-$ co-transporter on the basolateral membrane (Greger & Schlatter, 1981; Haas, 1989), the mechanism by which frusemide provides its protective effect in the airways is uncertain. A number of possible mechanisms by which frusemide exerts its effects have been suggested, including a reduction in afferent sensory neural activity (Anonymous, 1990; Chung & Barnes, 1992). Support for an effect of frusemide on afferent nerve endings comes from single nerve fibre recording studies in which Sant'Ambrogio and co-workers (1993) showed that frusemide, inhaled or instilled in the larynx, significantly attenuated dextrose solution (an isotonic solution which lacks ions)-induced stimulation of laryngeal "irritant receptors" in spontaneously breathing dogs.

Alternatively, frusemide may exert its protective effects by its ability to stimulate the production of PGE_2 and that PGE_2 through it's "inhibitory" properties protects against "indirect" bronchoconstrictor challenges (Pavord *et al.*, 1991b). There is some circumstantial support for this hypothesis. Frusemide stimulates PGE_2 release from renal tubular epithelium (Miyanoshita *et al.*, 1989), and PGE_2 is a cyclooxygenase metabolite of arachidonic acid in human tracheal epithelial cells, airway smooth muscle, alveolar macrophages and eosinophils (Hubscher *et al.*, 1975; MacDermot *et al.*, 1984; Haye-Legrand *et al.*, 1986; Churchill *et al.*, 1989). In addition, PGE_2 inhibits mast cell mediator release, neurally-mediated airway smooth muscle contraction and inflammatory cell activation (Peters *et al.*, 1982; Walters *et al.*, 1984; Christman & Christman, 1990; Giembycz *et al.*, 1990). There is also some indirect evidence to suggest that endogenous PGE_2 production is responsible for the refractory period commonly seen after exercise or osmolar challenge (O'Byrne & Jones, 1986; Mattoli *et al.*, 1987); the refractory period in these studies being the period of time during which further exercise or osmolar challenge of the same

intensity would have produced less bronchoconstriction. Furthermore, inhalation of PGE_2 attenuates the bronchoconstrictor responses to allergen, exercise, sodium metabisulphite and UNDW in some asthmatic subjects (Pasariglikan *et al.*, 1976, 1977; Pavord *et al.*, 1991b).

Although frusemide has been shown to inhibit low chloride ion-induced stimulation of laryngeal "irritant receptors" (Sant'Ambrogio *et al.*, 1993), the possibility of frusemide exerting its protective effects by a direct action on airway sensory nerve endings has not been examined. The present series of experiments were aimed at investigating and comparing the effects of frusemide (i.v. or as an inhaled aerosol) with 443C81, a peripherally acting, μ-opioid receptor agonist and a known inhibitor of RAR (lung irritant receptor) activity (Adcock *et al.*, 1987; Adcock & Smith, 1987), on the spontaneous discharge of RARs. To study the possibility that endogenous prostaglandins released from airway epithelial cells may modulate the spontaneous discharge of RARs, the effect of inhaled indomethacin (an inhibitor of the cyclooxygenase pathway of arachidonic acid metabolism) on RARs was also examined in a separate series of experiments.

6.2 Method

Male cats (2.8 - 3.7 kg) were anaesthetized and prepared for surgery as described in Chapter 2. Airflow (ml. s^{-1}) and transpulmonary pressure (cmH_2O) were measured. Identification and single afferent nerve fibre recording of the RARs was carried out as described in Chapter 2. Arterial blood pressure and heart rate were also recorded. Additional cannulae (Portex 4fg) were inserted into the right jugular vein and right carotid artery (pushed into the aortic arch) for administration of drugs. The animals were paralysed with DMT and artificially ventilated according to the protocol in Chapter 2. Drugs were administered by two routes, i.v. and inhalation (DeVilbiss

Ultrasonic nebuliser, Chapter 2).

Materials

Histamine diphosphate and 443C81 were dissolved in saline. Frusemide was available as a 10 mg ml^{-1} solution, further dilutions were made in saline. Indomethacin was dissolved in 1M Tris buffer, pH 8.8, then diluted with saline to give a 20 mg ml^{-1} solution. Indomethacin vehicle solution consisted of 1 ml of Tris buffer, pH 8.8, in 5 ml of saline. Saline was available as a 0.85% wt/vol solution.

Protocols

After surgery, the animals were allowed to stabilise for at least 30 mins, before the process of locating RARs was started. Single nerve fibres were dissected out from either the left or right vagus nerve and action potentials recorded. Each animal, and therefore, each nerve, was subjected to only one study protocol.

i.v. frusemide

Upon identification of a vagal fibre, originating from an intrathoracic RAR, saline and frusemide (1 & 3 mg kg^{-1}) were administered sequentially, by bolus i.v. injections, at 8 min intervals. This was later followed by bolus i.v. injections of 443C81 (1 mg kg^{-1}) and naloxone, an opiate receptor antagonist (0.3 mg kg^{-1}), 10 and 13 mins after administration of frusemide (3 mg kg^{-1}), respectively.

Frusemide aerosol

Following identification of a vagal fibre, originating from an intrathoracic RAR, frusemide (20 breaths generated from a 10 mg ml^{-1} solution) was administered by inhalation. This was followed later by i.v. bolus injections of 443C81 (1 mg kg^{-1}) and naloxone (0.3 mg kg^{-1}), 13 and 14 mins after administration of frusemide

aerosol, respectively. Inhalation of histamine aerosol (6 breaths generated from a 1 mg ml^{-1} solution) was repeated at the end of the experiment.

Indomethacin aerosol

When a vagal fibre originating from an intrathoracic RAR was found, indomethacin was administered sequentially as an inhaled aerosol (20 breaths from vehicle & 20 mg ml^{-1} solutions) at 12 min intervals. Inhalation of histamine aerosol (6 breaths from a 1 mg ml^{-1} solution) was repeated at the end of the experiment.

Data Analysis

Histamine aerosol

The effect of histamine aerosols (6 breaths from a 1 mg ml^{-1} solution) on RAR discharge are expressed as the Δ in impulses s^{-1} from baseline and compared with baseline controls using Student's "t" test for paired data. Statistical values of $P < 0.05$ were considered to be significant. In experiments where histamine aerosol was also administered at the end of study, histamine aerosol-evoked responses were compared using Student's "t" test for paired data. Statistical values of $P < 0.05$ were considered to be significant.

i.v. frusemide

Nerve impulses were counted in 5 second period "bins" and at each time point the average of a 25s period was taken and expressed as impulses s^{-1}. Arterial blood pressure, heart rate and transpulmonary pressure are expressed as absolute values. All of the variables were measured at intervals throughout the experiments before, during and after drug (saline, frusemide 1 & 3 mg ml^{-1} and 443C81 solutions) administration. Each variable was pooled to provide mean ± sem. Statistical analysis was performed using two-way analysis of variance (ANOVA). Statistical values of P

< 0.05 were considered to be significant. The effect of bolus i.v. naloxone (0.3 mg kg^{-1}), measured two mins post-administration, on arterial blood pressure, heart rate, transpulmonary pressure and spontaneous discharge of RARs, were compared with baseline controls using Student's "t" test for paired data. Statistical values of $P <$ 0.05 were considered to be significant.

Frusemide aerosol

The effect of frusemide aerosol on arterial blood pressure, heart rate, and transpulmonary pressure and the spontaneous discharge of the RARs, was measured and analysed in the same manner as for i.v. frusemide.. The effect of bolus i.v. 443C81 (1 mg ml^{-1}) and naloxone (0.3 mg kg^{-1}), measured 1 min post-administration of each drug, on arterial blood pressure, heart rate, transpulmonary pressure and spontaneous discharge of RARs, were compared with baseline controls using Student's "t" test for paired data. Statistical values of $P < 0.05$ were considered to be significant.

Indomethacin aerosol

The effect of indomethacin aerosols on arterial blood pressure, heart rate, and transpulmonary pressure and the spontaneous discharge of the RARs was measured and analysed in the same manner as for i.v. frusemide.

6.3 Results

6.3.1 Baseline Values

18 male cats were used. The mean ± sem of the resting values of pulmonary mechanics and cardiovascular parameters for 18 open chest animals (unless otherwise stated) are shown in Table 6.1.

Table 6.1 Baseline values for P_{Tp}, systolic and diastolic blood pressure and heart rate in anaesthetized, paralysed and artificially ventilated cats. Values are shown as mean ± sem, (n=18, unless otherwise stated).

parameters	baseline values
systolic blood pressure, mmHg	131 ± 9.5 (n=16)
diastolic blood pressure, mmHg	86 ± 6.0 (n=16)
Heart rate, beats min^{-1}	159 ± 6.4 (n=15)
P_{Tp}, cmH_2O	7.3 ± 0.44

6.3.2 i.v. frusemide on RARs

Six intrathoracic RARs were examined from 6 cats. The spontaneous activity in the fibres was 1.5 ± 0.4 imp s^{-1} (range 0.68 - 3.12 imp s^{-1}). The adaptation index of each receptor was 100%. Histamine aerosol (6 breaths from a 1 mg ml^{-1} solution) stimulated all six RARs (2.7 ± 0.6 imp s^{-1}, $P < 0.01$).

Bolus i.v. injection of saline and frusemide (1 & 3 mg kg^{-1}) had no significant effect on transpulmonary pressure, arterial blood pressure, heart rate or on the spontaneous discharge of the RARs (Figs 6.1 & 6.2). In contrast, bolus i.v. 443C81 (1 mg kg^{-1}) significantly reduced the spontaneous discharge of RARs (n=6) from 0.9 ± 0.2 imp s^{-1} (time zero) to 0.23 ± 0.13 imp s^{-1} (1 min post injection, $P < 0.05$) and 0.18 ± 0.03 imp s^{-1} (3 mins post injection, $P < 0.01$) (Fig. 6.1). An example of the effect of bolus i.v. 443C81 on the spontaneous discharge of an RAR is shown in Fig. 6.3. Bolus i.v. naloxone (0.3 mg kg^{-1}) reversed the inhibitory effect of bolus i.v. 443C81, mean discharge of the RARs increasing from 0.18 ± 0.03 to 1.5 ± 0.4 imp s^{-1} ($P < 0.05$, Fig. 6.2). Neither 443C81 nor naloxone had any significant effect on transpulmonary pressure, arterial blood pressure or heart rate (Fig. 6.4).

6.3.3 Frusemide aerosol on RARs

Six RARs were examined from 6 cats. The spontaneous activity of the RARs was 2.2 ± 0.4 imp s^{-1} (0.73 - 4.08 imp s^{-1}). All of the RARs had an adaptation index of 100%. Histamine aerosol (6 breaths generated from a 1 mg ml^{-1}) stimulated all 6 RARs, before and after the completion of the experimental protocol. (4.5 ± 1, $P < 0.01$ and 3.1 ± 0.8 imp s^{-1}, $P < 0.05$ respectively).

Inhalation of frusemide (20 breaths generated from a 10 mg ml^{-1} solution) had no significant effect on the spontaneous discharge of RARs, transpulmonary pressure or cardiovascular variables (Figs 6.5, 6.6). In contrast, bolus i.v. injection of 443C81 significantly ($P < 0.05$) attenuated the activity of the RARs from 1.6 ± 0.5 to 0.5 ± 0.3 imp s^{-1} (1 min post administration). This effect was reversed by bolus i.v. naloxone, with the spontaneous discharge of the RARs increasing to 3.0 ± 1 imp s^{-1}, $P < 0.05$ (1 min post administration).

6.3.4 Indomethacin aerosol on RARs

6 RARs were examined from 6 cats. The spontaneous activity was 1.2 ± 0.3 imp s^{-1} (0.17 - 2.32 imp s^{-1}). All of the RARs had an adaptation index of 100%. Histamine aerosol stimulated all of the RARs examined, before (2.5 ± 0.5 imp s^{-1}, $P < 0.01$) and after (2.5 ± 0.4 imp s^{-1}, $P < 0.001$) completion of the experimental protocol. There was no significant difference between histamine aerosol-evoked responses before and after the administration of indomethacin aerosols.

Indomethacin vehicle (20 breaths) had no effect on the spontaneous activity of RARs, transpulmonary pressure, arterial blood pressure or heart rate (Figs 6.7 & 6.8). Inhalation of indomethacin aerosol (20 breaths generated from a 20 mg ml^{-1} solution) significantly ($P < 0.05$), reduced the spontaneous activity of RARs 8 mins

post inhalation (Fig. 6.7). This effect was transient with the spontaneous discharge returning to baseline level 10 mins post-administration. Inhalation of indomethacin aerosol (20 breaths from a 20 mg ml^{-1} solution) had no significant effect on transpulmonary pressure, arterial blood pressure or heart rate (Fig. 6.8).

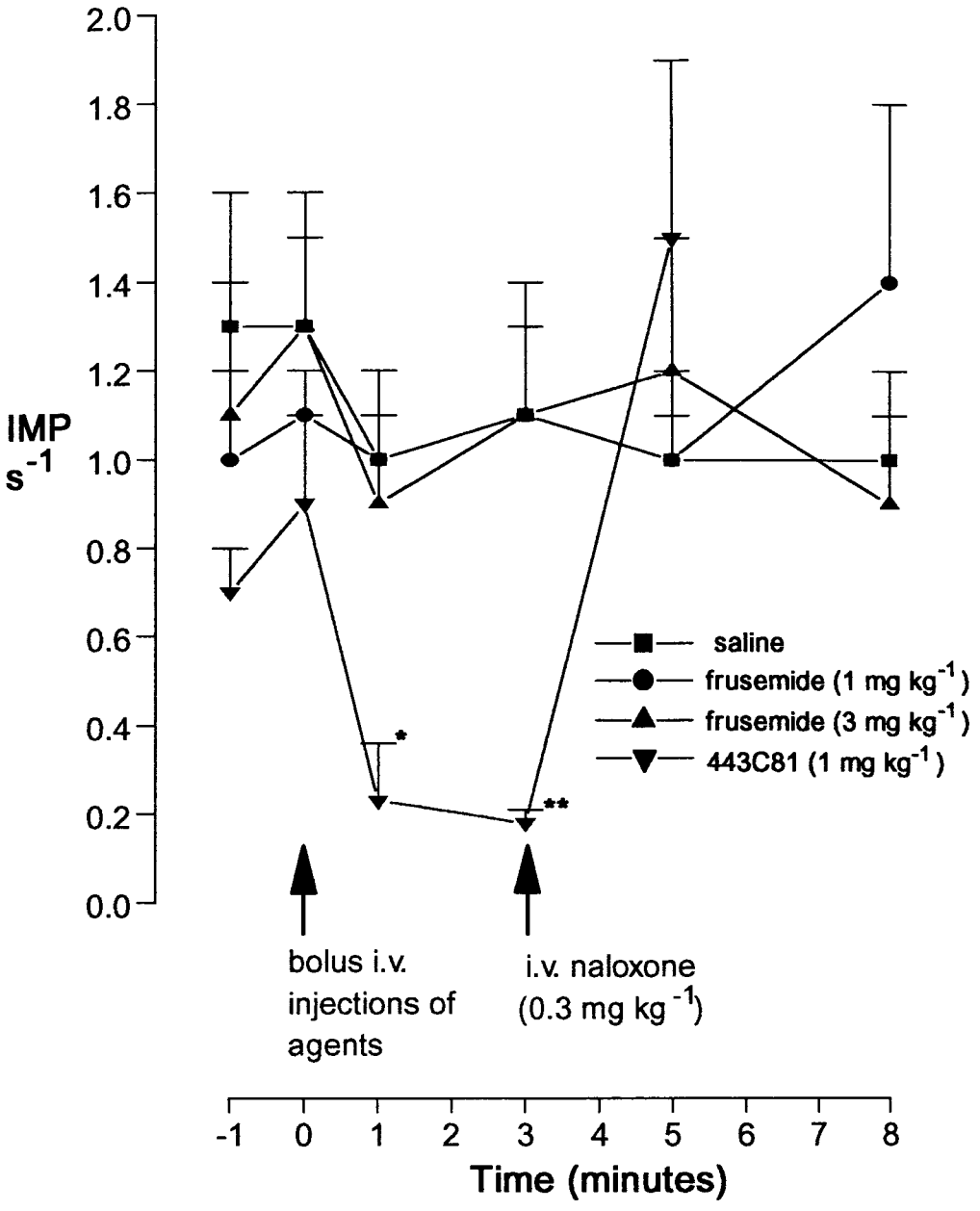


Fig. 6.1 Effect of administration of bolus i.v. injections, at 8 min intervals, of saline, frusemide (1 & 3 mg kg^{-1}) and 443C81 (1 mg kg^{-1}) on the spontaneous impulse activity of vagal fibres arising from intrathoracic RARs (n=6) in anaesthetized, paralysed and artificially ventilated cats. Naloxone (0.3 mg kg^{-1}, i.v.) was administered as shown, 3 mins after the administration of i.v. 443C81. Absolute values for the spontaneous impulse activity (IMP s^{-1}), expressed against time (minutes) are shown before, during and after administration of each agent. Vertical bars represent sem. All agents were administered as shown. Values following i.v. injection of agents, that were significantly different from those at time zero are shown by * $P \leq 0.05$, ** $P < 0.01$.

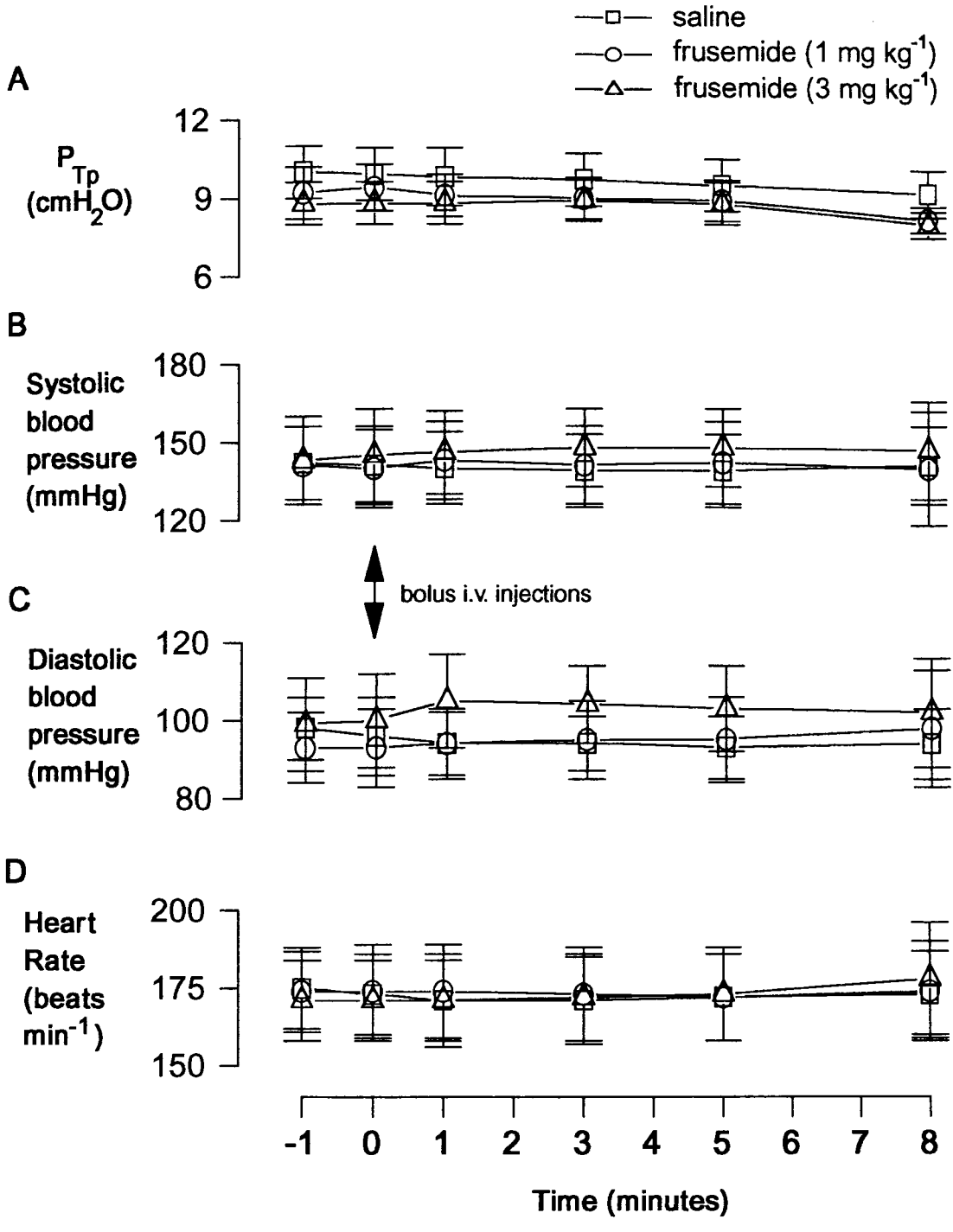


Fig. 6.2 Effect of administration of bolus i.v. injections, at 8 min intervals, of saline, frusemide (1 & 3 mg kg^{-1}) on transpulmonary pressure (**A**, P_{Tp}), arterial systolic (**B**) and diastolic (**C**) blood pressure and heart rate (**D**) in anaesthetized, paralysed and artificially ventilated cats (n=6). Absolute values for each variable, expressed against time (minutes) are shown before, during and after administration of each agent. Vertical bars represent sem. All drugs were administered as shown.

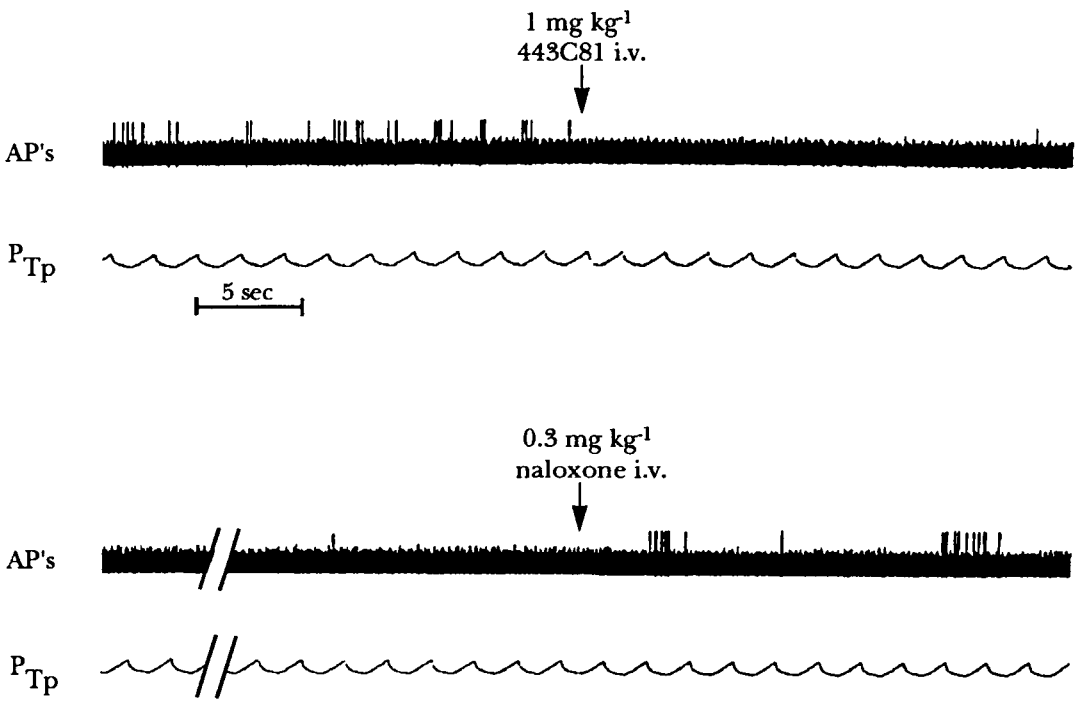


Fig. 6.3 Effect of bolus i.v. injections of 443C81 (1 mg kg^{-1}) and naloxone (0.3 mg kg^{-1}, 3 mins later) on the spontaneous discharge of a vagal fibre arising from an intrathoracic RAR in an anaesthetized, paralysed and artificially ventilated cat. Recording of transpulmonary pressure (P_{Tp}) is also shown.

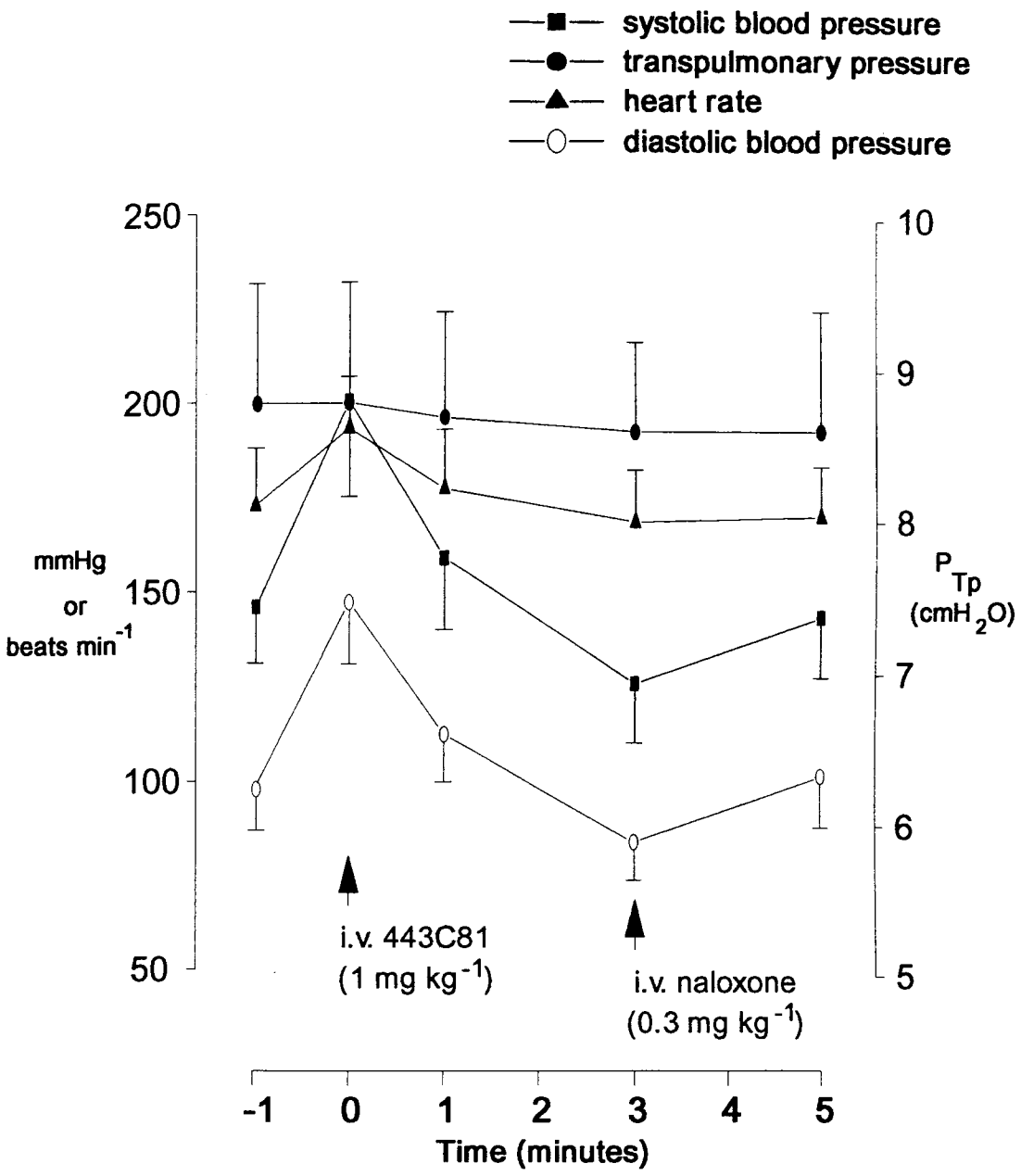


Fig. 6.4 Effect of bolus i.v. injections of 443C81 (1 mg kg $^{-1}$) and naloxone (0.3 mg kg^{-1}) at time zero and +3 mins respectively, on arterial systolic and diastolic blood pressure, heart rate and transpulmonary pressure (P_{Tp}) in anaesthetized, paralysed and artificially ventilated cats (n=6). Absolute values for each variable, expressed against time (minutes) are shown before, during and after administration of each agent. Vertical bars represent sem. All drugs were administered as shown.

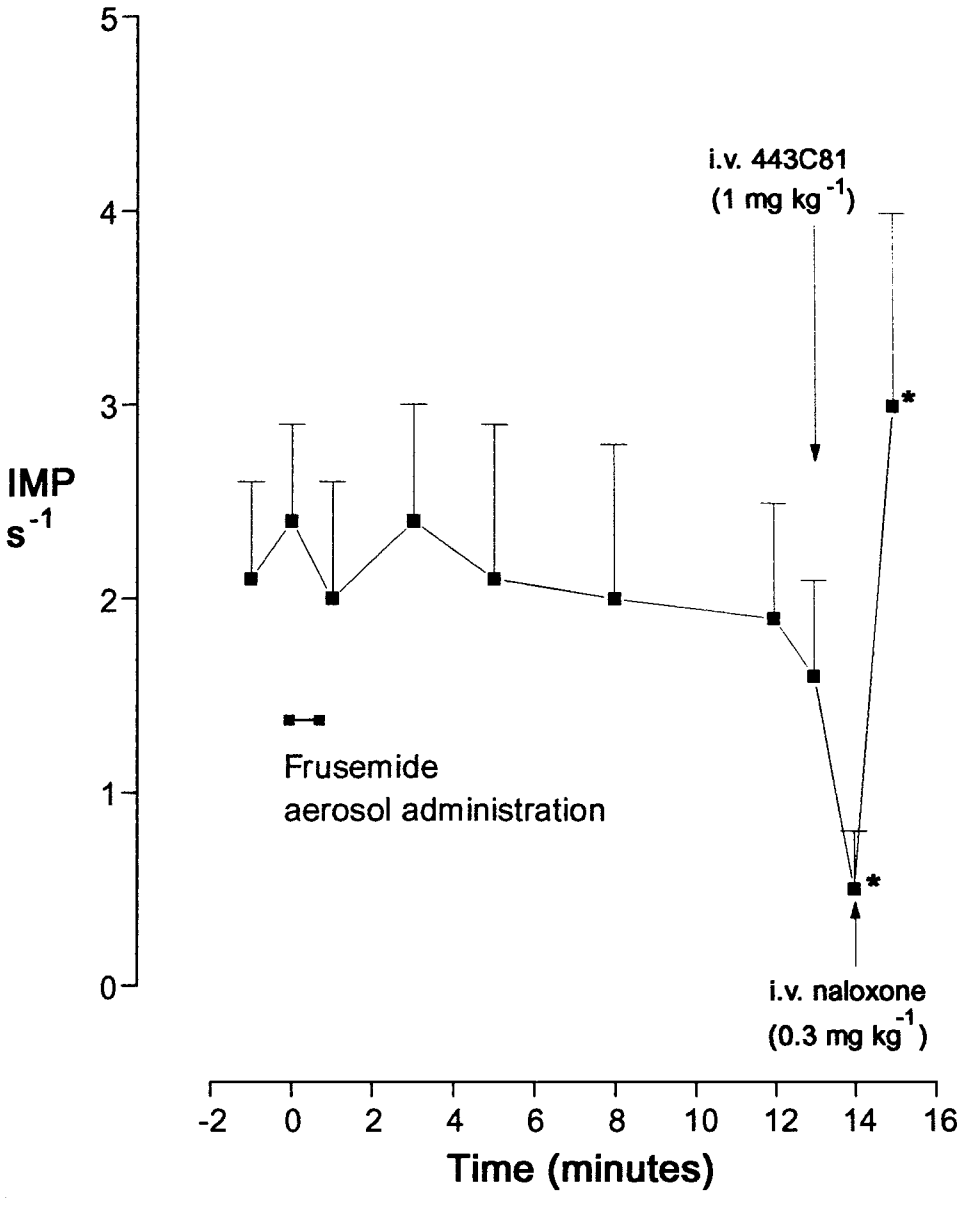


Fig. 6.5 Effect of inhaled frusemide (20 breaths, 10 mg ml^{-1} solution) and then followed by bolus i.v. injections of 443C81 (1 mg kg^{-1}) and naloxone (0.3 mg kg^{-1}) on the spontaneous impulse activity of vagal fibres arising from intrathoracic RARs (n=6) in anaesthetized, paralysed and artificially ventilated cats (n=6). Absolute values for the spontaneous impulse activity (IMP s^{-1}), expressed against time (minutes) are shown before, during and after administration of each agent. Vertical bars represent sem. All agents were administered as shown. 443C81 and naloxone responses that were significantly different from their respective control baselines are shown by * $P < 0.05$.

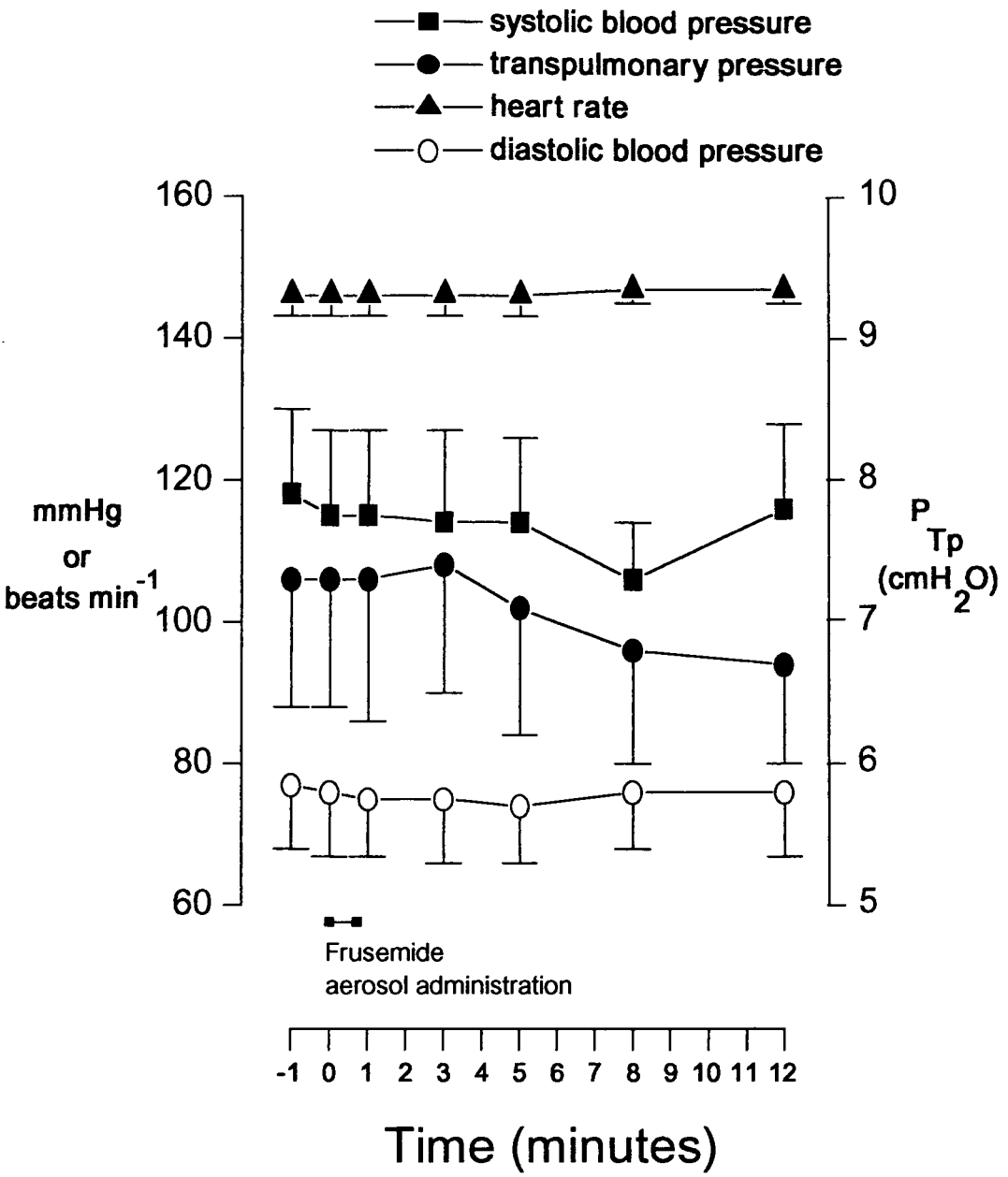


Fig. 6.6 Effect of inhaled frusemide (20 breaths from a 10 mg ml^{-1} solution) on arterial systolic and diastolic blood pressure, heart rate and transpulmonary pressure (P_{Tp}) in anaesthetized, paralysed and artificially ventilated cats (n=6). Absolute values for each variable, expressed against time (minutes) are shown before, during and after administration of inhaled frusemide. Vertical bars represent sem. Frusemide was administered as shown.

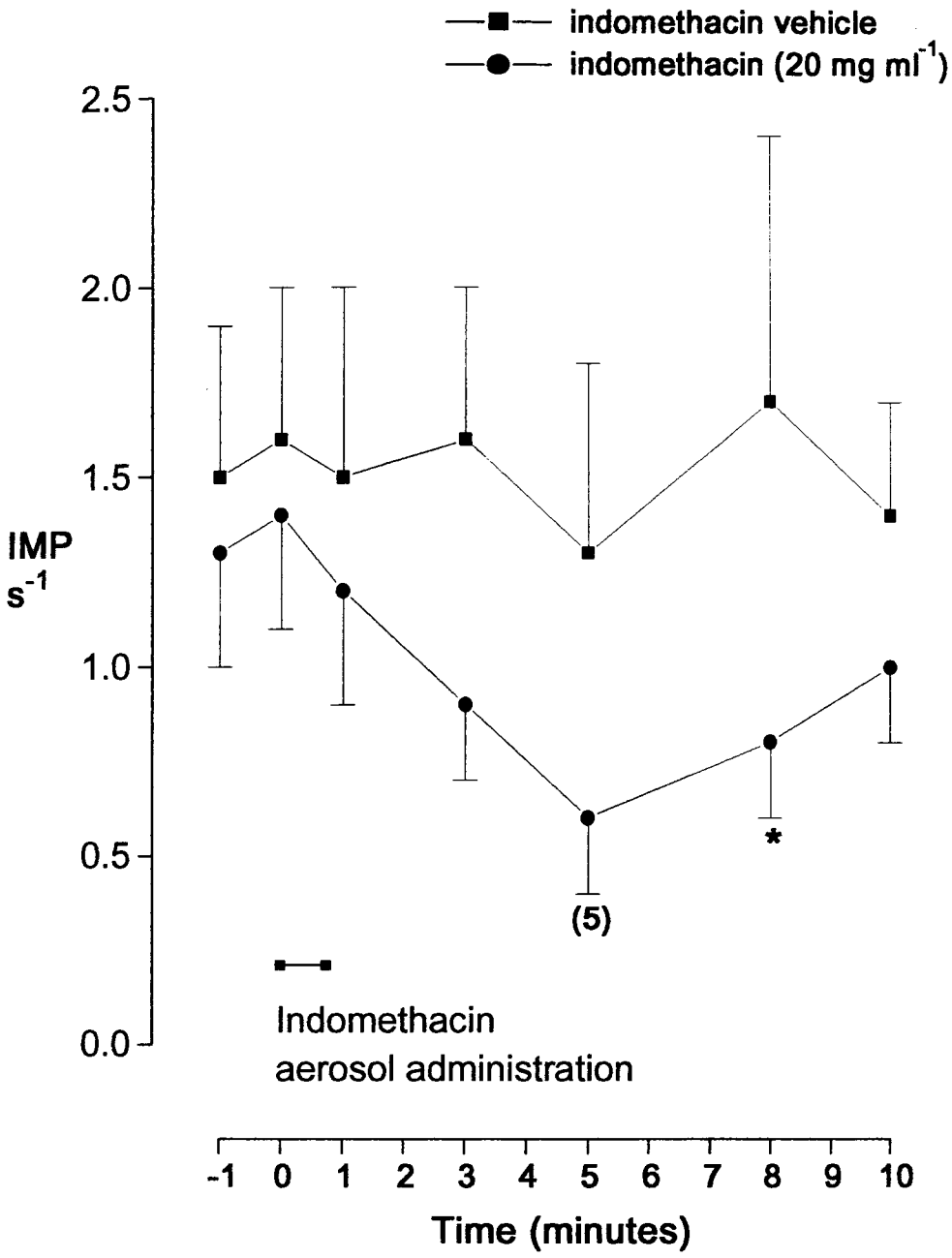


Fig. 6.7 Effect of inhaled aerosols of indomethacin (20 breaths vehicle, & 20 breaths of a 20 mg ml^{-1} solution, administered at 10 min intervals) on the spontaneous impulse activity of vagal fibres arising from intrathoracic RARs (n=6 unless otherwise stated) in anaesthetized, paralysed and artificially ventilated cats (n=6). Absolute values for the spontaneous impulse activity (IMP s^{-1}), expressed against time (minutes) are shown before, during and after administration of each agent. Vertical bars represent sem. All agents were administered as shown. Values following inhalation of indomethacin aerosols, that were significantly different from those at time zero are shown by * $P < 0.05$.

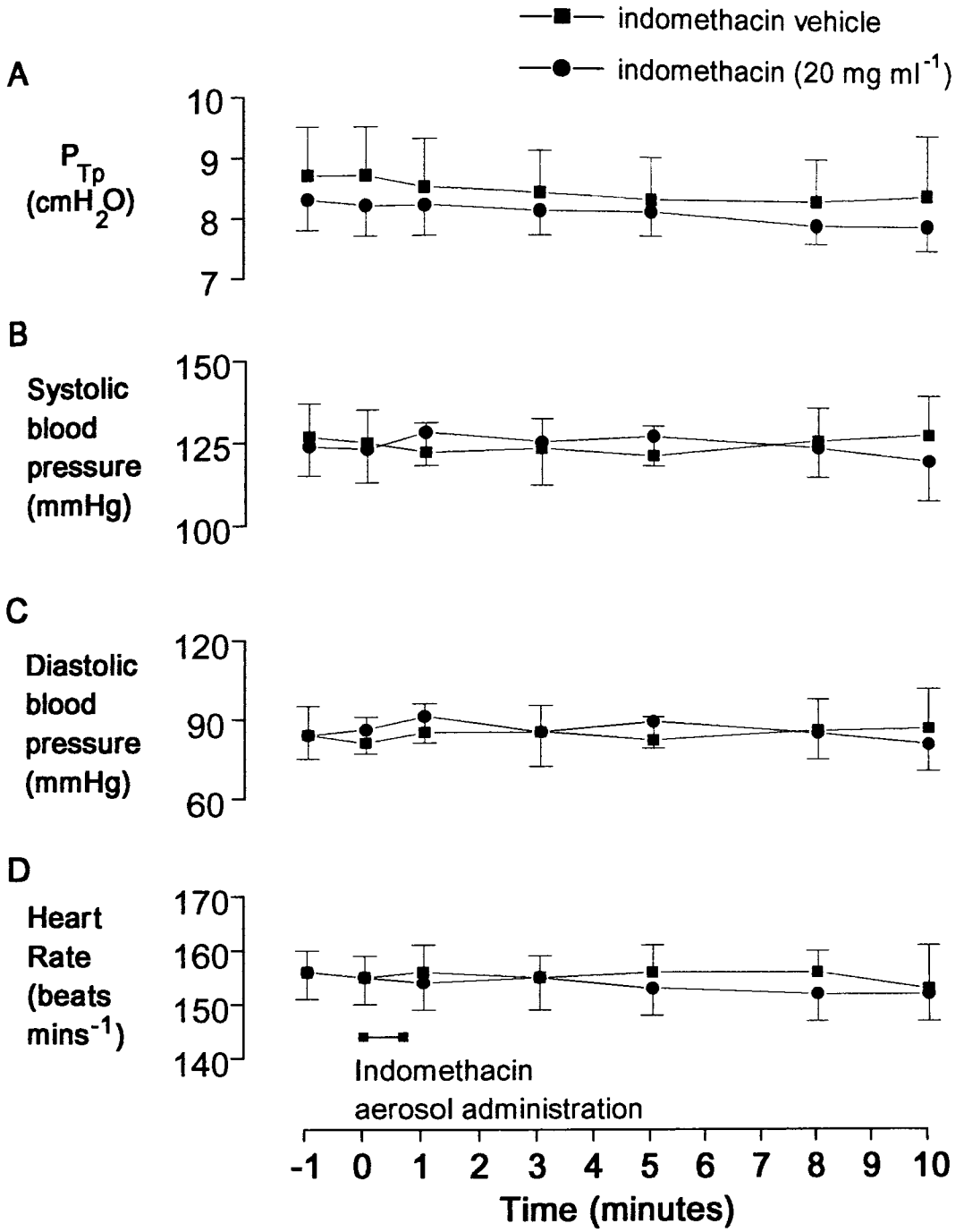


Fig. 6.8 Effect of inhaled aerosols of indomethacin (20 breaths vehicle & 20 breaths of a 20 mg ml^{-1} solution, administered at 10 min intervals) on transpulmonary pressure (**A**, P_{Tp}), arterial systolic (**B**) and diastolic (**C**) blood pressure and heart rate (**D**) in anaesthetized, paralysed and artificially ventilated cats (n=6). Absolute values for each variable, expressed against time (minutes) are shown before, during and after administration of each agent. Vertical bars represent sem. All agents were administered as shown.

6.4 Discussion

Frusemide, administered either intravenously (as a bolus injection) or as an inhaled aerosol, had no significant effect on the spontaneous impulse activity of intrathoracic RARs. By way of contrast, the peripherally acting μ-opioid receptor agonist, 443C81, significantly attenuated the spontaneous discharge of the RARs; an effect reversed by bolus i.v. naloxone. In a separate series of experiments, inhalation of indomethacin aerosol, but not vehicle, transiently decreased spontaneous RAR impulse activity.

Inhalation of UNDW causes cough and bronchoconstriction in asthmatic subjects and cough alone in normal subjects (Sheppard *et al.*, 1983; Eschenbacher *et al.*, 1984; Fuller & Collier, 1984; Godden *et al.*, 1986). The cough response is thought to be dependent on the lack of a permeant anion whereas the bronchoconstriction response is dependent on changes in osmolarity (Eschenbacher *et al.*, 1984). UNDW is believed to evoke cough by stimulation of airway sensory nerve endings. However, the mechanism by which UNDW and related aqueous solutions (low permeant [anion]) stimulate these sensory nerve endings is uncertain. The most likely explanation by which UNDW stimulates airway sensory nerve endings is related to the resting membrane potential of the sensory nerve endings. UNDW deposited on the airway epithelial surface could dilute [Cl^-] around airway sensory nerve endings, which would cause an efflux of intracellular chloride ions from the nerve endings and subsequent stimulation of the sensory nerve endings. Indeed, Hodgkin and Horowitz (1959) demonstrated that reduced extracellular [Cl^-] could depolarise skeletal muscle fibres. Earlier studies, conducted by Fenn *et al.*, (1934), also demonstrated that the Cl^- content inside frog sciatic nerves falls proportionately with decreases in [Cl^-] of the surrounding solution and Cl^- readily escapes from nerves immersed in isotonic sugar solutions. To the extent that relative intra- and extracellular [Cl^-] play a similar

role in nerve action potentials, as they do in muscle fibre potentials, this sort of Cl^- efflux could be expected to depolarise the nerve just as Hodgkin and Horowitz (1959) demonstrated in muscle.

Inhaled frusemide inhibits UNDW-induced cough in both asthmatic and normal subjects (Ventresca *et al.*, 1990; Wood *et al.*, 1990b) although in a recent study, frusemide failed to show any protection against the tussive effects of a chloride deficient solution in mild asthmatics (Stone *et al.*, 1993). In single nerve fibre recording studies, Sant'Ambrogio *et al.* (1993), demonstrated that frusemide instilled into the larynx inhibited dextrose solution-induced stimulation of myelinated laryngeal "irritant receptors". Therefore, it is possible that frusemide may attenuate UNDW-induced cough by an inhibitory action on myelinated laryngeal "irritant receptors". However, in the present study, frusemide administered either intravenously or as an inhaled aerosol, had no direct effect on the spontaneous impulse activity of intrathoracic RARs. When taken with the finding that frusemide has no effect on capsaicin aerosol-induced cough (Ventresca *et al.*, 1990), these observations would suggest that frusemide may be acting indirectly on sensory nerve endings, perhaps by acting on airway epithelial cells to prevent distilled water-induced changes in the $[Cl^-]$ around airway sensory nerve endings or, alternatively, by blocking chloride channels on the sensory nerve endings. In addition, the finding that frusemide has no effect on capsaicin-induced cough may simply reflect the fact that movement of chloride ions are not involved in capsaicin activation of airway sensory nerve endings. Frusemide also inhibits $PGF_{2\alpha}$-induced cough and the enhancement of capsaicin-induced cough by $PGF_{2\alpha}$ in normal volunteers, but is not active against $PGF_{2\alpha}$-induced bronchoconstriction (Ventresca *et al.*, 1990; Stone *et al.*, 1991). However, considering the possible mechanisms by which $PGF_{2\alpha}$ may evoke cough (direct and/or indirect stimulation of airway sensory nerve endings) it is

difficult to explain a means by which frusemide may be acting. Indeed, frusemide has no direct effect on the spontaneous activity of intrathoracic RARs (present study). Although, frusemide has a significant effect on methacholine challenge (Polosa *et al.*, 1990), other investigators have failed to show any effect with frusemide on direct smooth muscle challenges (Nichol *et al.*, 1990; O'Connor *et al.*, 1991a).

Inhaled frusemide inhibits bronchoconstrictor responses evoked by allergen challenge, UNDW or, as a consequence of hyperosmolarity, induced by exercise in asthmatic subjects (Bianco *et al.*, 1988, 1989; Robuschi, *et al.*, 1989, 1990). Since frusemide can inhibit both the early and late phase responses to allergen challenge, it is possible that frusemide may be inhibiting mediator release from various cell types, including mast cells. Indeed, UNDW can stimulate airway mast cells and other cell types (Findlay *et al.*, 1981; Eggleston *et al.*, 1983). There is some experimental evidence, from *in vitro* studies, to support the theory that frusemide acts by inhibition of inflammatory mediator release. Frusemide inhibits antigen-induced release of histamine and sulfidopeptide leukotrienes from sensitised human lung fragments (Anderson *et al.*, 1991). In addition, frusemide also inhibits the release of superoxide anions from human epithelial cells and alveolar macrophages stimulated by opsonised zymosan, *in vitro,* and frusemide, but not bumetanide, can inhibit the release of hydrogen peroxide from guinea-pig eosinophils which were activated by opsonised zymosan and leukotriene B_4 (Perkins *et al.*, 1991; Soloperto *et al.*, 1991). This effect of frusemide is also mimicked by drugs known to block chloride ion channels.

An alternative possible mechanism by which frusemide could be acting, may be related to its ability to reduce epithelial potential difference. Therefore, it is possible that frusemide may have a primary effect on airway epithelium. Indeed, it has been

demonstrated that topically applied frusemide reduces basal nasal potential difference and distilled water-induced changes in nasal potential difference in normal subjects, the latter effects being inhibited in a dose-dependent manner (Wood *et al.*, 1989a; Mialon, Regnard & Lockhart, unpublished observations). Although another research group failed to show any effect of inhaled frusemide on nasal potential difference (Nichol *et al.*, 1990), it should be noted that this may simply reflect differences in the method of administration of frusemide by each investigating group. The finding that frusemide has an effect when given by inhalation, but not by oral administration, suggests that frusemide may be acting on the airway epithelial surface. This is somewhat surprising since it is conventionally believed that frusemide acts on the basolateral surface of airway epithelial cells rather than on the mucosal surface (Welsh, 1983, 1987; Widdicombe *et al.*, 1983). Alternatively, the absence of any effect of oral frusemide may reflect the low drug concentrations found in the lung after oral administration (Cohen *et al.*, 1976).

The loop diuretics, frusemide and bumetanide, have been long considered specific inhibitors of the $Na^+/K^+/2Cl^-$ co-transporter system in a variety of cell types (Haas, 1989). Bumetanide has a higher potency than frusemide as an inhibitor of the $Na^+/K^+/2Cl^-$ co-transporter system. However, the observation that frusemide, but not bumetanide, inhibits the "indirect" bronchoconstrictor responses evoked by adenosine, exercise and sodium metabisulphite in asthmatic subjects, would suggest that inhibition of the $Na^+/K^+/2Cl^-$ co-transporter is not the underlying mechanism of the action of frusemide in the airways (Duggan *et al.*, 1990; O'Connor *et al.*, 1991a). Alternatively, the kinetics of inhaled bumetanide may be such that its transit through the airways is much more rapid than that of frusemide in the airways. It is also possible that frusemide may possess other properties which are not shared by bumetanide, such as inhibition of carbonic anhydrase or the Na^+/K^+/ATPase

(Chipperfield, 1985, Greger, 1985, Polosa *et al.*, 1990). Although the latter mechanism has been discounted by other investigators (Wood *et al.*, 1990a).

Water loss from the airways is known to provoke airway narrowing (Bar-Or *et al.*, 1977; Chen & Horton, 1977; Strauss *et al.*, 1978; Anderson *et al.*, 1982). The precise mechanism whereby the loss of water causes the airways to narrow is not known, but there have been several proposals for the mechanism of action: 1) that dehydration and the consequent increase in airway osmolarity results in the release of substances that cause airway smooth muscle to contract (Anderson, 1984; Anderson *et al.*, 1989); and 2) that airway cooling leads to a reactive hyperaemia of the bronchial circulation and oedema of the airway wall, both of which cause the airways to narrow (McFadden *et al.*, 1986; McFadden, 1990). Water loss through evaporation must be replaced instantaneously, if iso-tonicity of the airway surface liquid is to be maintained. The most readily available source of water is from the epithelial cells and the submucosa below the basement membrane. Water may also move onto the airway surface following active transport of Cl^- across the apical membrane of the airway epithelium. Frusemide is known to inhibit exercise-induced asthma, responses to hyperosmolar saline and hyperventilation challenge, and it may do so by reducing the water loss from the submucosa (Bianco *et al.*, 1988; Robuschi *et al.*, 1988; Grubbe *et al.*, 1990; Feather & Olsen, 1991; Seidenberg *et al.*, 1992; Rodwell *et al.*, 1993). When frusemide is added to sheep foetal lung, there is a decrease in foetal lung secretion, possibly as a result of frusemide blocking chloride ion channels on the apical surface of the epithelial cells (Cassin *et al.*, 1986). By blocking this channel, this would decrease the movement of water into the airway lumen thus protecting both the epithelial cell and the submucosa from dehydrating and increasing osmolarity. Further support for an action of frusemide on chloride ion channels comes from the observations that the chloride ion channel blocker 5-nitro-

2-(3-phenylpropylamino)-benzoate inhibits cholinergic neural responses in the guinea-pig trachea and the release of hydrogen peroxide from eosinophils activated by leukotriene B_4 in a similar fashion to frusemide (Chung & Barnes, 1992).

An alternative mechanism of action of frusemide may be related to its ability to generate PGE_2. Frusemide has been shown to stimulate PGE_2 release from renal tubular epithelium (Miyanoshita *et al.*, 1989). Therefore, one of the possible effects of frusemide in the airways may be to stimulate the production of PGE_2, particularly from the airway epithelium which is an important source of PGE_2 (Churchill *et al.*, 1989; Salari & Chan-Yeung, 1989). PGE_2 in turn, through its "inhibitory" properties, may protect asthmatics against "indirect" airway challenges. Inhaled PGE_2 has been shown to inhibit the bronchoconstrictor responses to allergen, exercise, sodium metabisulphite and UNDW in mild asthmatics (Pasargiklian *et al.*, 1976, 1977; Pavord *et al.*, 1991b). Furthermore, studies with cyclooxygenase inhibitors support a protective role for endogenous "inhibitory" prostanoids, showing a reduction in the refractoriness commonly observed after recovery from bronchoconstriction induced by exercise and other "indirect" challenges (Dorsch & Frey, 1981; O'Byrne & Jones, 1986; Mattoli *et al.*, 1987). The cyclooxygenase inhibitor indomethacin also appears to reverse the inhibitory effect of inhaled frusemide against exercise-induced bronchoconstriction (Pavord *et al.*, 1992). The suggestion that frusemide acts through a release of PGE_2 assumes that PGE_2 protects against bronchoconstrictor challenges through mechanisms other than a direct action on airway smooth muscle (for example on mast cell degranulation, neural pathways or inflammatory cells). Indeed, PGE_2 inhibits lung mast cell mediator release and eosinophil activation (Peters *et al.*, 1982; Giembycz *et al.*, 1990; Ito *et al.*, 1990).

PGE_2 a known "bronchodilator", however, has no effect on "direct" airway smooth muscle challenges such as methacholine (Pavord *et al.*, 1991b). Therefore, why PGE_2 should have a preferential effect on "indirect" but not "direct" bronchoconstrictor challenges is difficult to explain. On the basis of the hypothesis that there is a basal and/or stimulated release of PGE_2, for example from airway epithelial cells, which attenuates sensory nerve activity, then inhibition of PGE_2 generation should, in theory, result in an increase in the spontaneous activity of airway afferent sensory nerve endings. In the present study, however, indomethacin aerosol, although transiently, significantly reduced the spontaneous activity of intrathoracic RARs. Frusemide can also reduce the constrictor responses induced by electrical field stimulation in guinea-pig airway smooth muscle, the experiments being performed in the presence of indomethacin and *in vitro* (Elwood *et al.*, 1991). In addition, flurbiprofen another cyclooxygenase inhibitor, does not modulate the protection afforded by frusemide against sodium metabisulphite-induced bronchoconstriction (O'Connor *et al.*, 1991b). Furthermore, Bianco *et al.*, (1991) demonstrated that inhaled indomethacin protected patients with bronchial asthma against UNDW-induced bronchoconstriction. Taken together, these findings would suggest that local production of prostanoids, such as PGE_2, contribute to the pathogenesis of airways obstruction and sensory nerve stimulation rather than act as "inhibitory" agents. Indeed, PGE_2, administered i.v. or as an inhaled aerosol, is a potent non-selective stimulant of C-fibre endings and RARs, evoking airway reflexes such as cough and reflex bronchoconstriction (See Chapter 5).

443C81, a peripherally acting μ-opioid receptor agonist, has been shown to attenuate both spontaneous and chemically-induced impulse discharges in Aδ and C-fibres originating from intrathoracic RARs and pulmonary and bronchial C-fibre endings (Adcock *et al.*, 1987; Adcock & Smith, 1987). In the present series of experiments

443C81 was administered primarily to demonstrate that impulse discharges from the RARs could be attenuated. Indeed, 443C81 reduced the spontaneous discharge of all of the RARs examined, an effect that was reversed by the addition of naloxone, an opiate receptor antagonist.

Frusemide, administered intravenously or as an inhaled aerosol, appears to have no direct effect on the spontaneous activity of intrathoracic RARs. This would suggest that frusemide inhibits the cough response to UNDW via an indirect mechanism of action. However, it is possible that frusemide is only effective against an appropriate stimulus and has no effect on baseline activity of sensory nerve endings (Sant'Ambrogio *et al.*, 1993). The finding that indomethacin aerosol, in the present study, attenuates intrathoracic RAR activity, contributes towards the argument against the release of "inhibitory" prostanoids as a possible mechanism of action for frusemide in the lung. It is interesting to note that frusemide acts against the same "indirect" challenges as the anti-asthma agents nedocromil sodium and sodium cromoglycate (Busse *et al.*, 1989). Nedocromil sodium, like frusemide, is able to block UNDW-induced effects on nasal potential difference in normal subjects (Alison Wood, personal communication). Clearly the mechanisms by which all three of these agents inhibit "indirect" airway challenges require further investigation.

Chapter 7

Effects of glutamate and a glutamate-release inhibitor, lamotrigine, on RAR discharge and on airway tone

7.1 Introduction

Increased sensitivity (hyperreactivity) of the airways to physical, chemical, physiological and pharmacological stimuli is a characteristic feature of asthma and other broncho-respiratory disorders. The mechanism of airway hyperreactivity remains unknown and, although several theories have been advanced (Boushey *et al.*, 1980; Garland, 1984), it is unlikely that any one can explain the complex changes occurring in the airways of asthmatic patients. One theory receiving increasing attention is that the inflammatory component of asthma (Chung, 1986; Adcock & Garland, 1993) promotes damage to the airway epithelial cells, which either exposes or "sensitises" the sensory nerve endings to irritant stimuli, analogous to hyperalgesia in other inflamed tissues. This may result in an exaggerated vagal reflex bronchoconstriction (Empey *et al.*, 1976) and/or exaggerated local axon reflexes (Barnes, 1986a, b). The latter might be expected to exacerbate the condition by promoting neurogenic inflammation via the release of sensory neuropeptides.

The proposal that hyperalgesia may be analogous to the airway hyperreactivity seen in asthma leads to two separate lines of investigation. Firstly, whether inflammatory mediators that evoke hyperalgesia are capable of inducing airway hyperreactivity. Secondly, if analgesic agents which decrease hyperalgesia have any therapeutic benefit in asthma.

Co-existence of glutamate, an excitatory amino acid, and substance P in some primary sensory neurons, has raised the possibility that both agents may be released from terminals of primary afferents from the same dorsal root ganglia (Battaglia & Rustioni, 1988). Moreover, it has been shown that glutamate, injected into the rat hind paw, is a potent hyperalgesic agent (Follenfant & Nakamura-Craig, 1992) and thus may play an important role in the development of hyperalgesia.

The anti-convulsant effects of the anti-epileptic drug, lamotrigine, have been attributed to inhibition of the release of glutamate in the CNS (Leach *et al.*, 1986). Studies carried out in the rat hind paw model have also demonstrated that lamotrigine inhibits PGE_2-induced acute and sustained hyperalgesia and thus may represent a new class of analgesic agent (Nakamura-Craig & Follenfant, 1992).

There is evidence to suggest that N-methyl-D-aspartate (NMDA) receptors, which are stimulated by glutamate, may participate in the regulation of respiration (Foutz *et al.*, 1988a, b). The NMDA receptor is found in all areas throughout the brain, including the nucleus tractus solitarius (NTS) (Cotman *et al.*, 1987). Activation of NMDA receptors may contribute to excitatory afferent synaptic transmission in the NTS (Miller & Felder, 1988). The NTS is the site of the first central synapse for primary afferent fibres which originate from airway afferent nerve endings and plays an important role in the regulation of respiration (Jordan & Spyer, 1986). Moreover, the NTS is also adjacent to the "cough centre". There is evidence from several lines of research to suggest that some anti-tussive agents may be antagonists at NMDA receptors (Ferkany *et al.*, 1988; Wong *et al.*, 1988; Church, *et al.*, 1989). Indeed, both competitive and non-competitive NMDA receptor antagonists, when administered intracisternally, have been shown to significantly attenuate capsaicin aerosol-induced cough in rats (Kamei *et al.*, 1989). Thus it is possible that excitatory amino acids (such as glutamate) and NMDA receptors may be involved in the regulation of the coughing and other respiratory reflexes.

The above findings raise the possibility that glutamate may have a role in vagally-mediated airway reflexes and also in the development of airway hyperreactivity. Furthermore, the analgesic property of lamotrigine raises the possibility that glutamate-release inhibitors may represent a new class of anti-asthma/anti-tussive

therapy. The aims of the present studies, therefore, were to investigate the role that glutamate may have in the airways by examining the effects of glutamate on RAR discharge and on airway reflexes, evoked by capsaicin and histamine, in anaesthetized, paralysed and artificially ventilated cats. In addition, to determine if lamotrigine possessed any potential anti-asthmatic activity, the effect of this agent was examined on RAR discharge and on airway reflexes, evoked by capsaicin and histamine, in anaesthetized, paralysed and artificially ventilated cats.

7.2 Methods

Male cats (2.6-3.8 kg) were anaesthetized and prepared for surgery, as described in Chapter 2. Airflow (ml s^{-1}) and transpulmonary pressure (cmH_2O) were measured. Tidal volume, C_{Dyn} and R_L were computed from the above signals, on a breath-by-breath basis, with an on-line Buxco pulmonary mechanics analyser. Arterial blood pressure and heart rate were also recorded. An additional cannula (Portex 4fg) was inserted in the right femoral artery for periodic withdrawal of arterial blood. The animals were paralysed with DMT and artificially ventilated, according to the protocol set out in Chapter 2. Some experimental animals were set-up for recording of action potentials in Aδ-fibres (Chapter 2). Drugs were administered by two routes, i.v. and inhalation (DeVilbiss ultrasonic nebuliser, Chapter 2).

Measurement of Reflex "Bronchoconstriction"

A small rubber balloon (tip of a Durex Elite condom) was inserted into a section of the trachea above the endotracheal cannula and below the larynx (Fig. 7.1). The balloon was connected to a Statham P23 pressure transducer, via a short length of plastic tubing (Portex 4fg), for measurement of tracheal balloon pressure (P_{TB}, cmH_2O). This section of the trachea was not accessible to aerosols. Apart from where

the trachea was cut to insert the cannula, the blood and nerve supplies were essentially intact.

In reflex bronchoconstriction experiments, the Poh-ne-mah data acquisition and analysis system was used to obtain a continuous measurement of P_{TB} and derive pulmonary mechanics measurements. Airflow and transpulmonary signals were fed via a Beckman R611 chart recorder into the Poh-ne-mah system. From these signals, algorithms were used to derive C_{Dyn} and R_L measurements on a breath-by-breath basis. In addition, the signal from the Statham P23 transducer used to measure the pressure in the tracheal balloon was fed into the Poh-ne-mah, via the Beckman R611, to obtain a continuous measurement of P_{TB}. Data was stored initially onto the computer hard-disk and then later onto optical disk. Data were analysed after completion of the experiment.

Generation and Exposure to Ozone

The method of generating and exposing anaesthetized, paralysed and artificially ventilated cats to ozone is almost identical to that used by Adcock (1989). A schematic representation of the ozone generating system is shown in Fig. 7.2. Ozone was generated by passing 100% oxygen, at a rate of 70-100 mls min^{-1}, through a commercially available ozonizer (Sander, Type III). The effluent was subsequently passed into a 10 l chamber, where it was mixed with compressed air (6 l min^{-1}) to dilute the ozone to a concentration of 5 ± 0.5 ppm. The concentration of ozone was monitored before and during the experiments by sampling the gas in the mixing chamber with a Drager pump (Model 31) and ozone tube (Drager 10, 0,05/b). The gas in the mixing chamber flowed continuously through the chamber into an exhaust connected to an Aldasorber filter (Shirley Aldred & Co Ltd) to ensure equilibrium. At the relevant time, the inflow line to the ventilator was connected to the mixing

chamber so that the animal was artificially ventilated with ozone from the chamber (Fig. 7.2). Air from the lungs, via the ventilator, was passed through another Aldasorber to avoid contaminating the laboratory air. Ozone resistant tubing was used throughout. This method allowed continuous monitoring of pulmonary mechanics, whilst the animal was ventilated with ozone.

5-hydroxytryptamine (5-HT)-induced bronchoconstriction

The experimental set-up was essentially as that used for the measurement of reflex bronchoconstriction. 5-hydroxytryptamine (5-HT) was infused continuously, via the femoral vein, at a rate of either 10 or 20 $\mu g\ kg^{-1}\ min^{-1}$ (in a volume of 0.1 ml min^{-1}).

Compounds

Histamine, 5-HT, 443C81 and naloxone were dissolved in saline; capsaicin in an ethanol, Tween 80 and saline mixture in the ratio 1:1:23. A stock solution of capsaicin (1 mg ml^{-1}) was prepared and further dilutions made in saline. Lamotrigine isothionate was dissolved in distilled water to give a stock solution of 100 mg ml^{-1}, further dilutions were made in distilled water. Monosodium glutamate (L-glutamic acid) was dissolved in glutamate vehicle, pH 7.4, to give a stock solution of 100 mg ml^{-1} and diluted accordingly using glutamate vehicle. Glutamate vehicle consisted of a mixture of 40.5 mls of a 0.2 M solution of anhydrous di-sodium hydrogen orthophosphate (prepared in distilled water) and 9.5 mls of a 0.2M solution of sodium di-hydrogen orthophosphate (prepared in distilled water), which was then diluted to 100 mls with distilled water.

Protocols

i) Glutamate aerosols on RARs

After identification of a vagal fibre arising from an intrathoracic RAR, glutamate aerosols (20 breaths of vehicle & 0.1 - 100 mg ml^{-1} solutions) were administered sequentially at 6 min intervals. Histamine aerosol (6 breaths from a 1 mg ml^{-1} solution) administration was repeated at the end of the experiment.

ii) Glutamate aerosols on airway tone and tracheal balloon responses.

Reproducible control responses were obtained to capsaicin aerosol (6 breaths, 0.1 mg ml^{-1} solution) and histamine, administered intravenously (10 μg kg^{-1}) and as an inhaled aerosol (6 breaths, 1 mg ml^{-1} solution). This was then followed by sequential administration of glutamate aerosols (20 breaths vehicle, 1 & 100 mg ml^{-1} solutions) and in some experiments by bolus i.v. glutamate (10 mg kg^{-1}). Responses to histamine and capsaicin (at 15 min intervals) were also obtained after inhalation of glutamate aerosol (1 mg ml^{-1} solution), glutamate aerosol (100 mg ml^{-1} solution) and after i.v. glutamate administration.

iii) i.v. lamotrigine on spontaneous RAR discharge

Upon identification of a vagal fibre arising from an intrathoracic RAR, bolus i.v. injections of lamotrigine (1 - 30 mg kg^{-1}) were administered sequentially at 6 min intervals. This was then followed later, 6 & 8 mins respectively, by bolus i.v. injections of the peripherally acting μ-opioid receptor agonist, 443C81 (1 mg kg^{-1}) and the opiate receptor antagonist, naloxone (0.3 mg kg^{-1}).

iv) i.v. lamotrigine on i.v. histamine-induced RAR discharge

Following identification of a vagal fibre arising from an intrathoracic RAR, a dose of i.v. histamine (either 10 or 30 mg kg^{-1}) was administered until two reproducible

effects on RAR discharge were obtained. This was then followed 15 mins later by a bolus i.v. injection of lamotrigine (30 mg kg^{-1}). Bolus i.v. injections of histamine were then repeated, 3 and 12 mins post administration of lamotrigine. 443C81 (6 mg kg^{-1}, i.v.) and naloxone (0.3 mg kg^{-1}, i.v.) were also administered, each injection being followed by a bolus i.v. injection of histamine.

v) i.v. lamotrigine on i.v. histamine and capsaicin aerosol-induced increases in tracheal balloon pressure.

Reproducible control responses were obtained to capsaicin aerosol (6 breaths, 0.1 mg ml^{-1} solution) and histamine, administered intravenously (10 μg kg^{-1}) and as an inhaled aerosol (6 breaths, 1 mg ml^{-1} solution). Following this, the effects of bolus i.v. injections of lamotrigine (1 - 30 mg kg^{-1}) were examined on capsaicin aerosol and i.v. histamine-induced responses; lamotrigine was administered 3 mins before administration of either capsaicin or histamine.

vi) i.v. lamotrigine on ozone-induced hyperreactivity to inhaled histamine

After obtaining reproducible responses to inhaled histamine (6 breaths from a 1 mg ml^{-1} solution) each animal was exposed to ozone 5 ± 0.5 ppm, as described earlier, for a period of 90 mins, during which baseline pulmonary mechanics and cardiovascular parameters were monitored. Administration of histamine aerosol was repeated, whilst ozone exposure continued, until two reproducible responses were obtained. Following this, the effects of i.v. lamotrigine (1 - 30 mg kg^{-1}) were examined on histamine aerosol-induced responses, whilst ozone exposure continued; lamotrigine was administered 3 mins before administration of histamine.

vii) pre-treatment with i.v. lamotrigine on ozone-induced hyperreactivity to inhaled histamine

Control responses were obtained to inhaled histamine aerosol (6 breaths from a 1 mg ml^{-1} solution). Each animal was exposed to ozone (5 ± 0.5 ppm) for a period of 90 mins after pre-treatment, 3 mins before, with bolus i.v. injection of lamotrigine (30 mg kg^{-1}). This dose of lamotrigine was repeated 45 mins after commencing ozone administration. Histamine aerosol administration was repeated while ozone exposure continued. In some animals, the β_2 adrenoceptor agonist, salbutamol, was administered intravenously (1 μg kg^{-1}).

viii) effect of i.v. lamotrigine (1-30 mg kg^{-1}) on 5-HT induced bronchoconstriction.

Each animal was set-up for measurement of reflex bronchoconstriction and then continuously infused with 5-HT (10 or 20 μg kg^{-1} min^{-1}). When the increase in baseline tone stabilised, lamotrigine was administered intravenously (1 - 30 mg kg^{-1}) before and after bilateral vagotomy. In some animals, the β_2 adrenoceptor agonist, salbutamol, was administered intravenously (1 μg kg^{-1}).

Data Analysis

Single nerve fibre recording experiments involving:

histamine (i.v. and aerosol), 443C81 and naloxone: Nerve impulses were counted in 5 second period "bins". Control periods were counted for 1 min before administration of the chemical agent and expressed as the average impulses s^{-1} (imp s^{-1}). Impulse discharges during the period of the response were averaged (imp s^{-1}) and expressed as the Δ (imp s^{-1}) from the control period. In all cases the mean ± standard error of the mean (sem) is shown. The effect of chemical agents on arterial blood pressure and heart rate are expressed as the peak changes in absolute values, whereas

transpulmonary pressure responses are expressed as the maximum percentage change from pre-treatment values.

The effect of chemical agents on airway sensory nerve discharge, transpulmonary pressure, arterial blood pressure and heart rate were compared with baseline using Student's "t" test for paired data. In addition, comparison of histamine-evoked responses, before and after treatment, were analysed using Student's "t" test for paired data. Statistical values of $P < 0.05$ were considered to be significant.

i.v. lamotrigine and glutamate aerosols: Nerve impulses were counted in 5 second period "bins" and at each time point the average of a 25 s period was taken and expressed as impulses s^{-1} (imp s^{-1}). Transpulmonary pressure measurements are expressed as absolute values. All of the variables were measured at intervals throughout the experiments before, during and after drug administration. Each variable was pooled to provide mean ± sem. Statistical analysis was performed using two-way analysis of variance (ANOVA). Statistical values of $P < 0.05$ were considered to be significant.

Reflex bronchoconstriction and ozone experiments

Responses to histamine and capsaicin: C_{Dyn} and R_L are expressed as the peak percentage change from baseline values; P_{TB}, arterial blood pressure and heart rate are shown as changes in absolute values. Statistical significance of histamine and capsaicin responses were determined by comparing the responses with their respective control baselines using Student's "t" test for paired data, $P < 0.05$, was considered to be significant. In addition, the effects of treatment on histamine and capsaicin responses were determined by comparing the responses after treatment to those

before treatment using Student's "t" test for paired data; statistical values of $P < 0.05$, were considered to be significant.

glutamate aerosols and i.v. lamotrigine: effects on P_{TB} are shown as absolute changes, with significant changes from control baselines being analysed using Students "t" test for paired data. Statistical values of $P < 0.05$ were considered to be significant. C_{Dyn} and R_L are expressed as absolute values. Both variables were measured at intervals throughout the experiments before, during and after drug administration. Each variable was pooled to provide mean ± sem. Statistical analysis was performed using two-way analysis of variance (ANOVA). Statistical values of $P < 0.05$ were considered to be significant.

Effects of lamotrigine on cardiovascular parameters: arterial blood pressure and heart rate are shown as changes in absolute values. All of the variables were measured at intervals throughout the experiments before, during and after drug administration. Data for each variable were pooled from a number of experiments to provide mean ± sem. Statistical analysis was performed using analysis Student's "t" test for paired data. Statistical values of $P < 0.05$ were considered to be significant.

5-HT-induced bronchoconstriction: the effects of 5-HT, lamotrigine and salbutamol on P_{TB} are expressed as either absolute changes from control baselines or peak percentage change from baseline; whereas effects on C_{Dyn} and transpulmonary pressure are expressed as the peak percentage change from baseline. Each variable was pooled to provide mean ± sem. Statistically significant effects were determined by comparing the responses with their respective control baselines using Student's "t" test for paired data; statistical values of $P < 0.05$ were considered to be significant.

7.3 Results

7.3.1 Baseline Values

31 male cats were used. The mean ± sem of the resting values of pulmonary mechanics and cardiovascular parameters for 28 open chest and 3 closed chest animals, values pooled together, are shown in Table 7.1.

Table 7.1 Baseline values for C_{Dyn}, R_L, P_{Tp}, systolic and diastolic blood pressure and heart rate in anaesthetized, paralysed and artificially ventilated cats. Values are shown as mean ± sem.

parameters	baseline values
C_{Dyn}, ml cmH_2O^{-1}	6.0 ± 0.7 (n=18)
R_L, $cmH_2O\ l^{-1}\ s$	20.0 ± 2.6 (n=16)
P_{Tp}, cmH_2O	5.8 ± 0.4 (n=15)
systolic blood pressure, mmHg	134 ± 4.7 (n=30)
diastolic blood pressure, mmHg	94± 3.7 (n=30)
Heart rate, beats min^{-1}	161 ± 4.6 (n=30)

7.3.2 Effect of inhaled aerosols of glutamate (20 breaths of vehicle, 0.1 - 100 mg ml^{-1} solutions) on the spontaneous activity of RARs

Four intrathoracic RARs were examined in four cats. The spontaneous discharge of the RARs was 0.53 ± 0.25 imp s^{-1} (range 0.08 - 1.07 imp s^{-1}). The adaptation index of each receptor was 100%. Histamine aerosol (6 breaths, 1 mg ml^{-1} solution) evoked a significant increase in the rate of discharge of RARs (1.82 ± 0.5 imp s^{-1}, $P < 0.05$) and caused significant bronchoconstriction as shown by an increase in transpulmonary pressure (+27.6 ± 8.6%, $P < 0.05$). At the end of the protocol, histamine aerosol did not induce any significant change in RAR discharge (0.12 ± 0.24 imp s^{-1}, *NS*). In

addition, histamine aerosol had no effect on transpulmonary pressure (+14.1 ± 6.9%, *NS*).

In the same four animals, inhalation of glutamate vehicle (20 breaths) had no significant effect on the spontaneous discharge of RARs, transpulmonary pressure or cardiovascular parameters (Figs 7.3 & 7.4). Likewise, inhalation of increasing concentrations of glutamate aerosols (20 breaths, 0.1 - 100 mg ml^{-1} solutions) also had no significant effect on the spontaneous discharge of RARs, transpulmonary pressure or cardiovascular parameters (Figs 7.3 & 7.4).

7.3.3 Effect of glutamate on airway tone

All of the animals (n=3) in this study had closed chests and therefore transpulmonary pressure was measured using the oesophageal balloon method (See Chapter 2). Capsaicin aerosol (6 breaths, 1 mg ml^{-1} solution) and i.v. histamine (10 μg kg^{-1}) evoked bronchoconstriction as measured by increases in tracheal balloon pressure (P_{TB}) and R_L and decreases in C_{Dyn}, before and after inhalation of glutamate (aerosol, 20 breaths, vehicle, 1 & 100 mg ml^{-1} solutions & i.v., 10 mg kg^{-1}) (Figs 7.5 & 7.6). Histamine aerosol (6 breaths, 1 mg ml^{-1} solution) throughout the experiment, decreased C_{Dyn}, increased R_L but had no effect on the tracheal balloon pressure, before and after administration of glutamate (Figs 7.5 & 7.6).

Glutamate, administered as an inhaled aerosol (20 breaths, vehicle, 1 & 100 mg ml^{-1} solutions) had no effect on the baseline tracheal balloon pressure or on pulmonary mechanics (Figs 7.5 & 7.7). Likewise, i.v. glutamate (10 mg kg^{-1}, n=2) had no effect on tracheal balloon pressure, pulmonary mechanics or cardiovascular parameters. Administration of glutamate did not appear to potentiate any of the responses evoked by the bronchoconstrictor agents, capsaicin and histamine (Figs 7.5 & 7.6). Indeed the

i.v. histamine and capsaicin aerosol-induced P_{TB} responses appeared to decrease, but this was not significant (Fig. 7.5).

7.3.4 Effect of i.v. lamotrigine (1 - 30 mg kg^{-1}) on the spontaneous activity of RARs

Four intrathoracic RARs were examined in four cats. The spontaneous discharge rate in the fibres was 0.66 ± 0.36 imp s^{-1} (range 0.13 - 1.7 imp s^{-1}). The adaptation index of each receptor was 100%. Histamine aerosol (6 breaths, 1 mg ml^{-1} solution) evoked a significant increase in the rate of discharge of RARs (2.15 ± 0.9 imp s^{-1}, $P < 0.05$).

Administration of bolus i.v. injections of lamotrigine (1 - 30 mg kg^{-1}) had no significant effect on transpulmonary pressure or on the spontaneous activity of RARs (Figs 7.8 & 7.9). In contrast, i.v. 443C81 (1 mg kg^{-1}) significantly ($P < 0.05$) attenuated the spontaneous discharge of the RARs from 1.16 ± 0.32 imp s^{-1} to 0.29 ± 0.06 imp s^{-1}. This inhibitory effect of 443C81 was reversed by i.v. naloxone, with the spontaneous activity of the RARs increasing by 1.79 ± 0.92 imp s^{-1}.

7.3.5 Effect of i.v. lamotrigine (30 mg kg^{-1}) on i.v. histamine-induced discharges on RARs

Four intrathoracic RARs were examined in four cats. The spontaneous discharge rate in the fibres was 0.66 ± 0.36 imp s^{-1} (range 0.13 - 1.7 imp s^{-1}). The adaptation index of each receptor was 100%. Histamine aerosol (6 breaths, 1 mg ml^{-1} solution) evoked a significant increase in rate of discharge of RARs (2.15 ± 0.9 imp s^{-1}, $P < 0.05$).

Bolus i.v. injections of histamine (10 or 30 μg kg^{-1}) significantly ($P < 0.05$) increased the rate of RAR discharge (3.31 ± 0.87 imp s^{-1}). Lamotrigine (i.v., 30 mg kg^{-1}) had no significant effect on i.v. histamine-induced increases in RAR activity 3 mins (3.22 ±

0.58 imp s^{-1}, n=4, $P < 0.01$) and 12 mins (3.36 ± 0.78 imp s^{-1}, n=3, $P < 0.05$) post administration of lamotrigine (Fig. 7.10). After treatment with i.v. 443C81 (6 mg kg^{-1}) the response was significantly ($P < 0.05$) attenuated when compared with pre-treatment control responses. This inhibitory effect was reversed by the administration of i.v. naloxone (0.3 mg kg^{-1}) (Fig. 7.10). An example of the effect of i.v. lamotrigine, 443C81 and naloxone on i.v. histamine-induced stimulation of RARs is shown in Fig. 7.11

7.3.6 Effect of i.v. lamotrigine (1 - 30 mg kg^{-1}) on capsaicin aerosol and i.v. histamine-induced increases in tracheal balloon pressure

The effects of i.v. lamotrigine (1 - 30 mg kg^{-1}) on capsaicin aerosol and i.v. histamine-induced increases in P_{TB} was examined in 7 cats. All of the animals had open chests. In some experiments i.v. histamine and capsaicin aerosol were tested alone, in others both agents were tested together. The determining factor was the amplitude of the P_{TB} response (minimum of 2 cmH_2O) to each agent at the start of the experiment. For interpretation purposes, the effects of lamotrigine on i.v. histamine and capsaicin aerosol-induced responses are presented separately.

Inhalation of histamine aerosol (6 breaths from a 1 mg ml^{-1} solution, n=5) evoked bronchoconstriction as shown by an increase in R_L (+43 ± 17%, $P < 0.01$) and decrease in C_{Dyn} (-30 ± 8%, $P < 0.01$) but had no effect on P_{TB} (0.07 ± 0.01 cmH_2O). Administration of intravenous histamine (10 or 30 μg kg^{-1}, n=5) evoked bronchoconstriction and reproducible increases in P_{TB} (Figs 7.12 & 7.13A, C). Bolus i.v. lamotrigine at 1 & 10 mg kg^{-1}, had no effect on i.v. histamine-induced increases in P_{TB} (Fig. 7.12). In contrast, subsequent administration of i.v. lamotrigine, 30 mg kg^{-1}, significantly ($P < 0.05$) reduced i.v. histamine-evoked increases in P_{TB}, 3 and 33 mins post lamotrigine administration (Fig. 7.12). Intravenous lamotrigine (at all

concentrations) had no significant effect on i.v. histamine-induced increases in R_L and decreases in C_{Dyn} (Fig. 7.13A, C).

Capsaicin aerosol (6 breaths, 0.1 mg ml^{-1} solution, n=5)) evoked reproducible increases in P_{TB} (Fig. 7.14). Lamotrigine (i.v., 1 mg kg^{-1}) had no significant effect on capsaicin aerosol-evoked increases in P_{TB} (Fig. 7.14). Likewise i.v. lamotrigine (10 mg kg^{-1}) also had no effect on the increase in P_{TB} to inhaled capsaicin, 3 mins post-administration of lamotrigine. However, the increase in P_{TB} to inhaled capsaicin was significantly ($P < 0.05$) reduced 33 min after treatment with lamotrigine (Fig. 7.14). Capsaicin aerosol-induced increases were further attenuated after treatment with a higher dose of lamotrigine (30 mg kg^{-1}, i.v.) (Fig. 7.14). Intravenous lamotrigine (at all doses) had no significant effect on capsaicin aerosol-induced increases in R_L and decreases in C_{Dyn} (Fig. 7.13B, D).

Bolus i.v. lamotrigine (1 - 30 mg kg^{-1}) administration had no significant effect on baseline C_{Dyn} or R_L (Fig. 7.15). An example of the effects of capsaicin aerosol and histamine (i.v. and aerosol administration) on P_{TB} and pulmonary mechanics before and after i.v. lamotrigine administration are shown in Fig. 7.16.

7.3.7 Effect of i.v. lamotrigine (1 & 30 mg kg^{-1}) on ozone-induced hyperreactivity to inhaled histamine

Prior to ozone exposure, inhalation of histamine aerosol (6 breaths from a 1 mg ml^{-1} solution) evoked bronchoconstriction as shown by an increase in R_L and decrease in C_{Dyn} but had no effect on P_{TB} (Figs 7.17 & 7.18). After exposing the airways to ozone, 5 ± 0.5 ppm for 90 mins, and whilst exposure to ozone continued, histamine aerosol (6 breaths from a 1 mg ml^{-1}) significantly ($P < 0.05$) increased P_{TB} from 0.0 to 2.94 ± 1.03 cmH_2O, this response was reproducible (Figs. 7.17).

Administration of i.v. lamotrigine (1 mg kg $^{-1}$) had no effect on histamine aerosol-evoked increase in P_{TB} (Fig. 7.17). In contrast, i.v. lamotrigine (30 mg kg^{-1}) significantly ($P < 0.05$) reduced the effect of histamine aerosol on P_{TB} (ΔP_{TB}, 0.74 ± 0.36 cmH_2O) (Fig. 7.17). In addition, administration of i.v. lamotrigine (30 mg kg^{-1}) attenuated the increase in airway tone caused by exposure of the airways to ozone, as shown by a subsequent increase in C_{Dyn} and decrease in R_L (Fig. 7.19). However, this effect with lamotrigine (30 mg kg^{-1}) was approximately 30,000 fold less potent than that of the β_2-adrenoceptor agonist salbutamol (bolus i.v. injection, 1 μg kg^{-1}) in attenuating increases in airway tone induced by ozone exposure (Fig. 7.20).

An example of the effect of histamine aerosol on pulmonary mechanics and P_{TB} before and after ozone exposure and after i.v. lamotrigine treatment is shown in Fig. 7.21. In some experiments, i.v. administration of lamotrigine and salbutamol themselves evoked an increase in P_{TB}, examples of this are shown in Figs 7.21 & 7.22.

7.3.8 Effect of pre-treatment with i.v. lamotrigine (30 mg kg^{-1}) on ozone-induced hyperreactivity to inhaled histamine

Prior to ozone exposure, inhalation of histamine aerosol (6 breaths from a 1 mg ml^{-1} solution) evoked bronchoconstriction as shown by an increase in R_L (+46%, n=2) and decrease in C_{Dyn} (-38%, n=2) but had no effect on P_{TB} (Fig. 7.21). After pre-treating with bolus i.v. lamotrigine (30 mg kg^{-1}, 3 mins before and 45 mins after commencing ozone exposure) and exposing the airways to ozone, 5 ± 0.5 ppm for 90 mins, histamine aerosol (6 breaths from a 1 mg ml^{-1}) still significantly ($P < 0.05$) increased P_{TB}; an effect which was reproducible (Fig. 7.23).

7.3.9 Effect of i.v. lamotrigine (1 - 30 mg kg^{-1}) on 5-HT-induced bronchoconstriction

Continuous infusion of 5-HT (10 or 20 μg kg^{-1}, 0.1 ml^{-1} min^{-1}) caused a significant ($P < 0.05$) increase in P_{TB} (16.9 ± 3.4 cmH_2O, n=3), transpulmonary pressure (+ 54.5 ± 7.8%, n=3) and a decrease in C_{Dyn} (-43.6%, n=2).

After the increase in airway tone evoked by 5-HT stabilised, sequential administration of lamotrigine (1 & 10 mg kg^{-1}, i.v.) at 30 min intervals and before bilateral vagotomy, had no effect on P_{TB}, transpulmonary pressure or C_{Dyn} (Fig. 7.24). In contrast, i.v. lamotrigine (30 mg kg^{-1}) decreased P_{TB} and transpulmonary pressure and increased dynamic lung compliance (Fig. 7.24). After bilateral vagotomy, i.v. lamotrigine at 1 mg kg^{-1} had no effect on any of the three parameters (Fig. 7.24). However, i.v. lamotrigine at 10 & 30 mg kg^{-1} induced a dose-dependent fall in P_{TB}, and although not significant, a dose-dependent fall in transpulmonary pressure and an increase in C_{Dyn} (Fig. 7.24). Salbutamol (i.v., 1 μg kg^{-1}) also caused a fall in P_{TB} and transpulmonary pressure and an increase in C_{Dyn} (Fig. 7.24). However, it appears that lamotrigine at 30 mg kg^{-1} was approximately 30, 000 times less potent than i.v. salbutamol (1 μg kg^{-1}) in inducing relaxation of airway tone (Fig. 7.24).

7.3.10 Effects of bolus i.v. injections of lamotrigine (1 -30 mg kg^{-1}) on arterial blood pressure and heart rate

The effects of i.v. lamotrigine on arterial blood pressure and heart rate were pooled from a number of studies (7.3.4, 7.3.5, 7.3.6, 7.3.7).

Administration of i.v. lamotrigine (1 mg kg^{-1}) had no significant effect on either arterial blood pressure or heart rate (Fig. 7.25). At 10 mg kg^{-1}, lamotrigine evoked a small but transient fall in diastolic blood pressure 30 s after administration, but had no

effect on heart rate (Fig. 7.26). In contrast to the two lower doses, lamotrigine at 30 mg kg^{-1}, significantly reduced systolic and diastolic blood pressure up to three minutes after administration (Fig. 7.27). In addition there was a transient but significant ($P < 0.05$) fall in heart rate 30 s after administration. Time points after 3 mins were not recorded, since in most cases a bronchoconstrictor agent was usually administered, which in itself induced changes in arterial blood pressure and heart rate.

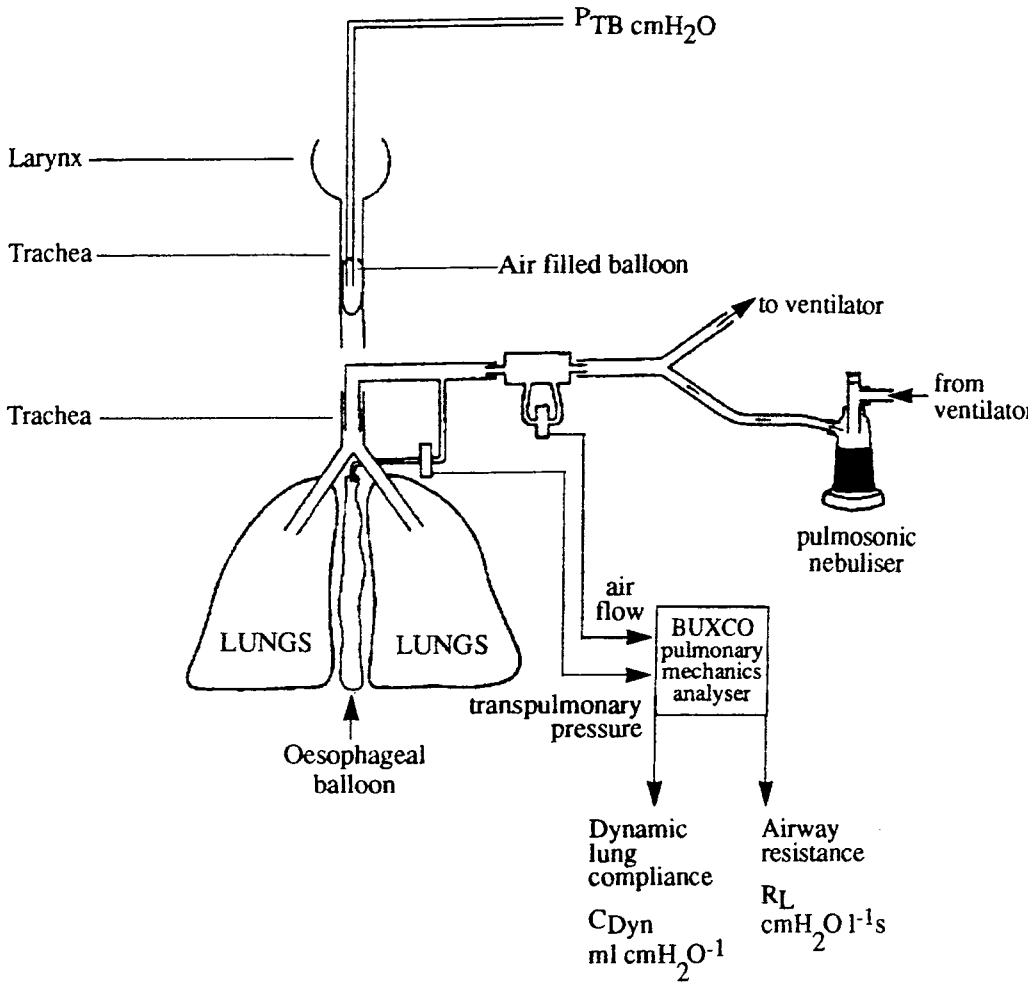


Fig. 7.1 Schematic representation of the experimental set-up for recording pulmonary mechanics and tracheal pressure (reflex "bronchoconstriction") in anaesthetized, paralysed, artificially ventilated cats. The cervical trachea is cannulated with a short length of endotracheal tubing and attached to a Fleisch pneumotachograph connected to a differential pressure transducer (as in Fig. 2.1). A side arm of the endotracheal cannula is connected to a differential pressure transducer; the other port of the transducer is connected to an oesophageal balloon (in the region of the thorax, inserted via the throat) in closed chest animals. The signals from the differential pressure transducers are input to a Buxco pulmonary mechanics analyser for determination, on a breath-by-breath basis, of dynamic lung compliance and lung resistance. A small air filled rubber balloon (connected to a pressure transducer) is inserted via the larynx into the upper trachea to measure pressure changes (P_{TB}) as an index of reflex "bronchoconstriction". A pulmosonic nebuliser is placed in the inflow line from the ventilator to the lower airways, for administration of agents by inhalation.

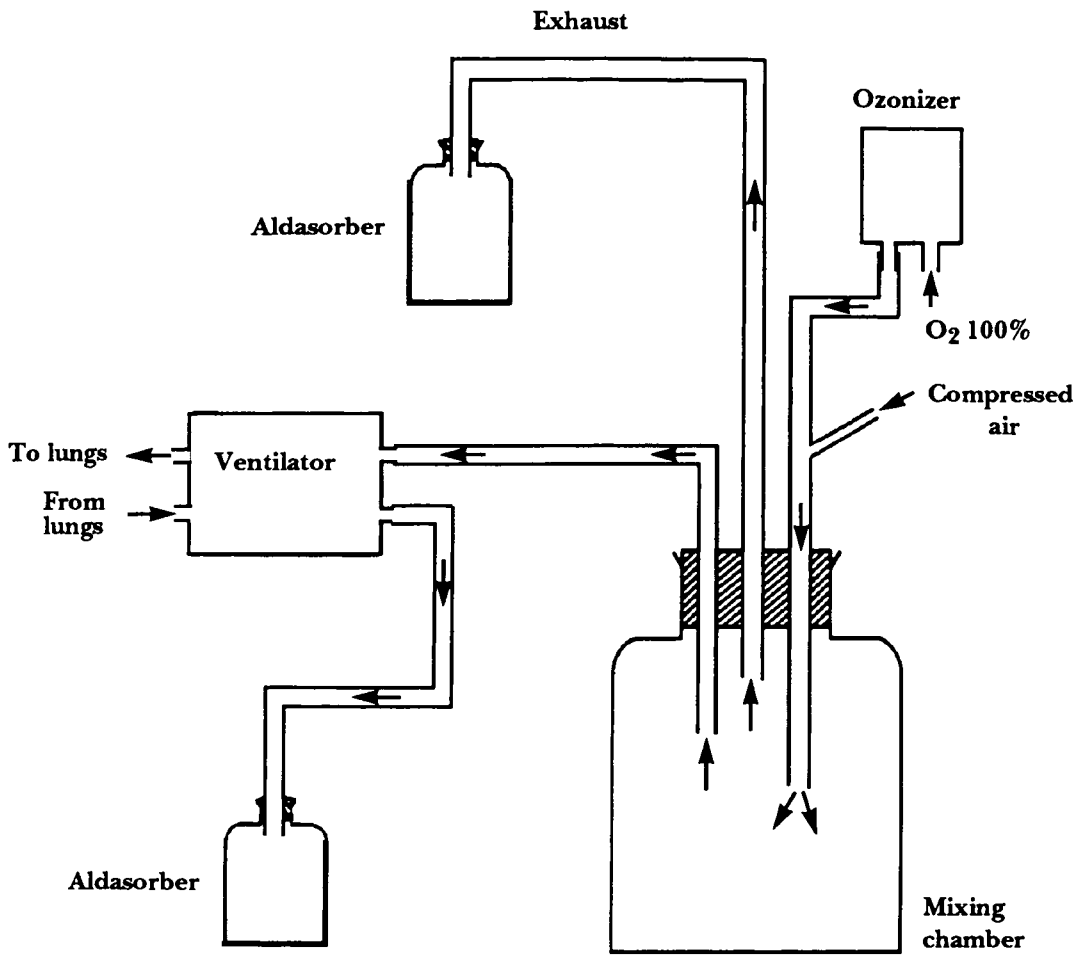


Fig. 7.2 Diagram showing the arrangement for generating ozone. Oxygen (100%) is passed through the ozonizer at 70 - 100 ml min^{-1}. The effluent from the ozonizer, together with compressed air (6 l min^{-1}), is then passed into a glass mixing chamber. An exhaust to an Aldasorber from this chamber allowed the gases to flow continuously, to ensure equilibrium. The air inflow line to the ventilator is connected to the mixing chamber so that the animal is artificially ventilated with ozone. Air from the lungs, via the ventilator, is passed through an Aldasorber to avoid contaminating the laboratory atmosphere. Ozone resistant tubing is used throughout.

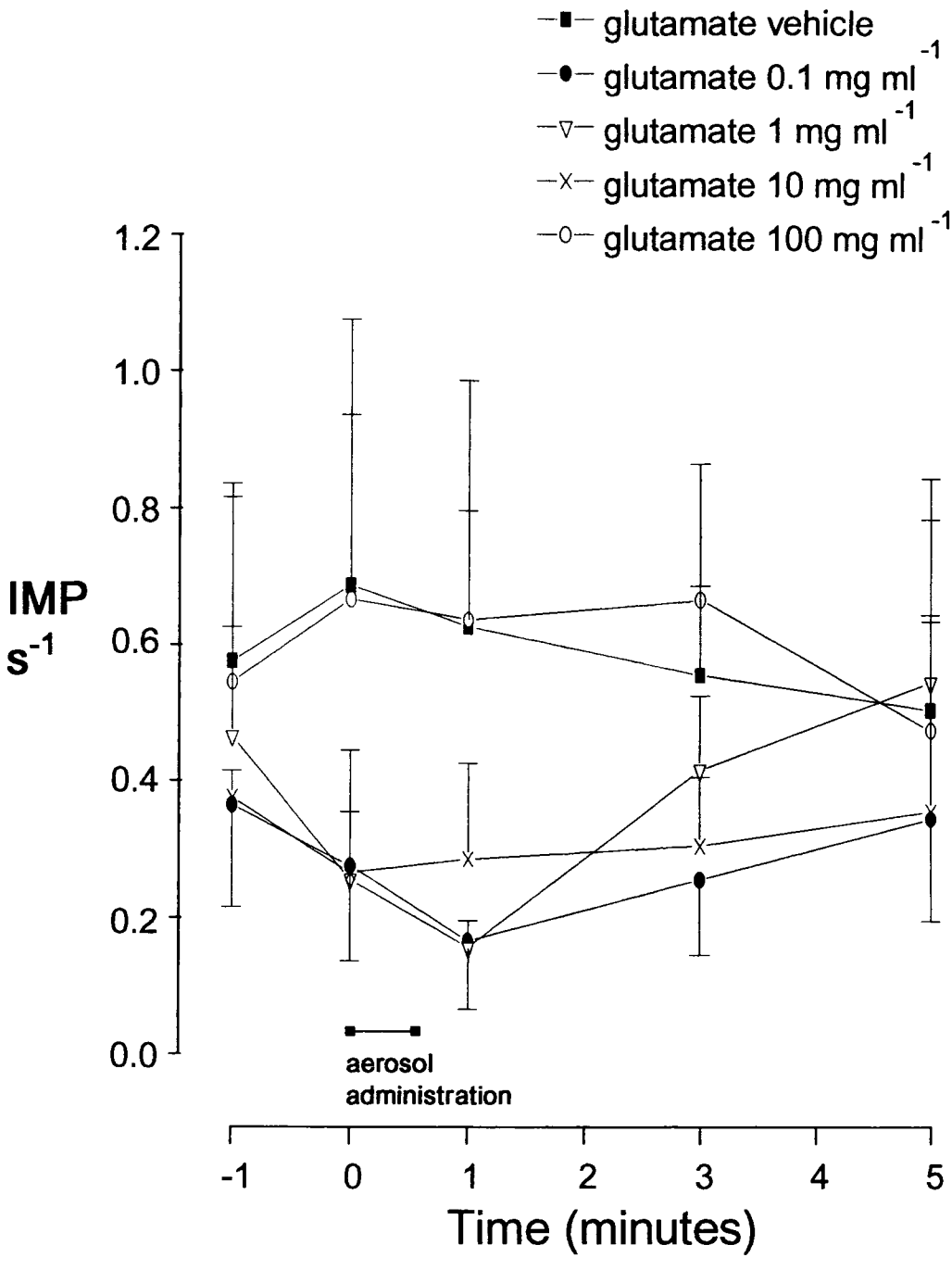


Fig. 7.3 Effect of administration of glutamate aerosols (20 breaths, vehicle, 0.1 - 100 mg ml^{-1} solutions; at 6 min intervals) on the spontaneous impulse activity of vagal fibres arising from intrathoracic RARs (n=4) in anaesthetized, paralysed and artificially ventilated cats. Absolute values for the spontaneous impulse activity (IMP s^{-1}), expressed against time (minutes) are shown before, during and after administration of each agent. Vertical bars represent sem. Aerosols were administered as shown.

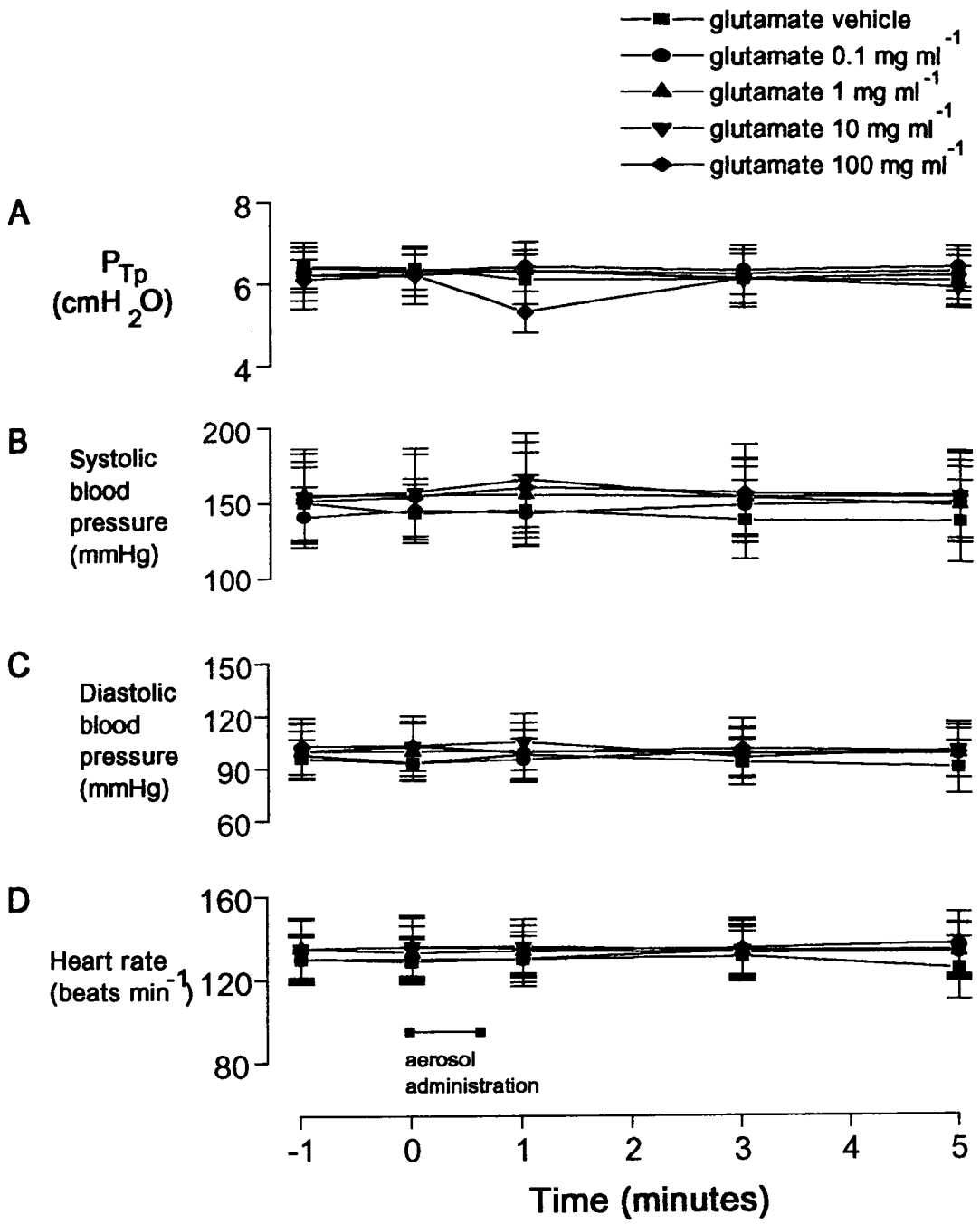


Fig. 7.4 Effect of administration of glutamate aerosols (20 breaths, vehicle, 0.1-100 mg ml^{-1} solutions; at 6 min intervals) on transpulmonary pressure (**A**, P_{Tp}), arterial systolic (**B**) and diastolic (**C**) blood pressure and heart rate (**D**) in anaesthetized, paralysed and artificially ventilated cats (n=4). Absolute values for each variable, expressed against time (minutes) are shown before, during and after administration of each agent. Vertical bars represent sem. Aerosols were administered as shown.

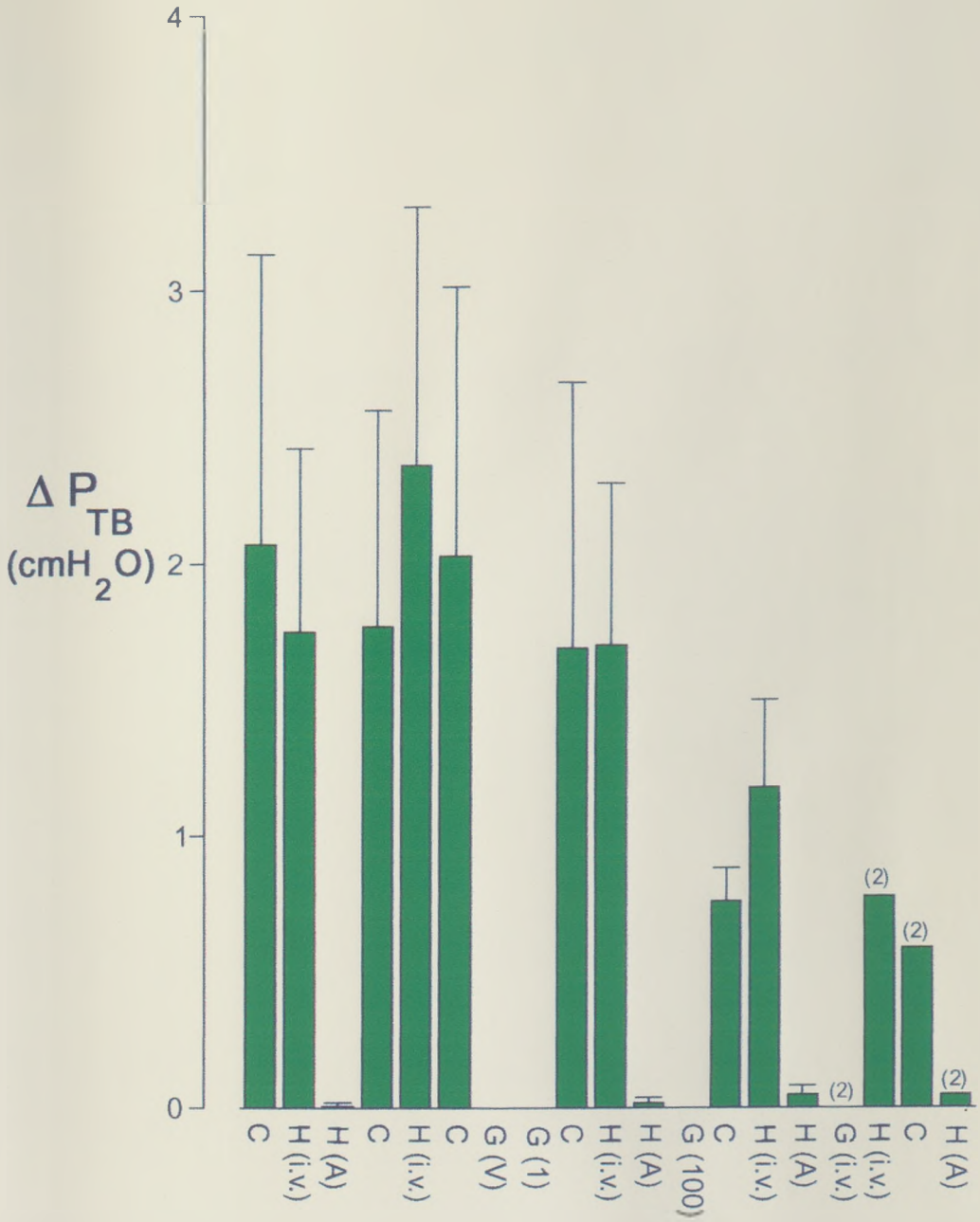


Fig. 7.5 Effect of glutamate aerosols (20 breaths, vehicle G (V), 1 mg ml^{-1} G (1) and 100 mg ml^{-1} G (100) solutions), glutamate i.v. (G (i.v.), 10 mg kg^{-1}), capsaicin aerosol (C, 6 breaths from a 0.1 mg ml^{-1} solution), histamine aerosol (H (A), 6 breaths from a 1 mg ml^{-1} solution) and intravenous histamine (H (i.v.), 10 μg kg^{-1}) on tracheal balloon pressure (P_{TB}) in anaesthetized, paralysed and artificially ventilated cats (n=3). The agents were administered sequentially, as shown on the horizontal axis, at 15 min intervals. Results are expressed as the mean Δ increase in P_{TB}. Vertical bars represent sem.

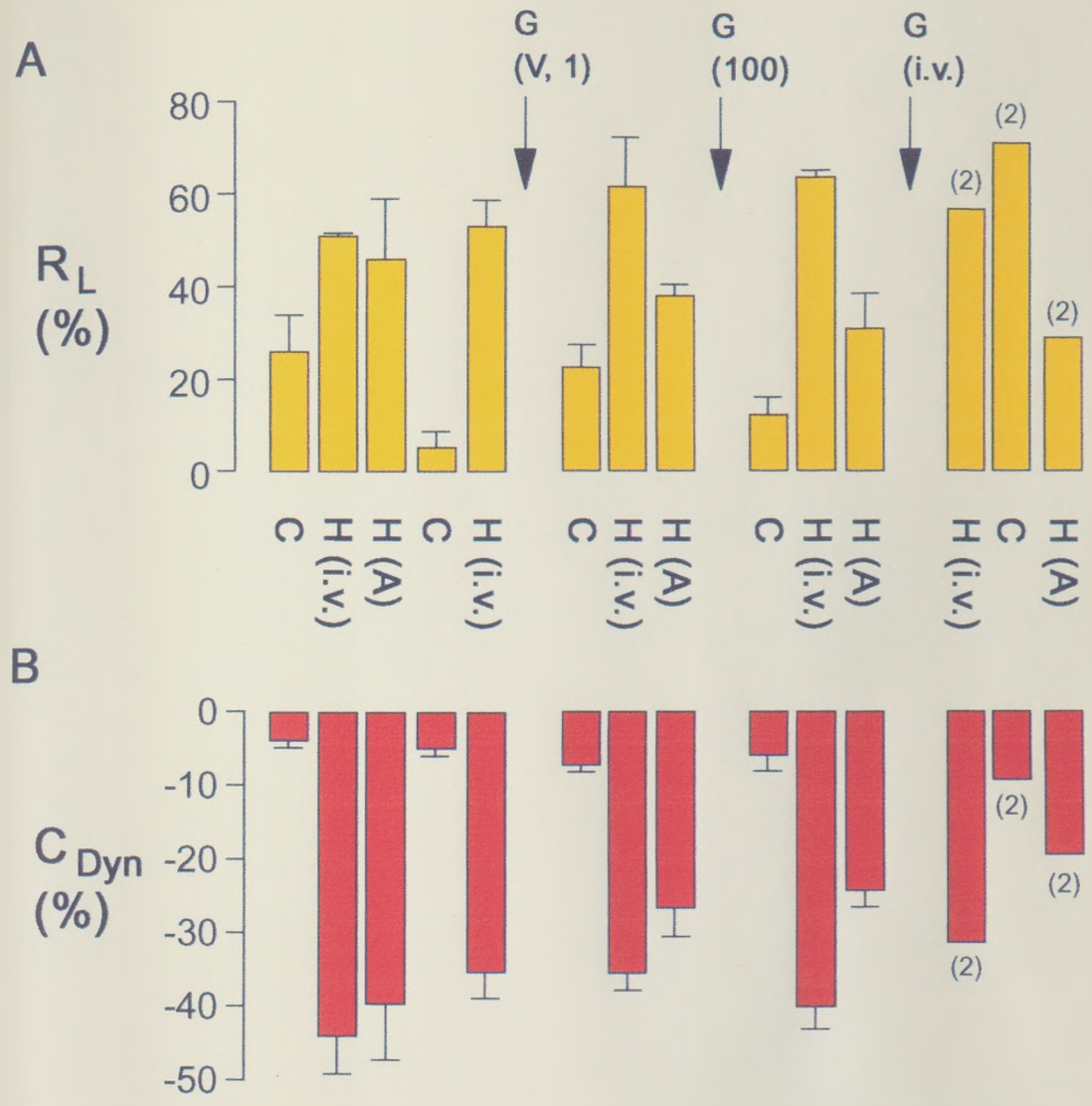


Fig. 7.6 Effect of administration of glutamate (20 breaths, vehicle and 1 mg ml^{-1} solutions G (V, 1); 20 breaths, 100 mg ml^{-1} G (100) solution; intravenously (G (i.v.), 10 mg kg^{-1}) on bronchoconstrictor responses evoked by capsaicin aerosol (C, 6 breaths from a 0.1 mg ml^{-1} solution), histamine aerosol (H (A), 6 breaths from a 1 mg ml^{-1} solution) and intravenous histamine (H (i.v.), 10 μg kg^{-1}) in anaesthetized, paralysed and artificially ventilated cats (n=3). Bronchoconstrictor responses evoked by capsaicin and histamine are expressed as the % increase in lung resistance (**A**, R_L) and the % decrease in dynamic lung compliance (**B**, C_{Dyn}). The agents were administered sequentially, as shown on the horizontal axis, at 15 min intervals. Vertical bars represent sem.

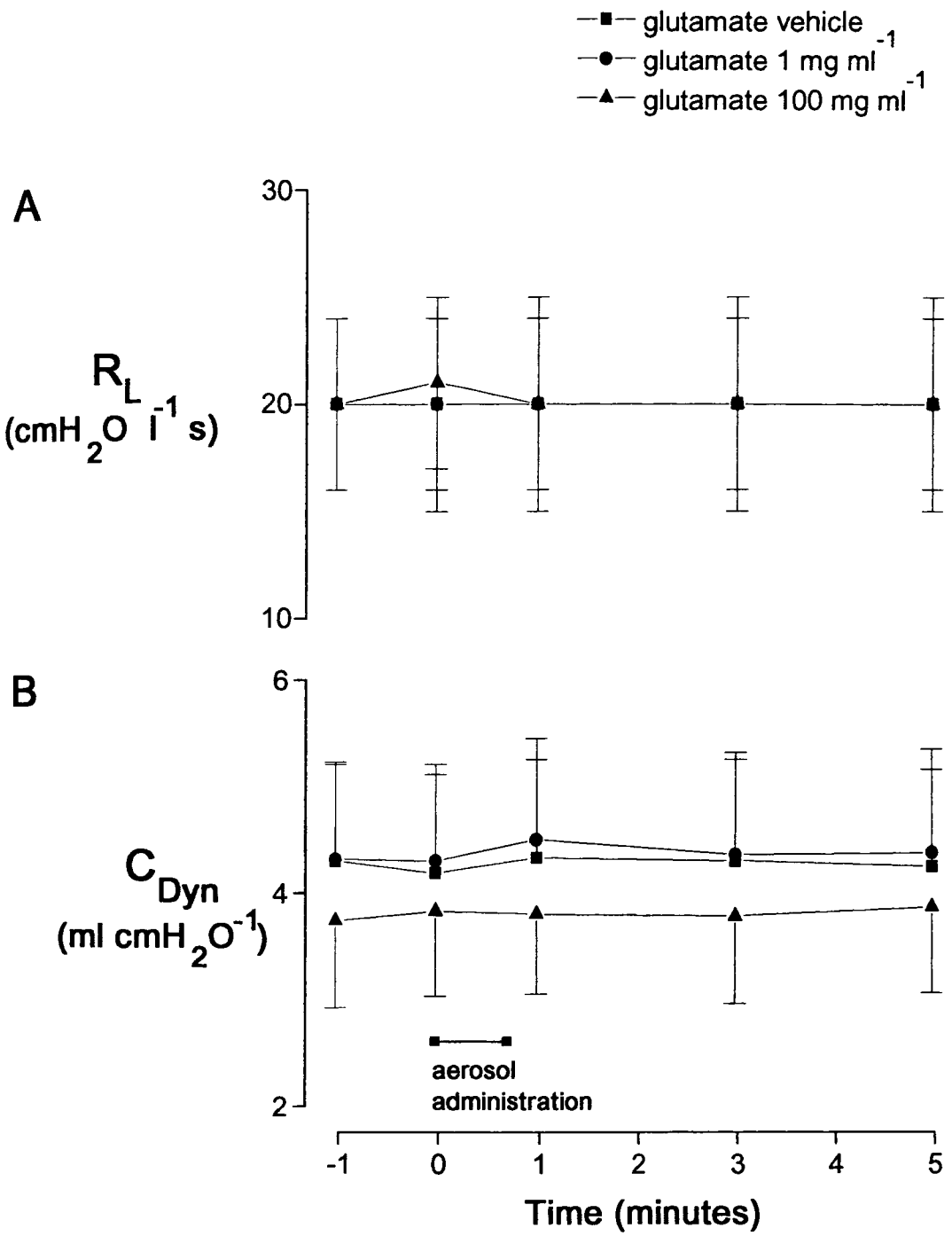


Fig. 7.7 Effect of glutamate aerosols (20 breaths, vehicle, 1 & 100 mg ml^{-1} solutions) on lung resistance (**A**, R_L) and dynamic lung compliance (**B**, C_{Dyn}) in anaesthetized, paralysed and artificially ventilated cats (n=3). Absolute values for each variable, expressed against time (minutes) are shown before, during and after administration of each agent. Vertical bars represent sem. Aerosols were administered as shown.

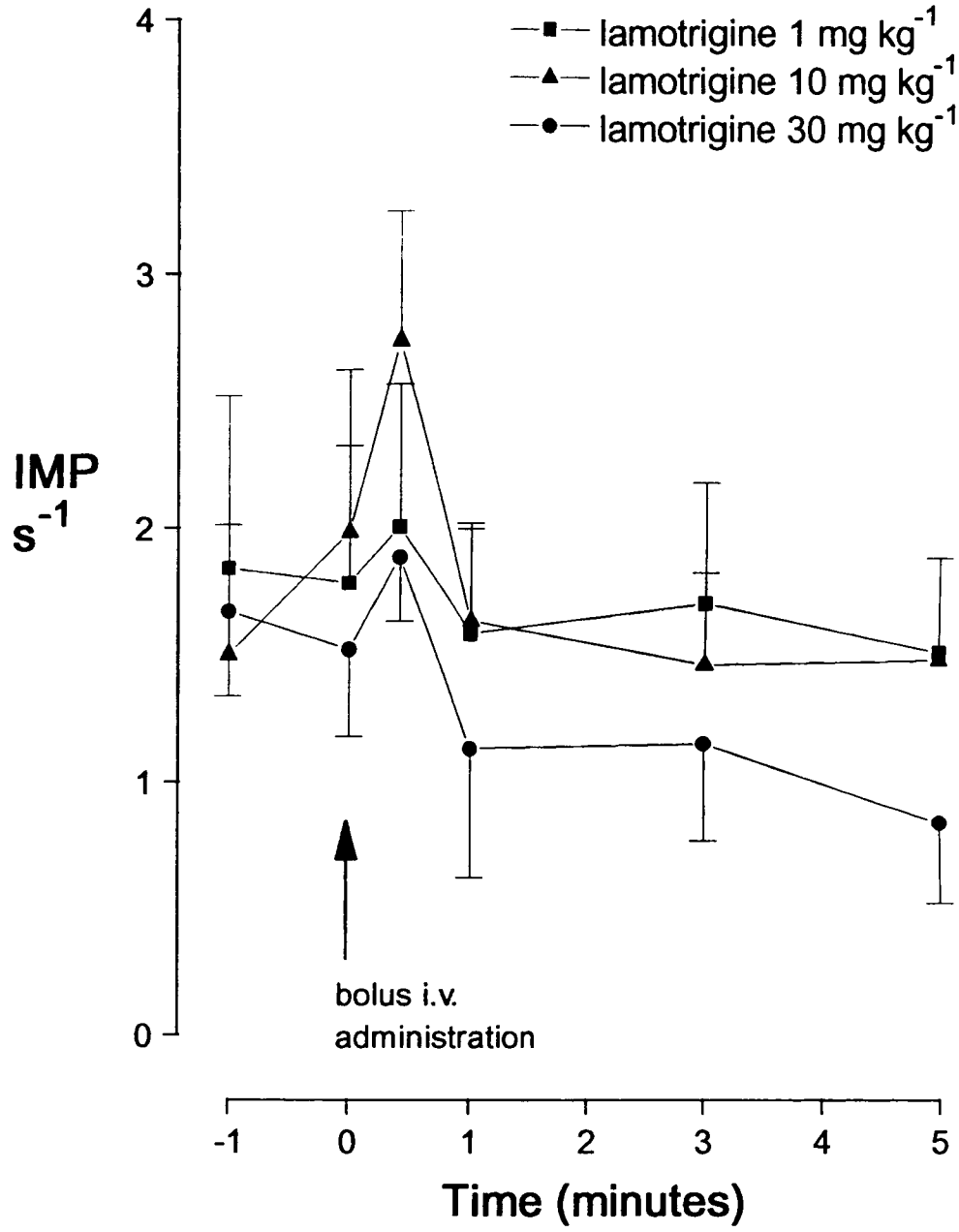


Fig. 7.8 Effect of administration of bolus i.v. injections of lamotrigine (1 - 30 mg kg^{-1} solutions; at 6 min intervals) on the spontaneous impulse activity of vagal fibres arising from intrathoracic RARs (n=4) in anaesthetized, paralysed and artificially ventilated cats. Absolute values for the spontaneous impulse activity (IMP s^{-1}), expressed against time (minutes) are shown before, during and after administration of each dose. Vertical bars represent sem. All agents were administered as shown.

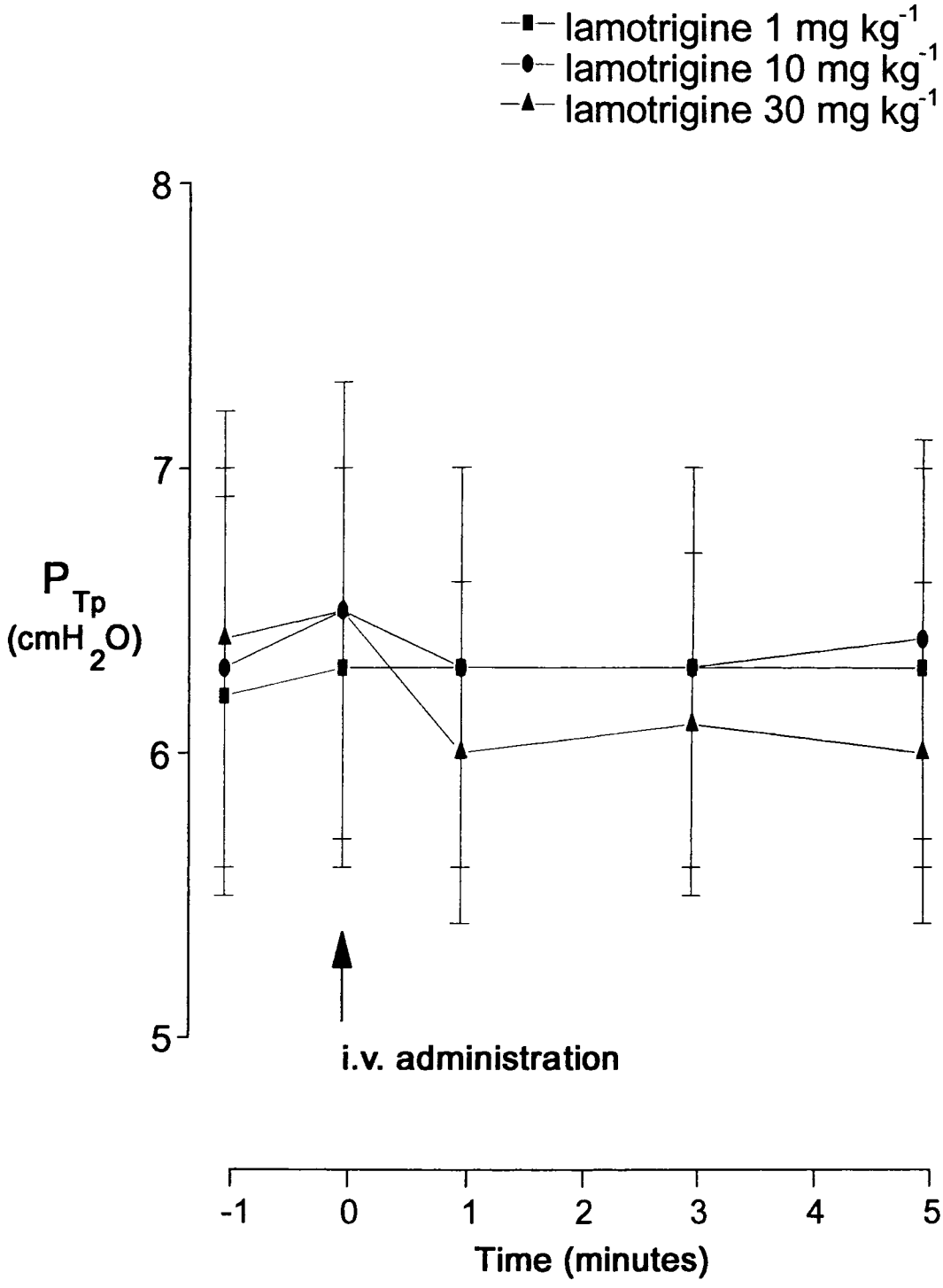


Fig. 7.9 Effect of administration of bolus i.v. injections of lamotrigine (1 - 30 mg kg^{-1} solutions; at 6 min intervals) on transpulmonary pressure in anaesthetized, paralysed and artificially ventilated cats (n=4). Absolute values for transpulmonary pressure (P_{Tp}, cmH_2O), expressed against time (minutes) are shown before, during and after administration of each dose. Vertical bars represent sem. All agents were administered as shown.

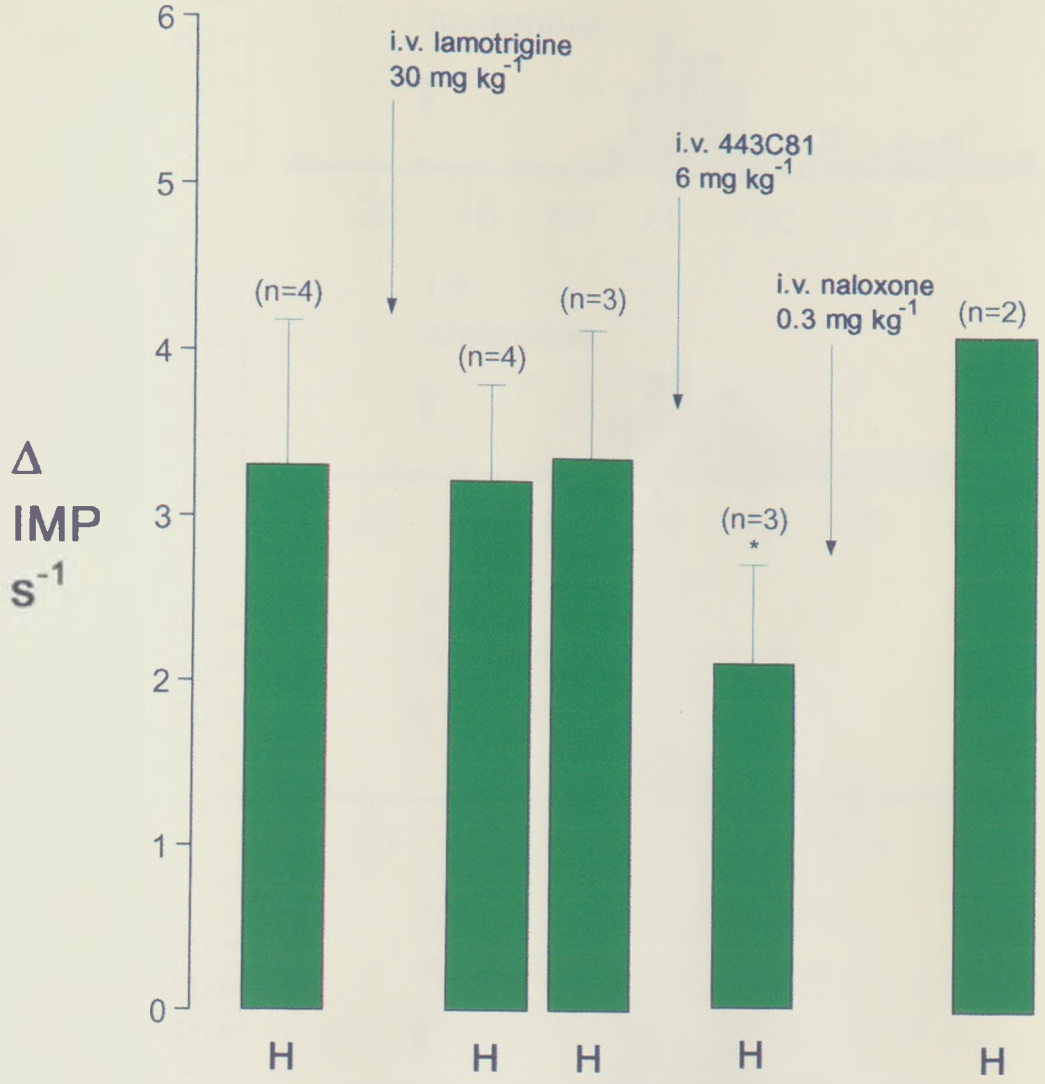


Fig. 7.10 Effect of i.v. lamotrigine (30 mg kg^{-1}), i.v. 443C81 (6 mg kg^{-1}) and i.v. naloxone (0.3 mg kg^{-1}) on i.v. histamine-evoked (H, 10 or 30 μg kg^{-1}) increases in discharge in vagal fibres arising from intrathoracic RARs (n=4) in anaesthetized, paralysed and artificially ventilated cats. Increases in the discharge of the RARs evoked by i.v. histamine are expressed as the mean Δ increase ± sem from control baselines. Lamotrigine, 443C81 and naloxone were administered as shown Significantly different i.v. histamine-evoked increases in RAR discharge, after treatment, from the control response are shown by * $P < 0.05$.

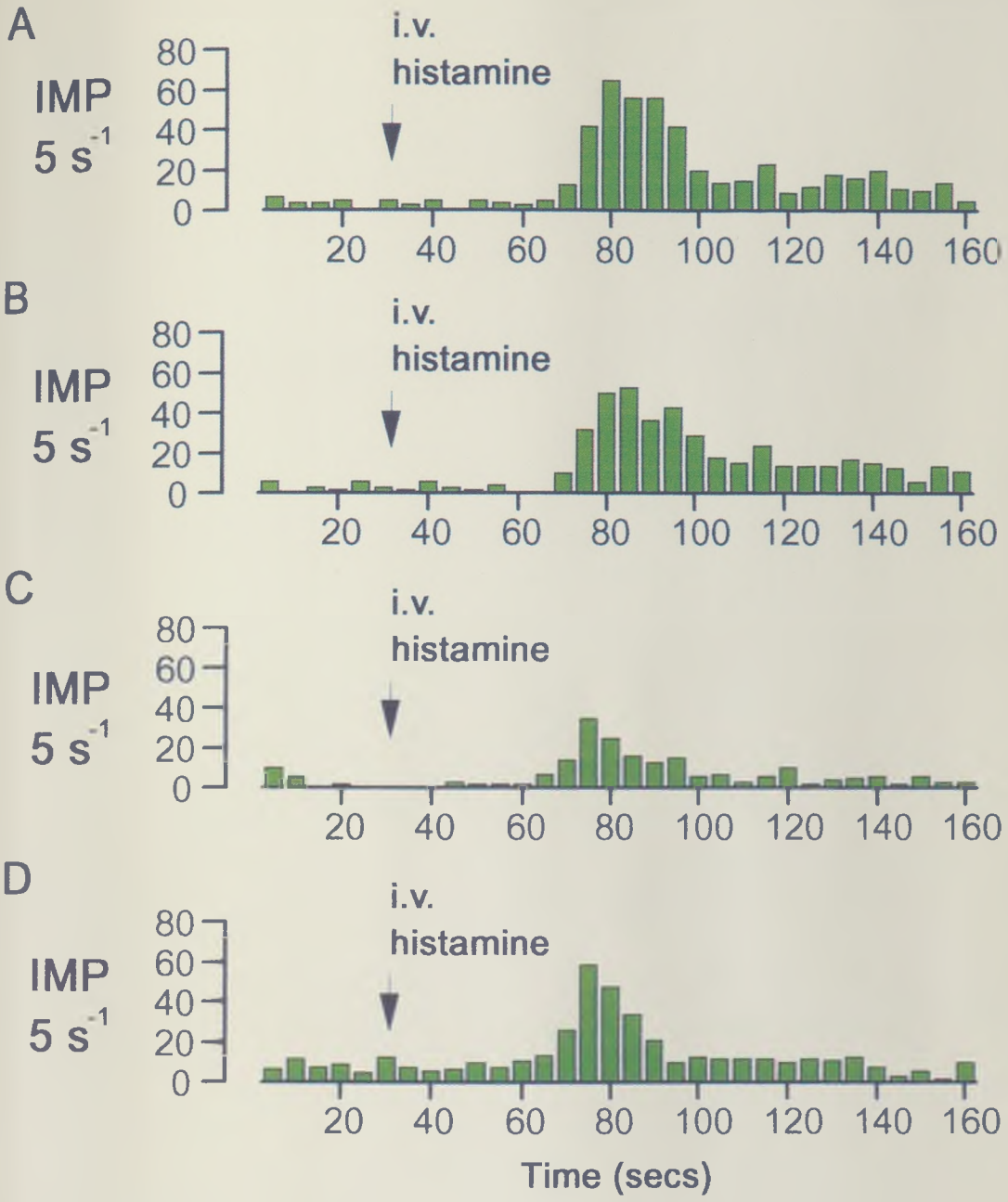


Fig. 7.11 Effect of i.v. lamotrigine (**B**, 30 mg kg^{-1}), i.v. 443C81 (**C**, 6 mg kg^{-1}) and i.v. naloxone after 443C81 administration (**D**, 0.3 mg kg^{-1}) on i.v. histamine-evoked (10 μg kg^{-1}) increases in discharge, in a vagal fibre arising from an intrathoracic RAR in an anaesthetized, paralysed and artificially ventilated cat. **A**, shows the control response to i.v. histamine (10 μg kg^{-1}). Impulse discharges were measured in 5 second bins.

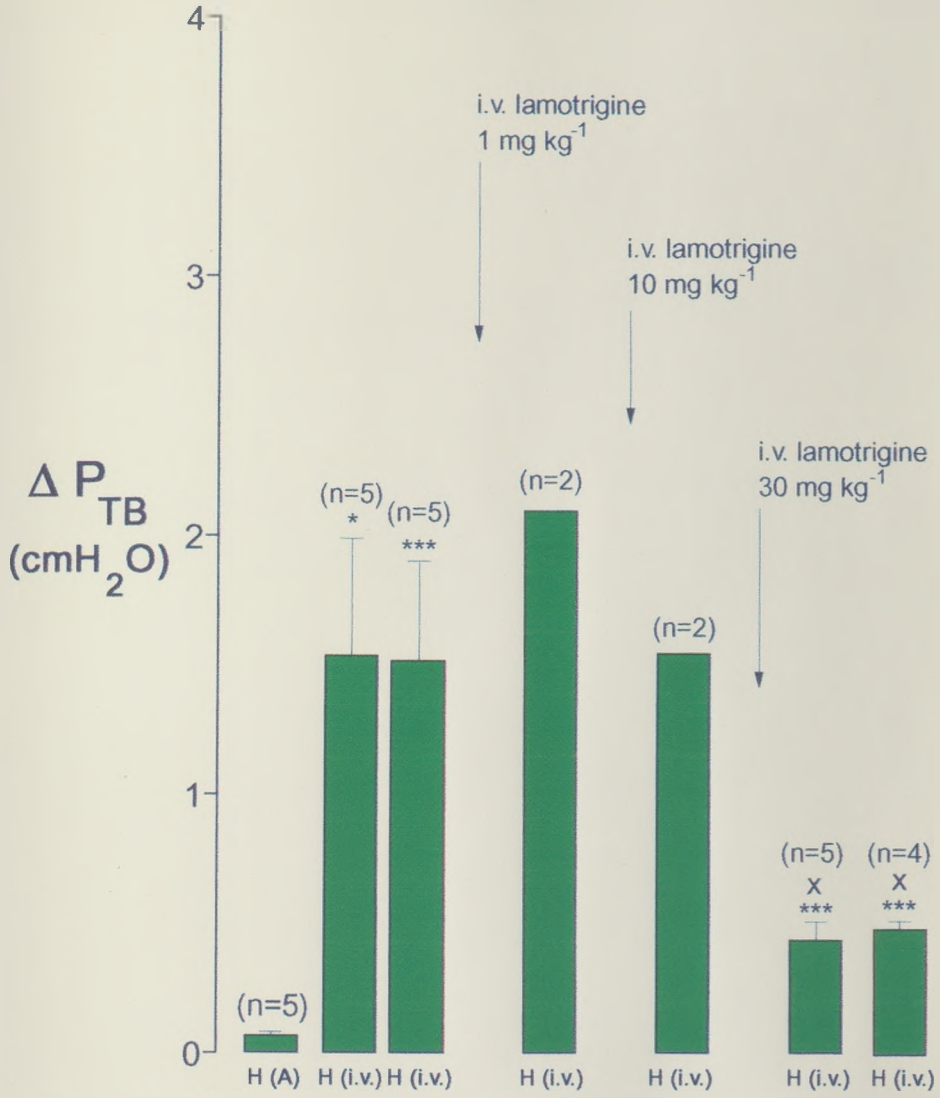


Fig. 7.12 Effect of histamine aerosol (**H**, **A**, 6 breaths 1 mg ml^{-1} solution) on tracheal balloon pressure and the effect of i.v. lamotrigine (1 - 30 mg kg^{-1}) on i.v. histamine-evoked (**H**, **i.v.**, 10 or 30 μg kg^{-1}) increases in tracheal balloon pressure (P_{TB}) in anaesthetized, paralysed and artificially ventilated cats (n=5). Agents were administered as shown on the horizontal axis. Results are expressed as the mean Δ increase in P_{TB} from baseline values. Vertical bars represent sem. Significant i.v. histamine-evoked responses in P_{TB} from baseline are shown as * $P < 0.05$, *** $P < 0.005$. Significantly different i.v. histamine-evoked responses, after treatment with lamotrigine, from the control responses are shown by X $P < 0.05$.

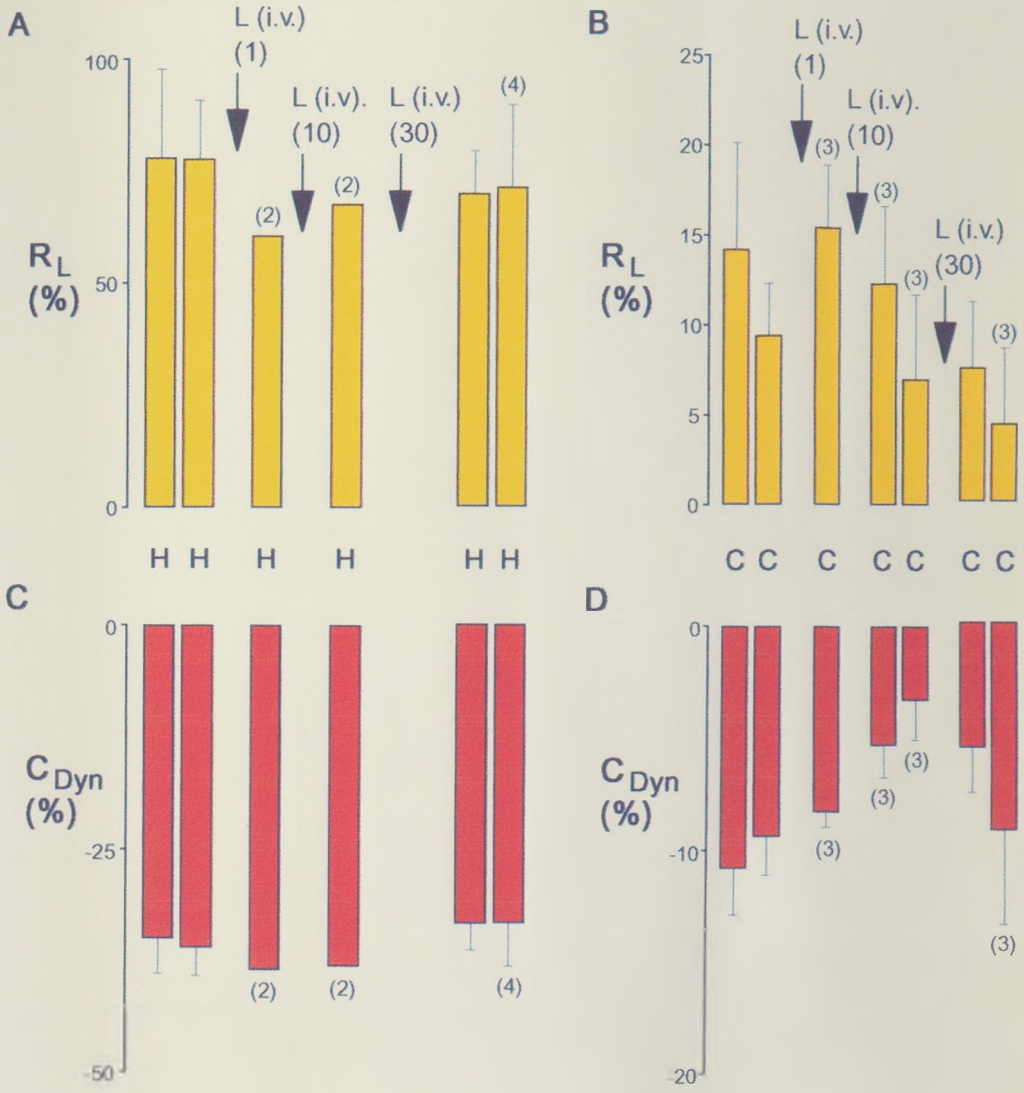


Fig. 7.13 Effect of i.v. lamotrigine (1, 10 & 30 mg kg^{-1}) on i.v. histamine-evoked (**H** in **A** & **C**; 10 or 30 μg kg^{-1}) and capsaicin aerosol-evoked (**C** in **B** & **D**; 6 breaths, 0.1 mg ml^{-1} solution) bronchoconstrictor responses in anaesthetized, paralysed and artificially ventilated cats (n=7). Agents were administered as shown on the horizontal axis. Bronchoconstrictor responses evoked by histamine and capsaicin, are expressed as the % increase in lung resistance (R_L) and the % decrease in dynamic lung compliance (C_{Dyn}). Vertical bars represent sem.

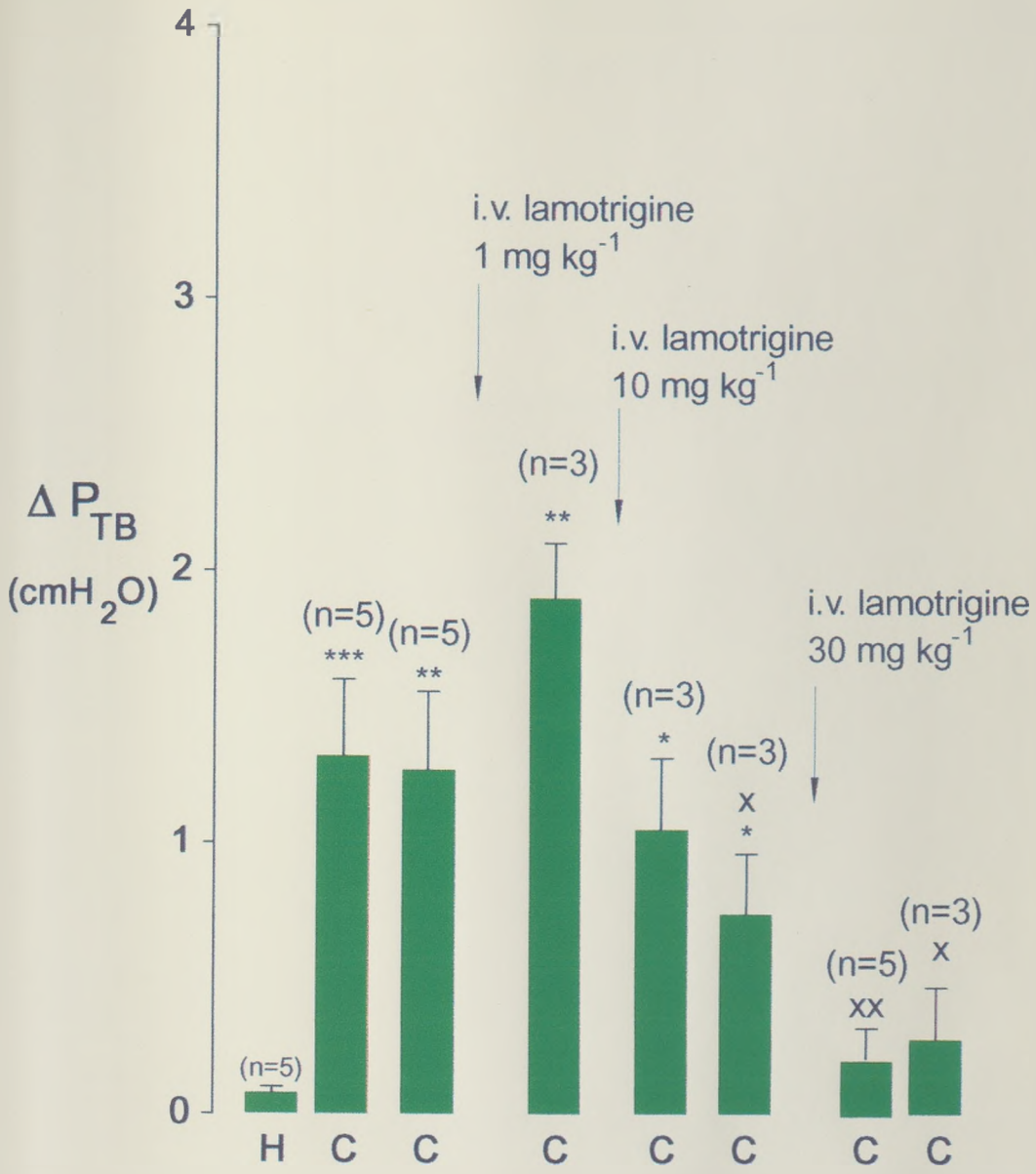


Fig. 7.14 Effect of histamine aerosol (**H**, 6 breaths 1 mg ml^{-1} solution) on tracheal balloon pressure and the effect of i.v. lamotrigine (1 - 30 mg kg^{-1}) on capsaicin aerosol-evoked (**C**; 6 breaths from a 0.1 mg ml^{-1} solution) increases in tracheal balloon pressure (P_{TB}) in anaesthetized, paralysed and artificially ventilated cats (n=5). Agents were administered as shown on the horizontal axis. Results are expressed as the mean Δ increase in P_{TB} from baseline values. Vertical bars represent sem. Significant capsaicin aerosol-evoked responses in P_{TB} from baseline are shown as * $P < 0.05$, *** $P < 0.005$. Significantly different capsaicin aerosol-evoked responses, after treatment with lamotrigine, from the control responses are shown by X $P < 0.05$, XX $P < 0.01$.

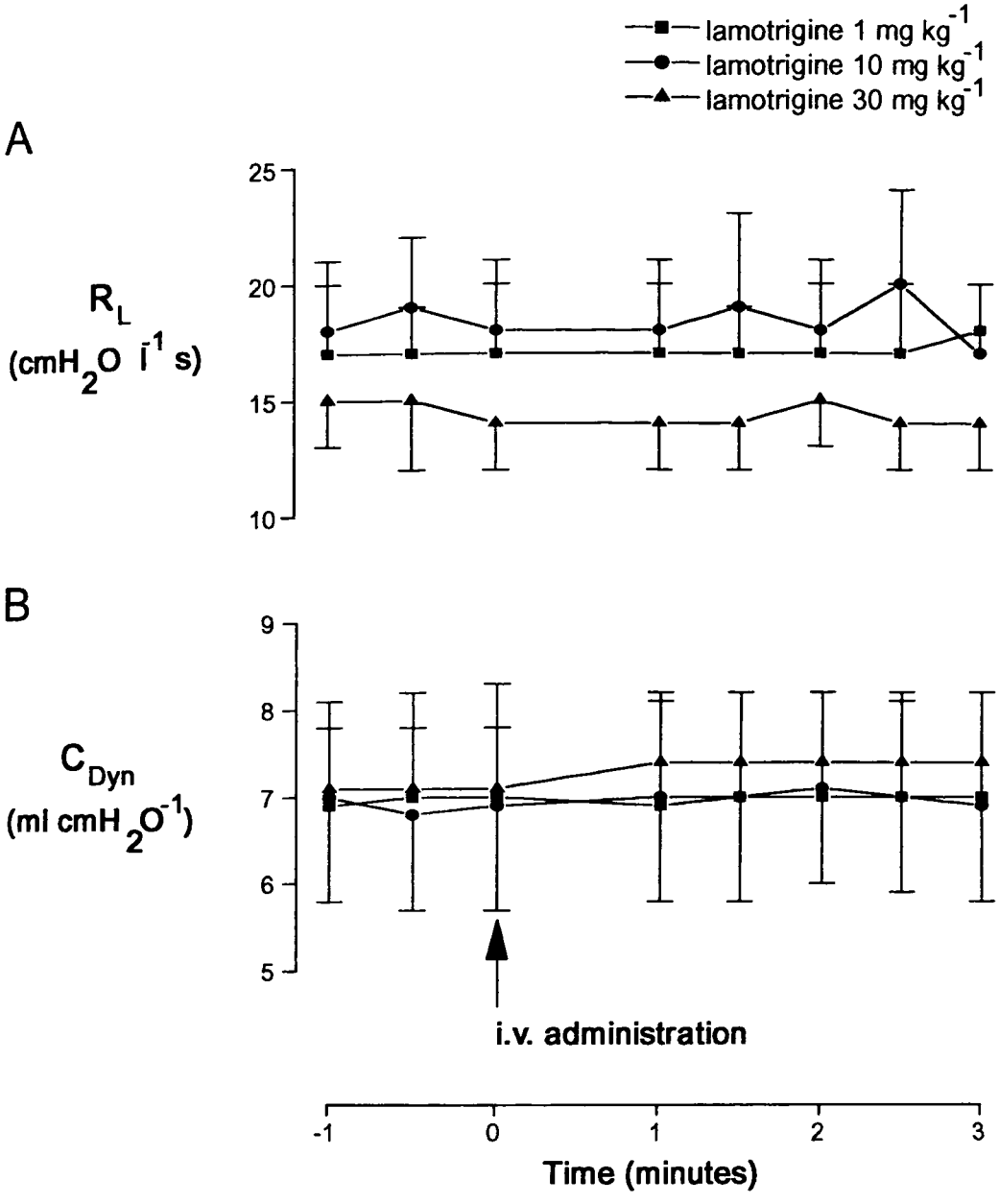


Fig. 7.15 Effect of administration of bolus i.v. injections of lamotrigine (1, 10 & 30 mg kg^{-1} solutions) on lung resistance (**A**, R_L) and dynamic lung compliance (**B**, C_{Dyn}) in anaesthetized, paralysed and artificially ventilated cats (n=7). Absolute values for R_L and C_{Dyn}, expressed against time (minutes) are shown before, during and after administration of each dose. Vertical bars represent sem.

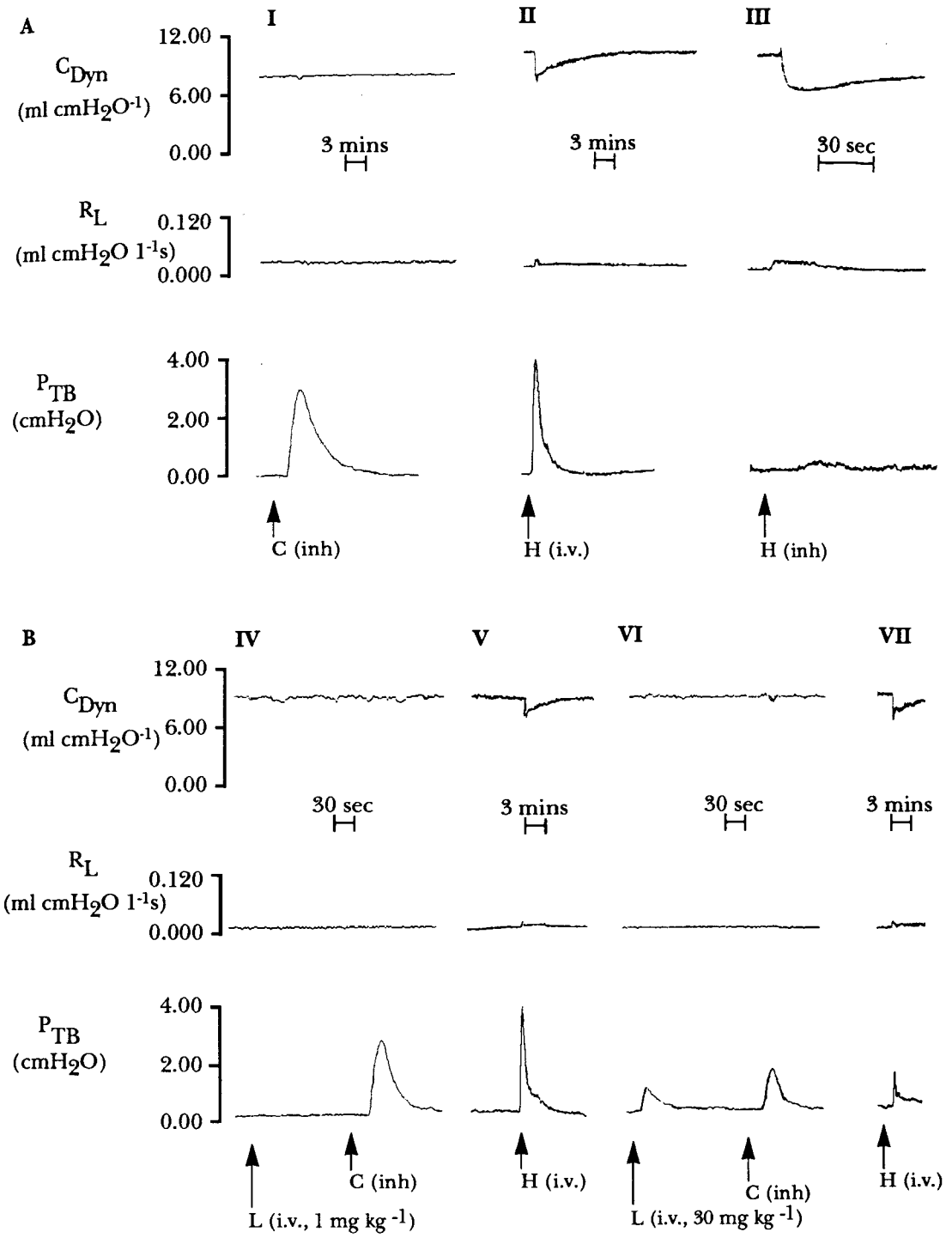


Fig. 7.16 Effect of intravenous lamotrigine on changes in dynamic lung compliance (C_{Dyn}), lung resistance (R_L) and tracheal balloon pressure (P_{TB}) evoked by inhalation of capsaicin aerosols (C inh) and intravenous injection of histamine (H i.v.) in an anaesthetized, paralysed and artificially ventilated cat. Administration of capsaicin (inh) and histamine (i.v.) were carried out in the same animal at 15-30 min intervals. **A)** Capsaicin aerosol (6 breaths, 0.1 mg ml^{-1} solution) **(I)**, histamine i.v. (10 μg kg^{-1}) **(II)**, histamine aerosol (6 breaths, 1 mg ml^{-1} solution; H inh) **(III)** before administration of i.v. lamotrigine. **B)** Capsaicin aerosol **(IV, VI)** and histamine **(V, VII)** after administration of intravenous lamotrigine (1 & 30 mg kg^{-1}).

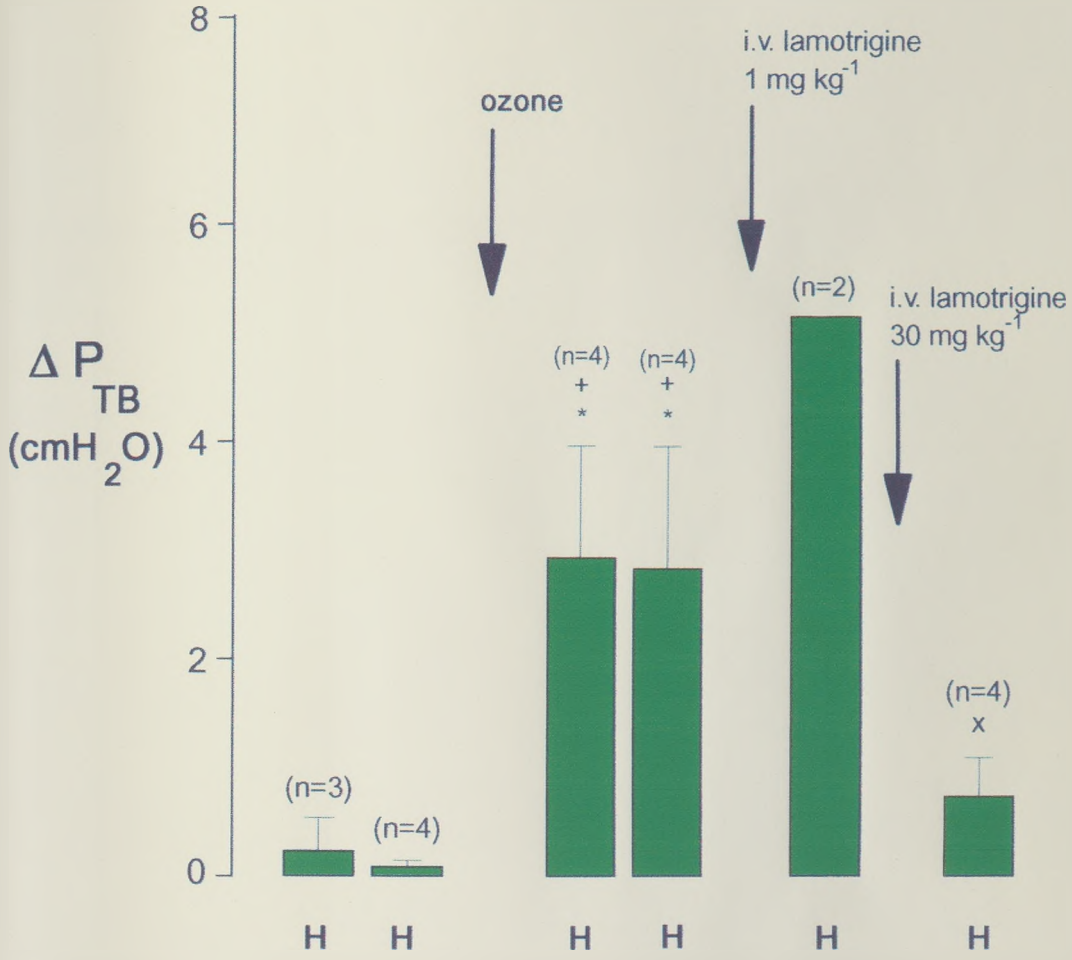


Fig. 7.17 Effect of histamine aerosol (H, 6 breaths 1 mg ml^{-1} solution) on tracheal balloon pressure (P_{TB}) before and after ozone administration (5 ± 0.5 ppm) and the effect of i.v. lamotrigine (1 & 30 mg ml^{-1}) on post-ozone histamine aerosol-induced responses in anaesthetized, paralysed and artificially ventilated cats (n=4). Agents were administered as shown. Results are expressed as the mean Δ increase in P_{TB} from baseline values. Vertical bars represent sem. Significant histamine aerosol-evoked responses are shown by * $P < 0.05$. Significantly different histamine aerosol-induced responses from control responses (pre-ozone) are shown by + $P < 0.05$. Significantly different histamine aerosol-induced responses (after administration of lamotrigine) from control responses (post-ozone) are shown by X $P < 0.05$.

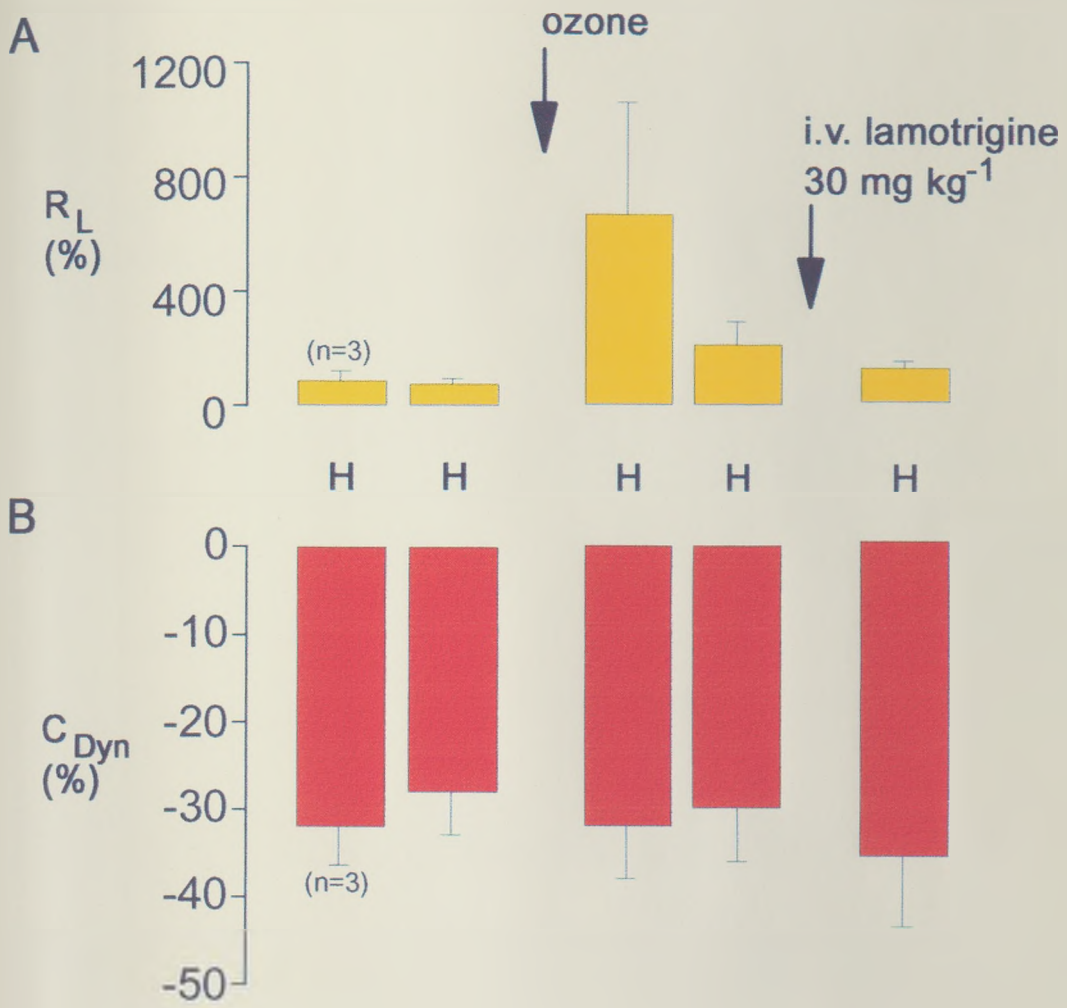


Fig. 7.18 Effect of histamine aerosol (H, 6 breaths 1 mg ml^{-1} solution) on lung resistance (**A**, R_L) and dynamic lung compliance (**B**, C_{Dyn}) before and after ozone administration (5 ± 0.5 ppm) and the effect of i.v. lamotrigine (30 mg ml^{-1}) on post-ozone histamine aerosol-induced responses in anaesthetized, paralysed and artificially ventilated cats (n=4) Agents were administered as shown. Results are expressed as the % change from baseline values. Vertical bars represent sem.

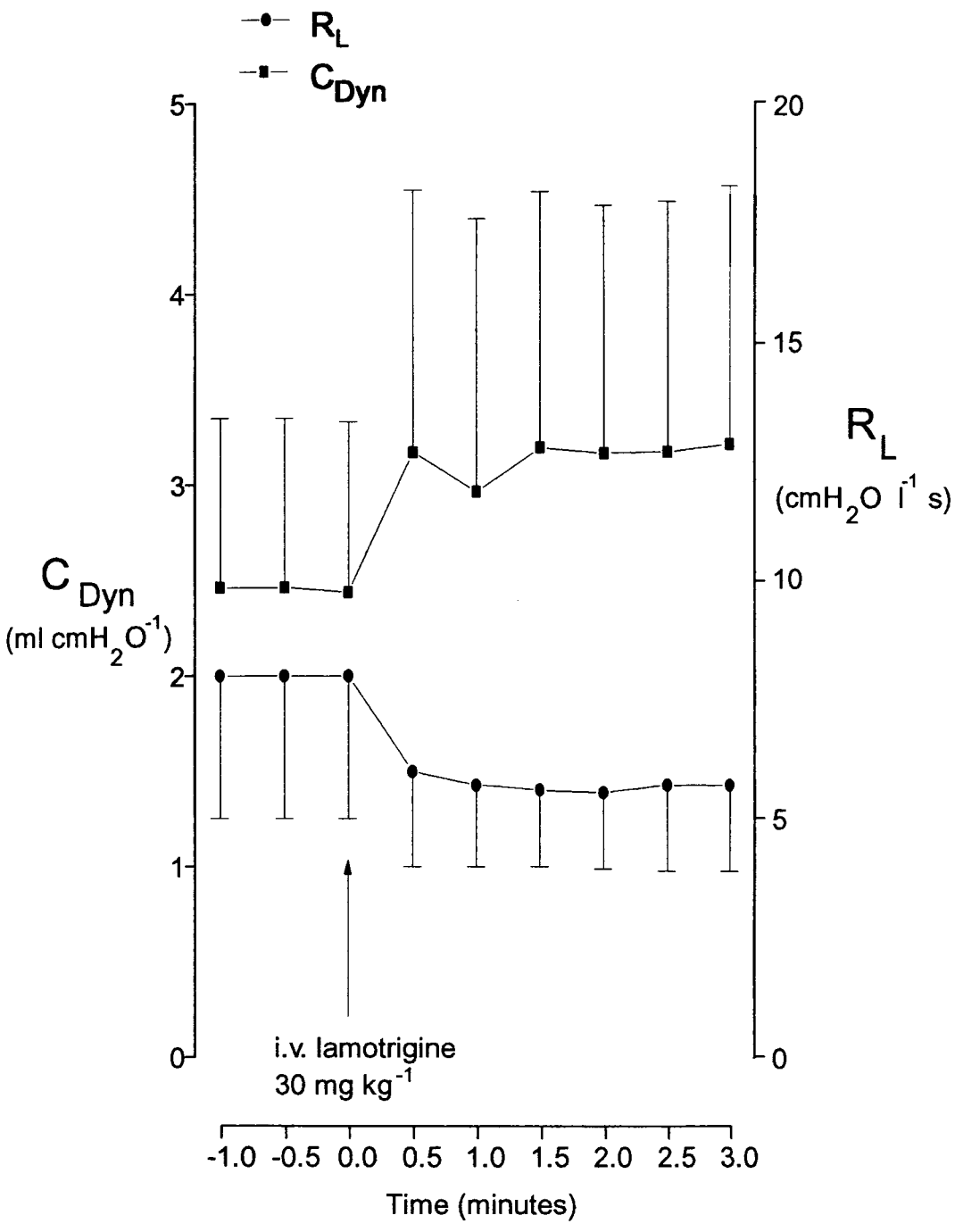


Fig. 7.19 Effect of i.v. lamotrigine (30 mg kg^{-1}) on lung resistance (R_L) and dynamic lung compliance (C_{Dyn}) in anaesthetized, paralysed and artificially ventilated cats (n=4) which have been exposed to ozone (5 ± 0.5 ppm). Results are expressed as mean absolute values. Vertical bars represent sem. Lamotrigine was administered at time zero.

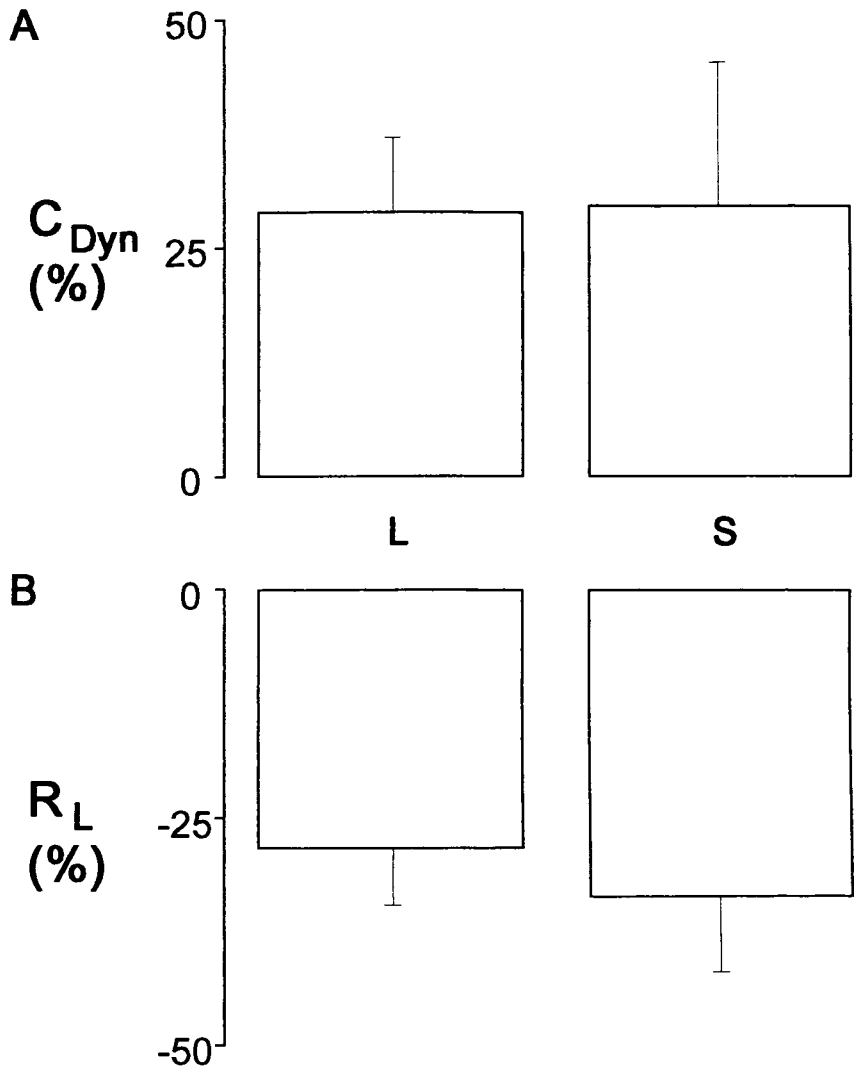


Fig. 7.20 Effect of i.v. lamotrigine (**L**, 30 mg kg^{-1}, n=4) and i.v. salbutamol (**S**, 1 μg kg^{-1}, n=3) on dynamic lung compliance (**A**, C_{Dyn}) and lung resistance (**B**, R_L) in anaesthetized, paralysed and artificially ventilated cats which have been exposed to ozone (5 ± 0.5 ppm). Results are expressed as the mean % increase in C_{Dyn} and the mean % decrease in R_L. Vertical bars represent sem.

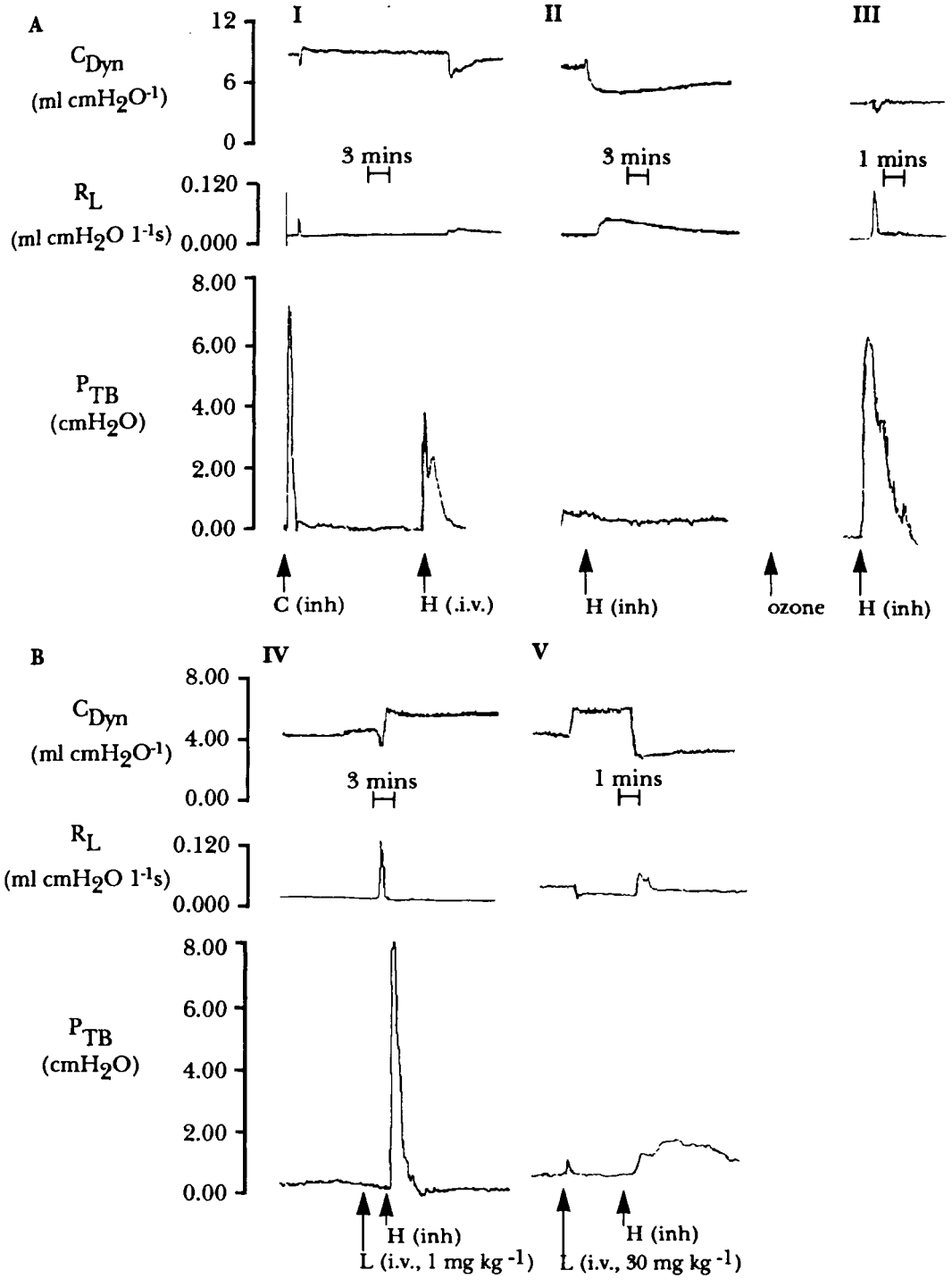


Fig. 7.21 Effect of intravenous lamotrigine on changes in dynamic lung compliance (C_{Dyn}), lung resistance (R_L) and tracheal balloon pressure (P_{TB}) evoked by inhalation of histamine aerosols (H inh) after exposure to ozone (5 ± 0.5 ppm) for 1.5 h in an anaesthetized, paralysed and artificially ventilated cat. **A)** Capsaicin aerosol (6 breaths, 0.1 mg ml^{-1} solution; C inh) and histamine i.v. (10 μg kg^{-1}, H i.v.) before ozone exposure **(I)**. Histamine aerosol (6 breaths, 1 mg ml^{-1} solution) before **(II)** and after **(III)** ozone; note increased responses to histamine after ozone, in particular P_{TB} **B)** Histamine aerosol responses after administration of intravenous lamotrigine (1 & 30 mg kg^{-1}) whilst exposure to ozone continued **(IV, V)**; note the fall in R_L and increase in C_{Dyn} after administration of i.v. lamotrigine (30 mg kg^{-1}).

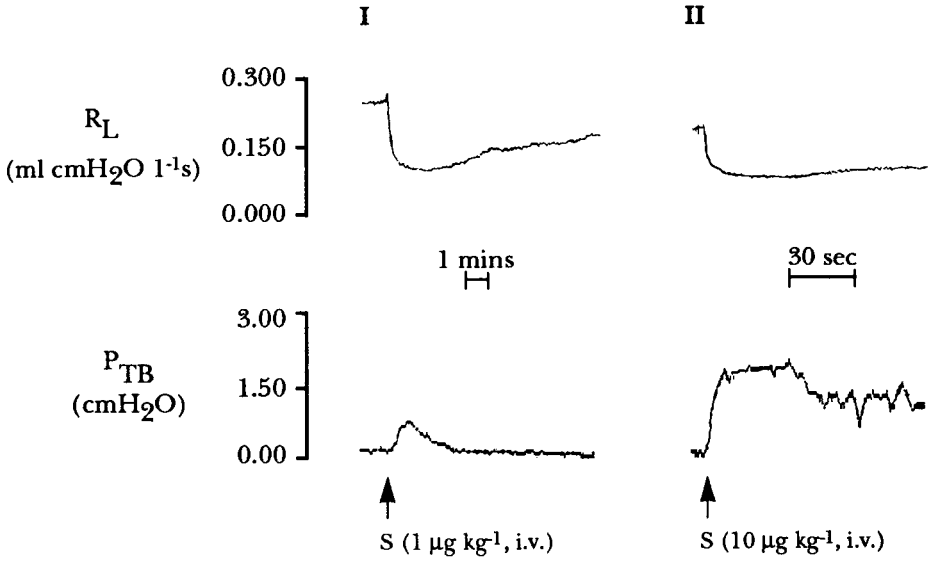


Fig. 7.22 Effect of intravenous salbutamol (**S**) on lung resistance (R_L) and tracheal balloon pressure (P_{TB}) after exposure of the lower respiratory tract to ozone (5 ± 0.5 ppm) for 1.5 hrs in an anaesthetized, paralysed and artificially ventilated cat. Intravenous salbutamol (1 & 10 μg kg^{-1}) after exposure to ozone exposure (**I, II**); in addition to the falls in R_L, note the dose-related increases in P_{TB}.

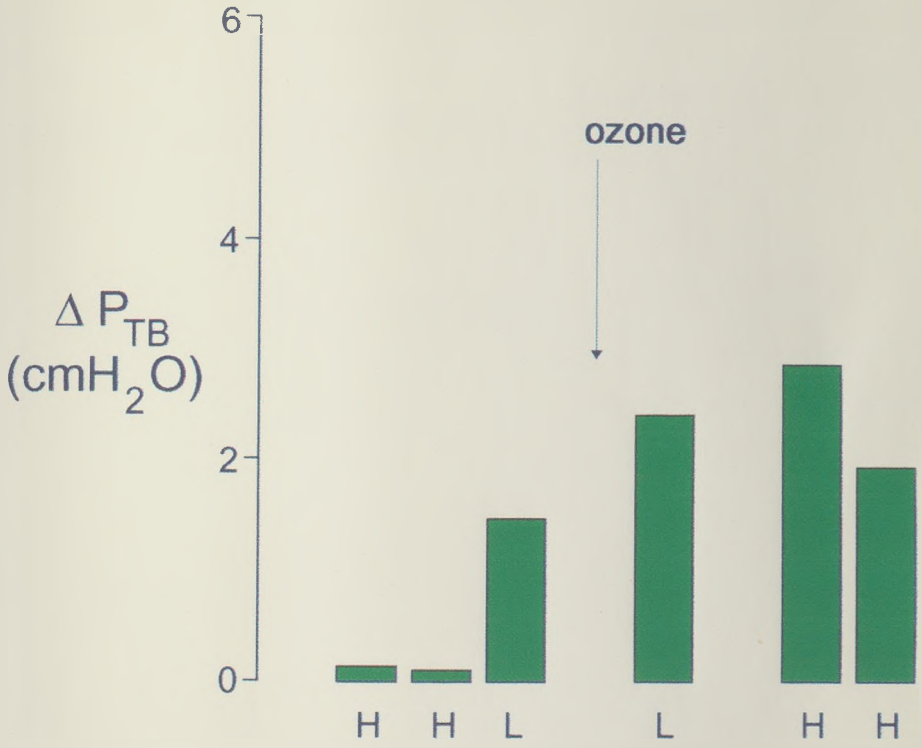


Fig. 7.23 Effect of histamine aerosol (**H**, 6 breaths 1 mg ml^{-1} solution) on tracheal balloon pressure (P_{TB}), before and after ozone exposure (5 ± 0.5 ppm) and the effect of i.v. lamotrigine (**L**, 30 mg kg^{-1}) treatment (3 mins before and 45 mins after commencing ozone exposure) on histamine aerosol-induced responses (post-ozone exposure) in anaesthetized, paralysed and artificially ventilated cats (n=2). Agents were administered as shown. Results are expressed as the mean Δ increase in P_{TB} from baseline values.

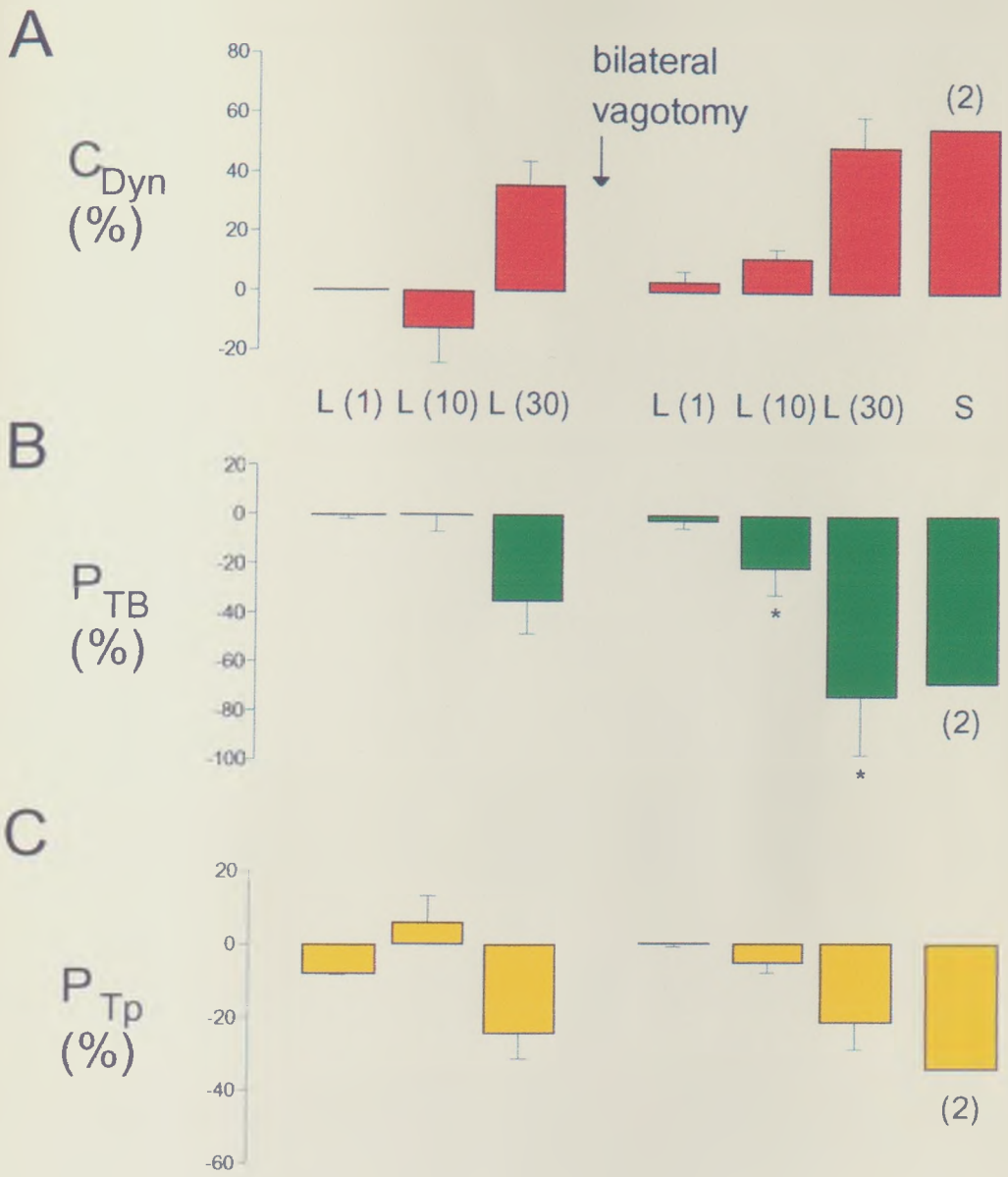


Fig. 7.24 Effects of the administration of i.v. lamotrigine (1 mg kg^{-1}, L (1); 10 mg kg^{-1}, L (10) and 30 mg kg^{-1}, L (30) at 30 min intervals) and i.v. salbutamol (S, 1 μg kg^{-1}, after bilateral vagotomy only) on serotonin (continuous infusion, 10 or 20 μg kg^{-1} min^{-1}, i.v.)-induced decreases in dynamic lung compliance (**A**, C_{Dyn}), increases in tracheal balloon pressure (**B**, P_{TB}) and transpulmonary pressure (**C**, P_{Tp}), before and after bilateral vagotomy, in anaesthetized, paralysed and artificially ventilated cats (n=3). Agents were administered as shown. Results are expressed as the mean % change in C_{Dyn}, P_{TB} and P_{Tp}. Vertical bars represent sem. Significantly different values from control baselines are shown by * $P < 0.05$.

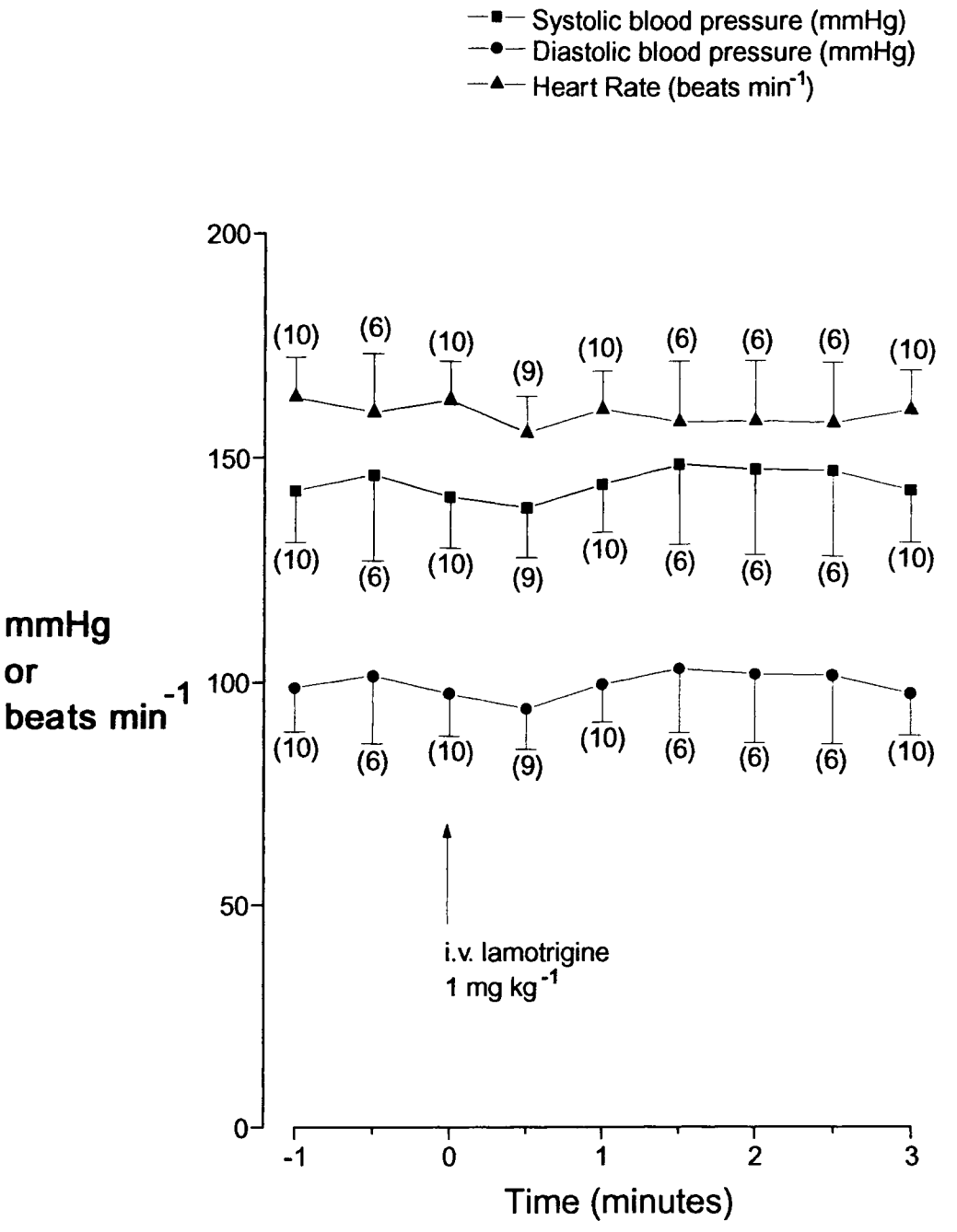


Fig. 7.25 Effect of i.v. lamotrigine (1 mg kg^{-1}) on absolute values of arterial blood pressure and heart rate in anaesthetized, paralysed and artificially ventilated cats. Results are expressed as the mean systolic and diastolic arterial blood pressure (mmHg), and mean heart rate (beats min^{-1}). Vertical bars represent sem. Lamotrigine was administered as shown.

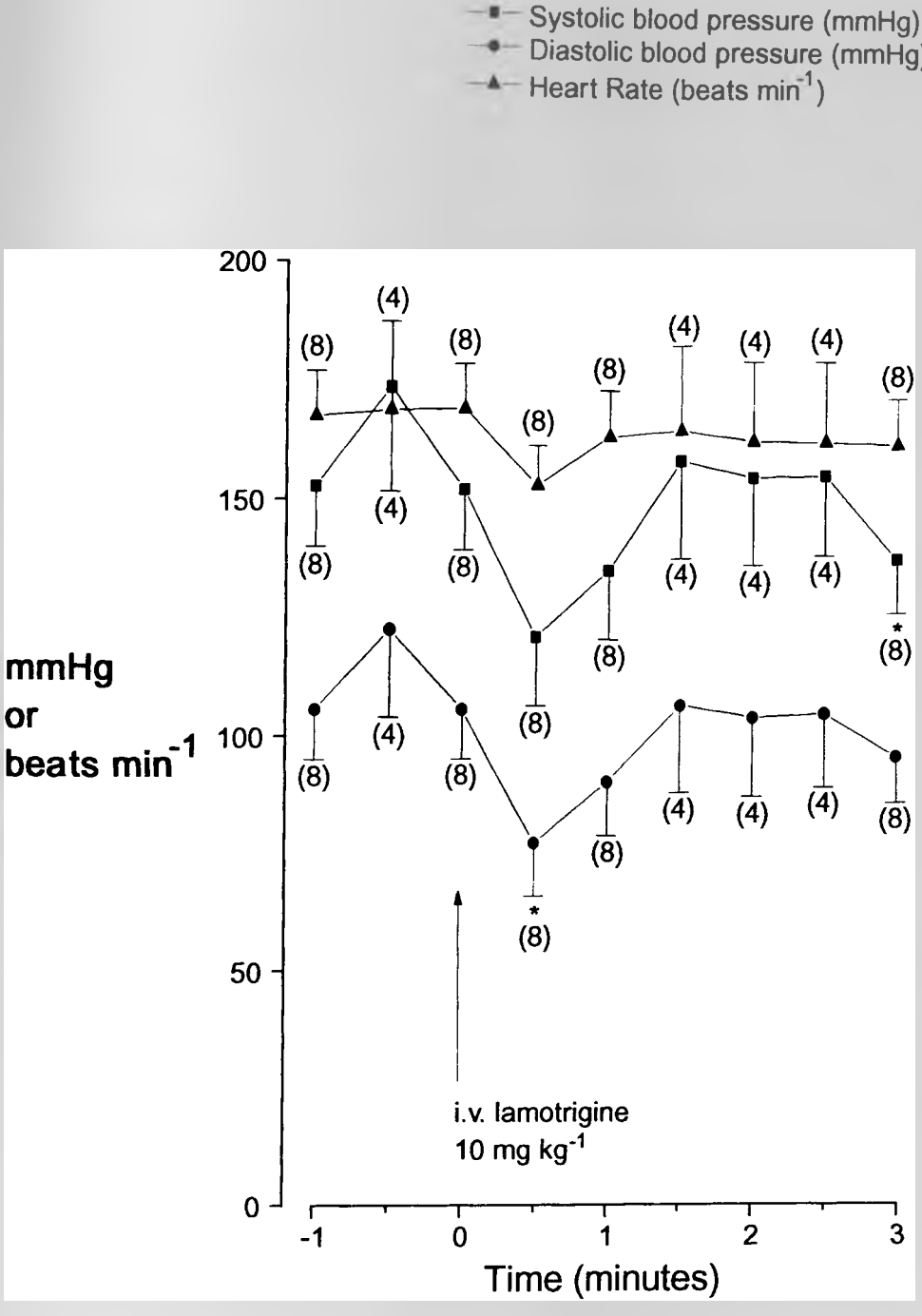


Fig. 7.26 Effect of i.v. lamotrigine (10 mg kg^{-1}) on absolute values of arterial blood pressure and heart rate in anaesthetized, paralysed and artificially ventilated cats. Results are expressed as the mean systolic and diastolic arterial blood pressure (mmHg), and mean heart rate (beats min^{-1}). Vertical bars represent sem. Lamotrigine was administered as shown. Significantly different values from control values (time zero) are shown by * $P < 0.05$.

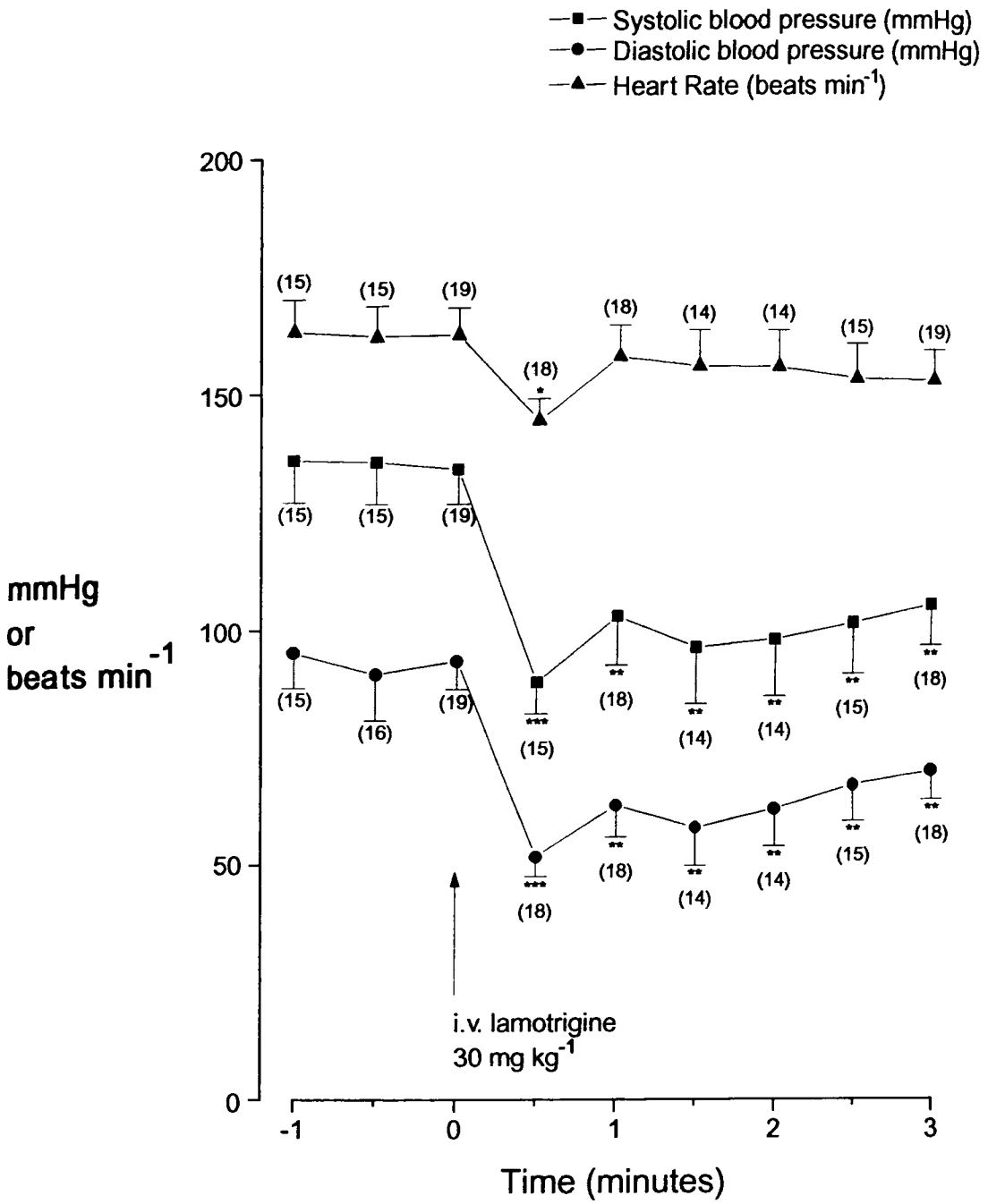


Fig. 7.27 Effect of i.v. lamotrigine (30 mg kg^{-1}) on absolute values of arterial blood pressure and heart rate in anaesthetized, paralysed and artificially ventilated cats. Results are expressed as the mean systolic and diastolic arterial blood pressure (mmHg), and mean heart rate (beats min^{-1}). Vertical bars represent sem. Lamotrigine was administered as shown. Significantly different values from control values (time zero) are shown by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

7.4 Discussion

Isolated segments of trachea (with or without a balloon) have been used extensively in studies of reflex bronchoconstriction (Kahn, 1907; Loofbourrow *et al.*, 1957; Nadel & Widdicombe, 1962; Green & Widdicombe, 1966; Graf *et al.*, 1975; Brown *et al.*, 1980; Roberts *et al.*, 1981; Adcock, 1989). This particular method has certain advantages in reflex studies; it permits investigation of an airway smooth muscle whose ultrastructural, mechanical and pharmacological properties are generally similar to those of smooth muscle in large bronchi (Stephens & Kroeger, 1980), but is more accessible; it also allows variation in smooth muscle tone and reflex bronchoconstriction to be recorded continuously. Moreover, the investigator is able to limit agents, or stimuli, to one area of the airways, whilst effects are studied in the other.

In the present series of experiments, capsaicin aerosol and i.v. histamine caused bronchoconstriction and increases in tracheal balloon pressure. The bronchoconstrictor responses evoked by inhaled capsaicin in the cat are mediated by a vagal cholinergic pathway; the responses being abolished after bilateral vagotomy or pre-treatment with atropine (Adcock, 1989). It is likely that both C-fibres and RARs are involved in evoking this reflex bronchoconstriction since capsaicin is not selective for either group of airway sensory nerve ending (Chapter 4). In contrast to inhaled capsaicin, the bronchoconstrictor responses, including the increases in tracheal balloon pressure, evoked by i.v. histamine are mediated by direct contraction of airway smooth muscle (Adcock, 1989).

The finding that sub-plantar injections of glutamate into the rat hind paw can induce both acute and sustained hyperalgesia (Follenfant & Nakamura-Craig, 1992) supports a possible role for this excitatory amino acid in the development of hyperalgesia.

Thus, when considering the hypothesis that hyperalgesia may be analogous to the airway hyperreactivity seen in asthma (Chapter 1, Section 4), it is possible that an agent such as glutamate may also have a role in the development of airway hyperreactivity. Indeed, an *in vitro* examination of rat bronchial smooth muscle has shown that low concentrations of exogenous glutamate can enhance electrical field stimulation-evoked cholinergic contractions in the rat, possibly as a result of glutamate acting pre-junctionally at a receptor for L-glutamate to enhance the release of acetylcholine (Aas & Fonnum, 1990). However, in the same study, higher concentrations of glutamate, although inhibiting electrical field stimulation-evoked contractions, enhanced the tone of the smooth muscle, possibly as a result of post-junctional potentiation of acetylcholine-induced contractions. From their findings, the authors suggested that glutamate may be acting, in the airways, pre and/or post-junctionally. In the present series of experiments, glutamate aerosols had no effect on either the spontaneous discharge of intrathoracic RARs or on pulmonary mechanics in cats with normal airways. Furthermore, glutamate (administered either intravenously or as an inhaled aerosol) did not appear to potentiate the bronchoconstrictor responses to inhaled capsaicin or to histamine (i.v. or aerosol). Indeed, the tracheal balloon responses to inhaled capsaicin and i.v. histamine, although not significantly, apparently decreased after administration of glutamate. However, histamine aerosol-induced stimulation of RARs was significantly attenuated by glutamate. A similar inhibitory effect with glutamate has also been demonstrated on primary auditory afferent nerves in the guinea-pig (Gleich *et al.*, 1990). In their study, Gleich and colleagues found that glutamate introduced into the perilymph of scala tympani reduced tone-evoked activity without a change in the spontaneous activity of primary auditory afferents. The mechanism, however, by which glutamate attenuates induced activity is unclear. Although microscopic analysis was not carried out at the end of the experiments, a cytotoxic effect on afferent nerve endings is unlikely since spontaneous

activity was unchanged. The lack of excitatory effects with glutamate (for example, sensitisation of airway nerve endings, potentiation of reflex bronchoconstrictor responses to inhaled capsaicin and/or histamine) in the present series of experiments, could be attributed to insufficient time given for the possible development of airway hyperreactivity; the experiments being completed within three hours of beginning glutamate administration. However this explanation is unlikely, since in rabbits with normal airways there is no change in airway function or histamine responsiveness following glutamate infusion (0.2 g kg^{-1} hr^{-1}, for 4 hours) for up to 12 hours after completion of the infusion (Nicholson *et al.*, 1988).

A widely used method for producing airway hyperreactivity has been the exposure to ozone, which induces airway hyperreactivity in a number of species, including the cat (Adcock, 1989). In cats with normal airways, histamine aerosol administered to the lower airways evoked bronchoconstriction (increase in lung resistance and decrease in dynamic lung compliance) but had no effect on tracheal balloon pressure (present study; Adcock, 1989). However, after the lower airways had been exposed to ozone there was a marked and reproducible hyperreactivity to inhaled histamine (increases in both lung resistance and tracheal balloon pressure), a response which is mediated by a vagal reflex pathway (Adcock, 1989). The lack of effect of histamine aerosol in evoking reflex bronchoconstriction, in cats with normal airways, is quite surprising considering that histamine aerosol stimulates both C-fibre endings and RARs (Adcock, 1989).

A number of mechanisms have been suggested to explain how ozone induces airway hyperreactivity including stimulation and/or sensitisation of afferent nerve endings by ozone (Adcock, 1989). Inhibition, by 443C81 (the peripherally acting μ-opioid receptor agonist), of the ozone-induced hyperreactivity to inhaled histamine and

capsaicin in cats would support the theory that ozone-induced hyperreactivity is mediated by an increase in the activity and/or sensitisation of vagal afferent nerves (Adcock, 1989). Support for a direct effect of ozone on airway afferent nerve endings comes from the finding that acute exposure of the lower airways to ozone stimulates some groups of airway afferent nerve endings (bronchial C-fibres and RARs but not pulmonary C-fibres or SARs) in dogs (Coleridge *et al.*, 1993). However, the effects of ozone in sensitising these nerve endings to an inhaled irritant (for example, histamine, capsaicin) were not studied. Although, Sampson and colleagues (1978) demonstrated in dogs exposed to ozone, that "irritant receptors" were no more sensitive to inhaled histamine than those in untreated animals (Sampson *et al.*, 1978), the time between ozone exposure and recording of "irritant receptor" activity was 24 hours whereas, in other studies, peak hyperreactivity to ozone in this species occurs in 1 hour (Holtzman *et al.*, 1983). Alternatively, ozone may act indirectly through epithelial damage and the release of inflammatory mediators, such as neuropeptides and prostaglandins, which may increase the accessibility and/or sensitise the vagal afferent nerve endings to inhaled irritants and endogenous mediators (Adcock, 1989).

The anti-convulsant effects of the anti-epileptic drug lamotrigine have been attributed to inhibition of the release of glutamate in the CNS (Leach *et al.*, 1986). Following the suggestion that glutamate may also be involved in the spinal mechanism of pain (Woolf & Thompson, 1991), glutamate release inhibitors have been examined for possible analgesic activity. Indeed, studies have shown that lamotrigine, when administered orally, inhibits both acute and sustained hyperalgesia induced by PGE_2 in the rat hind-paw model (Nakamura-Craig & Follenfant, 1992). These observations also raise the possibility that agents such as lamotrigine may represent a new approach to the attenuation of vagally-mediated airway reflexes.

In the present series of experiments, bolus i.v. injections of lamotrigine attenuated capsaicin aerosol-evoked increases in tracheal balloon pressure in cats with normal airways and histamine aerosol-evoked increases in tracheal balloon pressure in cats with hyperreactive airways. It is possible that lamotrigine may be acting at a number of sites to exert these effects. Since both of these bronchoconstrictor responses are vagal reflex in nature, one possible site of action includes a direct effect of lamotrigine on airway afferent sensory nerve endings. However, a direct effect on afferent sensory nerve endings is unlikely since lamotrigine had no effect on spontaneous or i.v. histamine-induced impulse activity in intrathoracic RARs. In contrast, the peripherally acting μ-opioid receptor agonist, 443C81, attenuated both the spontaneous and i.v. histamine-induced activity of RARs. Alternatively, if glutamate release is involved in mediating vagal reflex bronchoconstriction, lamotrigine may be inhibiting release of glutamate in the CNS and/or spinal cord. Other possibilities include lamotrigine acting on the efferent limb of the vagal reflex pathway or even directly on airway smooth muscle. The latter is more likely since i.v. histamine-induced increases in tracheal balloon pressure are inhibited by lamotrigine. Furthermore, although i.v. lamotrigine had no effect on pulmonary mechanics (lung resistance and dynamic lung compliance) in cats with normal airways, i.v. lamotrigine induced bronchodilatation in cats with hyperreactive airways. To explore this further, the effect of i.v. lamotrigine was examined in cats in which airway tone had been raised by infusion of 5-HT; the increase in airway tone by 5-HT being independent of vagal reflexes. In these studies, i.v. lamotrigine attenuated 5-HT-induced increases in tracheal balloon pressure and transpulmonary pressure and reductions in dynamic lung compliance before and after bilateral vagotomy.

Although lamotrigine appeared to have a more pronounced effect in inducing relaxation of airway tone after bilateral vagotomy, suggesting that lamotrigine may

itself be inducing a degree of airway tone by stimulation of airway afferent sensory nerve endings, the effect was not significant. Lamotrigine, in some nerve fibre recording experiments, evoked a transient stimulation of RARs, and in some reflex bronchoconstriction experiments an increase in tracheal balloon pressure, but overall these effects were also found not to be significant.

Despite its "anti-bronchoconstrictor" activity, pre-treatment with i.v. lamotrigine (30 mg kg^{-1}) was ineffective in inhibiting ozone hyperreactivity to inhaled histamine. Furthermore, when compared to the β_2-adrenoceptor agonist salbutamol (1 μg kg^{-1}, i.v.), lamotrigine is 30,000 times less potent in causing bronchodilatation. Although not examined in cats, it should also be noted that the anti-convulsant ED_{50} of lamotrigine in rats is 5 mg kg^{-1} p.o. and the ED_{50} to reduce electrically-induced afterdischarge in dogs is 4.5 mg kg^{-1} i.v. (Wheatley & Miller, 1989). Therefore, the dose of lamotrigine necessary to evoke bronchodilator responses in cats is in the region of 6 fold greater than that which produces anti-convulsant actions in other species, and at least 30,000 times higher than that of salbutamol in causing bronchodilatation.

Lamotrigine, administered intravenously, also evoked hypotension and a transient bradycardia. Taken together with the findings that lamotrigine reduces intestinal smooth muscle tone and peristaltic contractions *in vitro,* slows gastric emptying *in vivo* (J.J. Adcock & M. Leach, personal communication) and reduces raised airway tone (present study) it seems likely that lamotrigine, at high doses, has a non-selective effect on smooth muscle. The mechanism by which lamotrigine exerts these effects may be through inhibition of sodium ion channels (Lang & Wang, 1991).

From the present series of studies, it seems unlikely that glutamate has a role in the development of airway hyperreactivity in the cat. Although lamotrigine at high i.v. doses (approximately six times the anti-convulsant ED_{50}) has some "anti-bronchoconstrictor" actions, these are almost certainly the result of lamotrigine's non-selective action on smooth muscle. Due to its low potency (when compared with i.v. salbutamol) it seems highly unlikely that lamotrigine would have any therapeutic value in the treatment of asthma.

Chapter 8

General Discussion

8.1 General

Irritation of airway sensory nerve endings can evoke respiratory reflexes, such as cough and reflex bronchoconstriction. The reflex response to an inhaled irritant will be influenced not only by the afferent susceptibilities of airway sensory nerve endings but also by their anatomical distribution along the respiratory tract. Despite what is known about the morphology and chemical specificity of different groups of airway afferent sensory nerve endings, little is known about the distribution of the airway sensory nerve endings in the respiratory tract involved in evoking such respiratory reflexes, particularly in man.

The cough reflex is a suitable physiological response which allows the study of airway afferent sensory nerve endings involved in evoking airway reflexes, since it is not dependent on the conditioning of the vagal efferent system; a factor that has caused problems in the study of bronchoconstriction. There is considerable evidence, from studies in both experimental animals and man, to suggest regional differences in the sensitivity of the respiratory tract to inhaled tussive agents (Widdicombe, 1954b; Higenbottam *et al.*, 1989b; Forsberg *et al.*, 1990). In the present study, regional differences in the sensitivity of the respiratory tract of man were observed with inhaled capsaicin and PGE_2; the central airways being responsive to PGE_2 and the peripheral airways to inhaled capsaicin. It is, however, quite surprising to find differences in the regional sensitivity of the respiratory tract between these two agents, considering that capsaicin and PGE_2 are both believed to be "selective" C-fibre ending stimulants. Moreover, these findings raise the possibility that coughing evoked by capsaicin and PGE_2 aerosols may involve the activation of other airway afferent sensory nerve endings (for example, RARs).

The limitation of aerosol studies of cough in man, however, is the inability to precisely define which afferent nerve endings are stimulated by different mediators of inflammation. Studies of location and "adaptation and/or cross-adaptation" (present study; Higenbottam & Lowry, 1990) are no substitute for single nerve fibre recording studies. These can only be undertaken in animals which offer the opportunity to determine which nerve endings are responsive to various irritant agents and inflammatory mediators. Indeed, in the present study, aerosols of two supposedly selective C-fibre stimulants, capsaicin and PGE_2, increased the discharge of vagal fibres arising from intrathoracic RARs in anaesthetized, paralysed and artificially ventilated cats.

8.2 RARs and C-fibres in cough and reflex bronchoconstriction

There has been considerable speculation on the involvement of different groups of airway sensory nerve endings with respect to different airway reflex responses. Assigning any one group of airway sensory nerve endings to a specific reflex is difficult. However, considering the most recent evidence in the literature, and that in the present study, it seems reasonable to speculate on the contribution of RARs and C-fibre endings to cough and reflex bronchoconstriction.

Some reviewers believe the cough reflex to be mediated by RARs (Sant'Ambrogio, 1982, 1987; Coleridge & Coleridge, 1986; Widdicombe, 1986b). There is considerable evidence to support this view, including the observation that many tussigenic stimuli can stimulate or sensitise them to other stimuli (Mills *et al.*, 1970). Partial block of vagal conduction, limited to the myelinated fibres, has in several studies inhibited the cough reflex (Widdicombe, 1954a; Karczewski & Widdicombe, 1969a). RARs appear the more likely candidates if one also considers their abundance in the proximal tracheobronchial tree (Mortola *et al.*, 1975; Das *et al.*, 1978) and their

superficial location within the mucosa (Das *et al.*, 1978; Fisher & Sant'Ambrogio, 1982). The latter property explains their ability to respond to weak chemical and light mechanical stimuli.

Over the last decade, a number of investigators have suggested that cough can be induced by stimulation of C-fibre endings in the lungs and/or larynx (Collier & Fuller, 1984; Midgren *et al.*, 1986; Paintal, 1986; Forsberg & Karlsson, 1986, 1987; Fuller *et al.*, 1987a, b). Although these sensory nerve endings respond to many of the same stimuli that excite RARs, with some quantitative differences (Coleridge & Coleridge, 1984), the basis of the supporting evidence for an involvement of C-fibres in evoking cough has come from studies in which inhaled "selective" C-fibre stimulants (for example, capsaicin) cause cough in conscious animals and man (Karlsson *et al.*, 1988).

The results from the present study, however, do not support the view that C-fibre stimulation causes cough. Capsaicin and PGE_2 aerosols, when administered to the lower airways of anaesthetized, paralysed and artificially ventilated cats, do not appear to be "selective" stimulants of either RARs or C-fibre endings. It is possible, therefore, that the coughing evoked by inhalation of capsaicin aerosols in experimental animals and man (Karlsson *et al.*, 1988), is mediated by RARs and not by C-fibre endings. Although "selective" destruction of C-fibre endings by large doses of capsaicin in guinea-pigs can block some forms of induced cough (Forsberg & Karlsson, 1986; Forsberg *et al.*, 1986), large doses of capsaicin can also damage nerve endings with myelinated afferent fibres (Buck & Burks, 1986; Such & Jancso, 1986). The absence of the cough reflex in capsaicin-treated animals may, therefore, be due to damage of RARs which have myelinated vagal fibres with non-myelinated terminals in the airway epithelium (Das *et al.*, 1978; Sant'Ambrogio, 1987). This possibility is supported by

the observation that large doses of capsaicin destroy these epithelial non-myelinated nerve terminals (Hoyes *et al.*, 1981). It has been suggested that stimulation of C-fibre endings may even inhibit coughing. Indeed, nedocromil sodium, a drug that stimulates bronchial C-fibre endings (Jackson, 1989), inhibits cough induced by citric acid aerosols in dogs (Jackson, 1988); a mechanism that may be similar to the inhibitory action of capsaicin, via stimulation of pulmonary C-fibre endings, on the cough reflex in cats (Tatar *et al.*, 1988).

There is considerable evidence to suggest that activation of C-fibre endings is a powerful mechanism for evoking reflex bronchoconstriction (Coleridge & Coleridge, 1984, 1986). The possibility that RARs are involved in mediating this reflex response has been debated. A role for RARs in evoking reflex bronchoconstriction is supported by experiments carried out in rabbits in which the bronchoconstrictor effects of histamine are virtually abolished by cooling of the vagus nerves to temperatures sufficient to block myelinated fibres but not non-myelinated C-fibres (Karczewski & Widdicombe, 1969a). Furthermore, in the present study, inhaled capsaicin at doses known to evoke reflex bronchoconstriction in the cat activated a proportion of RARs tested. Since the activation of RARs by capsaicin is independent of intervening bronchoconstriction (present study), it is possible that RARs are involved in mediating this reflex response.

Thus, taking into account evidence from the existing literature and the results from the present study, it seems reasonable to propose the following: **i)** stimulation of RARs evokes coughing in response to mechanical or chemical stimuli, whereas activation of C-fibre endings (pulmonary and/or bronchial) may inhibit the cough response. **ii)** activation of either RARs and/or C-fibres endings can evoke reflex bronchoconstriction.

A direct involvement of SARs in the mechanism of coughing is improbable since these sensory nerve endings do not respond to recognised tussigenic stimuli, such as light mechanical probing of the extrapulmonary airways (Sant'Ambrogio, 1982, 1987). A more likely hypothesis is that SARs facilitate the cough reflex. Indeed, studies in rabbits, in which SAR activity was abolished by inhalation of sulphur dioxide but RAR activity was left intact, cough evoked by chemical or mechanical stimulation of the larynx was greatly reduced and cough evoked by mechanical stimulation of the trachea and extrapulmonary bronchi was abolished (Hanacek *et al.*, 1984). Furthermore, the cough reflex initiated by mechanical irritation of the carina is reduced in dogs with acrylamide neuropathy, which primarily affects thick myelinated fibres such as those of SARs (Hersch *et al.*, 1985).

It seems reasonable, from the information available, to hypothesise that RARs are the sensory nerve endings primarily responsible for evoking the cough reflex. Increased activity of RARs, as a result of chemical and/or mechanical stimulation, may explain the increased frequency of coughing seen in patients with airways disease. SARs and C-fibre endings on the other hand may provide the "fine-tuning" to this response. Indeed, changes in the frequency of SAR activity may influence the strength of the cough; the absence of SAR activity lessening the frequency and force of the coughing, whereas increased SAR activity may increase the frequency and force of coughing. Much of the discussion has centred on mechanisms by which cough is generated or augmented. However, it would also be reasonable to assume that a mechanism is present that may limit coughing, particularly if coughing is excessive. Such a possible mechanism may involve the activation of C-fibre endings. Stimulation of C-fibre endings may inhibit coughing in experimental animals (Tatar *et al.*, 1988; Jackson, 1989). Thus, even if the primary mechanism by which cough is evoked is by activation

of RARs, the resultant cough can be modified by the involvement of other airway afferent sensory nerve endings.

8.3 Afferent sensory nerve endings in diseased airways

Airway reflexes, such as cough and reflex bronchoconstriction, are often exaggerated and/or show an altered sensitivity in diseased airways. Viral infections, for example, can enhance the responsiveness to both bronchoconstrictor and tussive stimuli (Empey *et al.*, 1976). There is also an increased sensitivity of the cough reflex, to inhaled capsaicin, in patients with chronic cough (without any pathogenesis) (Hansson *et al.*, 1987), subjects with a dry cough (without expectorate) (Fuller & Choudry, 1988) and in patients who have cough in association with angiotensin converting enzyme (ACE) inhibitor therapy (Morice *et al.*, 1987; McEwan *et al.*, 1989). Increased sensitivity of the cough reflex could be explained by sensitisation of RARs by inflammatory mediators, analogous to the hyperalgesia seen in other inflamed tissues (Garland, 1984). There is evidence from both experimental animal and human studies to support this suggestion. Indeed, inhaled histamine, despite being able to stimulate both RARs and C-fibre endings, can only evoke reflex bronchoconstriction in anaesthetized, paralysed and artificially ventilated cats (a response which is mediated by a vagal pathway) after the airways have been exposed to ozone (Adcock, 1989). As discussed earlier, ozone may act directly to sensitise the airway nerve endings. Alternatively, it may be that ozone indirectly, through epithelial damage and the release of inflammatory mediators, increases the accessibility and/or sensitises the airway nerve endings to the effects of inhaled irritants and endogenous mediators (Chapter 7, Section 4). The suggestion that inflammatory mediators may be involved in the sensitisation process comes from a number of observations: Firstly, inflammatory mediators (for example, PGE_2, 15-HPETE, PAF, substance P) injected into the rat hind paw can cause both acute and sustained hyperalgesia (Adcock &

Garland, 1993). Acute and sustained hyperalgesia, for example with 15-HPETE, is not blocked by cyclooxygenase inhibitors or 5-lipoxygenase inhibitors, and is not accompanied by oedema or marked infiltration of polymorphonuclear leukocytes. It is possible, therefore, that 15-HPETE may act directly to decrease the stimulation threshold of sensory nerves. Indeed, after 14 daily injections of 15-HPETE (1 ng) into the rat hind paw, the basal activity of sensory C-fibres in the saphenous nerve was no different to controls that received sterile water. However, topical administration of mustard oil to the paw significantly increased impulse traffic in C-fibres from paws treated with 15-HPETE, but not in the controls (Follenfant *et al.*, 1990). Secondly, several studies have linked the use of ACE inhibitors in hypertension with a dry, non-productive cough (Semple & Herd, 1986; Coulter & Edwards, 1987). ACE, in addition to converting angiotensin I to angiotensin II, hydrolyses other substrates such bradykinin and substance P. It is therefore possible that the cough associated with ACE inhibitor therapy could be attributed to sensitisation of afferent sensory nerve endings by inflammatory mediators such as substance P and bradykinin. Thirdly, cough responses to inhaled capsaicin in normal subjects are potentiated after inhalation of $PGF_{2\alpha}$ and PGE_2 (Stone *et al.*, 1992).

The mechanism by which inflammatory mediators may sensitise airway nerve endings, however, is unclear. It is possible that sensitisation of the airway nerve endings may be mediated by specific pharmacological receptors located on the nerve endings. Although, sensitisation of nerve endings does not necessarily involve changes in the basal discharge of the nerve endings, much of the supporting evidence for pharmacological receptors being located on airway nerve endings has come from studies which have principally been aimed at determining whether RARs can be activated by inflammatory mediators independently of changes of pulmonary mechanics.

Investigations into whether RARs can be activated by inflammatory mediators have been complicated by the finding that most of the inflammatory mediators known to stimulate RARs, are themselves powerful constrictors of airway smooth muscle. Nonetheless, there is evidence in the present study and in the literature to suggest that RARs can be activated directly. In some experiments, inhaled PGE_2, at doses that did not cause bronchoconstriction, stimulated some of the RARs tested (present study). Furthermore, AH13205, an EP_2 receptor agonist, also stimulated a proportion of the RARs tested without affecting pulmonary mechanics (present study). Further supporting evidence for inflammatory mediators having a direct effect on RARs comes from the findings of Vidruk, *et al.*, (1977). These researchers showed that histamine, applied onto the receptive field of RARs via a bronchoscope, was still capable of activating the RARs even after administration of isoprenaline, a β-adrenoceptor agonist, which was used to block changes in pulmonary mechanics.

It is ironic that capsaicin, which until now has been principally regarded as being a "selective" stimulant of C-fibre endings, should be instrumental in determining whether RARs can be activated directly. In cats, inhaled capsaicin, as well as activating C-fibre endings and a proportion of RARs, evokes a bronchoconstriction which is mediated solely by a vagal cholinergic reflex mechanism, a response which can be completely abolished by either bilateral vagotomy or pre-treatment with atropine. Thus, by examining the effects of inhaled capsaicin on RARs, in cats which are either bilaterally vagotomised or pre-treated with atropine, experimental conditions are created in which it is possible to determine if RARs can be activated by a direct chemical stimulation. In the present study, inhaled capsaicin activated 4 out of the 11 RARs tested, in cats which were either bilaterally vagotomised or pre-treated with atropine.

Stimulation of RARs, by a range of inflammatory mediators and foreign materials, raises the possibility that there may be different pharmacological receptors on the nerve endings of RARs. Indeed, there is considerable evidence to suggest the existence of a capsaicin receptor (Holzer, 1991). Considering the excitatory effects of inhaled AH13205 and PGE_2 on RARs (present study), it is possible that the irritant properties of AH13205 and PGE_2 may be mediated by EP_2 receptors located on RAR nerve endings. The effects of histamine on RARs, total lung resistance and dynamic lung compliance can be blocked by chlorpheniramine, a H_1 receptor antagonist, but not by cimetidine, a H_2 receptor antagonist (Dixon *et al.*, 1979b). It is possible, therefore, that the direct effect of histamine on RARs (Vidruk *et al.*, 1977) may be mediated by H_1-receptors, located on RAR nerve endings. Considering that the nerve terminals of RARs, in the airway epithelium, are non-myelinated (Das *et al.*, 1978), the possibility that pharmacological receptors may be present on afferent sensory nerve endings should, therefore, not be restricted to RARs. Indeed, C-fibre endings are responsive to many of the stimuli that activate RARs (Coleridge & Coleridge, 1984, 1986).

Although, much of the discussion has centred on chemical stimuli that increase airway afferent sensory nerve activity, it is also possible that there may be pharmacological receptors located on these nerve endings which inhibit their activity. Indeed, inhibition of airway reflexes, mediated by stimulation of Aδ and C-fibre endings, by 443C81 (the peripherally acting μ-opioid receptor agonist) and the classical opiate, codeine, are likely to be mediated by opioid receptors located on these sensory nerve endings (Adcock, 1989).

The concept that pharmacological receptors may be located on sensory nerve endings (RARs and C-fibre endings) may be of considerable importance in pathological

conditions. Indeed, the production of inflammatory mediators and the release of neuropeptides, by acting on specific pharmacological receptors on sensory nerve endings, may stimulate and/or sensitise sensory nerve endings in the lung and thus contribute to the development of airway hyperreactivity. As discussed previously (Chapter 1, Section 4), hyperalgesia, as seen in other inflamed tissues, may be analogous to airway hyperreactivity (Adcock & Garland, 1993). Thus, it can be envisaged, that a vicious circle of hyperalgesia and sensory neurogenic activity may be associated with non-specific hyperreactivity of the inflamed lung (Fig. 8.1). However it should also be noted that agents which induce hyperalgesia may not necessarily be involved in the development of airway hyperreactivity. In the present study, glutamate, a neurotransmitter that provokes acute and sustained hyperalgesia in the rat hind paw model (Follenfant & Nakamura-Craig, 1992), did not appear to modulate the afferent arm of vagally-mediated airway reflexes and is, therefore, unlikely to have a role in the development of airway hyperreactivity.

If the above hypothesis is correct, then attenuation of hyperalgesia and sensory nerve function would be expected to reduce airway hyperreactivity. Of the drugs used in the present study, only 443C81, which has proven analgesic activity, has the ability to reduce airway afferent sensory nerve activity. However, in spite of the effectiveness of 443C81 in inhibiting airway reflexes in experimental animals (Adcock, 1989), clinical studies in man using 443C81 have not been as promising (Choudry *et al.*, 1990; Pavord *et al.*, 1991a). It should be noted, however, that although 443C81 did not appear to be effective in these clinical trials, the results also did not clearly demonstrate that 443C81 was unequivocally ineffective. This may have been due to a number of reasons, arguably because of study design. Alternatively, it may have been that 443C81, a peptide, was rapidly metabolised in the lung and/or that insufficient concentrations were being deposited for 443C81 to be therapeutically active.

Therefore, it remains that 443C81, or an agent with similar properties, may still represent effective agents to test the hypotheses that airway reflexes are important in airways disease.

8.4 Future studies

Further investigations to determine whether either RARs or C-fibre endings are exclusively responsible for evoking one particular airway reflex are limited until an agent is either discovered or developed which will specifically stimulate one group of airway sensory nerve endings but not the other. Alternatively, the question could be answered by the development of an agent that will specifically inhibit either RARs or C-fibre endings. Therefore, until an agent is found which, categorically either stimulates or inhibits either RARs or C-fibre endings alone, the debate into whether RARs or C-fibre endings are solely responsible for evoking one particular airway reflex, such as cough, will continue.

Clearly, the hypothesis that hyperalgesia may be analogous to airway hyperreactivity merits further investigation. Indeed, it would be reasonable to examine the effects of sub-threshold concentrations of inflammatory mediators, such as PGE_2 and 15-HPETE, on the activity of airway sensory nerves and also on vagally-mediated airway reflexes. Furthermore, the possibility that pharmacological receptors may be located on airway afferent sensory nerve endings also merits further study. Such studies should examine the effects of inflammatory mediators and also their antagonists, if available, when applied directly onto the receptive field of RARs and C-fibres, both *in vivo* and *in vitro*. Moreover, radioligand studies would also be necessary to verify the presence of pharmacological receptors on the airway nerve endings.

Although frusemide and lamotrigine, in the present study, failed to have any significant effect in attenuating airway afferent sensory nerve activity, other agents may be more rewarding.

Following acute administration, capsaicin provokes an initial stimulation which is followed by reversible anti-nociception (Hayes, 1984). Anti-nociceptive activity has also been demonstrated with capsaicin analogues (NE-19550 and NE-21610) (Campbell *et al.*, 1989; Holroyde *et al.*, 1989). Furthermore, cutaneous plasma extravasation in the rat, evoked by stimulation of the saphenous nerve, is inhibited by NE-21610 (Holroyde, *et al.*, 1989). Unlike capsaicin, these analogues desensitise sensory nerves without an initial stimulation which suggest that they may be useful agents to attenuate sensory nerve function.

Moguisteine ((R,S)-2-(2-methoxyphenoxy)-methyl-3-ethoxycarbonyl-acetyl-1,3-thiazolidine) has recently been described as a novel, non-narcotic anti-tussive agent (Gallico *et al.*, 1990). The anti-tussive activity of this compound is not due to activation of opioid receptors and appears to be through an effect on peripheral nerve fibres. Moguisteine also blocks hyperreactivity to acetylcholine, eosinophil infiltration, epithelial cell desquamation and plasma leakage induced by exposure to smoke. These actions suggest that moguisteine may act on sensory nerves in the airways, but electrophysiological evidence to support this has not yet been reported.

Compounds that prevent the activation of sensory nerves would also inhibit the release of sensory neuropeptides from local axon reflexes. Several agents have been described which inhibit the release of neurotransmitters, including neuropeptides, via an action on pre-junctional receptors (Adcock, & Garland, 1993). For example, binding studies show that histamine H_3-receptors appear to be present in low density

in lung tissue (Arrang *et al.*, 1987) and it has been shown that the H_3-receptor agonist (R)-α-methylhistamine inhibits the cholinergic component of vagally mediated contraction of isolated guinea-pig and human airways *in vitro* (Ichinose *et al.*, 1989; Ichinose & Barnes, 1989a). This agent also inhibits non-cholinergic bronchospasm and neurogenic plasma extravasation in guinea-pig airways (Ichinose & Barnes, 1989b, Ichinose *et al.*, 1990) and thus probably blocks the release of neuropeptides from NANC nerves. Whether such agents as H_3-receptor agonists would also prevent activation of the sensory nerves is not yet known.

There are a wide variety of pharmacological agents, including those mentioned above, which could be used to attenuate airway afferent sensory nerve function and thereby inhibit airway reflexes and neurogenic inflammation (Adcock & Garland, 1993). However, the use of such agents remains speculative until definite electrophysiological experiments are carried out on airway afferent sensory nerve endings.

8.5 Conclusions

1) There appears to be differences in the regional sensitivity of the human respiratory tract to inhaled capsaicin and PGE_2; the central airways being more responsive to PGE_2 whereas the peripheral airways are more responsive to capsaicin.

2) Capsaicin aerosols, at doses known to evoke reflex bronchoconstriction, activated a proportion of intrathoracic RARs in the cat independently of reflex bronchoconstriction.

3) Prostaglandin E_2 aerosols evoked a dose-related stimulation of intrathoracic RARs discharge and bronchoconstriction in the cat. In addition the EP_2 receptor agonist, AH13205, activated a proportion of the RARs tested without affecting pulmonary mechanics.

4) Frusemide, administered intravenously or as an inhaled aerosol, had no effect on the spontaneous discharge of intrathoracic RARs. Furthermore, it is unlikely that the protective effects of frusemide are mediated by a release of inhibitory prostanoids.

5) Glutamate does not seem to have any role in the development of hyperreactivity in the airways of the cat. In addition, lamotrigine, a glutamate release inhibitor, appears to have no therapeutic potential in the treatment of asthma.

6) RARs are likely to be the airway afferent sensory nerve endings primarily responsible for evoking the cough reflex. In addition, RARs may also be involved in evoking reflex bronchoconstriction.

7) Capsaicin and PGE_2, when administered by inhalation to the lower respiratory tract of cats, are not "selective" stimulants of either non-myelinated C-fibre endings or myelinated RARs and should, therefore, not be relied upon to investigate the airway reflexes evoked by one particular group of airway afferent sensory nerve ending. Indeed, studies in which these agents have been used to investigate the airway reflexes evoked by one particular group of airway sensory nerve ending, particularly in man, could result in misleading conclusions.

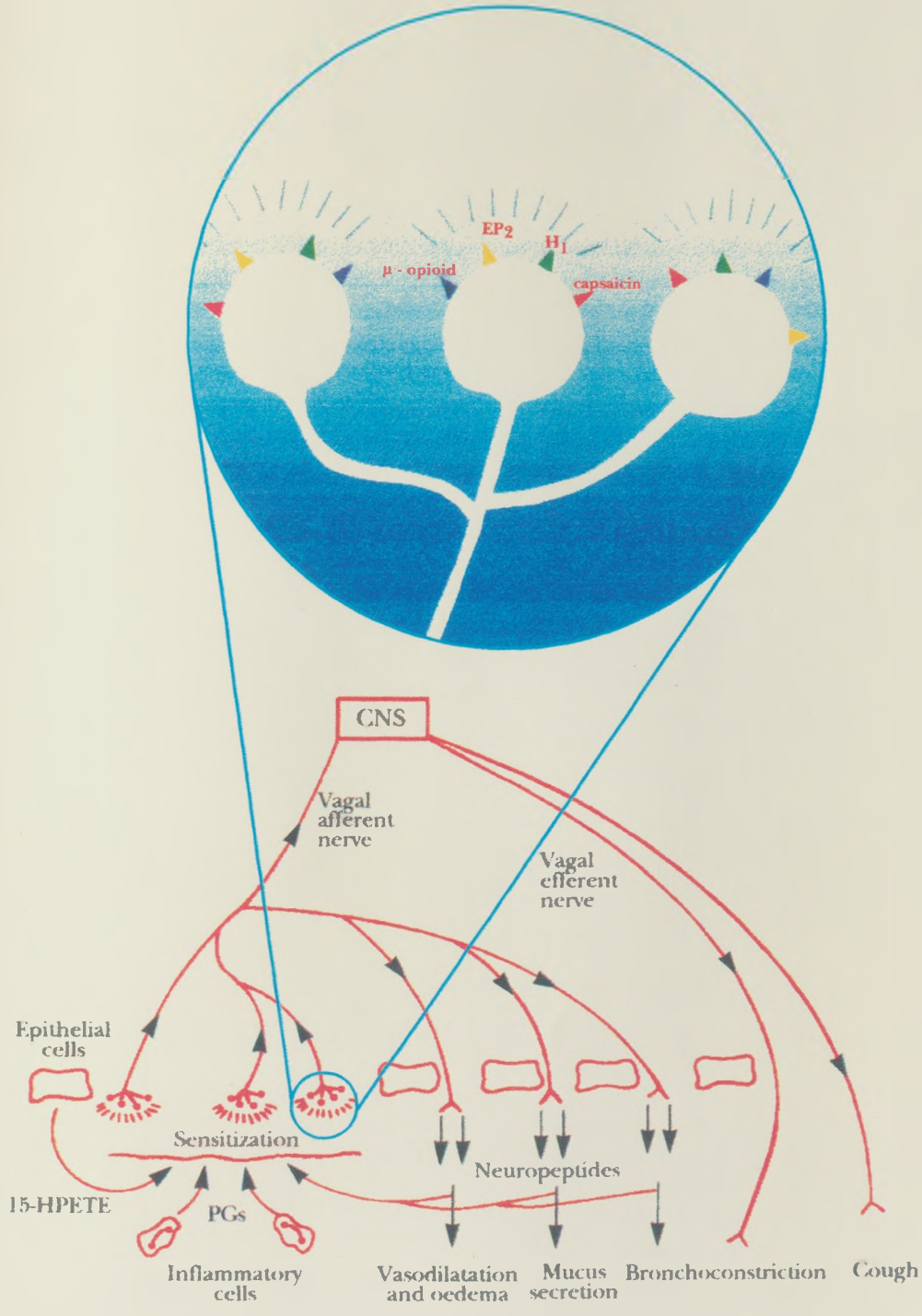


Fig. 8.1 *Main*: Schematic representation of the vicious cycle of hyperalgesia and sensory neurogenic activity that may be associated with non-specific hyperreactivity of the inflamed lung. Stimulation of airway afferent sensory nerve endings can evoke vagally mediated central reflexes and local axon reflexes. Inflammatory mediators, released from axon reflexes and inflammatory cells, may act on the airway sensory nerve endings to sensitise or stimulate them, resulting in a vicious cycle. *Insert*: shows the possibility that specific pharmacological receptors are located on airway afferent sensory nerve endings. (Modified from Adcock & Garland, 1993.)

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