J. Pharm. Pharmacol. 1989, 41: 316–321
Received July 4, 1988

Non-specific Hyperreactivity to Pharmacological Stimuli in Tracheal and Lung Parenchymal Strips of Actively Sensitized Guinea-pigs

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Abstract—The responsiveness of tracheal and lung parenchymal strips isolated from actively sensitized guinea-pigs to $CaCl_2$ (in K^+-depolarized tissue), KCl, acetylcholine and histamine was compared with that of strips from unsensitized animals. The concentration-response curves to the mentioned agonists exhibited, in the sensitized group, a left upward displacement (greater maximal effect, lesser effective concentration 50% and a steeper slope) compared with those obtained in the unsensitized group. These results indicate the existence of a non-specific increase in responsiveness in the airway smooth muscle from sensitized animals.

Airway hyperreactivity to physical, chemical, immunological and pharmacological stimuli is a characteristic feature of asthma (Boushey et al 1980). Focusing upon pharmacological agents, the heightened responsiveness to autonomic drugs (cholinomimetics) and endogenous autacoids (histamine, 5-hydroxytryptamine, bradykinin and prostaglandin $F_{2\alpha}$) is well documented in asthmatics (Boushey et al 1980).

The sensitization of laboratory animals, in particular of the guinea-pig, has become a widely adopted method used to create in-vivo and in-vitro models of allergic asthma that, as closely as possible, mimic the disease state in man (Giembycz & Rodger 1987).

The characteristics of the antigen-induced contraction of isolated airway smooth muscle, the mediators involved in its generation and its modification by a wide array of antagonists have been extensively studied in various animal species (Chand et al 1979; Yen & Kreutner 1980; Fleisch et al 1982; Burka 1983; Songsiridej et al 1983; Creese & Temple 1986a, b; Mansour & Daniel 1986). By contrast, there are fewer studies comparing the response to a certain agonist in normal vs sensitized animals (Brink et al 1981; Souhrada & Souhrada 1981; Turner et al 1983; Nakagoshi et al 1987) and very few with a systematic approach, considering the main physiological and pharmacological agents under the same experimental conditions (Saad & Burka 1983; Morcillo et al 1984; Mansour & Daniel 1987).

The present work was designed to see if trachea and lung parenchyma isolated from actively sensitized guinea-pigs exhibit hyperresponsiveness to four distinct agonists: $CaCl_2$ (in K^+-depolarized tissue), KCl, acetylcholine and histamine.

Materials and Methods

Isolated preparations and sensitization procedure

Male guinea-pigs (300–400 g) were randomly allocated to one of two groups, control (non-sensitized) and sensitized. The sensitization procedure was: on day 0 the animals were injected subcutaneously with 0·25 mL of Freund's complete adjuvant plus 1·25 $\mu g\ g^{-1}$ body weight of bovine serum albumin (BSA) dissolved in 0·25 mL saline, on day 2 and day 4 the animals received the same amount of Freund's complete adjuvant and BSA by the intramuscular route. The animals were used for experiments on days 21 to 25. The control group was subjected to the same protocol but received only saline. The guinea-pigs were transported from the animal house to the laboratory at least two weeks before entering the sensitization or sham procedures, kept in individual cages under controlled conditions of light and temperature and with supplementary amount of ascorbic acid included in their diet, according to Hitchcock (1980), until killed.

Each animal was killed by stunning and exsanguination, the thorax opened and its contents rapidly transferred to a Petri dish with oxygenated physiological salt solution (PSS). The trachea was excised, cleaned of adhering tissues, opened by cutting longitudinally through the cartilage rings diametrially opposite the trachealis and divided into 4 mm wide segments. Parenchymal strips, 20 mm in length with 3×3 mm cross section, were trimmed from the pleural edge of the right and left lower lobes of the lung. The tissue strips were mounted in jacketed 20 mL organ baths containing a PSS of the following composition (mM): NaCl 118·4, KCl 4·7, $NaHCO_3$ 25·0, $CaCl_2$ 2·5, $MgSO_4$ 0·6, KH_2PO_4 1·2, dextrose 11·1, maintained at 37°C and continuously gassed with 5% CO_2 in oxygen (pH $7{\cdot}4 \pm 0{\cdot}1$). An isometric recording was obtained with force transducers (Hewlett-Packard FTA 100–1 or Grass FT 0·3 C) connected through amplifiers to a polygraph. Baths and tranducer holders were wall-mounted to minimize mechanical disturbances which may interfere with the recording of the small changes in force generated by the strips.

The strips were gently stretched, by using a precision micrometer, up to 1 g of initial isometric force and a 90 min equilibration period was permitted with changes of the PSS at 15 min intervals before any pharmacological intervention. During this period the tracheal strips normally tended to increase in tone whereas the parenchymal strips relaxed slightly, but care was taken to maintain the resting tension constant until the end of the equilibration period. The

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loading tension of 1 g imposed on the preparation was found by preliminary experiment to allow optimal responses to agonists.

Concentration-response curves to agonists

The agonists were added to the bath in a cumulative fashion to obtain concentration-response curves (CRC). Only one complete CRC to a given agonist was obtained from each strip. In those experiments in which $CaCl_2$ was the contractile agonist the following protocol was used. The strip was initially set up in Tris-buffer solution of this composition (mM): NaCl 134·8, KCl 4·7, $CaCl_2$ 2·5, $MgSO_4$ 1·2, Tris 5·0, HCl 5·0, dextrose 10·1 (pH 7·4). After 60 min of equilibration, this medium was changed for a Ca^{2+}-free (neither Ca^{2+} nor a Ca^{2+} chelating agent was present) Tris solution where the strips remained for 30 min. Then, this medium was replaced by a K^+-rich (55 mM KCl in equimolar replacement for NaCl), Ca^{2+}-free Tris solution and after an equilibration period of 30 min, $CaCl_2$ was added cumulatively to the bath. Tris-buffer solution was aerated with pure oxygen and allowed the construction of complete (up to 100 mM) CRC to $CaCl_2$. In previous experiments it was found that CRC to $CaCl_2$ obtained in depolarizing, Ca^{2+}-free, Tris solution did not significantly differ from those constructed in depolarizing, Ca^{2+}-free, PSS.

Responses to BSA

The strips from actively sensitized guinea-pigs were tested, once the experiment was terminated, with BSA 1 mg mL^{-1}, to confirm the existence of an antigen-induced contraction. This was also done in the strips from non-sensitized animals to check the lack of responsiveness. All tracheal strips from sensitized animals reacted with a rapidly developing and sustained contraction which reached a peak of 174 ± 44 mg mg^{-1} ($n = 33$). All lung parenchymal strips from sensitized guinea-pigs reacted with a rapidly developing contraction which reached a peak of 101 ± 27 mg mm^{-2} ($n = 24$) usually decaying to a plateau representing 30 to 40% of the peak.

Calculation of results and data analysis

The changes in force resulting from drug addition were determined directly from the recordings and transformed into force (mg)/tissue dry weight (mg) for the tracheal strips, or force (mg)/cross-sectional area (mm^2) for the lung parenchymal strips. The cross-sectional area was determined by dividing the tissue wet weight by the tissue length which was measured at the end of the equilibration period by means of a graduated magnifying lens ($\pm 0{\cdot}1$ mm). Immediately after completion of the pharmacomechanical studies, the strips were removed from the bath, blotted and the wet and dry (24 h at 64°C) weights of the sample obtained on a precision balance ($\pm 0{\cdot}1$ mg).

The pD_2 values were defined as the negative logarithm (base 10) of the EC50 values which were interpolated from individual CRC. EC50 is the drug concentration causing 50% of maximal effect. The slope of the steepest portion of CRC was measured and expressed as % per log unit (Foster et al 1984). Data are presented as mean ± s.e.m. and were analysed for statistical significance by the use of Student's *t*-test for unpaired data. The difference between groups was considered significant when $P < 0{\cdot}05$.

Drugs and solutions

Drug concentrations are expressed as final molar (M) bath concentrations of the active species. The following chemicals were used: acetylcholine chloride (Sigma), bovine serum albumin (Sigma), Freund's complete adjuvant (Difco) and histamine dihydrochloride (Sigma).

Stock solutions were prepared in twice-distilled water just before experimentation and serially diluted as necessary. Drug solutions were kept on ice and added to the organ bath in small volumes (0·1 mL).

Results

In the non-sensitized group of animals, addition to the bath of cumulative concentrations of $CaCl_2$ (acting on K^+-de polarized tissue), KCl, acetylcholine and histamine resulted in concentration-related contraction of tracheal and lung parenchymal strips (Fig. 1). The parameters of the CRC to these agonists are shown in Table 1. When the responses to these agonists were determined in strips from sensitized animals, it was found that their CRC exhibited a left upward displacement and a steeper slope compared with those obtained in the unsensitized group (Fig. 1) which translates

Table 1. Characteristics of concentration-response curves to $CaCl_2$ (in K^+-depolarized tissues), KCl, acetylcholine (ACh) and histamine (HA) in tracheal and lung parenchymal strips obtained from control (non-sensitized) and actively sensitized guinea-pigs.

	Non-sensitized			Sensitized		
Trachea	E_{max}	pD_2	S	E_{max}	pD_2	S
$CaCl_2$	171 ± 39	$2{\cdot}64 \pm 0{\cdot}05$	$63{\cdot}2 \pm 4{\cdot}6$	356 ± 38*	$2{\cdot}79 \pm 0{\cdot}05$*	$114{\cdot}0 \pm 5{\cdot}6$*
KCl	212 ± 30	$1{\cdot}79 \pm 0{\cdot}02$	$61{\cdot}6 \pm 1{\cdot}6$	541 ± 36*	$1{\cdot}87 \pm 0{\cdot}02$*	$132{\cdot}8 \pm 7{\cdot}7$*
ACh	337 ± 70	$5{\cdot}02 \pm 0{\cdot}11$	$44{\cdot}0 \pm 6{\cdot}7$	649 ± 99*	$5{\cdot}45 \pm 0{\cdot}13$*	$77{\cdot}4 \pm 9{\cdot}2$*
HA	363 ± 31	$4{\cdot}85 \pm 0{\cdot}06$	$57{\cdot}2 \pm 6{\cdot}1$	488 ± 47*	$6{\cdot}06 \pm 0{\cdot}16$*	$71{\cdot}4 \pm 5{\cdot}3$*
Parenchyma						
$CaCl_2$	82 ± 6	$2{\cdot}63 \pm 0{\cdot}07$	$49{\cdot}2 \pm 3{\cdot}2$	174 ± 11*	$2{\cdot}79 \pm 0{\cdot}05$*	$100{\cdot}8 \pm 4{\cdot}3$*
KCl	140 ± 10	$1{\cdot}71 \pm 0{\cdot}03$	$91{\cdot}2 \pm 2{\cdot}7$	230 ± 17*	$1{\cdot}82 \pm 0{\cdot}03$*	$135{\cdot}4 \pm 3{\cdot}0$*
ACh	87 ± 5	$4{\cdot}75 \pm 0{\cdot}14$	$51{\cdot}0 \pm 4{\cdot}8$	168 ± 8*	$5{\cdot}79 \pm 0{\cdot}17$*	$71{\cdot}8 \pm 3{\cdot}4$*
HA	136 ± 14	$5{\cdot}37 \pm 0{\cdot}09$	$72{\cdot}5 \pm 5{\cdot}4$	229 ± 29*	$5{\cdot}55 \pm 0{\cdot}07$*	$122{\cdot}4 \pm 8{\cdot}1$*

The number of experiments was 12 in each group; E_{max} is the maximal effect of the agonist expressed as mg mg^{-1} (trachea) or mg mm^{-2} (parenchyma): pD_2 is $-\log$ EC50: S indicates the slope of steepest portion of curve (% per log unit); data are mean ± s.e.m. All values in the sensitized group were found to be significantly different (* is $P < 0{\cdot}05$) from those in the non-sensitized group.

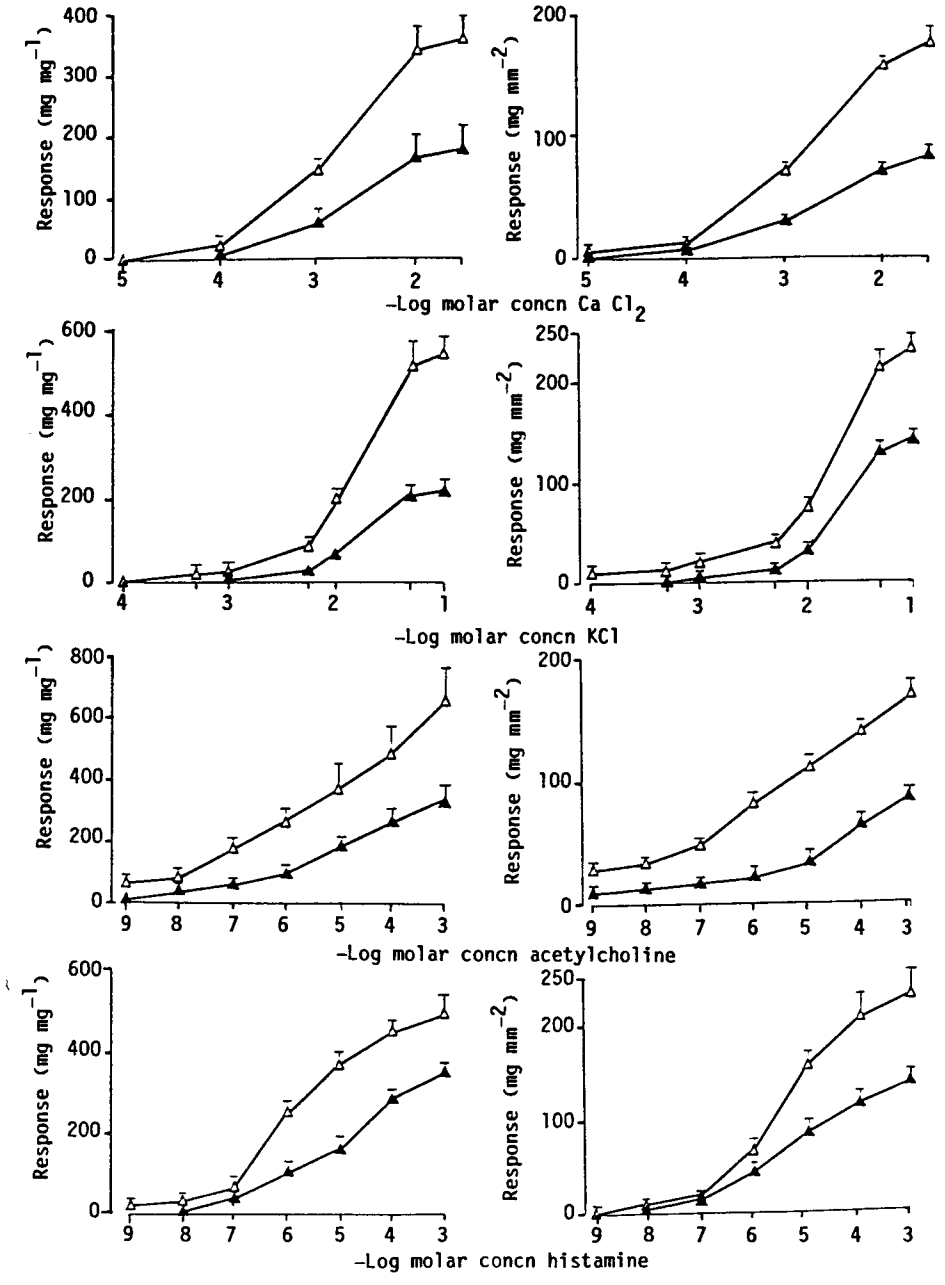

FIG. 1. Concentration-response curves for contraction of tracheal (left panels) and lung parenchymal (right panels) strips by $CaCl_2$ (in K^+-depolarized tissues), KCl, acetylcholine and histamine in control (▲) and sensitized (△) guinea-pigs.

into the differences of the parameters of CRC shown in Table 1.

This kind of displacement of the CRC for the four agonists used in this study indicates the existence of a non-specific hyperresponsiveness in the preparations obtained from the sensitized guinea-pigs. To further analyse this finding, the two components of the shift of the CRC, i.e. the horizontal and vertical shift, have been measured according to the procedure of Kalsner (1974) which gives the results of Table 2.

Discussion

The results from this study show the existence of an increase in responsiveness to $CaCl_2$ (in K^+-depolarised tissue), KCl, acetylcholine and histamine in tracheal and lung parenchymal strips obtained from actively sensitized guinea-pigs. This finding confirms a previous report from this laboratory using various pharmacological stimuli in lung parenchymal strips (Morcillo et al 1984) and extends the observation to other agents and to the central airways of the same animal species.

The agonists used in the present study were selected to be representative of two, theoretically possible, agonistical procedures (Giembycz & Rodger 1987): (i) opening of potential sensitive calcium channels (PSC) by K^+-induced membrane depolarization leading to the influx of extracellular Ca^{2+} present in the medium (electromechanical coupling)

Table 2. Analysis of the sensitization-induced displacement of the concentration-response curves to $CaCl_2$ (in K^+-depolarized tissue). KCl, acetylcholine (ACh) and histamine (HA) in tracheal and lung parenchymal strips isolated from control and actively sensitized guinea-pigs.

	Horizontal shift			Vertical shift			
	Dose-ratio at fixed percentages of the maximal response (control/sensitized)			Effect-ratio at fixed concentrations producing a determined percentage of the control maximal response (sensitized/control)			
	25%	50%	75%	25%	50%	75%	100%
Trachea							
$CaCl_2$	1·74	1·41	1·13	2·79	2·33	2·17	2·07
KCl	1·22	1·20	1·11	2·98	2·71	2·41	2·46
ACh	9·65	2·37	0·82	2·90	2·14	1·90	1·93
HA	3·81	16·09	7·74	2·67	2·13	1·60	1·36
Parenchyma							
$CaCl_2$	1·26	1·44	1·30	2·34	2·24	2·21	2·10
KCl	1·55	1·19	1·03	2·40	1·86	1·71	1·64
ACh	15·62	10·94	2·94	3·77	2·72	2·14	1·95
HA	1·06	1·51	1·48	1·57	1·88	1·86	1·69

or exogenously supplied (Ca^{2+}-induced contraction), and (ii) activation of receptor-operated ion channels (ROC) or of release mechanisms from intracellular calcium stores following occupation of cell-surface receptors (muscarinic, H_1) by their specific agonists (acetylcholine, histamine), with or without simultaneous depolarization permitting extracellular Ca^{2+} entry and/or promoting intracellular Ca^{2+} release (pharmacomechanical coupling). The situation in the airway smooth muscle has not yet been fully elucidated. The existence of PSC in trachealis seems well established whereas there is little experimental evidence for the presence of ROC (Small & Foster 1986; Giembycz & Rodger 1987).

Nevertheless, the contractile responses elicited by the four distinct agonists used in the present study were all augmented in the sensitized specimens compared with the non-sensitized. This, together with previous data (Morcillo et al 1984), indicates the existence of a non-specific increase in responsiveness, with CRC to the agonists lying on the left upper side of their controls. The analysis of this displacement, according to Kalsner (1974), shows that while the degree of horizontal shift is variable, depending on the level of the response at which it was evaluated, the degree of vertical shift remains reasonably constant at the four levels of response considered. If a pure horizontal shift (equal dose-ratio at all response levels) is known as type I (Kalsner 1974), prejunctional (Fleming et al 1973) or deviation (Westfall 1981) supersensitivity and a pure vertical shift as type II, postjunctional or non-deviation supersensitivity, the results from this study indicate that the type of hyperresponsiveness observed fits better with a type II supersensitivity mechanism, having a component of type I. It is known that both types coexist in various experimental models (Westfall 1981).

The airway response of asthmatics is characterized both by a leftward shift of CRC and by an increased maximal response (steeper slope) to various agonists (Orehek et al 1977; Woolcock et al 1984), i.e. a non-specific hyperreactivity that is also compatible with a mixed type of supersensitivity. Many factors could contribute in-vivo to produce hyperreactivity (Boushey et al 1980; Morley 1982; Moreno et al 1986) and among them, in accord with the presence of a postjunctional supersensitivity, an alteration in the intrinsic properties of airway smooth muscle has been postulated.

However, attempts to demonstrate hyperresponsiveness in-vitro of airway smooth muscle obtained from patients with hyperreactivity in-vivo have failed (Roberts et al 1983; Vincenc et al 1983; Armour et al 1984; Taylor et al 1985), with few exceptions (Schellenberg & Foster 1985), although conclusions from these experiments should be accepted with caution because of the methodological difficulties inherent to the use of human samples (Moreno et al 1986; Mansour & Daniel 1987).

Unfortunately, studies using sensitized animals have reported conflicting results. Thus, Krell et al (1976), Brink et al (1981), Saad & Burka (1983), Turner et al (1983) and Mansour & Daniel (1987) found no increase of responsiveness or sensitivity to agonists in sensitized tissues which is in contrast with the results from the present study. This discrepancy may be explicable in a number of ways such as the different animal species or strain used, the sensitization procedure (differences in the antigen and coadjuvant used, route of administration, time elapsed between sensitization and death of the animal, resensitization, challenge with antigen before testing the agonists), the additional supply of ascorbic acid in the diet of animals (Hitchcock 1980), the tissue preparation (type of strip mounted in the organ bath, presence or absence of intact epithelium, size of loading tension imposed to the tissue), and the type of recording (isometric vs isotonic, etc.) Notwithstanding, other authors (Popa et al 1973; Weiss & Viswanath 1979; Kleinstiver & Eyre 1980; Souhrada & Souhrada 1981; Iwayama et al 1982; Rubinfeld et al 1982; Morcillo et al 1984) have found increased airway responses to various agonists in sensitized animals.

The characteristics of the hyperresponsiveness observed in the present study closely resemble those reported for the reserpine-induced supersensitivity in rabbit aorta (Kalsner 1974) which has been associated with an enhanced ability of the tissue to retain and utilize calcium (Carrier & Hester 1976; Krishnamurty & Mukherjee 1981). Results with $CaCl_2$ and KCl in this study may be interpreted as due to more extracellular Ca^{2+} being able to enter the cell and/or more PSC opening after depolarization by K^+. Studies in trachea isolated from actively sensitized guinea-pig have demonstrated an increased sensitivity to extracellular Ca^{2+} (Weiss & Viswanath 1979; Martorana & Rodger 1981; Dhillon & Rodger 1981). Studies with ^{45}Ca show that although basal

Ca^{2+} uptake is not modified (Reaburn et al 1987), the K^+-stimulated Ca^{2+} uptake was enhanced in sensitized tissues (unpublished observation).

On the other hand, acetylcholine and histamine, and also in part KCl, promote mobilization of Ca^{2+} of intracellular origin (Giembycz & Rodger 1987) and, therefore, their enhanced contractile responses in sensitized tissues may be due to augmented intracellular Ca^{2+} release. An additional piece of evidence in favour of this is that caffeine, a drug producing intracellular Ca^{2+} release, elicits a contraction in guinea-pig trachealis under certain experimental conditions (Small et al 1988) which is increased in sensitized tissues (Ortiz et al 1988). The efficacy of trifluoperazine, an intracellular calcium antagonist, is augmented in lung parenchymal strips from sensitized guinea-pigs (Perpiñá et al 1987; Sanz et al 1988). Finally, an alteration of the contractile proteins in the sensitized state should not be discarded although preliminary evidence from this laboratory shows equal responses to calcium in skinned trachea (Cortijo et al 1987) from sensitized and control guinea-pigs.

The biochemical basis of these alterations in the characteristics of the membrane of airway smooth muscle cells and in the intracellular Ca^{2+} movements remain uncertain (Nath et al 1983; Souhrada & Souhrada 1984; Weiss & Bellino 1985) but warrants further research since a future therapeutic approach to asthma may be the finding of more selective intracellular calcium antagonists.

Acknowledgements

The authors wish to express their gratitude to Mr A. Crespo and Mr P. Santamaria for technical assistance. This project was supported by a grant from Fondo de Investigaciones Sanitarias (87/1376) of Ministerio de Sanidad and Comisión Asesora de Investigación Científica y Técnica (PB85-0228 and PB86-0428) of Ministerio de Educación y Ciencia (Spain).

References

Armour, C. L., Lazar, N. M., Schellenberg, R. R., Taylor, S. M., Crane, N., Hogg, J. C., Pare, P. D. (1984) A comparison of *in vivo* and *in vitro* human airway reactivity to histamine. Am. Rev. Respir. Dis. 129: 907–910

Boushey, H. A., Holtzman, M. J., Sheller, J. R., Nadel, J. A. (1980) Bronchial hyperreactivity. Ibid. 121: 389–413

Brink, C., Duncan, P. G., Douglas, J. S. (1981) The response and sensitivity to histamine of respiratory tissues from normal and ovalbumin-sensitized guinea-pigs: Effects of cyclooxygenase and lipoxygenase inhibition. J. Pharmacol. Exp. Ther. 217: 592–601

Burka, J. F. (1983) Effects of selected bronchodilators on antigen- and A23187-induced contraction of guinea-pig trachea. Ibid. 225: 427–435

Carrier, J., Hester, R. K. (1976) The relationship between calcium and increased sensitivity of rabbit aorta four hours after reserpine. Br. J. Pharmacol. 56: 449–455

Chand, N., Deroth, L., Eyre, P. (1979) Pharmacology of Schultz-Dale reaction in canine lung strip *in vitro*: Possible model for allergic asthma. Ibid. 66: 511–516

Cortijo, J., Dixon, J. S., Foster, R. W., Small, R. C. (1987) Influence of some variables in the Triton X-100 method of skinning the plasmalemmal membrane from guinea-pig trachealis muscle. J. Pharmacol. Meth. 18: 253–266

Creese, B. R., Temple, D. M. (1986a) Anaphylactic responses of guinea-pig lung parenchymal strips: Antigen concentration dependence and pharmacological modification. Arch. Int. Pharmacodyn. 281: 252–264

Creese, B. R., Temple, D. M. (1986b) The mediators of allergic contraction of human airway smooth muscle: a comparison of bronchial and lung parenchymal strip preparations. Clin. Exp. Pharmacol. Physiol. 13: 103–111

Dhillon, D. S., Rodger, I. W. (1981) Hyperreactivity of guinea-pig isolated airway smooth muscle. Brit. J. Pharmacol. 74: 180P–181P

Fleisch, J. H., Haisch, K. D., Spaethe, S. M. (1982) Slow reacting substance of anaphylaxis (SRS-A) release from guinea-pig lung parenchyma during antigen- or ionophore-induced contraction. J. Pharmacol. Exp. Ther. 221: 146–151

Fleming, W. W., McPhillips, J. J., Westfall, D. P. (1973) Postjunctional supersensitivity and subsensitivity of excitable tissues to drugs. Ergeb. Physiol. 68: 55–119

Foster, R. W., Okpalugo, B. I., Small, R. C., (1984) Antagonism of Ca^{2+} and other actions of verapamil in guinea-pig isolated trachealis. Br. J. Pharmacol. 81: 499–507

Giembycz, M. A., Rodger, I. W. (1987) Electrophysiological and other aspects of excitation-contraction coupling and uncoupling in mammalian airway smooth muscle. Life Sci. 41: 111–132

Hitchcock, M. (1980) Effect of variation in endogenous levels of ascorbic acid on the *in vitro* immunological release of histamine and slow reacting substance of anaphylaxis from actively sensitized guinea-pig lung fragments. Br. J. Pharmacol. 71: 539–543

Iwayama, Y., Chung, C. Z., Takayangi, I. (1982) Effects of antiasthmathic drugs on airway resistance and plasma level of cyclic AMP in guinea-pig. Jpn. J. Pharmacol. 32: 329–334

Kalsner, S. (1974) A new approach to the measurement and classification of forms of supersensitivity of autonomic effector responses. Br. J. Pharmacol. 51: 427–434

Kleinstiver, P. W., Eyre, P. (1980) The lung parenchymal strip preparation of the cat and dog. Responses to anaphylactic mediators and sympathetic bronchodilators. Res. Comm. Chem. Phathol. Pharmac. 27: 451–467

Krell, R. D., Chakrin, L. W., Christian, P. (1976) Canine airway responses to acetylcholine, prostaglandin $F_{2\alpha}$, histamine and serotinin after chronic antigen exposure. J. Allergy Clin. Immunol. 58: 664–675

Krishnamurty, V. S. R., Mukherjee, A. (1981) Effect of reserpine on Mg^{2+}-induced calcium fluxes and reactivity of the rat aorta. Arch. Int. Pharmacodyn. 251: 180–191

Mansour, S., Daniel, E. E. (1986) Mechanisms of response to antigen in isolated guinea-pig trachea after active sensitization. Can. J. Physiol. Pharmacol. 64: 1566–1571

Mansour, S., Daniel, E. E. (1987) Responsiveness of isolated tracheal smooth muscle from normal and sensitized guinea-pigs. Ibid. 65: 1942–1950.

Martorana, M. G., Rodger, I. W. (1981) Effects of Ca^{2+} withdrawal and verapamil on excitation-contraction coupling in sensitized guinea-pig airway smooth muscle. Br. J. Pharmacol. 72: 175P–176P

Morcillo, E., Perpiñá, M., Esplugues, J. (1984) Hyperresponsiveness to autacoids and autonomic drugs in lung parenchymal strips from sensitized guinea-pigs. Am. Rev. Respir. Dis. 129: 948–951

Moreno, R. H., Hogg, J. C., Paré, P. D. (1986) Mechanics of airway narrowing. Ibid. 133: 1171–1180

Morley, J. (ed) (1982) Bronchial hyperreactivity. Academic Press, London.

Nakagoshi, A., Koike, K., Takayanagi, I. (1987) Characteristics of beta-adrenoceptors in tracheal smooth muscle of guinea-pig sensitized by egg albumin. Can. J. Physiol. Pharmacol. 65: 2163–2167

Nath, P., Joshi, A. P., Agrawal, K. P. (1983) Biochemical correlates of airway hyperreactivity in guinea-pigs: role of lysophosphatidylcholine. J. Allergy Clin. Immunol. 72: 351–358

Orehek, J., Gayrard, P., Smith, A. P., Grimaud, C., Charpin, J. (1977) Airway response to carbachol in normal and asthmatic subjects. Am. Rev. Respir. Dis. 115: 937–943

Ortiz, J. L., Sanz, C., Cortijo, J., Perpiñá, M., Palau, M., Sarriá, B., Morcillo, E., Esplugues, J. (1988) Differences between calcium antagonists to inhibit caffeine-induced contraction of tracheal smooth muscle in control and sensitized guinea-pigs. Ibid. 137 (4): 180 (abstract)

Perpiñá, M., Cortijo, J., Sanz, C., Esplugues, J., Morcillo, E. (1987) Active sensitization dicriminates between groups of calcium

antagonists in lung parenchyma strips. Bull. Eur. Physiopathol. Respir. 23: 255–260

Popa, V., Douglas, I. S., Bouhuys, A. (1973) Airway responses to histamine, acetylcholine and propranolol in anaphylactic hypersensitivity in guinea-pigs. J. Allergy Clin. Immunol. 51: 344–356

Reaburn, D., Hay, D. W. P., Fedan, J. S. (1987) Calcium uptake into guinea-pig trachealis: the effect of epithelium removal. Cell. Calcium 8: 429–436

Roberts, J. A., Reaburn, D., Rodger, I. W., Thompson, N. C. (1983) Relationship between *in vivo* airway responsiveness and *in vitro* muscle sensitivity to methacholine in man. Thorax 38: 705–706

Rubinfeld, A. R., Rinard, G. A., Mayer, S. E. (1982) Responsiveness of isolated tracheal smooth muscle in a canine model asthma. Lung 160: 99–107

Saad, M. H., Burka, J. F. (1983) Effect of immunological sensitization on the responses and sensitivity of guinea-pig airways to bronchoconstrictors, modulation by selective inhibition of arachidonic acid metabolism. Can. J. Physiol. Pharmacol. 61: 876–887

Sanz, C., Cortijo, J., Perpiñá, M., Esplugues, J., Morcillo, E. J. (1988) Different ability of trifluoperazine to inhibit agonist-induced contraction of lung parenchyma strips from control and sensitized guinea-pigs. J. Pharm. Pharmacol. 40: 120–125

Schellenberg, R. R., Foster, A. (1985) *In vitro* responses of human asthmatic airway and pulmonary vascular smooth muscle. Int. Arch. Allergy Appl. Immunol. 75: 237–241

Small, R. C., Foster, R. W. (1986) Airways smooth muscle: An overview of morphology, electrophysiology and aspects of the pharmacology of contraction and relaxation, in: Kay, A. B. (ed) Asthma: Clinical Pharmacology and Therapeutic Progress, Blackwell Scientific, Oxford. pp. 101–113

Small, R. C., Boyle, J. P., Cortijo, J., Davies, J. M., Foster, R. W., Hofer, P. (1988) The relaxant and spasmogenic effects of some xanthine derivatives acting on guinea-pig isolated trachealis muscle. Br. J. Pharmacol. 94: 1091–1100

Songsiridej, V., Busse, W. W., Buckner, C. K. (1983) Pharmacological alteration of antigen-induced contraction of lung parenchymal strips isolated from actively sensitized guinea-pig. Eur. J. Pharmacol. 92: 215–222

Souhrada, M., Souhrada, J. F. (1981) Reassessment of electrophysiological and contractile characteristics of sensitized airway smooth muscle. Respir. Physiol. 46: 17–27

Souhrada, M., Souhrada, J. F. (1984) Immunologically induced alterations of airway smooth muscle cell membrane. Science 225: 723–725

Taylor, S. M., Pare, P. D., Armour, C. L., Hogg, J. C., Schellenberg, R. R. (1985) Airway reactivity in chronic obstructive pulmonary disease. Failure of *in vivo* methacholine responsiveness to correlate with cholinergic, adrenergic or non-adrenergic responses *in vitro*. Am. Rev. Respir. Dis. 132: 30–35

Turner, A. J., Seale, J. P., Shaw, J. (1983) Antigen sensitization does not alter response of guinea-pig lung strips to noradrenaline. Eur. J. Pharmacol. 87: 141–144

Vincenc, K. S., Black, J. S., Yan, K., Armour, C. L., Donnelly, D. D., Woolcock, A. J. (1983) Comparison of *in vivo* and *in vitro* responses to histamine in human airways. Am. Rev. Respir. Dis. 128: 875–879

Weiss, E. B., Viswanath, S. G. (1979) Calcium hypersensitivity in airway smooth muscle. Respiration 38: 266–272

Weiss, E. B., Bellino, I. R. (1985) Interaction of leukotriene D_4 superoxide anion induce airways hyperreactivity. Am. Rev. Respir. Dis. 131: A2 (Abstract)

Westfall, D. P. (1981) Supersensitivity of smooth muscle, in: Bulbring, E., Brading, A. F., Jones, A. W., Tomita, T. (eds) Smooth muscle: an assessment of current knowledge, University of Texas Press, Austin, pp. 285–309

Woolcock, A. J., Salome, C. M., Yan, K. (1984) The shape of the dose-response curve to histamine in asthmatic and normal subjects. Am. Rev. Respir. Dis. 130: 71–75

Yen, S. S., Kreutner, W. (1980) Effect of inhibition of arachidonic acid metabolism on anaphylaxis of guinea-pig lung strips. Agents Actions 10: 274–278