Br. J. Pharmac. (1980), **68**, 467–472

ATROPINE INHIBITS THROMBOXANE A_2 GENERATION IN ISOLATED LUNGS OF THE GUINEA-PIG

F. BERTI, G.C. FOLCO, A. GIACHETTI, S. MALANDRINO, C. OMINI & T. VIGANÒ

Institute of Pharmacology and Pharmacognosy, School of Pharmacy, University of Milan, 20129 Milan, Italy

1 Histamine (0.5 to 5 μg) and slow reacting substance of anaphylaxis (SRS-A, 0.05 to 0.3 u), injected in the isolated, perfused lungs of normal and ovalbumin-sensitized guinea-pigs, promote formation and release of thromboxane A_2(TXA_2) and other arachidonate metabolites, the effect being more pronounced in sensitized lungs.

2 Carbachol injected (1 to 10 μg) or perfused (1 μg ml^{-1} min^{-1}) through normal or sensitized lungs does not elicit formation of TXA_2 and prostaglandins. Furthermore the increased generation of arachidonate metabolites due to histamine is not altered by carbachol.

3 Atropine and ipratropium bromide (1 μg ml^{-1} min^{-1}) reduce significantly the increased rate of production of TXA_2 caused by histamine and SRS-A both in normal and sensitized lungs, whereas hexamethonium (10 to 25 μg ml^{-1} min^{-1}) is ineffective.

4 The mechanism of action of atropine in inhibiting the increased generation of TXA_2 is clearly not related to its antimuscarinic or antihistaminic properties. The drug might act at the early events, involved in the activation of arachidonic acid metabolism. The results suggest new sites of action for atropine which, besides the control of the vagal bronchomotor tone, interferes directly with the primary mediators of anaphylaxis.

Introduction

The efficacy of atropine in alleviating either human asthma (Yu, Galant & Gold, 1972) or experimental anaphylaxis (Gold, Kessler & Yu, 1972) has been attributed entirely to blockade of the cholinoceptor which is reflexly activated by stimulation of epithelial irritant receptors localized in the airways. This interpretation of atropine's activity does not take into consideration that tissues from asthmatic patients and sensitized animals, particularly lungs, bronchi and leukocytes, release on exposure to specific antigen, large amounts of local mediators (Brocklehurst, 1960; Lichtenstein, 1971) which are more powerful than acetylcholine in eliciting bronchoconstriction.

Recently it has become clear that the primary mediators of anaphylaxis, namely histamine and slow reacting substance of anaphylaxis (SRS-A), released by the activated cells of the sensitized lungs, promote a rapid formation of thromboxane A_2 (TXA_2) along with other arachidonate metabolites (Piper & Vane, 1969; Engineer, Morris, Piper & Sirois, 1978). Indeed it appears that histamine and SRS-A owe part of their bronchoconstrictor effect to the accelerated synthesis of TXA_2, one of the most potent contractile agents on smooth muscles of vascular and respiratory systems (Gryglewski, Dembinska-Kiec & Grodzinska, 1977; Seale & Piper, 1978).

Therefore whilst the anti-asthmatic action of atropine is generally considered to be due to its anti-cholinoceptor activity, the possibility that the release of cyclo-oxygenase products may be involved in the pathogenesis of asthma has been investigated.

Methods

Guinea-pigs (male 300 to 400 g) were used in this study. Sensitization of animals was performed with ovalbumin according to Engineer, Piper & Sirois (1976). The lungs were removed and perfused with Krebs-bicarbonate solution (10 ml/min, 37°C) as described by Piper & Vane (1969). The pulmonary outflow was used to superfuse a cascade of isolated tissues including spirally cut strips of a rabbit coeliac (or mesenteric) artery (RbCA, RbMA), rabbit aorta (RbA), rabbit stomach (circular fibres) strip (RbSS) and rat stomach (RSS) or rat colon strips (RC) in order to detect stable and unstable arachidonate metabolites. The bioassay tissues were treated with a

0007-1188/80/030467-06 $01.00

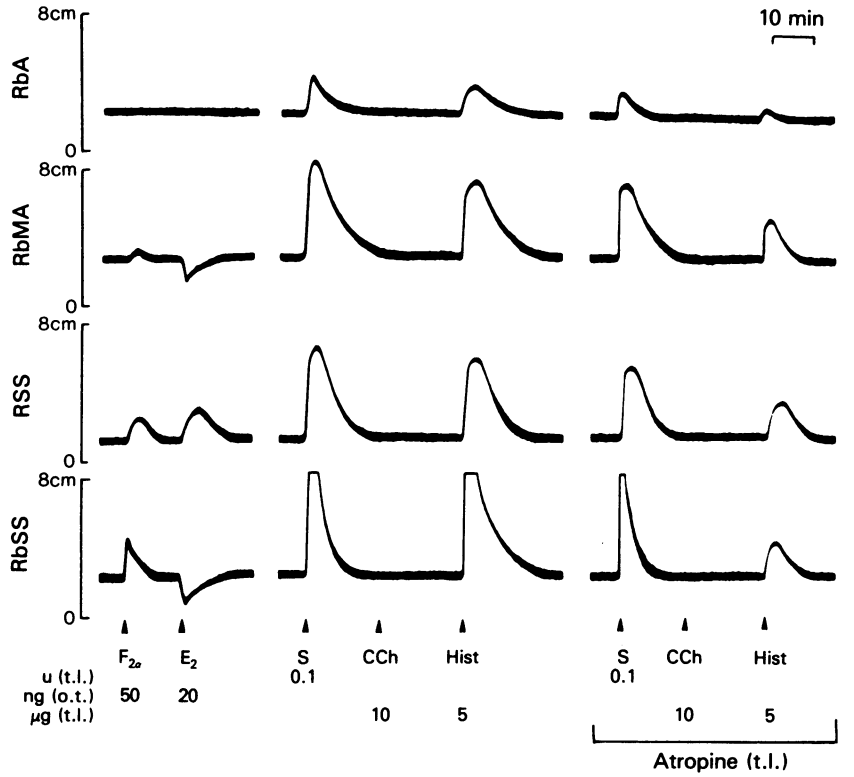

Figure 1 Effect of atropine on the generation of prostaglandin-like substances and thromboxane A_2 in normal perfused lungs of the guinea-pig. Lung outflow superfused a bank of assay tissues consisting of: rabbit aorta (RbA), rabbit mesenteric artery (RbMA), rat stomach strip (RSS), and rabbit stomach strip (RbSS). The following agonists were injected as a bolus through the lungs (t.l.): Slow reacting substance of anaphylaxis (S) 0.1 u; carbachol (CCh) 10 μg; histamine (Hist) 5 μg. Atropine was perfused t.l. at a concentration of 1 μg ml^{-1} min^{-1}. Prostaglandin $F_{2\alpha}$ ($F_{2\alpha}$, 50 ng) and prostaglandin E_2 (E_2, 20 ng) were injected directly over the tissues (o.t.) in cascade.

mixture of antagonists as suggested by Gilmore, Vane & Wyllie (1968) and with indomethacin (1 μg ml^{-1} min^{-1}) in order to increase the sensitivity of the tissues and to prevent endogenous prostaglandin generation. Changes of the tone of the tissues in cascade were measured with isotonic transducers connected to a multichannel recorder (Watanabe-Mark III).

Unsensitized and sensitized lungs were challenged with histamine and SRS-A obtained from the peritoneal cavity of rats previously sensitized to rat anti-ovalbumin, following the procedure described by Orange, Murphy, Karnovsky & Austen (1973).

The supernatant of rat peritoneal fluid was pooled, mixed with 4 volumes of absolute ethanol, sedimented at 40,000 *g* for 30 min, decanted and evaporated at 60°C. The dried ethanol extract was stored at −80°C.

One unit of SRS-A activity was arbitrarily defined as the concentration required to produce a contraction of the guinea-pig isolated ileum (treated with atropine and mepyramine) equal to 5 ng of histamine (Stechschulte, Austen & Bloch, 1967).

Up to 0.5 u of SRS-A prepared in our laboratory were tested as direct standard agonists over the tissue in cascade, but no biological activity was observed.

The amount of TXA_2 present in the pulmonary effluent was evaluated by the radioimmunoassay method described by Granström, Kindahl & Samuelsson (1976). The lung perfusates were collected for 30 s after injection or perfusion of the lungs with the different agonists and antagonists. During this time, biological activity was monitored by the tissues in cascade.

Experimental data were processed according to the method of factorial analysis of variance for completely randomized design with two factors at two levels. Multiple comparison according to Duncan (1955) was also performed.

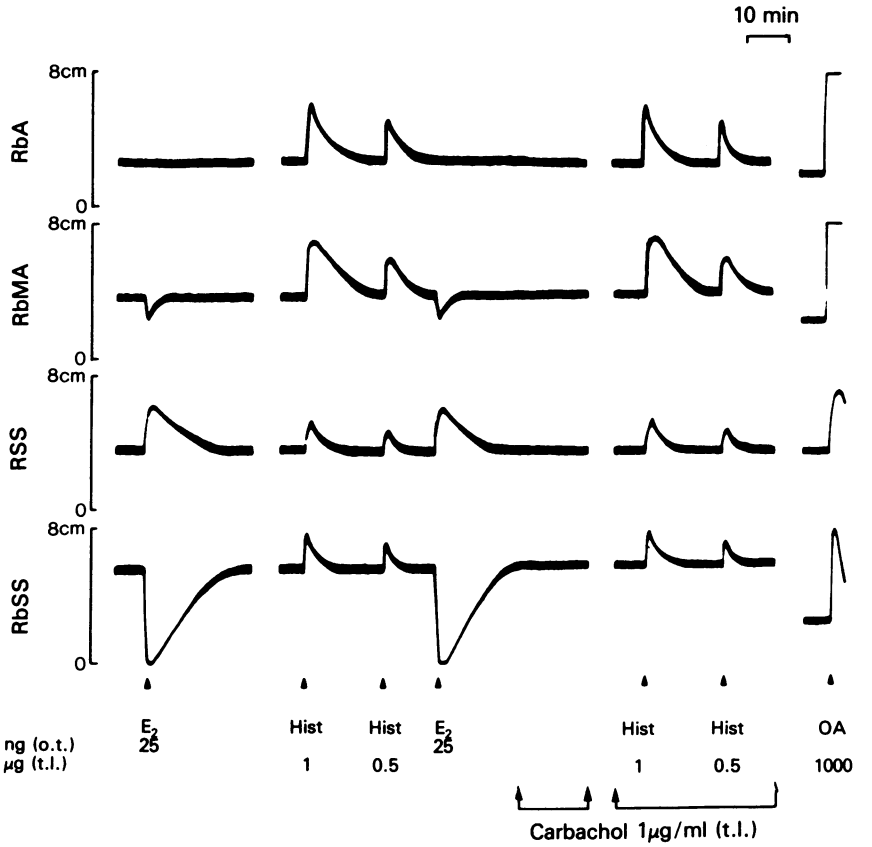

Figure 2 Effect of carbachol on the generation of prostaglandin-like substances and thromboxane A_2 in ovalbumin-sensitized lung of the guinea-pig. The bank of tissues in cascade was as described in Figure 1. Lungs were challenged with histamine (Hist) 0.5 and 1 μg. Ovalbumin (OA) was injected through lungs (t.l.) (1000 μg) at the end of the experiment to check the sensitization of the tissue. Prostaglandin E_2 (E_2, 25 ng) was injected directly over the tissue (o.t.) in cascade..

Drugs

The following compounds were used: histamine dihydrochloride (Carlo Erba), acetylcholine bromide (Sigma), carbamylcholine chloride (Sigma), neostigmine bromide (Sigma), hexamethonium bromide (Sigma), atropine sulphate (Sigma), ipratropium bromide (Valeas), hyoscine hydrobromide (BDH), pyrilamine dihydrochloride (M & B), methysergide hydrogen maleate (Sandoz), Propranolol hydrochloride (ICI), phenoxybenzamine hydrochloride (SKF), indomethacin (MSD), ovalbumin grade 3 (Sigma).

Results

The injection of histamine (0.5 to 5 μg) or SRS-A (0.05 to 0.3 u) in the isolated lungs of unsensitized or ovalbumin sensitized guinea-pigs, promoted a dose-dependent formation and release of arachidonate metabolites, which was monitored by the bank of tissues in cascade. The lung perfusates induced contraction of aorta, mesenteric (or coeliac) artery and stomach strip of the rabbit as well as of the stomach strip of the rat indicating the generation of TXA_2 and prostaglandins (Figures 1, 2 and 3).

Pretreatment of the lungs with eicosatetraynoic acid (ECTA) (1 μg ml^{-1} min^{-1}) inhibited formation of arachidonic acid metabolites and no contraction of the bioassay tissues was observed following injection of histamine or SRS-A.

When carbachol was injected (1 to 10 μg) or perfused (1 μg ml^{-1} min^{-1}) through normal or sensitized lungs, the bioassay tissues did not change their

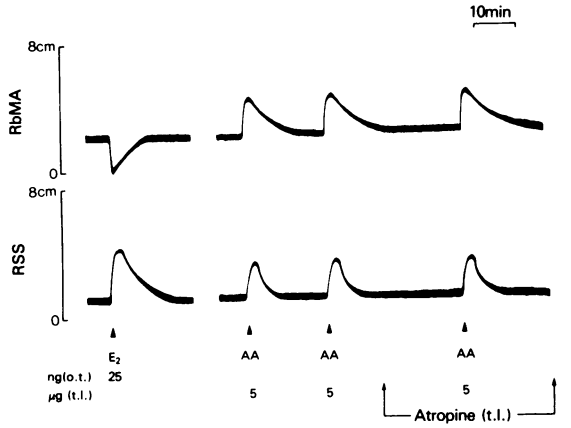

Figure 3 Effect of atropine on the capacity of the isolated lungs of the guinea-pig to metabolize arachidonic acid into prostaglandin-like substances and Thromboxane A_2. Lung outflow superfused a bank of tissues consisting of: rabbit mesenteric artery (RbMA) and rat stomach strip (RSS). Arachidonic acid (AA, 5 μg) was injected through lungs (t.l.) before and during perfusion with atropine (5 μg ml^{-1} min^{-1}). Prostaglandin E_2 (E_2, 20 ng) was injected directly over the tissues (o.t.) in cascade.

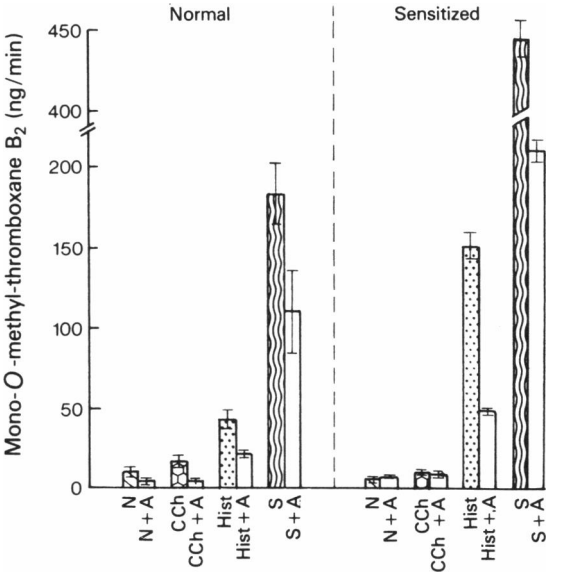

Figure 4 Effect of atropine (A) on thromboxane A_2 (TXA_2) generation (measured as mono-*O*-methyl TXB_2) induced by carbachol (CCh), histamine (Hist) and slow reacting substance of anaphylaxis (S) in perfused lungs of normal and ovalbumin-sensitized guinea-pigs. Columns represent the amount (ng/min) of TXA_2 in 5 ml of perfusate 30 s after the bolus injection of the various challengers. Carbachol (10 μg), histamine (1 μg) and SRS-A (0.1 u) were injected separately and after 15 min perfusion of the lungs with atropine (1 μg ml^{-1} min^{-1}) N denotes control lungs and each bar represents the s.e. mean of six experiments. Duncan test gives the following results for the comparison between each two means: Normal and sensitized; N vs CCh = not significant ($P > 0.5$); N vs Hist = highly significant ($P < 0.01$); N vs S = highly significant ($P < 0.01$); Hist vs Hist + A = highly significant ($P < 0.01$); S vs S + A = highly significant ($P < 0.01$); Normal Hist vs sensitized Hist = highly significant ($P < 0.01$); Normal S vs sensitized S = highly significant ($P < 0.01$).

tone indicating the absence of TXA_2 or other arachidonate metabolites in the pulmonary outflow (Figures 1 and 2).

Atropine perfused at a concentration of 1 μg ml^{-1} min^{-1} reduced the ability of histamine and SRS-A to release TXA_2 and other prostaglandins from the lungs. The drug appeared more effective in inhibiting the release induced by histamine than that of SRS-A (Figure 1). A synthetic anticholinoceptor compound, ipratropium bromide (1 μg ml^{-1} min^{-1}) was as effective as atropine in normal and sensitized lungs, while the ganglion blocker hexamethonium (10 to 25 μg ml^{-1} min^{-1}) was completely ineffective.

The conversion of arachidonic acid in the lungs to prostaglandin-like substances and TXA_2 was not affected by atropine.

When arachidonic acid (5 μg) was injected through the lungs during perfusion with atropine (5 μg ml^{-1} min^{-1}), the pulmonary outflow induced contraction of the bioassay tissues (Figure 3).

The presence of TXA_2 in lung perfusate of normal and sensitized animals has also been quantitatively measured by radioimmunoassay procedure. The results of these experiments, which were in agreement with those obtained with the bioassay, are illustrated in Figure 4.

The basal release of TXA_2 (measured as mono-*O*-methyl-TXB_2) by normal and sensitized lungs was in the range of 6.5 to 10 ng/min and the difference between the two experimental conditions was not significant. Histamine (1 μg) increased the formation of TXA_2 in both normal and sensitized lungs. In normal lungs this agent augmented the production of TXA_2 4 times (from 10 to 43 ng/min) whereas, in sensitized tissues, the increase was 25 times above the control levels (from 6.4 to 152 ng/min).

SRS-A (0.1 u) induced a massive generation of TXA_2 in normal (184 ng/min) and in sensitized lungs (445 ng/min), the difference between the two groups being highly significant.

The injection of carbachol (10 μg) failed to alter the formation of TXA_2 both in normal and sensitized lungs, and did not reverse the effect of atropine.

Although atropine (1 μg ml^{-1} min^{-1}) did not change the basal rate of formation of TXA_2 of normal and sensitized lungs, it modified profoundly the ability of both histamine and SRS-A to generate TXA_2 in this organ (Figure 3). The reduction of TXA_2 formation caused by atropine was in the order of 50% in tissues exposed to histamine and SRS-A, except for sensitized lungs challenged with histamine, where the inhibition was in the order of 70%.

Discussion

Previous papers from our laboratory (Berti, Folco, Nicosia, Omini & Pasargiklian, 1979) and others (Piper & Vane, 1969; Dawson, Boot, Cockerill, Mallen & Osborne, 1976) have described the capacity of primary mediators of anaphylaxis, histamine and SRS-A, to promote formation and release of TXA_2 and prostaglandins from normal and sensitized lungs of guinea-pig. This release, which is significantly increased in sensitized lungs, seems to be rather specific as it does not correlate with changes of the tone of bronchial smooth muscles and other contractile proteins present in alveolar interstitial cells of lung parenchyma (Kapanci and Gabbiani, 1976). In fact, in our experiments, carbachol or neostigmine (not shown) did not induce generation of arachidonate metabolites in spite of their contractile activity. Although the immunological release of histamine and SRS-A from human lung tissue can be enhanced by stimulation with acetylcholine-like agents (Kaliner, Orange & Austen, 1972), the secondary formation of TXA_2 and other prostaglandins from guinea-pig lungs does not involve an activation of muscarinic receptors. The possibility that the effect of histamine in TXA_2 generation might be mediated through stimulation of nicotinic receptors in ganglia can also be excluded because hexamethonium does not modify histamine activity. The finding that atropine and ipratropium bromide reduce the generation of TXA_2 and prostaglandin-like substances adds a new dimension to the bronchodilator and anti-asthmatic activity of anticholinoceptor compounds and raises the question of their mechanism of action.

In our experimental conditions, the observed inhibition by atropine, is clearly not related to its antimuscarinic properties. It could be attributed to the antihistaminic activity of this compound (Innes & Nickerson, 1975); however, the concentrations of atropine used in these experiments were devoid of anti-histaminic activity when tested in guinea-pig lung-strips and tracheal-chain preparations. Furthermore, in agreement with Vane (1973) we found that atropine did not interfere with cyclo-oxygenase activity since a normal conversion of arachidonic acid to TXA_2 took place in atropine-treated guinea-pig lungs.

It is only possible to speculate on the mechanism of action of atropine in decreasing lung TXA_2 at the moment: the drug could diminish the leakage of phospholipase A_2 from lung lysosomes or could interfere with the action of this enzyme which cleaves arachidonic acid from membrane phospholipids. Another possibility is that atropine could induce biosynthesis of a phospholipase A_2 inhibitor which prevents TXA_2 and prostaglandin generation. Such a possibility has already been proposed by Flower & Blackwell (1979) for the mechanism of action of anti-inflammatory steroids. Whatever the mechanism, our results provide evidence for a new site of action for atropine, which interferes directly with the primary mediators of anaphylaxis.

Besides the control of the increased vagal bronchomotor tone, the clinical usefulness of atropine and related compounds in human asthma should also be considered in view of their ability to inhibit generation of TXA_2 and other arachidonate metabolites in the lung during anaphylaxis.

The authors are indebted to Mr G. Rossoni and Mr G. Brunelli for their invaluable technical assistance. Thanks are due to Dr L. Campio, Upjohn S.p.A., Italy for prostaglandins and to Dr E. Granström and Dr H. Kindhal, Karolinska Institutet, Stockholm, Sweden, for a generous gift of antibody.

References

Berti, F., Folco, G.C., Nicosia, S., Omini, C. & Pasargiklian, R. (1979). The role of histamine H_1 and H_2 receptors in the generation of thromboxane A_2 in the perfused guinea-pig lungs. *Br. J. Pharmac.*, **65**, 629–633.

Brocklehurst, W.E. (1960). The release of histamine and formation of a slow-reacting substance (SRS-A) during anaphylactic shock. *J. Physiol.*, **151**, 416–435.

Dawson, W., Boot, J.R., Cockerill, A.F., Mallen, D.N.B. & Osborne, D.J. (1976). Release of novel prostaglandins and thromboxanes after immunological challenge of guinea-pig lung. *Nature, Lond.*, **262**, 699–702.

Duncan, D.B. (1955). Multiple range and multiple F. tests. *Biometrics*, **II**, 1–42.

ENGINEER, D.M., MORRIS, H.R., PIPER, P.J. & SIROIS, P. (1978). The release of prostaglandins and thromboxanes from guinea-pig lung by slow-reacting substance of anaphylaxis, and its inhibition. *Br. J. Pharmac.*, **64**, 211–218.

ENGINEER, D.M., PIPER, P.J. & SIROIS, P. (1976). Interaction between the release of SRS-A and of prostaglandins. *Br. J. Pharmac.*, **57**, 460–461P.

FLOWER, R.J. & BLACKWELL, G.J. (1979). Antiinflammatory steroids induce a biosynthesis of a phospholipase A_2 inhibitor which prevents prostaglandin generation. *Nature*, **278**, 456–459.

GILMORE, N., VANE, J. R. & WYLLIE, J.H. (1968). Prostaglandins released by the spleen. *Nature*, **218**, 1135–1140.

GRANSTRÖM, E., KINDAHL, H. & SAMUELSSON, B. (1976). A method for measuring the unstable thromboxane A_2: radioimmunoassay of the derived mono-*O*-methyl-thromboxane B_2. *Prostaglandins*, **12**, 929–941.

GRYGLEWSKI, R.J., DEMBINSKA-KIEC, A. & GRODZINSKA, L. (1977). Generation of prostaglandin and thromboxane-like substances by large airways and lung parenchima. In *Prostaglandins and Thromboxane*, ed. Berti, F., Samuelsson, B. & Velo, G.P. pp. 165–178. New York: Plenum Press.

GOLD, W.M., KESSLER, G.F. & YU, D.Y.C. (1972). Role of vagus nerves in experimental asthma in allergic dogs. *J. appl. Physiol.*, **33**, 719–725.

INNES, I.R. and NICKERSON, M. (1975). Atropine, scopolamine and related antimuscarinic drugs. In *The Pharmacological Basis of Therapeutics*. ed. Goodman, L.S. & Gilman, A. pp. 514–532. New York: MacMillan Publishing Co., Inc.

KALINER, M., ORANGE, R.P. & AUSTEN, F.K. (1972). Immunological release of histamine and slow-reacting substance of anaphylaxis from human lung. *J. exp. Med.*, **136**, 556–567.

KAPANCI, Y. & GABBIANI, G. (1976). Location and function of contractile interstitial cells of the lung. *Pneumologie*, **153**, 141.

LICHTENSTEIN, L.M. (1971). The immediate allergic response: *in vitro* separation of antigen activation, decay and histamine release. *J. Immun.*, **107**, 1122–1130.

ORANGE, R.P., MURPHY, R.C., KARNOVSKY, M.L. & AUSTEN, F.K. (1973). The physicochemical characteristics and purification of slow-reacting substance of anaphylaxis. *J. Immun.*, **110**, 760–770.

PIPER, P.J. & VANE, J.R. (1969), Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. *Nature, Lond.*, **223**, 29–35.

SEALE, J.P. & PIPER, P.J. (1978). Stimulation of arachidonic acid metabolism by human slow-reacting substance. *Eur. J. Pharmac.*, **52**, 125–128.

STECHSCHULTE, D.J., AUSTEN, K.F. & BLOCH, K.J. (1967). Antibodies involved in antigen-induced release of Slow Reacting Substance of Anaphylaxis (SRS-A) in the guinea-pig and rat. *J. exp. Med.*, **125**, 127–147.

VANE, J.R. (1973). Prostaglandins and aspirin-like drugs. In *Proceedings of the Fifth International Congress of Pharmacology, San Francisco*, 1972, Pharmacology and the future of man. pp. 352–378. Basel: Karger.

YU, D.Y.C., GALANT, S.P. & GOLD, W.M. (1972). Inhibition of antigen-induced bronchoconstriction by atropine in asthmatic patients. *J. appl. Physiol.*, **32**, 823–828.

(Received February 16, 1979.
Revised June 4, 1979.)