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MEDIATORS OF PASSIVE LUNG ANAPHYLAXIS IN THE RAT

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1 Passive lung anaphylaxis (PLA) was investigated in rats sensitized by the intravenous injection of high titre reaginic antiserum prepared in rats.

2 The effect of various pharmacological antagonists on anaphylactic bronchoconstriction *in vivo* were examined. An antihistamine (mepyramine), a kallikrein inactivator (aprotinin) or a prostaglandin synthesis inhibitor (aspirin) did not inhibit PLA, whereas an anti-5-hydroxytryptamine agent (methysergide) and an anti-slow reacting substance-A agent (FPL 55712) significantly reduced the response.

3 Isolated perfused lungs taken from sensitized rats released, on challenge with the sensitizing antigen, histamine, 5-hydroxytryptamine, slow reacting substance of anaphylaxis (SRS-A) and prostaglandins, but no rabbit aorta contracting substance (RCS).

4 Disodium cromoglycate inhibited both anaphylactic bronchoconstriction *in vivo* and the anaphylactic release of mediators *in vitro*. Inhibition *in vivo* was dose-related.

5 Mediators from the intestine, the primary shock organ of anaphylaxis in the rat, did not contribute to the lung response.

6 Vagal reflex pathways were found not to be important in PLA *in vivo*.

7 The relationship between the mediators released following antigen challenge of passively sensitized rat lung *in vitro* and passive lung anaphylaxis *in vivo* is discussed.

Introduction

One of the tests which has been widely used in the search for new compounds effective in the treatment of bronchial asthma, is passive cutaneous anaphylaxis (PCA) in the rat induced by homologous reagin (IgE)-like antibodies (Goose & Blair, 1969). These antibodies have properties (e.g. they are heat labile, non-precipitating and act with long latency) similar to human reagins which are associated with immediate hypersensitivity in man. No study has been reported of anaphylactic bronchoconstriction in rats passively sensitized with rat IgE. A study of this reaction with regard to mediator release and activity of the anti-asthma agent, disodium cromoglycate, both *in vitro* and *in vivo* has therefore been made. A preliminary report of this work was communicated to a Joint Meeting of the British Pharmacological Society and the Deutsche Pharmakologische Gessellschaft (Farmer, Richards, Sheard & Woods, 1973).

Methods

Passive sensitization

Female Sprague Dawley rats weighing 200-250 g were sensitized by the injection of conalbumin 5 mg/kg intramuscularly and 0.5 ml *Bordetella pertussis* vaccine 2×10^{10} organisms intraperitoneally, on day 0. On day 10 the animals were injected with 4×10^{3} larvae *Nippostrongylus brasiliensis* subcutaneously to potentiate the production of antibodies to conalbumin (Orr & Blair, 1969). On day 24 or 25, the blood was collected by cardiac puncture under anaesthesia and the antisera collected, pooled and stored at -20°C. Groups of 100 or 200 rats were used to provide a large bulk of antiserum. The titre (or highest effective dilution which gave a mean 72 h PCA response of 5 mm in diameter in a rat) of the antiserum used was 1:512. Wistar rats were passively sensitized by intravenous injection of 0.5-1 ml of this high titre antiserum.

Passive lung anaphylaxis in vivo

Twenty-four hours after sensitization, rats were anaesthetized with intraperitoneal pentobarbitone sodium 80-100 mg/kg, a dose sufficient to abolish spontaneous respiration. The tail vein of each rat was cannulated with a hypodermic needle cut from its mount and inserted in narrow polythene tubing to provide a flexible connection to a

syringe for intravenous injection. Airways resistance was then measured by the overflow technique of Konzett & Rossler (1940) as modified by Burden, Parkes & Gardiner (1971). The trachea was cannulated and the animal ventilated by means of a Starling miniature respiration pump at a rate of 72 strokes/minute. Inflation pressure was kept constant at 9 cm water by means of a water valve. The air overflow from the water valve passed through a pneumotachograph tube connected to a differential air pressure transducer. Changes in overflow were displayed on a Devices recorder. The animals were challenged with egg albumin 25 mg/kg. Anaphylactic bronchoconstriction was recorded as % of the maximal overflow (obtained by clamping off the trachea). The inhibitory effect of antagonists was given by the formula:

$$\frac{\%\text{ bronchoconstriction in control group} - \%\text{ bronchoconstriction in test group}}{\%\text{ bronchoconstriction in control group}} \times 100.$$

Evisceration of rats

The abdomen was opened, the viscera exposed and 3 branches of the superior mesenteric artery (ileo-colic, colic and pancreatic-duodenal) tied off. The intestines were then removed from just below the stomach to the anus. In 'sham' operations, the intestines and blood vessels were left intact.

Passive lung anaphylaxis in vitro

Rats were killed 24 h after sensitization and the lungs removed and perfused via the pulmonary artery with Krebs solution gassed with 95% O_2 and 5% CO_2, as described by Piper & Vane (1969) for guinea-pig lungs. The Krebs solution had the following composition (mM): NaCl 118, $MgSO_4 7H_2O$ 1.17, $NaHCO_3$ 25.0, glucose 5.6, KCl 4.7, $CaCl_2 6H_2O$ 2.5, KH_2PO_4 1.2. The effluent was superfused over six tissues at 6 ml/min using a Watson-Marlow flow inducer. The tissues were suspended in polycarbonate chambers and arranged in two banks of three. The actions of acetylcholine, histamine, 5-hydroxytryptamine (5-HT) and catecholamines were eliminated, except where indicated, with a combination of antagonists (referred to as combined antagonists), consisting of atropine sulphate 0.1 μg/ml, mepyramine maleate 0.1 μg/ml, methysergide bimaleate 0.1 μg/ml, phenoxybenzamine hydrochloride 0.1 μg/ml and propranolol hydrochloride 2 μg/ml. These antagonists were infused at 0.1 ml/min into the superfusing fluid so that the antagonists reached some or all of the assay tissues.

Histamine was assayed on the cat terminal ileum treated with the combined antagonists excluding mepyramine and methysergide.

Several tissues were tested for sensitivity to 5-HT. In preliminary experiments rat stomach strips were used, but it proved difficult to separate the actions of 5-HT and prostaglandins. The most useful tissue for 5-HT assay was the rabbit aorta. After it had been verified that no rabbit aorta contracting substance (RCS) was released from sensitized rat lungs by antigen challenge, as found by Piper & Walker (1973) in actively sensitized rats, the rabbit aorta was used regularly for the detection of 5-HT. Higher sensitivity was found in spirally cut strips taken from young female rabbits. The superfusion fluid for this tissue contained the combined antagonists excluding methysergide.

A guinea-pig ileum treated with the combined antagonists was used to detect slow-reacting substance (SRS)-A. Standard responses were obtained with crude SRS-A prepared from guinea-pig lungs.

Prostaglandins were assayed on the rat stomach strip, chick rectum and rat colon treated with the combined antagonists (Piper & Vane, 1969).

Standard solutions of agonists were injected either in known volumes (1 ml or less as a bolus injection) before the roller pump to avoid any injection artefacts, or by slow infusion (0.1 ml/min) for 1 or 3 min over the assay tissues. Bolus injection was the most useful procedure when a series of bench samples were to be assayed, while slow infusion produced tissue responses which more closely resembled those obtained after challenge of sensitized lungs suspended above the assay tissues. The isolated lungs were challenged with 400 μg of 5 x crystallized egg albumin solution.

Drugs and reagents

The drugs and other agents used were histamine acid phosphate (BDH), 5-hydroxytryptamine sulphate (BDH), bradykinin (Sandoz), prostaglandin $F_{2\alpha}$ (Upjohn), prostaglandin E_2 (Cambrian Chemicals), methysergide bimaleate (Sandoz), mepyramine maleate (May & Baker), aprotinin (Trasylol, Bayer), disodium cromoglycate (Fisons), FPL 55712 (Fisons), atropine sulphate (Sigma), phenoxybenzamine hydrochloride (ICI), propranolol hydrochloride (ICI), sodium pentobarbitone (May & Baker), acetylsalicylic acid (BDH), egg albumin (BDH), conalbumin Type II (Sigma), 5 x crystallized egg albumin (Koch-Light) and *B. pertussis* vaccine (Burroughs Wellcome).

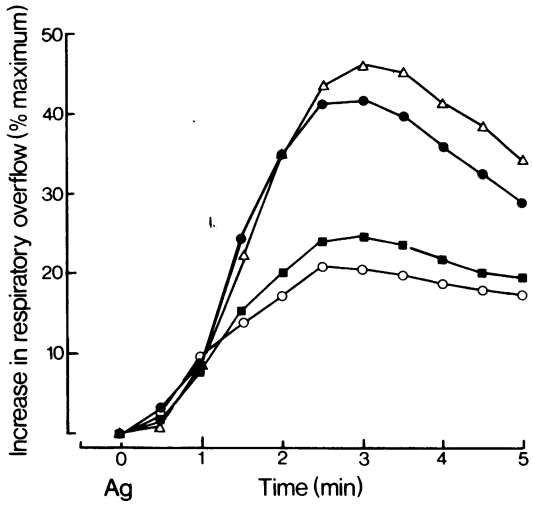

Figure 1 Time course of anaphylactic bronchoconstriction in passively sensitized rats. (•) Control response; (△) response in animals pretreated with mepyramine (10 mg/kg, i.p. 30 min before antigen); (▪) methysergide (4 mg/kg, i.v. 15 min before antigen) or (○) FPL 55712 (10 mg/kg, i.v. immediately before antigen). (n = 9).

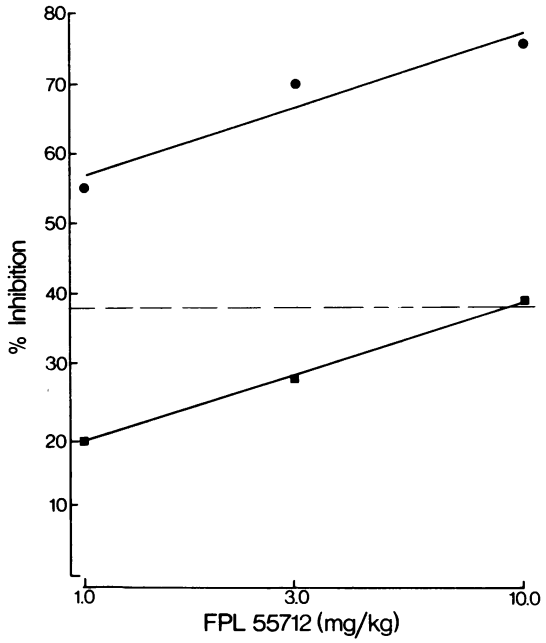

Figure 2 Inhibition of anaphylactic bronchoconstriction in passively sensitized rats after pretreatment with methysergide (4 mg/kg, i.v. 15 min before antigen) indicated by dashed line; (▪) FPL 55712, and (•) FPL 55712 + methysergide (4 mg/kg) (n = 7).

Results

Effect of heat treatment of reaginic antiserum on its ability to sensitize rats for anaphylactic bronchoconstriction in vivo

One group of 7 rats was sensitized with unheated serum and another with serum which had been heated at 56°C for 1 hour. The increase in respiratory overflow (% maximum mean ± s.e. mean) was, in the first group 58 ± 8.3 and in the second group 10.7 ± 1.2 (n = 7). These results suggested that a heat labile reagin-like antibody was responsible for the greater part of the lung anaphylactic responses produced in these experiments.

Effect of pharmacological antagonists on anaphylactic bronchoconstriction in vivo

Figure 1 shows the time course of anaphylactic bronchoconstriction in untreated rats and in rats after pretreatment with an antihistamine (mepyramine 10 mg/kg i.p. 30 min before antigen), an anti-5-HT agent (methysergide, 4 mg/kg i.v. 15 min before antigen) or a selective antagonist of SRS-A (FPL 55712 10 mg/kg i.v. immediately before antigen; Augstein, Farmer, Lee, Sheard & Tattersall, 1973). The increase in respiratory overflow (% maximum) was as follows: control group 42.7 ± 7.2, mepyramine-treated group 47.4 ± 7.3, methysergide-treated group 25.0 ± 1.7 and FPL 55712-treated group 21.7 ± 4.4. The % inhibition produced by the drugs was nil, 41.3 ($P < 0.05$) and 49.2 ($P < 0.25$), respectively.

The dose of FPL 55712 used in these experiments inhibits PCA in the rat (unpublished results). A further experiment was therefore performed to determine if the effects of FPL 55712 and methysergide were additive in PLA. FPL 55712, 1, 3 and 10 mg/kg was tested against anaphylactic bronchoconstriction in the presence and absence of methysergide 4 mg/kg. The results (Figure 2) show that the effects of the compounds were additive at each dose of FPL 55712.

FPL 55712 (10 mg/kg) did not inhibit bronchoconstrictor responses produced by intravenous injection of 5-HT (12.5 μg/kg) in the rat, which confirmed the selectivity found *in vitro* with FPL 55712 (Augstein *et al.*, 1973). Intravenous injection of other possible mediators of anaphylaxis (histamine, bradykinin, prostaglandins E_2 or $F_{2\alpha}$ at doses up to 100 μg/kg) did not produce bronchoconstriction in the rat, although all of these agents produced a fall in blood pressure.

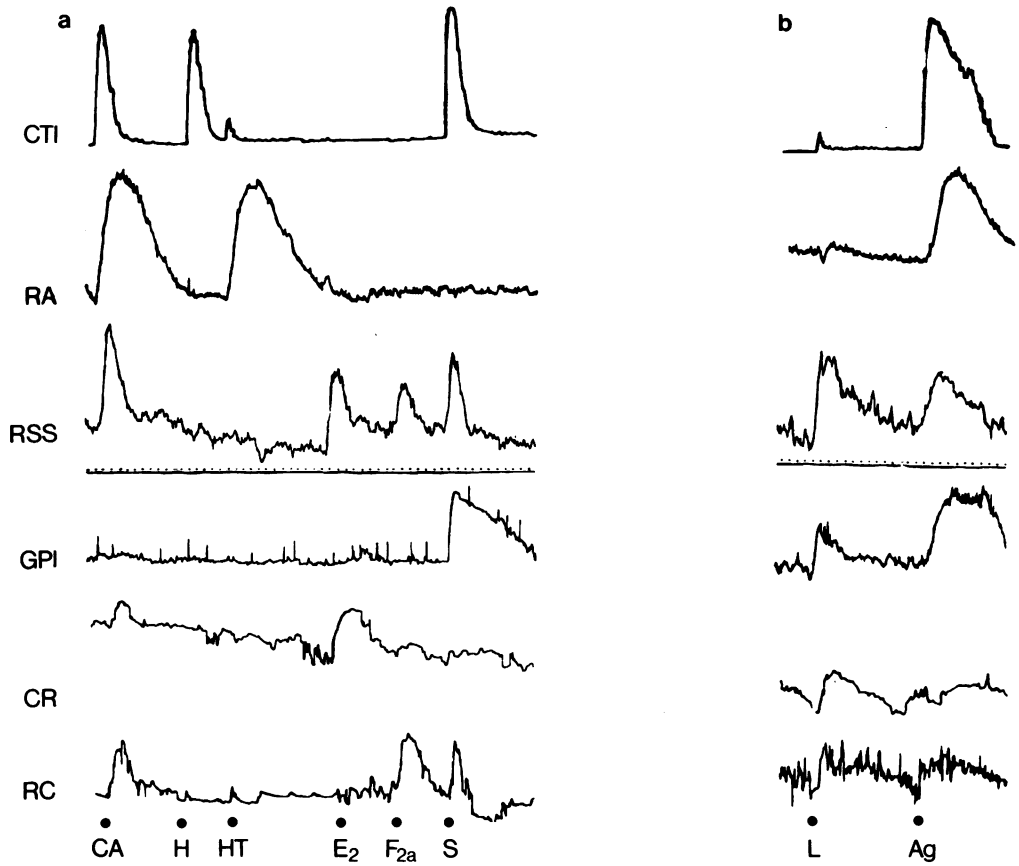

Figure 3 Mediators released after antigen challenge of lungs taken from passively sensitized rats: (a) standard solution of individual agonists, histamine 20 ng/ml (H), 5-hydroxytryptamine 20 ng/ml (HT), prostaglandins E_2 2 ng/ml and $F_{2\alpha}$ 2 ng/ml and crude SRS-A 1.0 ml (S); and combined agonists (CA) excluding SRS-A; superfused over cat terminal ileum (CTI), rabbit aorta (RA), rat stomach strip (RSS), guinea-pig ileum (GPI), chick rectum (CR) and rat colon (RC); (b) the effluent from isolated lungs superfused over the same assay tissues, L = lungs suspended above the assay tissues, Ag = injection of 400 μg of 5 x crystallized egg albumin. Selective antagonists were superfused over the assay tissues as described in the text.

Aspirin pretreatment in vivo

Following treatment with aspirin (5 mg/kg i.v.) 10 min before challenge, the % maximum bronchoconstriction (mean ± s.e. mean) was 86.0 ± 2.9 (n = 5) compared to 67.1 ± 16.2 (n = 5) for controls. The difference between these responses was not significant ($P > 0.05$).

Aprotinin pretreatment in vivo

Intraperitoneal injection of aprotinin, 100,000 kallikrein-inhibitor units/kg 10 min before challenge, produced a slight inhibition of the bronchoconstrictor response, but this was not significant ($P > 0.05$). The % maximum bronchoconstriction (mean ± s.e. mean) was 55.0 ± 11.5 (n = 5) compared to 63.6 ± 7.0 (n = 5) for control rats.

Influence of mediators released from the intestine in vivo

There was no significant difference ($P > 0.05$) in the anaphylactic bronchoconstriction obtained in eviscerated or 'sham' operated animals. The % maximum bronchoconstriction (mean ± s.e. mean) was 44.9 ± 10.2 (n = 7) in eviscerated rats and 42.8 ± 7.1 (n = 7) in 'sham' eviscerated rats.

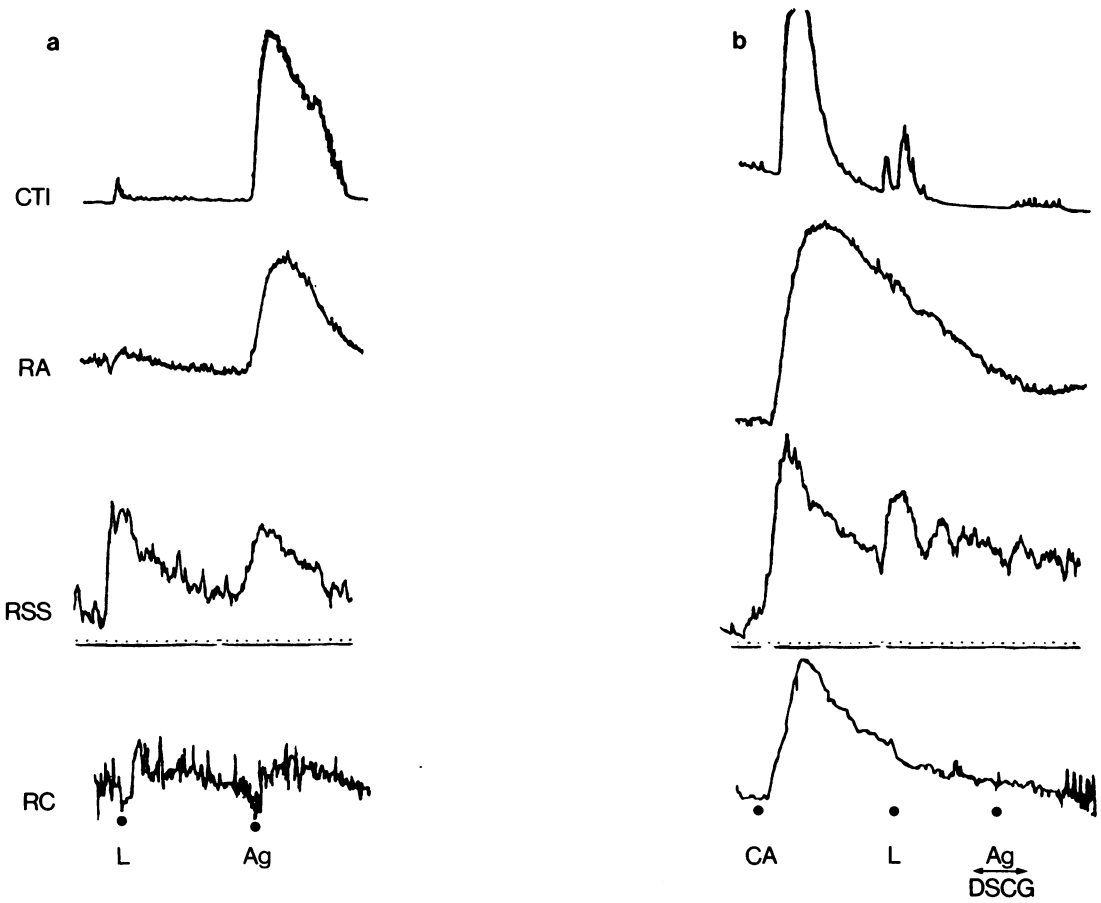

Figure 4 Inhibition by disodium cromoglycate (DSCG) of release of mediators after antigen challenge of lungs taken from passively sensitized rats; (a) control lungs; (b) lungs perfused with DSCG 10 μg/ml for 1 min before and 5 min after antigen challenge. Other details as in Figure 3.

Vagal involvement in vivo

In groups of 6 rats there was no significant difference ($P > 0.05$) in the anaphylactic bronchoconstriction obtained in bilaterally vago-sympathectomized animals (64.5 ± 6.1) or in 'sham' operated animals (71.0 ± 10.8).

Mediators released from passively sensitized lung in vitro

After challenge of isolated perfused lungs taken from passively sensitized rats, the perfusing fluid was allowed to superfuse a series of assay tissues (Figure 3). The release of histamine was demonstrated by a contraction of the cat terminal ileum; of 5-HT by a methysergide-sensitive contraction of the rabbit aorta; of SRS-A by a slow, prolonged contraction of the guinea-pig ileum superfused with combined antagonists; and of prostaglandins by contractions of the rat stomach strip, chick rectum and rat colon. It was noticeable that prostaglandins could only be detected after severe shock when large amounts of the other mediators were also released. In some experiments only histamine and 5-HT and no prostaglandins were released. After a second antigen challenge no mediators could be detected in the perfusing fluid.

No contraction of a rabbit aortic strip superfused with a combination of antagonists including methysergide was seen after challenge of 8 sets of sensitized rat lungs. That lung shock had actually taken place was shown by the presence of the other mediators and the contraction of a second aortic strip which was not superfused with

methysergide. These results agree with the findings of Piper & Walker (1973) that RCS is released after mechanical stimulation, but not after antigen challenge of lungs taken from actively sensitized rats.

Effects of disodium cromoglycate on PLA in vivo *and* in vitro

In vivo dose-dependent inhibition of passive lung anaphylaxis was obtained by disodium cromoglycate (DSCG) given intravenously 1 min before challenge in groups of 7 rats. Doses of 0.4, 1, 2 and 5 mg/kg DSCG gave respectively 28.2, 46.6, 52.3 and 84.5% inhibition of PLA, the ID_{50} being 1.25 mg/kg.

In vitro DSCG (10 or 100 μg/ml) was infused through the isolated lungs from 1 min before antigen challenge until 5 min after challenge. DSCG at 100 μg/ml totally inhibited mediator release in 5 out of 6 rats and at 10 μg/ml in 3 out of 7 rats (Figure 4). Sixteen control sets of lungs all showed some mediator release.

Discussion

A chemical substance can be accepted as a mediator of a biological reaction only if several criteria are satisfied (Vane, 1972a). The present experiments allow some conclusions about the contribution of various potential mediators to anaphylactic bronchoconstriction in rats passively sensitized with reaginic antiserum.

The evidence that 5-HT participates as a mediator of PLA in the rat is conclusive. 5-HT injection into the normal rat produced bronchoconstriction. Methysergide, an antagonist of 5-HT, reduced anaphylactic bronchoconstriction *in vivo*. Rat lungs contain relatively high quantities of 5-HT (Weissbach, Waalkes & Udenfriend, 1958, 3.5 μg/g; Sadavongvivad, 1970, 1.91 μg/g) and we have shown that 5-HT can be released from rat lungs during PLA *in vitro*.

Although histamine is present in rat lungs (Aviado & Sadavongvivad, 1970, 5.0 μg/g) and can be released during anaphylactic shock *in vitro*, histamine injection did not produce bronchoconstriction *in vivo*, nor did mepyramine reduce anaphylactic bronchoconstriction *in vivo*. It appears therefore that histamine is not an important mediator of anaphylactic bronchoconstriction in the rat, in contrast to conditions in the guinea-pig (Parrat & West, 1957; Austen, 1963).

The lungs of actively sensitized guinea-pigs release SRS-A upon challenge with specific antigen (Brocklehurst, 1960). We have demonstrated the release of SRS-A following antigen challenge of lungs from passively sensitized rats *in vitro*. In addition, the partial inhibition of anaphylactic bronchoconstriction in passively sensitized rats *in vivo* by FPL 55712 suggests that SRS-A is involved in this response. FPL 55712 has some anti-PCA activity in the rat, which might suggest that the inhibitory effect on anaphylactic bronchoconstriction which we obtained was due to a general anti-allergic property of this compound. However, the additive effects of FPL 55712 and methysergide in the present experiments suggest that FPL 55712 acts mainly as an antagonist of SRS-A. Conclusive evidence for SRS-A involvement in rat PLA will be possible only when rat SRS-A has been purified.

Main (1964) and Horton (1969) have suggested that prostaglandins are normally involved in the local control of bronchial smooth muscle tone. This view is supported by Vane (1972b) who suggests that prostaglandins function as homeostatic regulators in the lung by modulating the effects of other mediators rather than by producing anaphylactic bronchoconstriction themselves. Our results to some extent support this view. Aspirin pretreatment did not modify PLA *in vivo*. During *in vitro* experiments prostaglandins were detected in the perfusates from lungs only after severe anaphylactic shock. Moreover, the ratio of prostaglandins to other mediators released was far higher during the mechanical stimulation involved in setting up the lungs than during anaphylactic shock (Figure 3). During anaphylactic shock in rats bradykinin is found in the blood (Csaba & Went, 1971), bradykininogen levels fall and kinin-forming activity is increased (Dawson, Starr & West, 1966; Csaba & Went, 1971). Because no true bradykinin antagonists were available, aprotinin which inhibits kinin formation was used. Aprotinin did not modify anaphylactic bronchoconstriction. In addition, the injection of bradykinin into rats did not induce bronchoconstriction. This argues against bradykinin being a mediator of anaphylactic bronchoconstriction in the rat.

In the experiments reported here, DSCG produced a dose-related inhibition of PLA *in vivo* and complete inhibition of mediator release *in vitro* (Figure 4). This is in contrast to the 'bell-shaped' dose-inhibitory response curve produced against anaphylactic bronchoconstriction in actively sensitized rats (Church, Collier & James, 1972).

DSCG is thought to act primarily at the cellular level by preventing the release of tissue damaging substances following antigen interaction with mast cells sensitized with IgE (Cox, 1971).

Evidence has been presented that DSCG blocks cyclic adenosine 3′,5′-monophosphate (AMP)

phosphodiesterase *in vitro* (Roy & Warren, 1974). However, the drug has no spasmolytic activity on smooth muscle and therefore, probably has no effect on smooth muscle phosphodiesterase. Taylor, Francis, Sheldon & Roitt (1974), carried out experiments using rat PCA and peritoneal mast cell degranulation which led them to suggest that the anti-asthmatic activity of DSCG might be due to its inhibitory effect on a specific mast cell phosphodiesterase iso-enzyme. However, experiments of Koopman, Orange & Austen (1970) had previously suggested that the action of DSCG in inhibiting antigen-induced release of SRS-A from the rat IgE-sensitized peritoneal cavity of rats was independent of the cyclic AMP system. Thus, the relevance of phosphodiesterase inhibition by DSCG to its anti-asthmatic effect, particularly when considered in relation to the respective doses needed to exert these effects, is still uncertain.

The unusual bell-shaped dose-response curve with DSCG in actively sensitized rats *in vivo* can probably best be explained by the presence of antibodies other than IgE, which may contribute to, or initiate events leading to, anaphylactic bronchoconstriction in these animals. In contrast, in the passively sensitized rat, the long latency and heat lability of the antibodies involved in the anaphylactic bronchoconstriction suggest that the reaction is primarily due to interaction of antigen with IgE attached to the lung tissue. Thirteen other compounds (each of which produced a dose-related inhibition of rat PLA) had almost identical activity, relative to DSCG, both in rat PLA and PCA and therefore, PLA was no more predictive as a screen for anti-allergic activity than PCA (Farmer *et al.*, 1973).

Orange, Stechschulte & Austen (1970), have demonstrated the release of histamine and/or SRS-A during passive peritoneal anaphylaxis in the rat. Mediators released were dependent on the immunoglobulins involved. As our experiments with eviscerated animals showed, mediators released from the intestine, considered to be the primary 'shock' organ of anaphylaxis in the rat (Sanyal & West, 1958; West, 1959), did not contribute to anaphylactic bronchoconstriction. The mediators which produce bronchoconstriction *in vivo* are probably released from the lungs themselves.

It has been suggested that vagal reflexes are partly responsible for anaphylactic bronchoconstriction in guinea-pigs (Mills & Widdicombe, 1970), dogs (Gold, Kessler & Yu, 1972) and rabbits (Karczewski & Widdicombe, 1969). In our experiments, vagotomy did not modify PLA in the rat. This species difference could be significant in the search for a predictive animal test for anti-allergic activity in man. However, the involvement of vagal reflexes in human bronchial asthma has not been clearly established. Some workers have shown atropine to be an effective inhibitor of antigen-induced bronchoconstriction in asthmatic patients (Yu, Galant & Gold, 1972) while others report little (Itkin & Anand, 1970), or no effect (Orie, Van Lookeren Campagne, Knol, Booij-Noord & De Vries, 1973; Rosenthal, Summer, Permutt & Norman, 1974). The relevance of anaphylactic bronchoconstriction in rats (or other animals) to asthma in man, with respect to vagal reflex involvement, cannot be resolved at present.

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References

AUGSTEIN, J., FARMER, J.B., LEE, T.B., SHEARD, P. & TATTERSALL, M.L. (1973). Selective inhibitor of slow-reacting substance of anaphylaxis. *Nature, New Biol.*, **245**, 215-217.

AUSTEN, K.F. (1971). Histamine and other mediators of allergic reactions. In *Immunological Diseases*. Vol. 1, 2nd edition, pp. 332-355, ed. Samter, M. Boston: Little Brown.

AVIADO, D.M. & SADAVONGVIVAD, C. (1970). Pharmacological significance of biogenic amines in the lungs: histamine. *Br. J. Pharmac.*, **38**, 366-373.

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.

BURDEN, D.T., PARKES, M.W. & GARDINER, D.G. (1971). Effect of β-adrenoceptive blocking agents on the response to bronchoconstrictor drugs in the guinea-pig air overflow preparation. *Br. J. Pharmac.*, **41**, 122-131.

CHURCH, M.K., COLLIER, H.O.J. & JAMES, G.W.L. (1972). The inhibition by dexamethasone and disodium cromoglycate of anaphylactic bronchoconstriction in the rat. *Br. J. Pharmac.*, **46**, 56-65.

COX, J.S.G. (1971). Disodium cromoglycate. Mode of action and its possible relevance to the clinical use of the drug. *Br. J. Dis. Chest.*, **65**, 189-204.

CSABA, B. & WENT, M. (1971). Role of histamine, 5-hydroxytryptamine and bradykinin in the rat anaphylaxis. *Acta Physiol. acad. scient. Hung.*, **39**, 369-374.

DAWSON, W., STARR, M.S. & WEST, G.B. (1966). Inhibition of anaphylactic shock in the rat by antihistamines and ascorbic acid. *Br. J. Pharmac. Chemother.*, **27**, 249-255.

FARMER, J.B., RICHARDS, I.M., SHEARD, P. & WOODS, A.M. (1973). Passive lung anaphylaxis in the rat. Inhibition by disodium cromoglycate. *Naunyn-Schmiedebergs Arch. Pharmac.*, **279**, (Suppl.) R35.

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

GOOSE, J. & BLAIR, A.M.J.N. (1969). Passive cutaneous anaphylaxis in the rat, induced with two homologous reagin-like antibodies and its specific inhibition with disodium cromoglycate. *Immunology*, **16**, 749-760.

HORTON, E.W. (1969). Hypotheses on physiological roles of prostaglandins. *Physiol. Rev.*, **49**, 122-161.

ITKIN, I.H. & ANAND, S.C. (1970). The role of atropine as a mediator blocker of induced bronchial obstruction. *J. Allergy*, **45**, 178-186.

KARCZEWSKI, W. & WIDDICOMBE, J.G. (1969). The role of the vagus nerves in the respiratory and circulatory reactions to anaphylaxis in rabbits. *J. Physiol.*, **201**, 293-304.

KONZETT, H. & ROSSLER, R. (1940). Versuchsanordnung zu Untersuchungen an der Bronchialmuskulatur. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.*, **195**, 71-74.

KOOPMAN, W.J., ORANGE, R.P. & AUSTEN, K.F. (1970). Immunochemical and biologic properties of rat IgE. III Modulation of the IgE-mediated release of slow-reacting substance of anaphylaxis by agents influencing the level of cyclic 3',5' adenosine monophosphate. *J. Immunol.*, **105**, 1096-1102.

MAIN, I.H.M. (1964). The inhibitory actions of prostaglandins on respiratory smooth muscle. *Br. J. Pharmac. Chemother.*, **22**, 511-519.

MILLS, J.E. & WIDDICOMBE, J.G. (1970). Role of the vagus nerves in anaphylaxis and histamine-induced bronchoconstrictions in guinea-pigs. *Br. J. Pharmac.*, **39**, 724-731.

ORANGE, R.P., STECHSCHULTE, D.G. & AUSTEN, K.F. (1970). Immunochemical and biologic properties of rat IgE. II Capacity to mediate the immunologic release of histamine and slow reacting substance of anaphylaxis (SRS-A). *J. Immunol.*, **105**, 1087-1095.

ORIE, N.G.M., VAN LOOKEREN CAMPAGNE, J.G., KNOL, K., BOOIJ-NOORD, H. & DE VRIES, K. (1973). Late reactions in bronchial asthma. In *Intal in Bronchial Asthma*, pp. 17-29. Ed. Pepys, J. & Yamamura, Y. Loughborough: Fisons Ltd.

ORR, T.S.C. & BLAIR, A.M.J.N. (1969). Potentiated reagin response to egg albumin and conalbumin in *Nippostrongylus brasiliensis* infected rats. *Life Sci.*, **8**, 1073-1077.

PARRAT, J.R. & WEST, G.B. (1957). 5-hydroxytryptamine and tissue mast cells. *J. Physiol.*, **137**, 169-178.

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.

PIPER, P.J. & WALKER, J.L. (1973). The release of spasmogenic substances from human chopped lung tissue and its inhibition. *Br. J. Pharmac.*, **47**, 291-304.

ROSENTHAL, R.R., SUMMER, W.R., PERMUTT, S. & NORMAN, P.S. (1974). The effect of atropine on antigen mediated bronchospasm. *J. Allergy Clin. Immunol.*, **53**, 73.

ROY, A.C. & WARREN, B.T. (1974). Inhibition of cAMP phosphodiesterase by disodium cromoglycate. *Biochem. Pharmac.*, **23**, 917-920.

SADAVONGVIVAD, C. (1970). Pharmacological significance of biogenic amines in the lungs: 5-hydroxytryptamine. *Br. J. Pharmac.*, **38**, 353-365.

SANYAL, R.K. & WEST, G.B. (1958). Anaphylactic shock in the albino rat. *J. Physiol.*, **142**, 571-584.

TAYLOR, W.A., FRANCIS, D.H., SHELDON, D. & ROITT, I.M. (1974). The anti-anaphylactic actions of disodium cromoglycate, theophylline, isoprenaline and prostaglandins. *Int. Arch. Allergy*, **46**, 104-120.

VANE, J.R. (1972a). In: *Inflammation, Mechanisms and Control*, p. 264. Ed. Lepow, I.H. & Ward, P.A. New York: Academic Press.

VANE, J.R. (1972b). Prostaglandins and the aspirin-like drugs. *Hosp. Practice*, 7, 61-71.

WEISSBACH, H., WAALKES, T.P. & UDENFRIEND, S. (1958). A simplified method for measuring serotonin in tissues; simultaneous assay of both serotonin and histamine. *J. biol. Chem.*, **230**, 865-871.

WEST, G.B. (1959). Some factors involved in anaphylactic shock. *Int. Arch. Allergy*, **15**, 231-236.

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.

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