Br. J. Pharmac. (1978), **64**, 211–218.

THE RELEASE OF PROSTAGLANDINS AND THROMBOXANES FROM GUINEA-PIG LUNG BY SLOW REACTING SUBSTANCE OF ANAPHYLAXIS, AND ITS INHIBITION

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1 Rabbit aorta contracting substance (RCS; consisting mainly of thromboxane A_2) and prostaglandin-like material were released from guinea-pig isolated perfused lungs by injection of slow reacting substance of anaphylaxis (SRS-A).

2 SRS-A was resistant to boiling and proteolytic enzymes and was therefore distinguished from rabbit aorta contracting substance releasing factor (RCS-RF).

3 The release of RCS and prostaglandin-like material by SRS-A was anatagonized by indomethacin (1 μg/ml), betamethasone and dexamethasone (4 to 50 μg/ml).

4 Imidazole (200 μg/ml) inhibited the formation of thromboxane A_2 but not that of prostaglandins.

5 The activity of SRS-A on guinea-pig ileum and its ability to release RCS and prostaglandins were destroyed by incubation with arylsulphatase (0.83 μg to 1 mg/ml) and with lipoxidase (16.5 to 50 μg/ml): SRS-A lost activity on incubation with bovine serum albumin (9 μg/ml) due to protein binding.

Introduction

Prostaglandins and rabbit aorta contracting substance (RCS), which is a mixture of endoperoxides, prostaglandin G_2 and H_2, and thromboxane A_2 (Hamberg, Svensson & Samuelsson, 1976) are released from guinea-pig lungs by a variety of stimuli. These include anaphylaxis, injection into the pulmonary artery of the anaphylactic mediators, slow reacting substance of anaphylaxis (SRS-A), histamine and bradykinin; also infusion of arachidonic or dihomo-γ-linolenic acids or mild mechanical damage (Piper & Vane, 1969; 1971; Vargaftig & Dao Hai, 1971). After the RCS activity of the perfusate from lungs has decayed, another more stable substance remains which, when injected into perfused lungs, releases RCS; this substance is RCS-releasing factor (RCS-RF) (Piper & Vane, 1969). In 1976, Nijkamp, Flower, Moncada & Vane partially purified RCS-RF, suggested that it is a peptide and showed that its action was blocked by both non-steroid and steroid anti-inflammatory drugs.

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The purpose of this investigation was to study the SRS-A-induced release of RCS from guinea-pig lungs, and its inhibition by various pharmacological agents.

Part of this work has been communicated to the British Pharmacological Society (Engineer, Piper & Sirois, 1977).

Methods

Preparation of SRS-A

Since non-steroid anti-inflammatory drugs increase the release of SRS-A by up to five fold (Engineer, Niederhauser, Piper & Sirois, 1978), SRS-A was prepared from indomethacin-treated isolated perfused lungs from guinea-pigs sensitized to ovalbumin (Sigma Grade II) by intraperitoneal and subcutaneous injection of 100 mg at least 3 weeks before use. After antigen challenge with 10 mg ovalbumin (Sigma Grade II) injected into the pulmonary artery, the effluent from the lung was collected for 10 min, the histamine removed and SRS-A concentrated by

adsorption onto activated charcoal. The SRS-A was eluted with 80% ethanol. After evaporation under reduced pressure, the resulting aqueous solution of SRS-A was freeze-dried and stored at −20°C in ampoules containing nitrogen until required. The activity of SRS-A was expressed in arbitrary units based on the potency of an initial batch; 0.068 ± 0.004 u were equivalent to two units of SRS-A measured by the method of Stechschulte, Austen & Bloch (1967). The contents of each ampoule (7 to 10 u) were dissolved in 3 ml distilled water and kept on ice during use.

Criteria for identification of STS-A

Since the time course and conditions of release of SRS-A and RCS-RF are similar, it was possible that our samples of SRS-A might have been contaminated with RCS-RF. Therefore, various procedures were carried out in an attempt to differentiate between the two substances. As SRS-A has not been completely purified or identified, we adopted the following criteria to show that we were examining the action of SRS-A. The substance under test (a) contracted the guinea-pig ileum in the presence of mepyramine and hyoscine (Brocklehurst, 1960); (b) was destroyed by arylsulphatase (Orange, Murphy & Austen, 1974); (c) had its myotropic action on the guinea-pig ileum antagonized by sodium 7-(3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroproxy-4-oxy-8-propyl-4H-1-benzopyran-2-carboxylate (FPL 55712) (50 ng/ml to 1 μg/ml) (Augstein, Farmer, Lee, Sheard & Tattersall, 1973); (d) was stable to base hydrolysis in 0.1 N NaOH and (e) was labile in 1 N HCl (Orange & Austen, 1969).

Differentiation between SRS-A and RCS-RF

Boiling Samples of SRS-A were boiled for up to 1 h to destroy any RCS-RF present in the partially purified SRS-A (Nijkamp *et al.*, 1976).

Enzyme studies Since RCS-RF has been described as a peptide (Nijkamp *et al.*, 1976) the actions of proteolytic enzymes which inactivate it together with those of other peptidases on SRS-A were investigated. Arylsulphatase was used to inactivate SRS-A as Nijkamp *et al.* (1976) found that it inactivated RCS-RF in 2 out of 4 experiments.

Release of RCS and prostaglandins from perfused lungs

In experiments showing RCS release, lungs from unsensitized or sensitized guinea-pigs were perfused with Tyrode solution (at 5 ml/min) via the pulmonary artery and the effluent superfused over isolated strips of smooth muscle selected from rabbit aorta, coeliac and mesenteric arteries, rat stomach strip and colon, chick rectum and longitudinal smooth muscle stripped from the guinea-pig ileum. The arterial tissues of the rabbit were used to differentiate between thromboxane A_2 and prostaglandins G_2 and H_2 (Bunting, Moncada & Vane, 1976). All three tissues contracted to thromboxane A_2, whereas the rabbit coeliac artery and mesenteric artery responded to prostaglandin G_2 or H_2 with a small contraction followed by a large relaxation.

The tissues were treated with a combination of antagonists infused to give a final concentration of mepyramine, hyoscine and phenoxybenzamine 0.1 μg/ml, propranolol 2 μg/ml and methysergide 0.2 μg/ml. Indomethacin 1 μg/ml was infused together with the antagonists listed above to prevent synthesis of prostaglandins in the assay tissues.

When SRS-A activity was assayed on the guinea-pig ileum, a solution containing mepyramine and hyoscine was infused continuously to give a final concentration of each drug of 0.1 μg/ml.

Release of prostaglandin-like material was detected and estimated by bioassay (on rat stomach, chick rectum and rat colon) and radioimmunoassay (Jose, Niederhauser, Piper, Robinson & Smith, 1976).

Radioimmunoassay of prostaglandins

The concentration of prostaglandins in the effluent was estimated in terms of prostaglandin $F_{2\alpha}$ by radioimmunoassay (Jose *et al.* 1976). The cross-reaction of the antiserum to prostaglandin $F_{2\alpha}$ was 6% with 15-keto, 13,14-dihydro-15-keto and 13,14-dihydro metabolites of $F_{2\alpha}$; 17.5% with $F_{1\alpha}$ and 0.02% with prostaglandin E_2; SRS-A had very low cross-reaction to the antisera used.

Release of RCS and prostaglandins by SRS-A

In most experiments SRS-A which had been boiled for 1 h was injected into the pulmonary artery of perfused lungs in doses of 0.01 to 0.1 u; the action of this material was compared with unboiled SRS-A. Release of RCS was detected by contraction of rabbit aorta and prostaglandins by simultaneous contraction of rat stomach strip, chick rectum and rat colon. Prostaglandins were quantitated by radioimmunoassay.

Inhibition of release of RCS

The following drugs were investigated as potential inhibitors of release of thromboxanes and prostaglandins induced by SRS-A: indomethacin, imidazole, dexamethasone, betamethasone and FPL 55712. In these experiments, guinea-pig isolated lungs were perfused with Tyrode solution via the pulmonary artery.

Prostaglandin G_2 and, in some experiments, prostaglandin H_2 or noradrenaline were used to assess the sensitivity of the rabbit arterial tissues. A control release of RCS and prostaglandins was obtained by giving intra-arterial injections of bradykinin and/or arachidonic acid or SRS-A. The lung effluent was diverted away from the assay tissues in experiments when indomethacin and FPL 55712 were used, since indomethacin sometimes desensitized the guinea-pig ileum to SRS-A, and FPL 55712 antagonized the responses to SRS-A. In these experiments indomethacin or FPL 55712 was infused through the pulmonary circulation for 15 min and the lungs then washed with drug-free Tyrode solution for 5 min before the effluent was once more superfused over the assay tissues. Imidazole, 200 μg/ml was infused directly over the assay tissues and a control release of thromboxane and prostaglandins obtained with an intra-arterial injection of SRS-A. The infusion of imidazole was then given intra-arterially for 20 min and the dose of SRS-A repeated.

In experiments with steroids, intra-arterial infusions of drug solutions were started as soon as the lungs had been cannulated because it was necessary for the steroids to be in contact with the lung for at least 30–45 min.

Drugs

The following drugs were used: arachidonic acid (Sigma), bradykinin (Parke Davis), indomethacin (Merck, Sharp & Dohme), hyoscine hydrobromide (BDH.), mepyramine maleate (May & Baker), methysergide maleate (Sandoz), phenoxybenzamine hydrochloride (ICI), propranolol hydrochloride (ICI), dexamethasone (Devlab), FPL 55712 (Fisons), arylsulphatase from limpets Type V (Sigma), elastase (prepared by Dr D. Shotton, Lab. of Molecular Biology, Cambridge), pepsin (Sigma), carboxypeptidase A (Sigma), leucine aminopeptidase (Sigma), prostaglandin H_2 (Ran Chemicals), lipoxidase Type IV (Sigma).

Results

Identification of SRS-A

After partial purification the standard preparation of SRS-A fulfilled all the criteria listed for SRS-A activity: it contracted guinea-pig ileum in doses of 0.01 to 0.10 u and this effect was antagonized by PFL 55712 0.05 to 1 μg/ml; it was stable to base hydrolysis but labile in acid; incubation of SRS-A with arylsulphatase (0.83 μg/ml to 1 mg/ml) at pH 5.0 caused a dose-dependent destruction of the myotropic action of SRS-A and its ability to release RCS (Table 1).

Differentiation between SRS-A and RCS-RF

As shown in Figure 1, both boiled and unboiled SRS-A when injected directly over the tissues (dir) contracted the guinea-pig ileum and released RCS when given intra-arterially to the lungs. Boiling did not affect the action of SRS-A on guinea-pig ileum but in the experiment in Figure 1 caused a reduction in its ability to release RCS: this was not always seen in other experiments (2 out of 4). The ability of SRS-A to release RCS was not eliminated by boiling but may have been reduced, perhaps indicating contamination with RCS-RF.

As already mentioned, incubation with arylsulphatase (0.83 μg/ml to 1 mg/ml) at pH 5.0 caused a dose-dependent destruction of the myotropic action of SRS-A and its ability to release RCS (Table 1). In 4 experiments, arylsulphatase (1 mg/ml) completely destroyed the activity of SRS-A. The action of arylsulphatase was reduced by incubation in non-optimal conditions at pH 8.6 and prevented at 0°C.

Actions of proteolytic enzymes on SRS-A

Incubation with elastase (0.55 to 10.0 μg/ml) and pepsin (100 μg/ml) did not affect the contraction of the guinea-pig ileum or the release of RCS by SRS-A. In low doses carboxypeptidase A (0.4 to 3.6 μg/ml) and leucine aminopeptidase (0.017 to 0.15 μg/ml) did not affect the action of SRS-A but at 39 μg/ml and 1.65 μg/ml respectively, they reduced the action on the guinea-pig ileum by up to 40% (4 experiments) and up to 100% (5 experiments). The ability of SRS-A to release RCS was similarly inhibited by both

Table 1 Inactivation of slow reacting substance of anaphylaxis (SRS-A) by arylsulphatase and lipoxidase

Enzyme	*Conc. (μg/ml)*	*% inactivation of SRS-A activity measured on GP ileum*	*Release of RCS*
Arylsulphatase	0.83	0	0
	7.5	27	22
	50	44	NT
	83	73–79	75
	1 mg	100	100
Lipoxidase	0.16	0	0
	1.5	0	0
	16.5	71–100	71–100
	50	100	100

Each result is from 2 to 4 experiments.

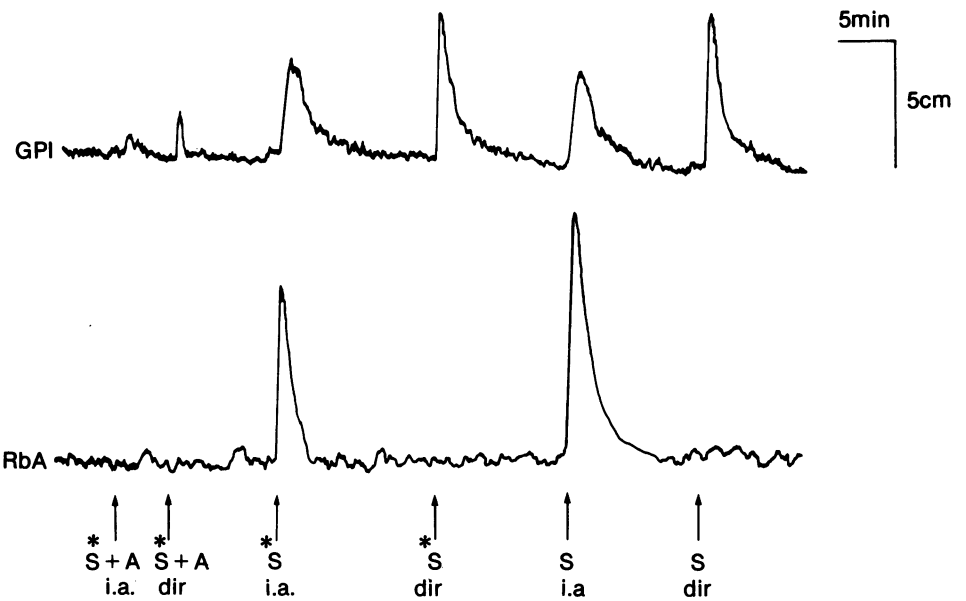

Figure 1 Release of rabbit aorta contracting substance (RCS, thromboxane A_2) from guinea-pig lung by slow reacting substance of anaphylaxis (SRS-A). Effluent from guinea-pig isolated perfused lungs superfused smooth muscle stripped from guinea-pig ileum (GPI) and a strip of rabbit aorta (RbA). Both tissues were continuously blocked with mepyramine and hyoscine. Doses of SRS-A(S), 110 mu were given either directly (dir) to GPI and RbA or injected into the pulmonary artery (i.a.). S was incubated with arylsulphatase (1 mg, S* + A) at pH 5 for 1 h and the reaction terminated by boiling*. This almost completely destroyed the action of S given intra-arterially or directly. S was boiled for 1 h (S*). When given intra-arterially S* caused contraction of GPI and RbA showing the release of RCS but when given directly only contracted GPI. Unboiled S given intra-arterially or directly produced the same responses. Vertical scale 5 cm, horizontal scale 5 minutes.

enzymes. The same apparent inactivation of SRS-A by aminopeptidase and carboxypeptidase occurred at both 0°C and 37°C. Similarly, incubation of SRS-A with bovine serum albumin (9 μg/ml) caused more than 80% inhibition of SRS-A action.

Action of lipoxidase on SRS-A

Incubation with lipoxidase (16.5 to 50 μg/ml in ammonium bicarbonate at pH 8.6 for 1 h) also destroyed the activity of SRS-A. (Table 1). The action of lipoxidase was reduced by incubation in non-optimal conditions of pH 5.0. Incubation at 0°C did not affect inactivation of SRS-A by lipoxidase.

Release of RCS and prostaglandins by SRS-A

As shown in Figure 2, a single intra-arterial injection of SRS-A releases RCS and other prostaglandin-like material from guinea-pig lungs. This occurred both in lungs from guinea-pigs sensitized to ovalbumin and from unsensitized animals. In 10 experiments doses of SRS-A (0.01 to 0.05 u) given into the pulmonary artery repeatedly at 15 min intervals caused contractions of rabbit aorta of similar height (maximum variation 10 to 15%) over the same duration as test experiments (2 to 3 h), presumably indicating the repeatable output of similar quantities of RCS. The total prostaglandin-like material released during RCS-induced contraction of rabbit aorta was equivalent to 0.1 to 1.0 ng/ml prostaglandin $F_{2\alpha}$. SRS-A caused release of thromboxane A_2 as shown in Figure 2. The contrasting effects of thromboxane A_2 and prostaglandins G_2 and H_2 on the rabbit coeliac and mesenteric arteries helped to differentiate between the release of the two substances but the contractile effect of thromboxane A_2 on the vascular tissues used would mask any relaxation due to the endoperoxides.

Inhibition of thromboxane formation with imidazole

As shown in Figure 2, imidazole, 200 μg/ml, selectively inhibited SRS-A-induced release of thromboxane A_2 (6 experiments); the relaxation of rabbit coe-

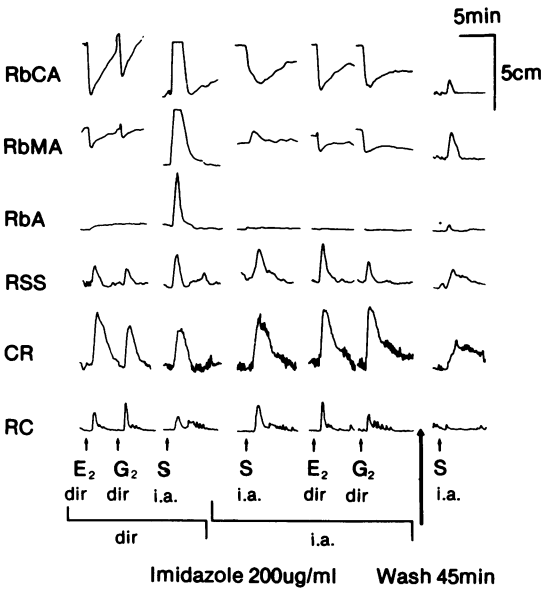

Figure 2 Inhibition of thromboxane formation by imidazole. Effluent from guinea-pig perfused lungs was superfused over rabbit coeliac artery (RbCA), mesenteric artery (RbMA), rabbit aorta (RbA), rat stomach strip (RSS), chick rectum (CR) and rat colon (RC). Figure arranged and labelled as Figure 1 except for prostaglandins E_2 and G_2 (E_2 and G_2). In the first part of the experiment, imidazole 200 μg/ml was infused directly (dir). Prostaglandin E_2, 50 ng given directly relaxed RbCA and RbMA, had little action on RbA but contracted RSS, CR, RC. Prostaglandin G_2 50 ng caused an initial contraction followed by relaxation of RbCA and RbMA, did not affect RbA but contracted RSS, CR, RC. After slow reacting substance of anaphylaxis (S) intra-arterially (i.a.) all tissues contracted, showing release of thromboxane A_2 and prostaglandin-like substances. In the second part of the figure imidazole was infused intra-arterially for 20 min before dosing and then continuously. After S intra-arterially, RbCA relaxed, RbMA, RSS, CR and RC contracted, showing that the release of thromboxane A_2 had been inhibited, whereas that of prostaglandin-like material was unchanged and possibly increased slightly. Calibrating doses of E_2 and G_2 were given. After washing imidazole out of the lungs for 45 min, the ability of S given intra-arterially to release rabbit aorta contracting substance (RCS) and prostaglandin-like substances began to return.

liac artery in the presence of imidazole might have been caused by prostaglandin G_2 or E_2 since they could not be distinguished in this experiment. In Figure 2 and other experiments when thromboxane release had been inhibited, the release of other prostaglandin-like material, as detected on the rat stomach strip, chick rectum and rat colon, appeared to be increased. The SRS-A-induced release of thromboxane started to return after imidazole had been washed out of the lungs for 30–45 min.

Inhibition of RCS release

Indomethacin Release of RCS and prostaglandin-like substances stimulated by both SRS-A and arachidonic acid was completely inhibited by indomethacin 1 μg/ml infused into the pulmonary artery (6 experiments). The release of RCS by SRS-A started to return after indomethacin had been washed out for 50 min.

Anti-inflammatory steroids. The release of RCS and prostaglandin-like substances by SRS-A was prevented by treating the lungs with dexamethasone and betamethasone 4 to 50 μg/ml (12 experiments). Figure 3 shows the time-dependent inhibition of RCS release by betamethasone 25 and 50 μg/ml. In other experiments the release of prostaglandin-like material was also blocked. The release of RCS by bradykinin was unaffected by steroids. Control releases of RCS by bradykinin were obtained before and after steroid treatment to ensure that the lungs were able to release RCS. The effect of steroids was persistent and lasted at least one hour. Infusion of arachidonic acid (1 μg/ml) reversed the steroid block of RCS release by SRS-A (Figure 3).

FPL 55712 Infusion of FPL 55712, 0.5 to 1 μg/ml for 15 min antagonized the SRS-A-induced release of RCS (10 experiments). The action of FPL 55712 was reversed by perfusion of the lungs with Tyrode solution for 20 min.

Discussion

As both SRS-A and RCS-RF are released at about the same time from the lungs of sensitized guinea-pigs during anaphylaxis (Piper & Vane, 1969), it is important to examine the similarities between these two substances since contamination of one with the other could mask or potentiate the activities of either of the two substances.

Similarities between SRS-A and RCS-RF include: a similar time course of release from sensitized guinea-pig lungs during challenge; solubility in methanol; destruction by large changes in pH, both being more easily destroyed by acid rather than alkaline conditions; inactivation by arylsulphatase; loss of activity after incubation with high concentrations of carboxypeptidase or aminopeptidase for one hour at 37°C; ability to release RCS and prostaglandins from

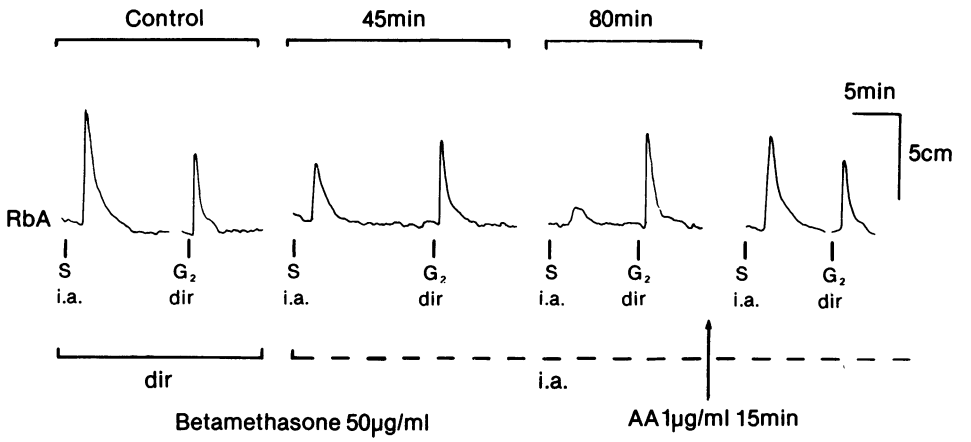

Figure 3 Time-dependent steroid inhibition of rabbit aorta contracting substance (RCS) release and its reversal by arachidonic acid. The effluent from guinea-pig isolated lungs was perfused over rabbit aorta (RbA). Tracing arranged and labelled as in Figures 1 and 2. In the first panel betamethasone 50 μg/ml was infused directly (dir). Slow reacting substance of anaphylaxis (SRS-A, 79 mu) intra-arterially (i.a.) caused contraction of RbA, showing release of thromboxane A_2. Prostaglandin G_2 (G_2) 10 ng was given directly for calibration. In the second and third panels betamethasone was infused continuously intra-arterially and doses of S intra-arterially and G_2 directly repeated. After 45 min the release of thromboxane A_2 was reduced and after 80 min almost abolished. Effects of calibrating doses of G_2 were unchanged. In the fourth panel arachidonic acid (AA) was infused intra-arterially for 15 min while the infusion of betamethasone continued. This restored the ability of S given intra-arterially to release thromboxane.

guinea-pigs lungs; inhibition of RCS release induced by SRS-A and RCS-RF by both steroid and non-steroid anti-inflammatory drugs. Hence there appear to be a number of similarities between these two substances. However, 75% of the activity of RCS-RF can be destroyed by boiling: when reduction (by boiling) in the ability of SRS-A to release RCS occurred, this could indicate that some RCS-RF had been present. The release of SRS-A can be inhibited by diethylcarbamazine, 1 mg/ml (Orange, Valentine & Austen, 1968) but that of RCS-RF is not (Nijkamp *et al.*, 1976). These authors suggested that RCS-RF is a peptide of less than 10 amino acids. The structure of SRS-A had not yet been elucidated, although it is not thought to be a peptide (Orange *et al.*, 1974). The results of incubating SRS-A with various enzymes emphasize the care needed in interpretation of enzymatic degradation studies with small amounts of impure biologically active substances. The results with carboxypeptidase, aminopeptidase and bovine serum albumin clearly show the phenomenon of the binding of SRS-A to proteins, since bovine serum albumin cannot act as an enzyme and the apparent action of carboxypeptidase and aminopeptidase was the same at 0°C and 37°C. The fact that protein binding occurred with lower concentrations of enzymes than those used by Nijkamp *et al.* (1976) suggests that the apparent destruction of RCS-RF might also have been due to protein binding which would throw doubt on the evidence that RCS-RF is a peptide. However, the results with arylsulphatase indicate genuine enzymatic inactivation since there was an increasing percentage of inactivation of SRS-A with increasing amounts of enzyme as well as decreased inactivation at 0°C or at pH 8.6. Thus, although RCS-RF and SRS-A show some similarities, any formal relationship between the two compounds awaits further investigation.

The action of lipoxidase on SRS-A is of interest since this enzyme caused marked deactivation of SRS-A which was decreased at non-optimal pH. The similar inactivation of SRS-A by lipoxidase at 0°C and 37°C may be explained by good binding to a very efficient enzyme. Although further investigations on the actions of this enzyme are necessary, these results provide further evidence that SRS-A is a lipid.

Demonstration of the release of thromboxane A_2 and prostaglandin-like substances, which possibly included prostaglandins G_2 and H_2 was obtained by the use of rabbit coeliac, mesenteric and aortic strips. Imidazole selectively inhibits thromboxane A_2 formation in platelets (Needleman, Raz, Ferrendelli & Minkes, 1977). Nijkamp, Moncada, White & Vane (1977) showed that in guinea-pig lungs, imidazole inhibited the metabolism of arachidonic acid to thromboxane and diverted it to prostaglandins, mainly prostaglandin $F_{2\alpha}$. The experiments described confirm these findings by showing selective inhibition of

SRS-A induced thromboxane release and increased synthesis of prostaglandins. Therefore it appears that imidazole would be a useful pharmacological tool to define the importance of thromboxanes in anaphylaxis but imidazole also activates phosphodiesterase (MacManus & Whitfield, 1971) and hence decreases cyclic adenosine 3',5'-monophosphate (cyclic AMP) levels and this has to be taken into account in a system such as anaphylaxis where cyclic AMP is also an important factor.

Whereas inhibition of SRS-A-induced RCS release by FPL 55712 is probably due to direct antagonism of the action of SRS-A (Augstein *et al.*, 1973), indomethacin probably inhibits RCS release by inhibiting the cyclo-oxygenase enzyme system and hence, the formation of endoperoxides, thromboxane A_2, prostaglandins E_2, $F_{2\alpha}$ and other prostaglandin-like materials (Vane, 1971). However, it has been suggested that these drugs may act on other enzymes to inhibit preferentially the formation of prostaglandins and have less action on the formation of thromboxanes (Boot, Brockwell, Dawson & Sweatman, 1977). The anti-inflammatory steroids, betamethasone and dexamethasone, caused a time-dependent inhibition of RCS release. The precise mode of action of steroid inhibition is not completely understood but it has been suggested by Gryglewski, Panczenko, Korbut, Grodzinska & Ocetkiewicz (1975) and Blackwell, Flower, Nijkamp & Vane (1977; 1978) that in the lung they may inhibit phospholipase A activation and prevent the supply of endogenous precursors to the arachidonic acid metabolizing enzymes. Exogenous [^{14}C]-arachidonic acid infused into guinea-pig lung is either rapidly metabolized by cyclo-oxygenase or incorporated into phospholipids. Although SRS-A mobilizes arachidonic acid from phospholipids, the newly incorporated [^{14}C]-arachidonic acid is not metabolized by subsequent doses of SRS-A (Al-Ubaidi, Bakhle, Jose & Seale, 1978). Therefore, if steroids somehow prevent phospholipase action, the apparent reversal of steroid block cannot be explained by an increased supply of precursor and remains to be elucidated. Since exogenous SRS-A released RCS and other prostaglandin-like material from perfused guinea-pig lungs, it is possible that SRS-A released in anaphylaxis similarly releases thromboxanes, endoperoxides and prostaglandins. Endogenous products of cyclo-oxygenase appear to depress release of histamine and SRS-A (Engineer *et al.*, 1978) suggesting that prostaglandin-related substances released in anaphylaxis may exert a negative feedback on further release of mediators, perhaps through an interaction with cyclic nucleotides.

We wish to thank Fisons Ltd., the Conseil de la Recherche en Santé due Quebec and the MRC for financial support; Dr J.E. Pike, Upjohn Co. for prostaglandin $F_{2\alpha}$ and its metabolites; Dr D. Shotton for elastase; Parke Davis for bradykinin; Merck, Sharp & Dohme for indomethacin and Fisons Ltd. for FPL 55712.

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(*Received December 12, 1977.*
Revised May 11, 1978.)