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NON-IMMUNOLOGICAL RELEASE OF SLOW-REACTING SUBSTANCE FROM GUINEA-PIG LUNGS

PRISCILLA J. PIPER & J.P. SEALE

Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, Lincoln's Inn Fields, London, WC2A 3PN

1 During incubation with calcium ionophore A23187, sensitized and unsensitized guinea-pig chopped lung released a slow-reacting substance (SRS) and histamine.

2 SRS possessed many characteristics of slow-reacting substance of anaphylaxis (SRS-A). It was inactivated by arylsulphatase, antagonized by FPL55712 (1 μg/ml) and more stable in alkali than acid. Furthermore, it behaved like SRS-A by stimulating arachidonic acid metabolism in guinea-pig isolated perfused lungs.

3 Maximal ionophore generation of SRS in sensitized lung was greater than maximal anaphylactic generation of SRS-A but the release of histamine was not significantly different. Simultaneous challenge with antigen and ionophore produced more SRS-like or SRS-A-like activity than either stimulus alone.

4 These results have shown a non-immunological release from guinea-pig lung of SRS which was similar (or possibly identical) to SRS-A. It is suggested that in addition to mast cells, other cell types may be involved.

Introduction

The formation of a 'slow-reacting muscle-stimulant substance' during anaphylactic shock in guinea-pig perfused lungs was first reported by Kellaway & Trethewie (1940) but its effects could not be clearly differentiated from those of histamine. With the development of anti-histamine drugs it became possible to describe this substance as causing a slow, prolonged contraction of the guinea-pig ileum which was resistant to anti-histamines (Brocklehurst, 1960) and it was referred to as slow-reacting substance of anaphylaxis (SRS-A). Subsequently SRS-A formation has been observed in many experimental models of immediate hypersensitivity (see review Orange & Austen, 1969; Kaliner, Wasserman & Austen, 1973; Grant & Lichtenstein, 1974) suggesting that it may play an important role in the pathophysiology of IgE- and IgG-mediated allergic reactions. Although its chemical structure remains unknown, SRS-A may be characterized as follows: it contracts guinea-pig terminal ileum treated with atropine and mepyramine (Brocklehurst, 1960) and this effect is antagonized by FPL55712 (Augstein, Farmer, Lee, Sheard & Tattersall, 1973); it is destroyed by limpet arylsulphatase (Orange, Murphy & Austen, 1974) and is stable to base hydrolysis (Orange & Austen, 1969). It has been reported that a slow-reacting substance (SRS) with the above characteristics may be released by a non-immunological stimulus (calcium ionophore A23187) from human leucocytes (Conroy, Orange & Lichtenstein, 1976), rat peritoneal cells (Bach & Brashler, 1974), rat lung cells dispersed by enzymatic digestion (Paterson, Leid, Said, Wasserman & Austen, 1976) and rat basophilic leukaemia cells (Jakschik, Kulczycki, MacDonald & Parker, 1977). The present experiments describe the release of SRS from guinea-pig lung during incubation with A23187. The abbreviations SRS-A and SRS are used in this paper to designate the slow-reacting substances as being anaphylactic and ionophore-induced respectively. A preliminary account of this work has been presented to the British Pharmacological Society (Piper & Seale, 1978).

Methods

Chopped lung

Unsensitized or sensitized (treated 3 weeks previously with ovalbumin grade II 100 mg subcutaneously and 100 mg intraperitoneally) male guinea-pigs (Dunkin-Hartley) were killed by cervical dislocation. The lungs were removed, perfused via the pulmonary artery with oxygenated Tyrode solution until free of blood and then cut into small pieces (approx. 2 mm^3). After washing three times, the chopped lung pieces (0.5 g) were incubated in 3 ml Tyrode solution (in duplicate)

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to which various agents had been added. The SRS-A or SRS activity in the supernatant was assayed (against a laboratory standard preparation of SRS-A) on smooth muscle stripped from guinea-pig ileum (Rang, 1964) and treated with mepyramine 7×10^{-7} M and hyoscine 7×10^{-7} M (for units see Engineer, Niederhauser, Piper & Sirois, 1978a).

Histamine in the supernatant and that remaining in the lung pieces (liberated by boiling for 10 min in 0.9% w/v NaCl solution) was measured fluorimetrically (Evans, Lewis & Thompson, 1973). Histamine release was expressed as a percentage of the total and was corrected for any 'spontaneous' release in control experiments.

Time course of mediator release

Sensitized chopped lung pieces were divided into two equal portions (2 g), one of which was incubated with ovalbumin grade III (10^{-2} mg/ml) in 12 ml Tyrode solution for 60 min at 37°C and the other incubated with A23187 (5 μg/ml) in 12 ml Tyrode solution for 60 min at 37°C. Repeated samples (0.5 ml) of supernatant were taken throughout the incubation period for determination of SRS-A, SRS and histamine content. After corrections for the volume removed, the cumulative totals of mediators at the various sampling times were calculated and expressed as a percentage of the maximum released into the supernatant during 60 min.

Effects of SRS in isolated perfused lungs

Sensitized chopped lung pieces (6 g) were incubated in 50 ml A23187 5 μg/ml in Tyrode solution containing cysteine 10^{-3} M for 45 min at 37°C. SRS in the supernatant was extracted and stored in ampoules as described for partially purified SRS-A (Engineer, Morris, Piper & Sirois, 1978b). Blank controls for the SRS extraction procedure were obtained using 50 ml A23187 (5 μg/ml in Tyrode solution) containing cysteine 10^{-3} M incubated at 37°C for 45 min without lung tissue. Unsensitized guinea-pig isolated lungs were perfused via the pulmonary artery (5 ml/min) with Krebs bicarbonate equilibrated with 5% CO_2 in O_2 at 37°C. The lung effluent superfused a rat stomach strip (RSS), rabbit aorta strip (RbA), rat colon (RC) and longitudinal muscle strips from guinea-pig ileum (GPI) which were made more specific for prostaglandin-like substances by the continuous infusion of combined antagonists to acetylcholine, histamine, 5-hydroxytryptamine and catecholamines (Piper & Vane, 1969). Contractions of these assay tissues following injections of partially purified SRS into the pulmonary artery were detected by Harvard 356 transducers and displayed on a multichannel pen-recorder (Watanabe).

Drugs and solutions

Calcium ionophore A23187 (gift from Lilly Research Centre) was initially dissolved in absolute ethanol and then diluted with Tyrode solution to concentrations of 1, 5 and 20 μg/ml (final concentrations of ethanol 0.2, 1 and 4% respectively). Other agents used were: indomethacin (Merck, Sharp & Dohme), ovalbumin grade II and III, cysteine and limpet arylsulphatase type V (Sigma).

Results

Release of SRS and histamine

During incubation for 45 min at 37°C with A23187, there was a dose-dependent release of SRS and histamine from chopped lung pieces (Figure 1), the peak release of both mediators occurring with 5 μg/ml A23187. At this concentration of ionophore, SRS production from sensitized lungs (1330 ± 290 mu/ml) was significantly greater ($P < 0.02$, $n = 5$ unpaired t test) than from unsensitized lungs (460 ± 50 mu/ml). Greater production of SRS from sensitized lungs was also apparent with A23187 1 μg/ml (sensitized 575 ± 150 mu/ml; unsensitized 215 ± 40 mu/ml, $P < 0.05$, $n = 3$ unpaired t test) but no difference was apparent with A23187 20 μg/ml ($P > 0.3$). The difference between histamine release from sensitized and un-

Table 1 Characterization of slow reacting substance (SRS)

SRS activity mu	*Procedure*	*Recovery mu (%)*	*Number of determinations*
460	Acetate buffer pH 5.0 (37°C × 60 min)	390 (85)	$n = 4$
460	Arylsulphatase type V (3.5 units) in acetate buffer pH 5.0 (37°C × 60 min)	0 (0)	$n = 4$
950	0.1 N NaOH (37°C × 30 min)	790 (83)	$n = 3$
950	0.1 N HCl (37°C × 30 min)	500 (53)	$n = 3$

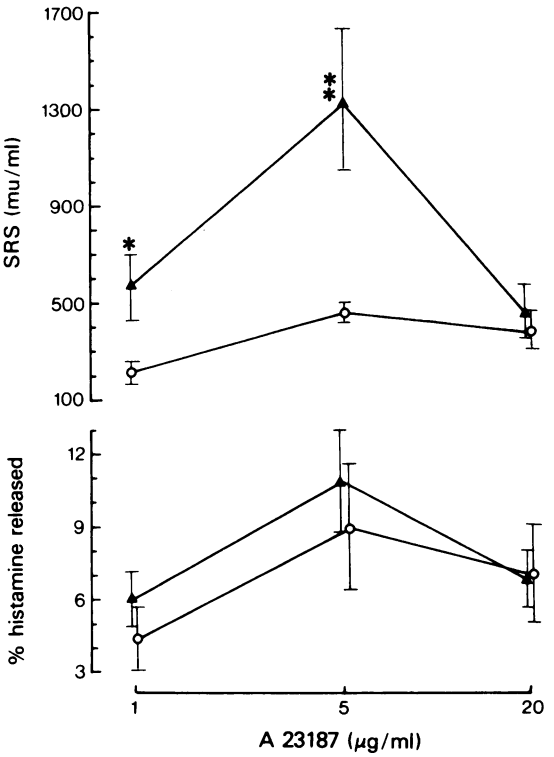

Figure 1 Release of slow-reacting substance (SRS) and histamine from guinea-pig chopped lung during 45 min incubation with calcium ionophore A23187. The release of SRS from sensitized lung (▲) was significantly greater than from unsensitized lung (○) at 1 and 5 μg/ml A23187 but the release of histamine was not significantly different. Mean values for results from 3 to 5 animals are shown. Vertical lines denote s.e. mean. * $P < 0.05$; ** $P < 0.02$.

sensitized lung was not significant at any concentration of ionophore ($P > 0.15$). In control experiments, lung pieces were incubated in ethanol (1 to 4%) in Tyrode solution for 45 min at 37°C. SRS was not detected but small amounts of histamine were occasionally present in the supernatant.

With sensitized lung, pre-incubation with indomethacin (1 μg/ml) for 60 min increased SRS production (mean 23%, $n = 5$) but did not significantly alter histamine release ($P > 0.4$). Similarly, cysteine 10^{-3} M added to the chopped lung pieces immediately before incubation with A23187 increased SRS production (mean 54%, $n = 5$) without significantly increasing histamine release.

Characterization of SRS

Ionophore-induced SRS was inactivated by arylsulphatase and was more stable at alkaline than at acid pH (Table 1). FPL55712 (1 μg/ml final concentration) infused over strips of guinea-pig ileum for 10 min antagonized the contractile effect of SRS.

Comparison of antigen- and ionophore-induced mediator release

Data from two preliminary experiments indicated that optimal release of SRS-A and histamine was achieved with ovalbumin 10^{-3} mg/ml to 10^{-1} mg/ml, so a concentration of 10^{-2} mg/ml was used in the experiments described here. The time courses for mediator release from sensitized lung with antigen and ionophore 5 μg/ml are shown (Figure 2). Whereas the content of SRS-A and histamine in the supernatant did not increase after 15 to 30 min with antigen challenge, both SRS and histamine increased up to 45 to 60 min with ionophore. With maximal antigen

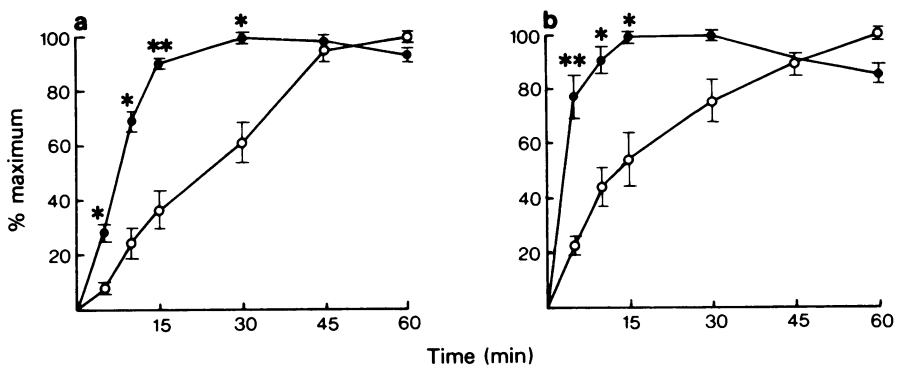

Figure 2 Time course for mediator release from guinea-pig chopped lung during incubation with antigen (●) and A23187 (○). At various time intervals slow-reacting substance (a) and histamine (b) in the supernatant were measured and expressed as a percentage of the maximum release in 60 min. Mean values for data from three guinea-pigs are shown. Vertical lines denote s.e. mean. ** $P < 0.01$ * $P < 0.025$ paired t-test.

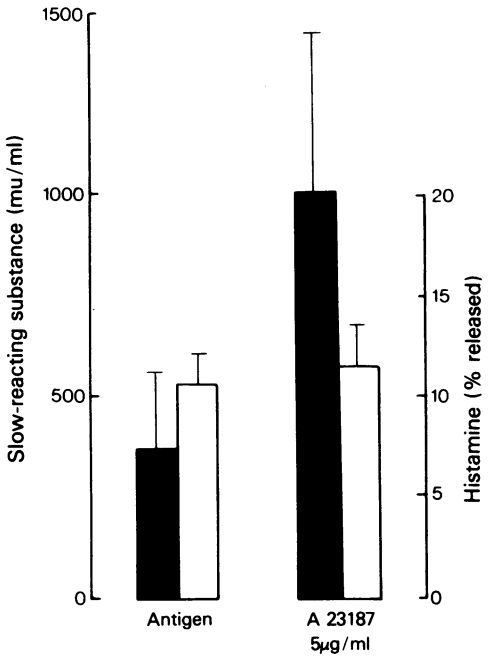

Figure 3 Maximal mediator release from guinea-pig sensitized chopped lung during 45 min incubation with antigen (10^{-2} mg/ml ovalbumin) or A23187. Mean values ($n = 6$) for slow-reacting substance (SRS, closed columns) and histamine (open columns) are shown and vertical lines represent s.e. mean. Maximal ionophore generation of SRS was significantly greater than maximal antigen generation of SRS-A ($P < 0.05$, paired t test) but the release of histamine was not significantly different.

challenge the formation of SRS-A was significantly less ($P < 0.05$, $n = 6$ paired t test) than with A23187 5 μg/ml but the release of histamine was not significantly different ($P > 0.35$) (Figure 3). Simultaneous challenge with antigen (10^{-2} mg/ml ovalbumin) and A23187 5 μg/ml produced more SRS-(A) than A23187 5 μg/ml ($P < 0.05$, $n = 5$ paired t test) or antigen ($P < 0.05$, $n = 5$ paired t test) alone.

Release of rabbit aorta contracting substance (RCS) and prostaglandin-like substances (PGLS) from isolated lungs

Injection of partially purified SRS (300 to 600 mu) into the pulmonary artery (t.p.) of isolated perfused lungs released RCS, which is mainly thromboxane A_2 (Svensson, Hamberg & Samuelsson, 1975; Bunting, Moncada & Vane, 1976) and PGLS (4 experiments) whereas injection directly over the assay tissues

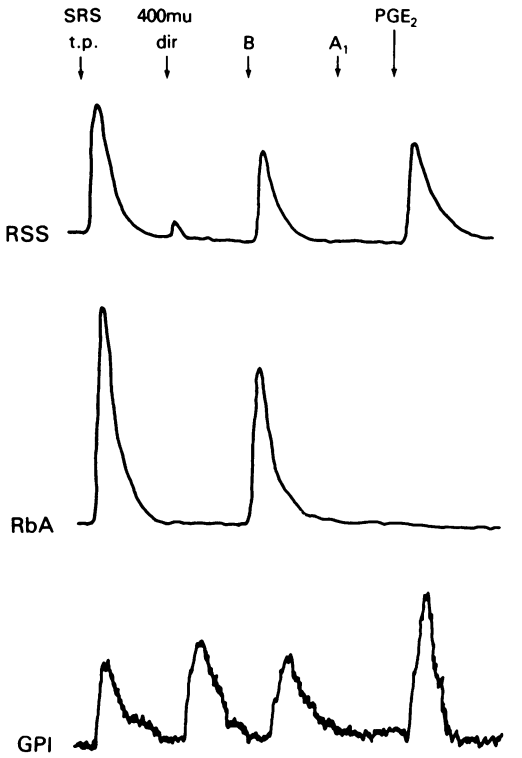

Figure 4 Effect of A23187-induced slow-reacting substance (SRS) in guinea-pig isolated lungs. SRS (400 mu), injected into the pulmonary artery (t.p.) released rabbit aorta contracting substance (RCS) and prostaglandin-like substances (PGLS) from the lungs. SRS (400 mu) given direct (dir) to the assay tissues caused a small contraction of rat stomach strip (RSS). Contractions of guinea-pig ileum (GPI) were similar with t.p. and dir doses of SRS. After SRS (400 mu) was incubated with acetate buffer pH 5.0 for 1 h, the release of RCS and PGLS was reduced (B). However, incubation with arylsulphatase in acetate buffer pH 5.0 destroyed the releasing activity of SRS (A_1). Effect of infusion of prostaglandin E_2 (PGE_2) 20 ng/ml for 1.5 min is given for comparison.

caused only a small contraction of RSS (Figure 4). SRS injections t.p. and direct caused similar contractions of GPI. Blank controls for SRS had no effect when given t.p. or direct to the tissues. Indomethacin (1 μg/ml for 15 min) inhibited the release of RCS and PGLS following t.p. injections of SRS. The releasing activity of SRS was destroyed by incubation with arylsulphatase (Figure 4). When samples of SRS were boiled for 15 min their ability to release RCS and PGLS from lungs was not diminished.

Discussion

The present experiments have shown that unsensitized or sensitized guinea-pig lung released SRS and histamine during incubation with A23187. From the evidence that A23187 initiates histamine release from rat peritoneal mast cells by transporting calcium into the cells (Foreman, Mongar & Gomperts, 1973) it is possible that this is also its mechanism of action in guinea-pig lung. SRS was inactivated by arylsulphatase (Orange *et al.*, 1974), more stable at alkaline than at acid pH (Orange & Austen, 1969) and antagonized by FPL55712 (Augstein *et al.*, 1973). When partially purified SRS was injected into the pulmonary artery of isolated perfused lungs it behaved like SRS-A by releasing RCS and PGLS (Engineer *et al.*, 1978b). This effect was inhibited by indomethacin 1 μg/ml confirming that the biological activity was due to metabolism of arachidonic acid by cyclo-oxygenase. Loss of releasing activity following incubation with arylsulphatase but stability to boiling implicated SRS as the releasing factor rather than RCS-releasing factor (Nijkamp, Flower, Moncada & Vane, 1976) or other known products. The reduced releasing activity of SRS after incubation in acetate buffer for 1 h indicated some instability at pH 5.0. There were also similarities between the mechanisms for release of SRS and SRS-A. Both indomethacin and cysteine, which have been shown to potentiate SRS-A production during antigen challenge of guinea-pig lung (Engineer *et al.*, 1978; Orange & Moore, 1976), increased ionophore-induced SRS production. These findings have augmented the existing evidence suggesting that slow-reacting substances generated by antigen challenge or calcium ionophore may be identical. Definitive comparison cannot be made until the chemical structure is known but analyses of highly purified samples have been unable to distinguish between them (Morris, Piper, Taylor & Tippins, 1979).

The peak release of mediators at A23187 5 μg/ml and rapid diminution of release at higher concentrations was comparable with the pattern observed in rat basophilic leukaemia cells (Jakschik *et al.*, 1977). The decreased release may have been due to increased cytotoxic effect of the ionophore (Jakschik *et al.*, 1977) or the higher concentrations of ethanol, since such effects have been reported with a variety of alcohols during antigen-induced mediator release (Fleisch, Calkins, Troxell & Hooker, 1976).

The greater production of SRS (but not histamine) by sensitized lung than by unsensitized lung was an interesting observation which requires further investigation. Two explanations for this consequence of sensitization seem possible: either there was an increase in lung of a cell type which produced SRS but little or no histamine during incubation with ionophore; or there was a qualitative change in the target cells such that after sensitization they responded to ionophore stimulation with greater SRS production but similar histamine release. It would appear that immunological sensitization of guinea-pig lung has many effects on cellular function since alterations in the synthesis and metabolism of prostaglandins (Palmer, Piper & Vane, 1973; Boot, Cockerill, Dawson, Mallen & Osborne, 1977) and cyclic adenosine 3′,5′-monophosphate (Mathé, Puri, Volicer & Sohn, 1976) have been reported previously.

Although there were similarities between the mechanisms for release of SRS and SRS-A (cf. effects of indomethacin and cysteine), the present investigations have also revealed qualitative and quantitative differences. Firstly, both SRS-A and histamine were released over a shorter time interval during antigen challenge than SRS and histamine with ionophore. Secondly, more SRS was released with maximal ionophore stimulation than SRS-A with maximal antigen challenge. Thirdly, the ratio of slow-reacting substances (mu/ml) produced to percent histamine was greater with ionophore than with antigen (ratio with ionophore 86:1; ratio with antigen 34:1). The slower mediator release with ionophore may have been partly related to its insolubility in aqueous media. However, when considered in conjunction with the other differences, a more likely explanation would be that antigen stimulated one cell population (presumably mast cells) and in addition to mast cells the ionophore stimulated another cell population. The additive effects in terms of greater release of slow reacting substances and histamine during simultaneous challenge with antigen and ionophore would be consistent with this hypothesis. Previous evidence has implicated cells other than mast cells (or basophils) in ionophore-induced SRS production from the rat peritoneal cavity (Bach & Brashler, 1974), human leucocytes (Conroy *et al.*, 1976) and dispersed rat lung cells (Paterson *et al.*, 1976) but the nature of the non-mast cell source of SRS in the lung remains speculative. Although the present data support the hypothesis that antigen and ionophore exerted their effects on predominantly different cell populations, they have not excluded the possibility that both stimuli activated a common cell population but via sufficiently independent mechanisms for additive effects to be apparent with simultaneous challenge. Further information may be provided by experiments with isolated lung cells.

This study has demonstrated the non-immunological release of SRS from the lungs and it seems likely that a non-mast cell source is involved. It is acknowledged that calcium ionophore is a non-physiological stimulus but its use has unmasked the potential for SRS formation in inflammatory processes other than IgE- and IgG-mediated allergic reactions. In particular, it supports the possibility that SRS may contrib-

ute to the pathophysiology of the poorly understood clinical entity of non-allergic asthma (Turnbull, Turnbull, Leitch, Crofton & Kay, 1977).

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