Differential Effects of a Partially Purified Preparation of Slow-Reacting Substance of Anaphylaxis on Guinea Pig Tracheal Spirals and Parenchymal Strips

JEFFREY M. DRAZEN, ROBERT A. LEWIS, STEPHEN I. WASSERMAN, ROBERT P. ORANGE,† and K. FRANK AUSTEN, *Department of Physiology, Harvard School of Public Health and the Departments of Medicine, Peter Bent Brigham Hospital, Robert B. Brigham Hospital, and Harvard Medical School, Boston, Massachusetts 02115*

ABSTRACT The contractile effects of partially purified slow-reacting substance of anaphylaxis (SRS-A) and histamine were compared on isolated guinea pig tracheal spirals and parenchymal strips. Histamine was equally active on both isolated tissues in a concentration-related fashion. SRS-A (0.1–10.0 U/ml) produced a concentration-related effect on parenchymal strips, whereas the tracheal spiral was 100 times less sensitive to this mediator. The contractile activity of SRS-A on parenchymal strips was diminished by incubation with limpet arylsulfatase and antagonized by FPL 55712, a known SRS-A antagonist. SRS-A, further purified by high pressure liquid chromatography, also demonstrated this preferential activity on guinea pig parenchymal strips. These data are consistent with the hypothesis, based on previous in vivo observations, that SRS-A is a selective peripheral airway constrictor.

INTRODUCTION

Slow-reacting substance of anaphylaxis (SRS-A),[1] an acidic lipid-like mediator with a molecular weight of ≅400 (1), is generated and released by a number of tissues after they have been prepared with antibody and subsequently incubated with specific antigen (2). Neither the material generated by immunologic reactions in the rat peritoneal cavity (1) or perfused guinea pig lung (3) nor that elicited by the action of divalent cation ionophore on preparations of rat peritoneal macrophages (4), leukemic basophils (5), or mast cells (6) has been structurally defined. We have previously demonstrated that intravenous infusion in the unanesthetized guinea pig of partially purified SRS-A results in a marked fall in dynamic pulmonary compliance but has little effect on pulmonary resistance (7). This apparent selective peripheral action of this mediator could be a result of uptake, distribution, or metabolism or of selective pharmacologic action on the peripheral airways compared to the central airways. To test this latter hypothesis, we have examined the comparative contractile effects of SRS-A on guinea pig central and peripheral airways in vitro (8). That the observed activity was a result of SRS-A was substantiated by showing inhibition with a preferential end-organ antagonist, FPL 55712 (9), by inactivation of the activity by arylsulfatases (10–12), and by maintenance of differential activity during considerable further purification.

METHODS

Histamine, atropine sulfate, limpet arylsulfatase, *p*-nitrocatechol sulfate (Sigma Chemical, Co., St. Louis, Mo.), mepyramine maleate (K & K Laboratories, Inc. Plainview, N. Y.), methanol, chloroform, silicic acid (CC-7), glacial acetic acid (Malinckrodt Inc., St. Louis, Mo.), Amberlite XAD-8 (Rohm and Haas Co., Philadelphia, Pa.), DE-52 cellulose (Whatman Ltd., Springfield Mill, Maidstone, Kent, England), C-18 Lychrosorb column (Altex Scientific, Inc., Berkeley, Calif.), and ammonium carbonate (Fisher Scientific Co., Medford, Mass.) were obtained as noted. FPL 55712 was a gift from Fisons Pharmaceutical, Ltd., Loughborough, Leicestershire, England.

† Deceased; former member of the Department of Immunology, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada. Dr. Lewis is the recipient of a Young Investigator Research Grant (HL-21089) from the National Heart, Lung, and Blood Institute. Dr. Wasserman is the recipient of an Allergic Diseases Academic Award (AI-00254) from the National Institute of Allergy and Infectious Diseases.

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[1] *Abbreviation used in this paper*: SRS-A, slow-reacting substance of anaphylaxis.

Comparative contractile activity of tracheal spirals and parenchymal strips. The spasmogenic potential of histamine and SRS-A was examined on guinea pig tracheal spirals and parenchymal strips. Male Hartley strain guinea pigs (300–350 g body wt) were killed by cervical dislocation and exsanguination. The trachea, heart, and lungs were removed en bloc. The trachea was cut spirally from the larynx to the carina according to the technique outlined by Constantine (13), and placed in an organ bath containing 37°C Tyrode's solution (14), continuously gassed with 95% oxygen and 5% CO_2. A strip of subpleural parenchyma (1.5 × 1.5 × 20 mm) was cut from the right lower lobe (8) and placed in an organ bath. One end of each tissue strip was fixed to a glass hook in the organ bath and the other end was connected to a force-displacement transducer (model FT 0.03C, Grass Instrument Co., Quincy, Mass.) by a silk thread. The initial tension in the tracheal spiral was adjusted to 8.0 g by turning a micrometer screw attached to the force transducer, and the initial tension of the parenchymal strip was set at 1.0 g.

Concentration-effect relationships for SRS-A and histamine were determined by adding appropriate amounts of concentrated solutions of each agent to the organ bath and waiting, usually 3–5 min, until a maximal contraction was reached. Geometrically increasing amounts of agonists were added to the bath until the maximally effective concentration was reached (100 μM histamine) (8) or until the maximum concentration permitted by the supply of material was used (SRS-A). The response of both tissues to 100 μM histamine was 85–95% of the response elicited by maximal depolarization with KCl. To normalize for varying amounts of contractile tissue in different preparations, the amplitude of the contraction of each type of tissue strip observed with 100 μM histamine was assigned a value of 100, and other contractile effects resulting from lower concentrations of histamine or SRS-A were scored relative to the maximal histamine contraction.

In initial experiments, the concentration-effect relationships for histamine and SRS-A were determined sequentially and repeated, allowing the tissues to relax to their initial tensions before another relationship was determined. In no case was tachyphylaxis or enhancement observed. In three experiments, after the initial histamine and SRS-A concentration-effect relationships were determined, the SRS-A antagonist, FPL 55712 (9), in a concentration of 0.1 μg/ml was added to all solutions, and SRS-A and histamine concentration-effect relationships were re-determined. In three additional experiments, concentration-effect relationships were determined for histamine, for SRS-A, for arylsulfatase-inactivated SRS-A (*vide infra*), for histamine in the presence of arylsulfatase, and for SRS-A to which arylsulfatase was added at the time of assay.

SRS-A preparation and assay. SRS-A was generated by ovalbumin challenge of the rat peritoneal cavity that had been prepared by injection of hyperimmune rat anti-ovalbumin serum. The SRS-A was harvested and partially purified by ethanol precipitation of proteins, desalting with Amberlite XAD-8 column chromatography, and stepwise elution from silicic acid as described (1, 7). This material, referred to as partially purified SRS-A, was used in the majority of experiments.

SRS-A activity was determined on the atropine and mepyramine-treated guinea-pig ileum (15). 1 U of SRS-A activity was defined as that amount of material yielding a contraction of the ileum equal in magnitude to that produced by 5 ng/ml of histamine in that bioassay (16).

To obtain a highly purified preparation, SRS-A was subjected to DEAE cellulose chromatography (17, 18) before stepwise elution from silicic acid (19, 20) and then further purified by high pressure liquid chromatography (3). 10,000 U of SRS-A in 0.9 ml H_2O:0.1 ml absolute methanol were applied to a 1.5-ml bed vol column of DE-52 cellulose that had previously been washed with 1 liter each of 1 N HCl, 1 N KOH, glacial acetic acid, methanol, and chloroform:methanol (2:1, vol/vol); the column was equilibrated with the final solution. After application of the SRS-A the column was eluted with 20-ml vol of chloroform:methanol (7:1, vol/vol), chloroform:methanol (7:3, vol/vol), absolute methanol, methanol:0.03 M ammonium carbonate (1:1, vol/vol), and methanol:0.3 M ammonium carbonate (1:1, vol/vol). These fractions were each flash evaporated to dryness, resuspended in distilled water, and assessed for SRS-A by bioassay on the guinea pig ileum. 5,000 U of SRS-A were detected in the ammonium carbonate-containing fractions with 90% of this amount in the final fraction. These two fractions were pooled, flash evaporated to dryness, and resuspended in 1.0 ml H_2O:methanol (9:1, vol/vol). The SRS-A was then applied to a 4-ml bed vol column of silicic acid that had been washed extensively with chloroform:methanol (7:1, vol/vol). The column was eluted sequentially at 1 ml/min with 20-ml vol of chloroform:methanol mixtures in the following ratios: 7:1, 7:3, 7:5, 1:1, and 1:5 (vol/vol). All fractions were flash evaporated to dryness and resuspended in distilled H_2O. 3,000 U of SRS-A were recovered in the final two fractions with 90% of the activity in the 1:1 chloroform:methanol mix. These two fractions were pooled, flash evaporated to dryness, and resuspended in 0.2 ml of 0.01 M phosphate buffer, pH 7.8:methanol (1:1, vol/vol) and applied to a C18 Lychrosorb column, equilibrated with 94% phosphate buffer (0.01 M, pH 7.8):6% methanol (vol/vol) in a high pressure liquid chromatography apparatus (Altex model 100, Altex Instruments, Berkeley, Calif.). The column was washed with the equilibrating buffer until the optical density of the eluate at 254 nm reached a stable base line. A linear gradient from the starting buffer to 100% methanol was applied to the column at a flow rate of 0.5 ml/min and nine fractions of 10-ml vol each were collected. Each fraction was flash evaporated to dryness, resuspended in water and assayed for SRS-A. Biologically active SRS-A (1,000 U) eluted in the range of 56–75% methanol was termed highly purified SRS-A because this material had <10% of the OD 254 activity of the combined material eluted from the high pressure liquid chromatography column.

Limpet arylsulfatase (10) possessing 9 U arylsulfatase activity/mg protein as defined by cleavage of 0.01 M *p*-nitrocatechol sulfate in Tyrode's buffer was used in all experiments. The use of Tyrode's buffer (pH 7.4–7.5) reduced the activity of the limpet arylsulfatase to ≅25% of its maximum for either cleavage of *p*-nitrocatechol sulfate or inactivation of SRS-A, but had the advantage of minimizing spontaneous inactivation of SRS-A at acid pH and permitting direct bioassay of SRS-A during the inactivation study. SRS-A, dissolved in 2–4 ml Tyrode's buffer, was inactivated with arylsulfatase in a ratio of 1 U arylsulfatase activity per 10 U SRS-A activity for 4 h at 37°C. Additional arylsulfatase was added hourly at a ratio of 1 U arylsulfatase to 10 U residual SRS-A. This procedure resulted in ≅80% inactivation of SRS-A. Equivalent amounts of SRS-A alone and arylsulfatase alone were incubated in parallel.

RESULTS

The contractile effects of partially purified SRS-A and histamine on tracheal spirals and parenchymal strips are compared in Fig. 1. Histamine contracted both strips; the effective concentration for one-half maximal contraction in both preparations was ≅3 μM. On the

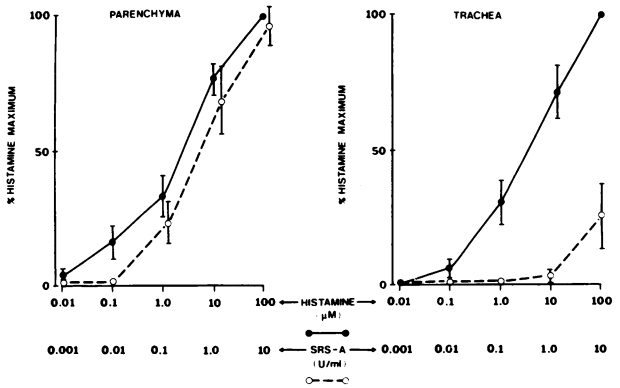

FIGURE 1 Contractile effects of histamine and partially purified SRS-A on parenchymal strip and tracheal spiral preparations. The contraction of each strip as a result of 100 μM histamine was given a value of 100 and all other contractions of that strip scored as a percent of the maximal histamine contraction. The results shown are the mean of three experiments. Vertical bars represent ±1 SEM. The absolute tensions developed in response to 100 μM histamine ranged from 1–3 g for tracheal spirals and 0.3–1.2 g for parenchymal strips.

parenchymal strip, concentrations of 10 U/ml of SRS-A achieved contractions of similar magnitude to those observed with 100 μM histamine. In contrast, SRS-A had little contractile effect on the tracheal spiral; 10 U/ml of SRS-A produced a contraction that was only 26% of that observed with 100 μM histamine. A 100-fold greater concentration of SRS-A was required to elicit a significant contraction of the tracheal spiral than was required to produce a contraction of the parenchymal strip.

The effects of the SRS-A antagonist, FPL 55712 (0.1 μg/ml), on parenchymal strip contractions induced by histamine or SRS-A are shown in Fig. 2. FPL 55712 inhibited the response of the parenchymal strip to SRS-A such that there were no contractions at 0.1 and 1.0

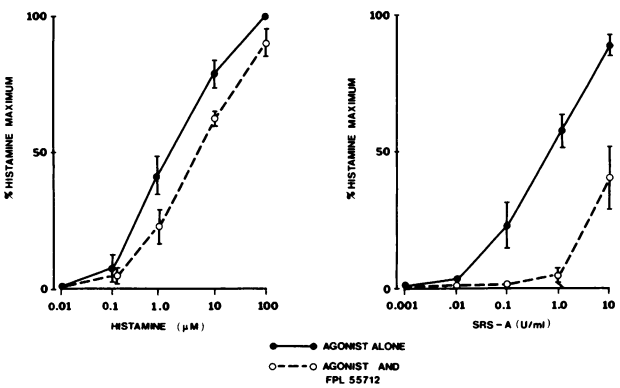

FIGURE 2 Effects of FPL 55712, 0.1 μg/ml, on the parenchymal strip contractions elicited by histamine or partially purified SRS-A. The results shown are the mean of three experiments. Vertical bars represent ±1 SEM.

U/ml and the contraction after 10 U/ml was significantly reduced ($P < 0.01$). There was a 43-fold shift in the response to SRS-A at 30% maximal contraction and a 2.5-fold shift in the histamine response. The effects of FPL 55712 on SRS-A were significantly greater than the effect on histamine-induced contractions ($P < 0.02$, Student's t test for paired variates).

The effects of incubation of SRS-A with arylsulfatase on the contractions elicited on the parenchymal strip were examined in three experiments. Arylsulfatase had no effect on the contractions elicited by histamine, nor did it affect contractions induced by SRS-A when the enzyme was added at the time of assay. The concentration-effect relationships for SRS-A incubated for 4 h in the presence or absence of arylsulfatase at 37°C in Tyrode's buffer are shown in Fig. 3. The concentration of the arylsulfatase-treated material required to elicit a 50% maximal contraction was 16.7 times greater than the untreated material, representing 94% inactivation. The inactivation observed in two additional experiments was 68 and 85%, respectively.

Highly purified SRS-A also demonstrated the differential contractile activity on the parenchymal strip and tracheal spiral. In the tracing shown in Fig. 4, cumulative additions of SRS-A to result in a final bath concentration of 0.03–1.0 U/ml had a graded contractile effect on the parenchymal strip, but these concentrations had no effect on the tracheal spiral. Both strips were contracted by 100 μM histamine. Therefore, with additional purification the differential contractile effects of SRS-A were retained.

DISCUSSION

SRS-A has a preferential contractile activity on the guinea pig parenchymal strip as compared to guinea

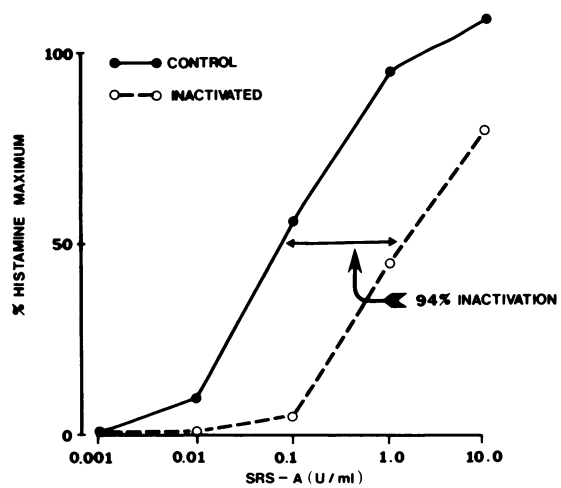

FIGURE 3 One experiment illustrating the effects of incubation of SRS-A with arylsulfatase (1 U arylsulfatase/10 U SRS-A) for 4 h at 37°C on the parenchymal strip response to SRS-A.

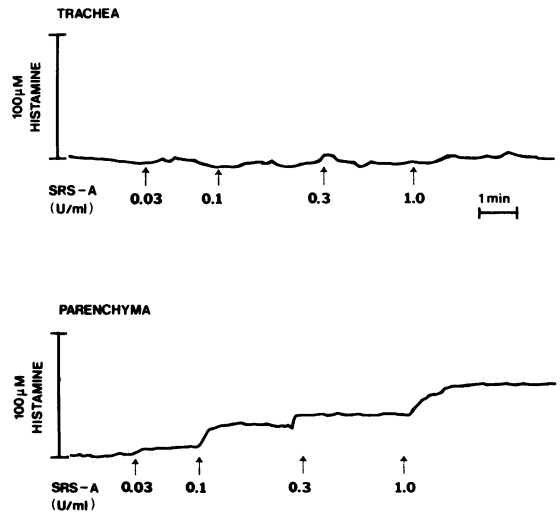

FIGURE 4 Tracing of the contractile effects of highly purified SRS-A on the tracheal spiral (upper panel) and parenchymal strip (lower panel). The magnitude of the contraction elicited by 100 μM histamine is shown by a bracket on the far left of each tracing.

pig tracheal spirals, whereas histamine has comparable activity on both preparations. Although SRS-A has not been structurally identified, it can be defined by a composite of biologic and physicochemical characteristics. Kellaway and Trethewie (21) identified SRS-A in anaphylactic diffusates from guinea pig lung based upon its prolonged onset and duration of spasmogenic action on the guinea pig ileum. Later, Brocklehurst (22) developed the current bioassay for SRS-A employing the guinea pig ileum in the presence of atropine and an antihistamine. Brocklehurst (22) also demonstrated that SRS-A had a specific profile of activity in various bioassay systems including exquisite sensitivity on the guinea pig ileum and human bronchiole with little contractile activity on the rat uterus or guinea pig trachea. More recently, Orange and coworkers established additional criteria including the physicochemical characteristics observed during isolation (1), lack of activity on the gerbil colon (16) and susceptibility to inactivation by crude limpet arylsulfatase (10), purified human arylsulfatase B (11, 12), and rat (23) arylsulfatase A or B. Finally, the antagonist FPL 55712 (9) preferentially antagonizes the contractile effects of SRS-A on the guinea pig ileum compared to other spasmogenic agents (9, 20, 24). The partially purified SRS-A employed in the present experiments elicited a concentration-effect relationship for contractile activity on the guinea pig parenchymal strip (Fig. 1), and this activity was inactivated by arylsulfatase (Fig. 3) and inhibited by FPL 55712 (Fig. 2). Further highly purified SRS-A obtained by high pressure liquid chromatography in a system similar to that used by others for purification of guinea pig SRS-A (3) also exhibited preferential contractile activity for the parenchymal strip. In addition, 1 U/ml of highly purified SRS-A and partially purified SRS-A, defined by their respective activity relative to histamine on the guinea pig ileum, had the same activity relative to histamine on the parenchymal strip. This finding supports the conclusion that the contractile activity of the partially purified SRS-A on the parenchymal strip was not a result of contaminants.

SRS-A, infused intravenously in the unanesthetized guinea pig, has been shown to produce a marked fall in dynamic compliance but only a small increase in pulmonary resistance (7). Based on the observation in the dog and in man that most of the resistance to airflow is in the larger more central airways (25, 26) and the similarity of the structure of the tracheobronchial tree of guinea pigs (27) and that of humans (28) and dogs (29), we interpreted these results to suggest that SRS-A had its predominant effects on the peripheral airways of the guinea pig. A similar analysis of the differential effects of histamine and acetylcholine in vivo showed that compared to histamine, acetylcholine has more predominant central airway effects (8, 30). It is noteworthy that the relative effects on pulmonary mechanics of these three pharmacologic agents were consonant with the subsequent in vitro findings. Acetylcholine is less active on the parenchymal strip than histamine and equally active on the tracheal spiral, whereas SRS-A is equally active with histamine on the parenchymal strip and has little activity on the tracheal spiral. Thus, analysis of both in vivo and in vitro data are consistent with the hypothesis that SRS-A is a selective peripheral airway constrictor. Because peripheral airway constriction is common in asthma (31, 32), it is tempting to speculate that SRS-A may be a major mediator of this peripheral airway constriction. An analysis of the relative amounts of SRS-A and histamine released during immunoglobulin E-dependent reactions in human lung tissues (33) supports this concept, because $\cong$100 U of SRS-A are released for each microgram of histamine.

The factors that may be responsible for this selective peripheral airway effect of SRS-A are a matter for speculation only. It is possible that the trachea lacks appropriate smooth muscle receptors for SRS-A, thus explaining the relatively small contractile effects of this mediator as compared to those of histamine on the trachea. It is also possible that substances released or synthesized upon activation of contractile elements by SRS-A act to modulate its effects differently in small and large airways. Such a hypothesis is not unreasonable because airway contractile responses have been shown in vitro to be modulated by secondarily synthesized prostaglandins (34, 35), and the arachidonic acid metabolic capabilities of guinea pig central and peripheral airways differ substantially (36).

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