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BRONCHOCONSTRICTOR ACTION AND ANTAGONISM OF A SLOW-REACTING SUBSTANCE FROM ANAPHYLAXIS OF GUINEA-PIG ISOLATED LUNG

BY

P. A. BERRY AND H. O J. COLLIER

From the Department of Pharmacological Research, Parke, Davis & Co., Hounslow, Middlesex

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Slow-reacting substance produced in anaphylaxis (SRS-A) increased resistance of the lungs to inflation in the guinea-pig *in vivo* and caused isolated preparations of its tracheobronchial muscle to contract. SRS-A also contracted human isolated bronchial muscle and some but not all preparations of rabbit trachea. Nonsteroid anti-inflammatory drugs, which antagonize bronchoconstriction induced by kinins, but not that by histamine, acetylcholine, 5-hydroxytryptamine, substance P, angiotensin or lung prostaglandin, also antagonized the bronchoconstrictor action of SRS-A. This antagonism resembled that of kinins in being surmounted by higher doses of agonist, in the potencies of active drugs and in the types of drugs which were inactive. However, receptors in guinea-pig tracheobronchial muscle for SRS-A seem to be distinct from those for bradykinin, since preparations of this muscle could become unresponsive to either agent *in vivo* and *in vitro*, while remaining responsive to the other.

When isolated lungs of sensitized guinea-pigs are treated with antigen, spasmogenic substances are obtained (Kellaway & Trethewie, 1940 ; Campbell & Nichol, 1940 ; Brocklehurst, 1953, 1955, 1960 ; Hawkins & Rosa, 1959 ; Chakravarty, 1960). One of these is histamine ; another is " slow-reacting substance," so named because the contraction it elicited from smooth muscle had a slower time-course than that elicited by histamine. Spasmogenic actions on several types of smooth muscle have been attributed to slow-reacting substance, and recent evidence shows that more than one unidentified spasmogen can be obtained from guinea-pig isolated lung treated with antigen (Änggård, Bergqvist, Högberg, Johansson, Thon & Uvnäs, 1963 ; Berry, Collier, Fawcett, Holgate, Jones & Lockhart, unpublished).

Brocklehurst (1953, 1955) described a " slow-reacting substance in anaphylaxis (SRS-A) " obtained by perfusing isolated lungs from sensitized guinea-pigs with antigen. This substance contracted guinea-pig ileum and human bronchioles *in vitro*, but failed to contract guinea-pig isolated trachea. However, in our hands (Berry, Collier & Holgate, 1963) material prepared by Brocklehurst's method also elicited a slow contraction from guinea-pig tracheobronchial muscle both *in vivo* and *in*

vitro. For reasons discussed below, we have attributed this bronchoconstrictor action to SRS-A, without implying that SRS-A necessarily contains only one spasmogenic principle. The present paper describes the characteristics of the bronchoconstrictor action of partially purified SRS-A in the guinea-pig and other animals.

Antagonism of the bronchoconstrictor action of SRS-A in the guinea-pig by acetylsalicylic acid and other nonsteroid anti-inflammatory drugs was also reported in our preliminary communication (Berry *et al.*, 1963). Since several of these drugs have antiasthmatic action in man (Herxheimer & Stresemann, 1961 ; Stresemann, 1963), we have explored their antagonism of SRS-A-induced bronchoconstriction more thoroughly.

Since drugs that antagonize the bronchoconstrictor action of SRS-A also antagonize that of kinins but not that of other bronchoconstrictor agents (Collier, Holgate, Schachter & Shorley, 1960 ; Collier & Shorley, 1960, 1963 ; Bhoola, Collier, Schachter & Shorley, 1962), we have investigated whether the receptors for bradykinin and SRS-A in tracheobronchial muscle are distinct and how far the potencies of drugs in antagonizing SRS-A and bradykinin are correlated.

METHODS

SRS-A

SRS-A was prepared by a method based on that of Brocklehurst (1953). Eight-week old albino guinea-pigs were sensitized by injecting a 10% suspension of powdered egg albumen (B.D.H.) in 0.9% saline (1 ml. subcutaneously and 1 ml. intraperitoneally). Three weeks later, the animals were killed, the lungs were removed and, after cannulating the pulmonary artery, the lungs were washed with a Tyrode solution (NaCl, 8 g ; KCl, 0.2 g ; $NaHCO_3$, 1 g ; NaH_2PO_4, 0.05 g ; $CaCl_2$, 0.2 g ; $MgCl_2$, 0.22 g ; and distilled water to 1 l.) to which cysteine (1.2 g/l.) and succinic acid (120 mg/l.) had been added. The perfusion fluid was kept at 40° C. After 5 to 10 min washing, the fluid was changed to a similar one with added egg albumen (0.75 to 4 g/l.). The perfusate was centrifuged for 10 min at 2,000 rev/min (relative centrifugal force, 1,100 to 1,200) ; the resulting supernatant solution was " crude SRS-A ". Removal of histamine from crude SRS-A by ion-exchange chromatography gave " histamine-free SRS-A." Absorption of histamine-free SRS-A on partially activated charcoal, followed by elution, gave " charcoal-purified SRS-A " (Berry, Collier, Fawcett, Holgate, Jones & Lockhart, unpublished). All preparations were freeze-dried and stored at −30° C. Unless otherwise stated, charcoal-purified SRS-A was used. For control purposes, the washing fluid collected before anaphylaxis was purified in the same way.

SRS-A preparations were assayed on guinea-pig isolated ileum, suspended in oxygenated Tyrode solution at 37° C, containing glucose (1 g/l.) and mepyramine (1 μg/ml.). Potency was expressed in U/mg, a unit being the activity of 0.5 mg of a laboratory reference sample of crude SRS-A. This unit is roughly equivalent to that of Brocklehurst (1960), who defined a unit as equivalent to the activity of 0.05 ml. of his standard sample of freeze-dried perfusate. Samples of crude and of histamine-free SRS-A usually assayed at 0.6 to 1.5 U/mg, and of charcoal-purified SRS-A at 40 to 200 U/mg.

Other substances

Synthetic bradykinin (Nicolaides & DeWald, 1961), lung prostaglandin $F_{2\alpha}$ (Bergström, Dressler, Krabisch, Ryhage & Sjövall, 1962), acetylcholine bromide, histamine acid phosphate, 5-hydroxytryptamine creatinine sulphate and a sample of substance P, prepared by the method of Pernow (1953) and estimated to contain 0.5 U/mg, were used for reference as broncho-

constrictor agents. As antagonists of some of these agents, atropine sulphate, hexamethonium bromide, mepyramine maleate and bromolysergic acid diethylamide were used. Drugs tested as antagonists of SRS-A are given in Table 2. These include the following new drugs: flufenamic acid (Winder, Wax, Serrano, Jones & McPhee, 1963), mefenamic acid (Winder, Wax, Scotti, Scherrer, Jones & Short, 1962), indomethacin (Winter, Risley & Nuss, 1963), ibufenac (Adams, Cliffe, Lessel & Nicholson, 1963) and α-(4-phenylphenoxy)propionic acid (Adams & Cobb, 1963). Weights of salts are expressed as active acid or base. Substances were administered *in vivo* by injecting a solution in 0.9% saline into a cannula tied into the jugular vein and washing in with 0.5 ml. of 0.9% saline containing heparin (10 U/ml.; Evans Medical). Urethane was used as anaesthetic except on one ocasion, when it was replaced by phenobarbitone.

In tests for inactivation by chymotrypsin, saline (0.9%) solutions of SRS-A were incubated for 20 to 30 min at 37° C in crystalline salt-free bovine chymotrypsin (3.3 μg/ml.; Armour, 957 U/mg).

Biological tests

With the small modifications mentioned below, methods of preparing lungs (Konzett & Rössler, 1940) and tracheobronchial muscle for recording the effects of SRS-A and the procedure for measuring minimal effective dose of antagonists were those used in previous studies on kinins (Collier *et al.*, 1960; Collier & Shorley, 1960, 1963; Bhoola *et al.*, 1962). For isolated preparations of guinea-pig and rabbit trachea, oxygenated Tyrode solution containing glucose (1 g/l.) was usually used. Occasionally, Krebs-Henseleit solution (NaCl, 9 g; KCl, 0.448 g; $NaHCO_3$, 0.13 g; $CaCl_2$, 0.36 g; $MgSO_4.7H_2O$, 0.38 g; glucose, 1 g; and distilled water to 1 l.) bubbled with oxygen containing 5% carbon dioxide was used. The rate of outflow from guinea-pig isolated lungs perfused through the trachea was recorded by a Thorp counter. For work with crude SRS-A, guinea-pigs were previously treated with mepyramine (1 to 5 mg/kg).

RESULTS

Action of SRS-A on tracheobronchial muscle

Intravenous injection of SRS-A increased resistance to inflation of guinea-pig lungs *in vivo*, 40 to 160 U of either crude or charcoal-purified material being effective. This dose-range of SRS-A corresponded in activity with 0.5 to 4 μg of histamine or 0.5 to 8 μg of bradykinin (Fig. 1). Charcoal-purified washing fluid, collected from isolated perfused lungs before anaphylaxis, had less than one-twentieth the activity, weight for weight, of SRS-A. Egg albumen (70 mg/kg) did not increase resistance to inflation. The tracing shown in the upper panel of Fig. 1 was obtained in an experiment comparing the latencies and rates of onset of the responses to SRS-A, histamine and bradykinin. The mean values (and their fiducial limits) are summarized in Table 1. This table shows that the mean latency of the response to SRS-A was significantly longer than that of histamine and possibly that of bradykinin, and that the mean time from onset to peak was significantly shorter for SRS-A than for bradykinin. In total time from injection to peak of response, SRS-A took significantly longer than histamine. In the lower panel of Fig. 1, the effect of SRS-A declined more slowly than that of histamine, but faster than that of bradykinin.

A similar response to SRS-A was obtained after pithing the spinal cord and crushing the sympathetic nerves and vagi in the neck (Fig. 7). In animals with

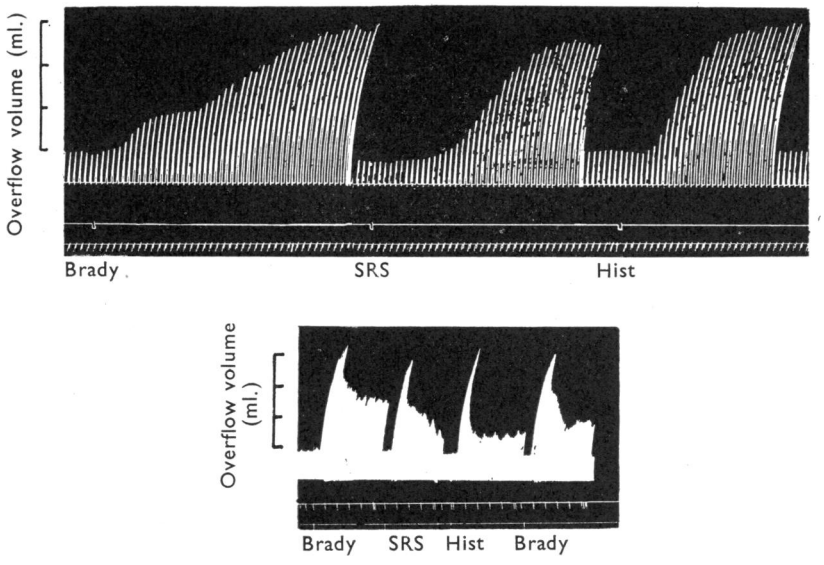

Fig. 1. Resistance to inflation of guinea-pig lungs *in vivo*. Konzett-Rössler preparation. Time course of action of SRS–A, histamine and bradykinin. The calibration (left) indicates the reduction in volume of air entering the lungs. Doses were injected into a jugular vein and the injection signal marks the time at which washing in of the dose began. After each response had been recorded, the drum was stopped and the lungs were forcibly reinflated. A unit of SRS–A is the activity of 0·5 mg of a laboratory standard preparation, assayed on guinea-pig ileum. The upper panel shows latency and rate of onset. Guinea-pig, 275 g ; time (lower signal), 1 sec.; Brady, 8 μg of bradykinin ; SRS, 40 U of SRS–A ; Hist, 2 μg of histamine. Doses were given at 5 min intervals. The lower panel shows decline of response. Guinea-pig, 416 g ; time (upper signal) 1 min ; Brady, 1 μg of bradykinin ; SRS, 120 U of SRS–A ; Hist, 0·8 μg of histamine. Doses were given at 10 min intervals.

TABLE 1

RATE OF DEVELOPMENT OF RESPONSES OF GUINEA-PIG LUNGS *IN VIVO* TO SRS–A, HISTAMINE AND BRADYKININ

Approximately equiactive doses are compared. Times are means with 95% fiducial limits in parentheses

Period	Agent	Time (sec)	Significance of difference from SRS–A: Histamine	Significance of difference from SRS–A: Bradykinin
Injection to onset of response	SRS–A	9·86 (5·9–13·8)		
	Histamine	5·35 (4·4– 6·3)	$P < 0·05$	$P < 0·10$
	Bradykinin	6·00 (4·6– 7·4)		
Onset to peak of response	SRS–A	16·71(13·7–19·7)		
	Histamine	13·05 (9·4–16·7)	$P \simeq 0·10$	$P < 0·01$
	Bradykinin	25·40(20·8–30·0)		
Injection to peak of response	SRS–A	26·57(22·4–30·7)		
	Histamine	18·40(15·2–21·6)	$P < 0·01$	$P \simeq 0·10$
	Bradykinin	31·40(27·1–35·7)		

the chest wall opened, resistance to pulmonary inflation could be increased by dropping a solution of SRS-A on to the pleural surface of the lungs.

The upper panel of Fig. 2 shows that mepyramine, atropine and bromolysergic acid diethylamide did not reduce responses to SRS-A, and that sodium acetylsalicylate abolished the response to SRS-A, but not that to substance P. The lower

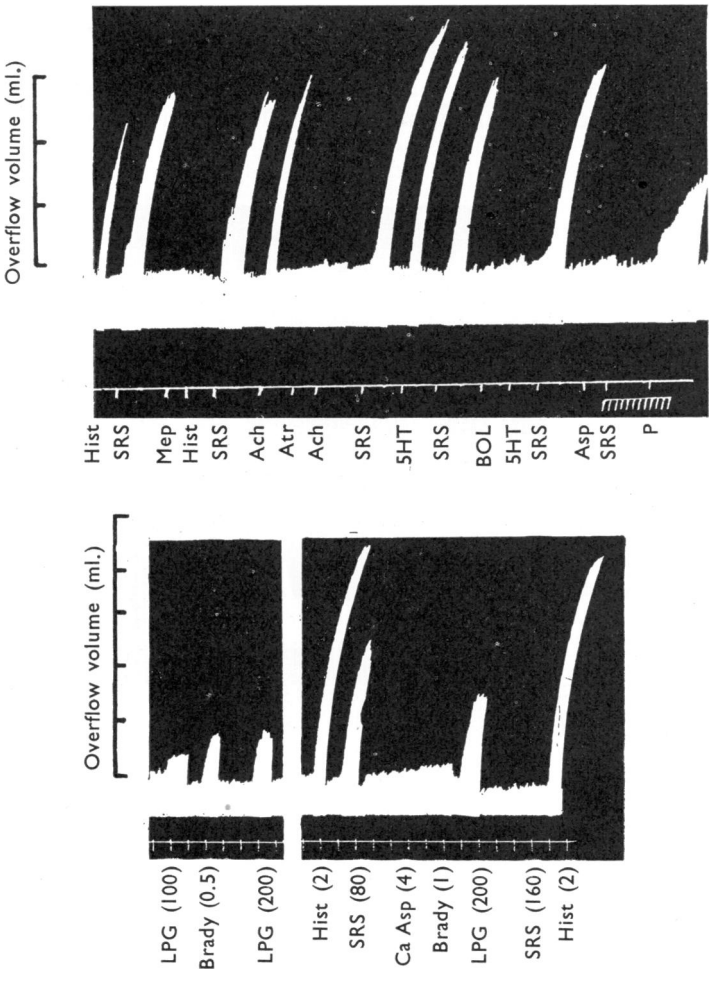

Fig. 2. Resistance to inflation of guinea-pig lungs *in vivo*. Distinction of SRS–A from histamine, acetylcholine, 5-hydroxytryptamine, substance P and lung prostaglandin $F_{2\alpha}$. Upper panel, guinea-pig 270 g ; time (lower signal) 10 sec ; Hist, 1 μg of histamine ; SRS, 40 U of SRS–A ; Mep, 1 mg/kg of mepyramine ; Ach, 5 μg of acetylcholine ; Atr, 1 mg/kg of atropine ; 5HT, 2 μg of 5-hydroxytryptamine ; BOL, 2 mg/kg of bromolysergic acid diethylamide ; Asp, 4 mg/kg of sodium acetylsalicylate ; P, 2·5 U of substance P. Lower panels, guinea-pig 330 g ; time, 30 sec ; LPG, 100 and 200 μg of lung prostaglandin ; Brady, 0·5 and 1 μg of bradykinin ; SRS, 80 and 160 U of SRS–A ; Hist 2 μg of histamine ; Ca Asp, 4 mg/kg of calcium acetylsalicylate. Doses were given at 5 min intervals ; other details as in Fig. 1.

panels of Fig. 2 show that relatively large intravenous doses of lung prostaglandin $F_{2\alpha}$ increased resistance of the lungs to inflation, 200 μg of prostaglandin being about as effective as 0.5 μg of bradykinin. After calcium acetylsalicylate, the response to prostaglandin appeared greater than before, whereas double the previous doses of SRS-A and bradykinin were ineffective, as expected. This was the only *in vivo* experiment with lung prostaglandin that supplies of the material permitted. Hexamethonium (10 mg/kg, intravenously) did not affect the response to SRS-A.

Since the effects of SRS-A and bradykinin were both abolished by acetylsalicylate, we investigated possible differences between them, other than that in onset of response shown in Table 1. Fig. 3 shows that incubation with crystalline chymotrypsin did not reduce the activity of SRS-A, although it inactivated bradykinin, as expected.

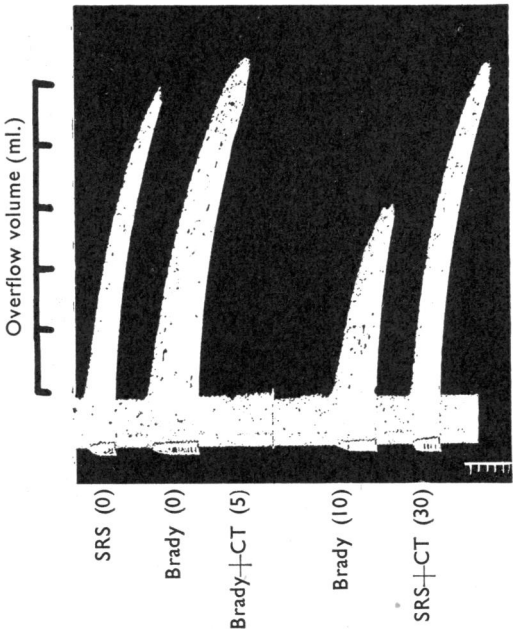

Fig. 3. Resistance to inflation of guinea-pig lungs *in vivo*. Failure of chymotrypsin to reduce responses to SRS–A. Figures in parentheses indicate min of incubation at 37° C in 0·2 ml of 0·9% saline. Guinea-pig, 800 g ; time, 10 sec ; SRS, 80 U of SRS–A ; Brady, 1 μg of bradykinin ; CT, 3·3 μg of chymotrypsin. Doses were given at 5 min intervals ; other details as in Fig. 1.

As with bradykinin, unresponsivenes to SRS-A sometimes developed *in vivo* and *in vitro*. Preparations of lungs *in vivo* and of guinea-pig isolated trachea, which had become unresponsive to SRS-A, remained sensitive to bradykinin (Fig. 4). Conversely, preparations becoming unresponsive to bradykinin remained sensitive to SRS-A (Fig. 5). On rare occasions, preparations became desensitized to both agents, while remaining responsive to histamine.

SRS-A elicited a slow contraction of the isolated tracheobronchial muscle of the guinea-pig. The lower panels of Figs. 4 and 5 show that, in this preparation, 7 to 20 U/ml. of SRS-A were about as effective as 0.2 to 1 μg/ml. of acetylcholine. In guinea-pig isolated lungs perfused through the trachea, SRS-A reduced the rate of flow of perfusion fluid, taking effect more slowly than did acetylcholine. In this preparation, 120 U of SRS-A were about equally effective as 16 μg of acetylcholine.

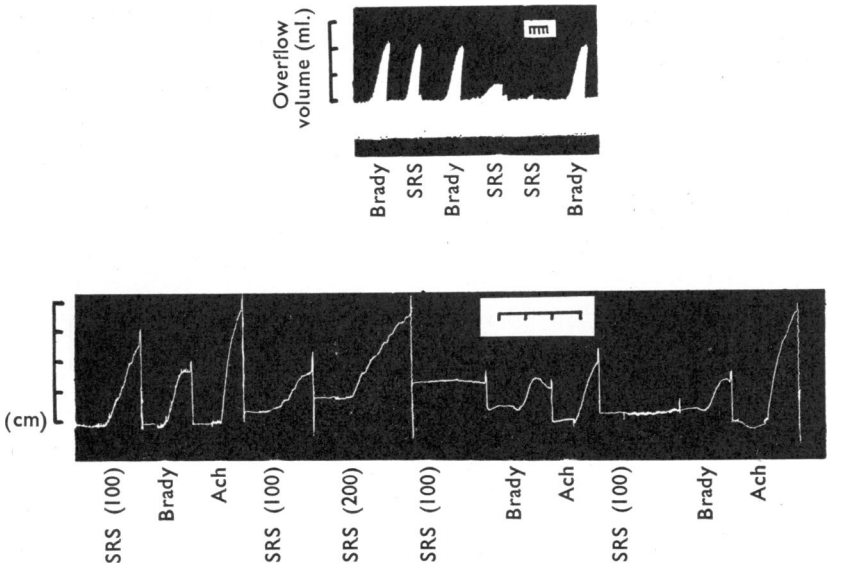

Fig. 4. Desensitization to SRS–A without corresponding desensitization to bradykinin. Upper panel, resistance to inflation of guinea-pig lungs *in vivo*. Guinea-pig, 440 g ; time, 10 sec ; Brady, 4 μg of bradykinin ; SRS, 160 U of SRS–A. Doses were given at 5 min intervals ; other details as in Fig. 1. Lower panel, guinea-pig isolated trachea in 15 ml. bath. Time, 1 min ; SRS, 100 and 200 U of SRS–A ; Brady, 100 μg of bradykinin ; Ach, 2·5 μg of acetylcholine. Doses were given at 10 min intervals ; lever magnification, × 12. The calibration (left) indicates distance traced by the writing point.

Of fourteen isolated preparations of rabbit trachea, eight contracted in response to SRS-A (1 to 32 U/ml.), whereas all responded to acetylcholine (0.1 to 4 μg/ml.). Histamine and bradykinin were inactive in these preparations, but 5-hydroxytryptamine was effective.

SRS-A (2 to 16 U/ml.) caused a contraction of all isolated preparations of human bronchial muscle. This response developed more slowly than that to acetylcholine (0.05 to 0.4 μg/ml.) which was used as a reference (see Fig. 9). Some human bronchial preparations were also tested with bradykinin. In low doses (4 μg/ml.), this peptide was inactive ; higher doses (20 to 40 μg/ml.) caused either slight relaxation, slight contraction, both of these responses consecutively, or neither.

In all the preparations mentioned above, response to SRS-A was related to dose. Fig. 6 gives the log dose/response curves obtained from four of these preparations

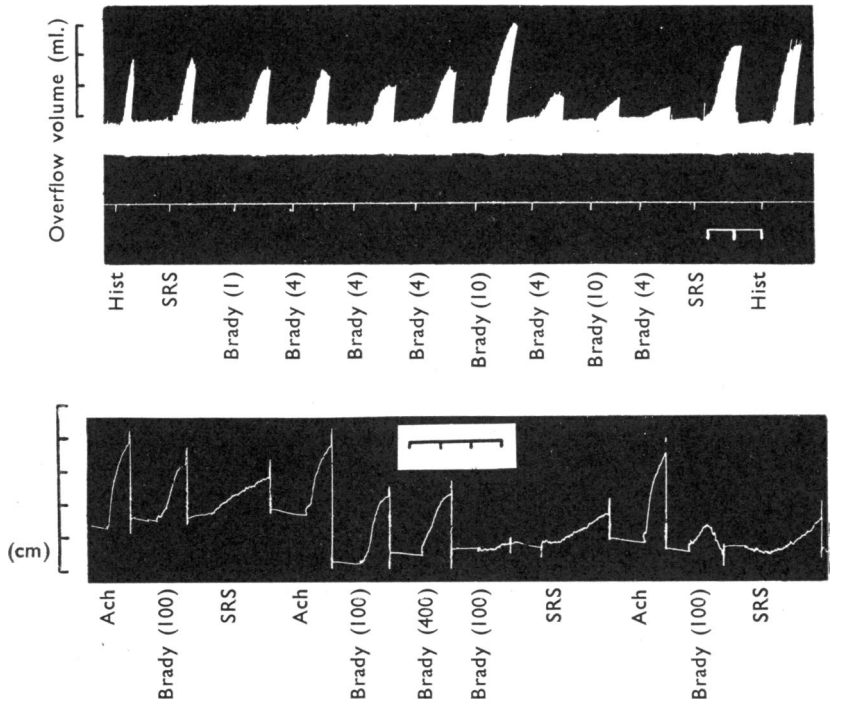

Fig. 5. Desensitization to bradykinin without corresponding desensitization to SRS–A. Upper panel, resistance to inflation of lungs *in vivo*. Guinea-pig, 350 g ; time, 10 sec; Hist, 1 μg of histamine ; SRS, 80 U of SRS–A; Brady, 1, 4 and 10 μg of bradykinin. Doses were given at 5 min intervals ; other details as in Fig. 1. Lower panel, guinea-pig isolated trachea in 5 ml. bath. Time, 1 min ; Ach, 5 μg of acetylcholine ; Brady, 100 and 400 μg of bradykinin ; SRS, 100 U of SRS–A. Lever magnification, $\times$ 19 ; other details as in Fig. 4.

and from guinea-pig isolated ileum preparations in comparable experiments using the same apparatus. In these, the human was the most and the guinea-pig the least sensitive to SRS-A of the *in vitro* preparations of tracheobronchial muscle. However, the sensitivity of human bronchial muscle to SRS-A was not always as great as shown in Fig. 6 (see Fig. 9). Guinea-pig lung was relatively sensitive to SRS-A *in vivo*.

Antagonism by drugs

In the guinea-pig, acetylsalicylic acid (1 to 4 mg/kg) injected intravenously before an effective dose of SRS-A, abolished the expected increase of resistance to pulmonary inflation *in vivo*. The response to bradykinin was affected in the same way, although that to histamine was not reduced (Fig. 2). The same effect was obtained when the sodium or calcium salt of acetylsalicylic acid was used, when the drug was given by the intraperitoneal instead of the intravenous route, or when

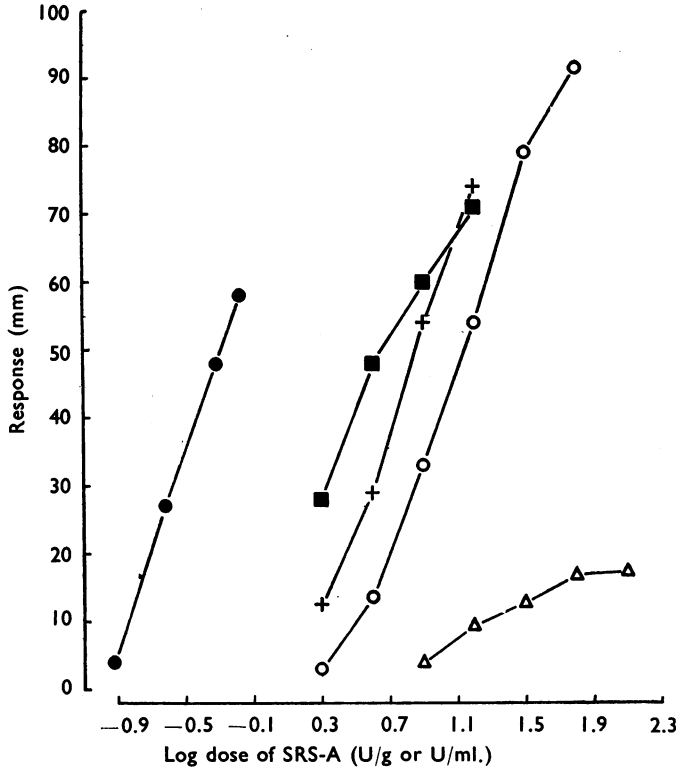

Fig. 6. Log dose/response curves of SRS–A ; ●——●, guinea-pig lungs *in vivo* ; ■——■, human isolated bronchial muscle ; +——+, guinea -pig isolated ileum ; ○——○, rabbit isolated trachea ; △——△, guinea-pig isolated trachea. The same 5 ml. organ-bath and lever magnification were used for all *in vitro* preparations.

phenobarbitone (210 mg/kg intraperitoneally) replaced urethane as anaesthetic. After pithing the spinal cord and crushing with tight ligatures both cervical sympathetic nerves and both vagi in the neck, acetylsalicylic acid was still effective (Fig. 7).

The antagonism of SRS-A by acetylsalicylic acid was surmounted by higher doses of agonist. Fig. 8 shows the parallel shift of the log dose/response curve of SRS-A caused by intravenous injection of calcium acetylsalicylate (1 mg/kg). In this experiment eleven-times the initial dose of SRS-A was needed to restore the pulmonary response to the same level as before the antagonist.

Table 2 summarizes the results of testing anti-inflammatory, antirheumatic and analgesic drugs as antagonists of SRS-A on guinea-pig lungs *in vivo*. It shows that the antipyretic drugs were effective, whereas quinoline antimalarials, corticosteroids and a narcotic analgesic were not. Table 2 also shows the close parallelism between the potencies of drugs against SRS-A and against bradykinin.

In some experiments on guinea-pig isolated trachea, acetylsalicylic acid (100 μg/ml.) reduced the response to SRS-A without reducing that to acetylcholine (Fig. 9.

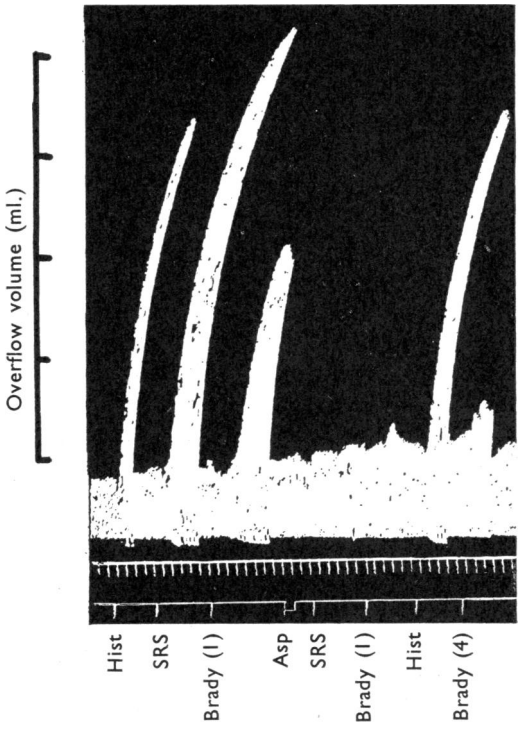

Fig. 7. Resistance to inflation of guinea-pig lungs *in vivo*. Action of SRS–A and bradykinin and their antagonism by acetylsalicylic acid after pithing the spinal cord and crushing the sympathetic cervical nerves and vagi. Guinea-pig, 700 g; time (upper signal), 10 sec ; Hist, 2 μg of histamine; SRS, 80 U of SRS–A; Brady, 1 and 4 μg of bradykinin ; Asp, 2 mg/kg of acetylsalicylic acid. Doses were given at 5 min intervals ; other details as in Fig. 1.

upper panel). The lower panel of this figure shows that, in human isolated bronchial muscle, acetylsalicylate depressed the response to SRS-A more than that to acetylcholine.

DISCUSSION

The work described concerns the action on tracheobronchial muscle of a partially purified material obtained by perfusing with antigen the isolated lungs of sensitized guinea-pigs. This material produced a slow contraction of guinea-pig isolated ileum and human bronchioles, and, when injected into the Konzett-Rössler preparation of the guinea-pig, it increased resistance of the lungs to inflation, acting more slowly than did histamine (Table 1). It also elicited a slow contraction of guinea-pig isolated trachea (Figs. 4 and 5). In origin and in actions on guinea-pig ileum and human bronchial muscle, this material resembles slow-reacting substance in anaphylaxis (SRS-A ; Brocklehurst, 1953, 1955) ; but the question arises whether its effects on guinea-pig tracheobronchial muscle are due to the presence of SRS-A or to that of some other endogenous substance, especially since Brocklehurst (1953) failed to obtain contraction of guinea-pig isolated trachea with SRS-A.

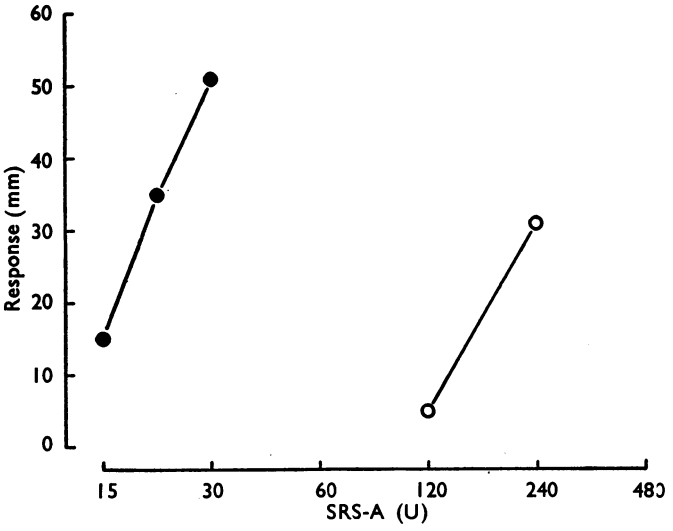

Fig. 8. Resistance to inflation of guinea-pig lungs *in vivo*. Dose/response curves of SRS–A before, ●——●, and after, ○ ——○, calcium acetylsalicylate (1 mg/kg). Guinea-pig wieght, 320 g. Doses were given intravenously at 5 min intervals. Crude SRS–A was used after treatment of the guinea-pig with mepyramine (5 mg/kg).

The failure of mepyramine, atropine and bromolysergic acid diethylamide to antagonize the material on guinea-pig lungs *in vivo* (Fig. 2) shows that its action cannot be due to the presence or release of histamine, acetylcholine or 5-hydroxytryptamine. The antagonism of the material by acetylsalicylic acid confirms this and excludes substance P, angiotensin (Bhoola *et al.*, 1962) and lung prostaglandin. The absence of desensitization to bradykinin by the material (Fig. 4) and *vice versa* (Fig. 5) exclude kinin as the substance involved, and this is confirmed by the failure of chymotrypsin to reduce the activity of the material (Fig. 3). The distinction from 5-hydroxytryptamine and bradykinin is further confirmed by the effectiveness of the material on human isolated bronchial muscle which is insensitive to the other two agents. Since the material produces a faster response than does bradykinin (Table 1), it presumably does not act by liberating bradykinin ; since the latency of the response to bradykinin is probably shorter and certainly not longer than that to the material, bradykinin presumably does not act by liberating the active constituent of the material. The above facts show that the material obtained from anaphylaxis of guinea-pig isolated lung does not owe its activity on guinea-pig tracheobronchial muscle to any of the other endogenous substances tested. We consider that this action may reasonably be attributed to SRS-A, without excluding the possibilities that SRS-A contains more than one active substance and that the principle that contracts ileum is distinct from that contracting bronchioles.

On the basis of *in vitro* studies, Brocklehurst (1962, p. 541) wrote " the dose of SRS-A necessary to produce a threshold contraction on guinea-pig bronchioles must be at least 10 times larger than that which contracts the corresponding human

TABLE 2

ANTAGONISM BY DRUGS OF SRS-A AND BRADYKININ IN GUINEA-PIG LUNG *IN VIVO*

The minimal effective dose (MED) is defined as the smallest intravenous dose of an antagonist, on the scale 1, 2, 4, 8 mg/kg etc., which reduces the response to a dose of agonist, which is twice the preceding dose, to less than half the preceding response, without reducing that to histamine. *Collier & Shorley (1960, 1963) ; Collier (1963)

Drug	MED (mg/kg) against SRS-A	MED (mg/kg) against Bradykinin
Acetylsalicylic acid	2	2*
Sodium acetylsalicylate	1	2*
Calcium acetylsalicylate	2	2*
Sodium salicylate	64	64–128*
Salicylamide	>64	>64
Sodium salicylamide-*o*-acetate	>64	>64
Sodium gentisate	>32	>32
Sodium 4-hydroxyisophthalate	>32	>64*
Paracetamol	64	16*
Cinchophen	16	32*
3-OH-Cinchophen	2	2
Sodium phenylbutazone	1	4*
Oxyphenbutazone	32	32
Amidopyrine	4	8*
Phenazone	16	8*
α-(4-Phenylphenoxy)propionic acid	4	4
Indomethacin	1	1
Ibufenac	16	16
Sodium anthranilate	>32	>32
Sodium flufenamate	1	1*
Sodium mefenamate	1	1*
Amodiaquine	>8	>16*
Chloroquine	>8	>16*
Amopyroquin	>8	>8
Hydrocortisone	>16	>32*
Dexamethasone	>16	>16
Paramethasone	>64	>64
Morphine sulphate	>32	>32*

tissue. It therefore follows that the anaphylactic bronchospasm of the guinea-pig is an imperfect model of human asthma." Our log dose/response curves for human and guinea-pig tracheobronchial muscle *in vitro* (Fig. 6) bear out the first part of Brocklehurst's contention. However, in this figure, the slope and position of the dose/response curve for guinea-pig lungs *in vivo* show that SRS-A is much more effective in the whole animal. Unless a corresponding increase in potency occurs in man *in vivo*, the guinea-pig may provide a better model of human asthma than Brocklehurst supposed.

Three findings suggest that SRS-A increases the resistance to inflation of guinea-pig lungs *in vivo* by contracting the bronchiolar muscle. First, the effect could be elicited by dropping SRS-A solution directly on to the lung surface. Secondly, SRS-A was fully effective after spinal pithing and crushing the vagi and sympathetic nerves (Fig. 7), or after administration of hexamethonium. Thirdly, SRS-A elicited a contraction from guinea-pig tracheobronchial muscle *in vitro* (Figs. 4 and 5).

Gjuris & Westermann (1963) have suggested that the increased resistance to inflation in the guinea-pig lung *in vivo*, produced by bradykinin, arises indirectly

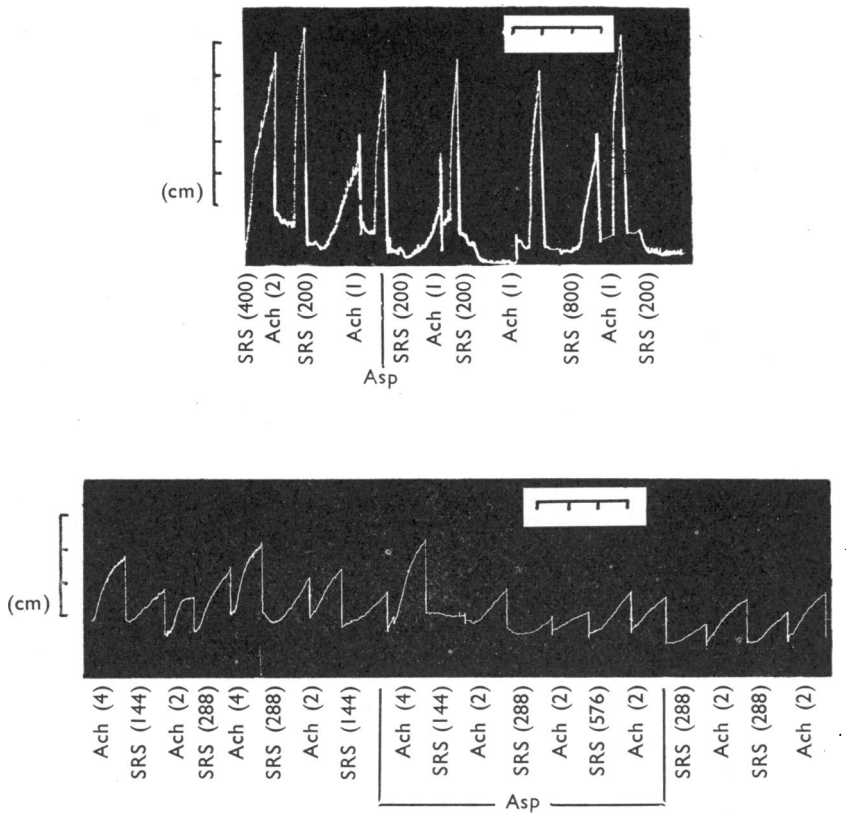

Fig. 9. Antagonism by acetylsalicylic acid of SRS–A on isolated tracheobronchial muscle. Upper panel, guinea-pig trachea in 5 ml. bath. SRS, 200, 400, and 800 U of SRS–A ; Ach, 1[and 2 μg of acetylcholine. From Asp, 500 μg acetylsalicylic acid was present in the bath. Doses were given at 10 min intervals ; lever magnification, × 15. Lower panel, human bronchial muscle in 5 ml. bath. Ach, 2 and 4 μg of acetylcholine ; SRS, 144, 288, and 576 U of SRS–A ; Asp, 500 μg of acetylsalicylic acid was present in the bath during the time shown. Doses were given at 10 min intervals ; lever magnification, × 19 ; other details as in Fig. 4.

through some effect on the nervous system outside the lung. The fact that bradykinin, which was used for reference in each of the experiments mentioned in the above paragraph, was active in each preparation appears to dispose of their suggestion.

The antagonism of SRS-A-induced bronchoconstriction by nonsteroid anti-inflammatory drugs resembles in five ways the antagonism of kinins by the same drugs (Collier *et al.*, 1960 ; Collier & Shorley, 1960, 1963 ; Bhoola *et al.*, 1962). First, antagonism is local, since it occurred *in vivo* in pithed guinea-pigs with vagi and sympathetic nerves crushed (Fig. 7) and since it could be demonstrated in the isolated trachea (Fig. 9). Secondly, the antagonism is probably competitive, since it was surmounted by higher doses of agonist and since acetylsalicylic acid caused a

parallel shift of the dose/response curve (Fig. 8). Thirdly, the same drugs were inactive against SRS-A and bradykinin (Table 2). Fourthly, the potencies of drugs active against SRS-A did not differ appreciably from their potencies against bradykinin (Table 2). Fifthly, active antagonists of SRS-A and kinins showed no antagonism towards bronchoconstrictor agents of other types. These facts would suggest that antagonism of SRS-A and kinins occurs at receptors for these agents in tracheobronchial muscle. The lack of cross-desensitization between SRS-A and bradykinin (Figs. 4 and 5) suggests that the receptors for these two agents are different. Two explanations in terms of receptor theory of how the same drug antagonizes both SRS-A and bradykinin in guinea-pig bronchial muscle appear consistent with the above facts: (1) the receptors for SRS-A and bradykinin may be supposed to be quite separate, but each to possess a similar site that can be blocked by the drug; or (2) the receptors may be supposed to be partly connected and to share a common site than can be blocked by the drug, although responding independently to their respective agonist.

If the first explanation is true, then kinin and SRS-A receptors might be expected each to possess a similar feature corresponding with a chemical configuration occurring on both bradykinin and SRS-A molecules. This would not be required by the second explanation, to visualize which the receptor apparatus might be pictured as a pit, having on its floor separate sites for SRS-A and bradykinin and at its mouth a common site for drug. Attachment or approach of a drug molecule to the mouth of the pit might be supposed to hinder access of SRS-A and kinin molecules to their sites within, such an obstacle being surmountable by higher concentrations of agonist.

The experiments described above show that the SRS-A obtained from guinea-pig isolated lung in anaphylaxis is a powerful bronchoconstrictor agent *in vivo* in that species. Since acetylsalicylic acid antagonizes this action, it might be expected to reduce the intensity of anaphylactic bronchospasm in the guinea-pig. An earlier attempt to show this in a relatively small group of animals failed (Collier & Shorley, 1960); but it has recently been clearly demonstrated in large-scale experiments (Collier, Hammond & Whiteley, 1963). However, since kinins are also bronchoconstrictor and are antagonized by acetylsalicylate in the guinea-pig, this experiment does not distinguish between release of kinins and of SRS-A in anaphylactic shock.

Acetylsalicylic acid, amidopyrine, phenazone and phenylbutazone are reported to have some antiasthmatic action in man (Herxheimer & Stresemann, 1961; Stresemann, 1963). The findings described above, together with those previously reported for kinins, raise the question whether antagonism by these drugs of released kinins and/or SRS-A may play a part in their action against human bronchial asthma.

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