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"SRS-A"

THE SLOW REACTING SUBSTANCE OF ANAPHYLAXIS

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Abstract—It is now recognized that histamine is only one of several pharmacologically active substances contributing to the phenomenon of anaphylaxis. SRS-A is one of these; it is an acidic substance of small molecular size, and present evidence indicates that it is important in human asthma. The detailed chemistry of SRS-A is not yet known, and no clues have been obtained from the i.r. and u.v. absorption spectra of relatively pure samples. Nevertheless such results show that SRS-A is unlikely to be a derivative of neuraminic acid, as has been postulated. Active SRS-A is not present in tissue in detectable amounts until after damage such as that resulting from an antigen–antibody reaction. The evidence concerning its pharmacological actions and the events by which it is formed in tissue, is briefly reviewed.

AT THE time when SRS-A was first shown to exist[1] all the substances which we are considering today would have come under the general title of "SRS". When it became clear that SRS released in anaphylaxis differed in its pharmacological properties from all such substances adequately characterized, the suffix A (for "anaphylaxis") was added to denote this particular material.[2] SRS-A is probably not a true lipid because when moderately pure it is not soluble or only minimally soluble in the usual lipid solvents, but when it is in contact with lipids, as *in vivo*, some sort of loose association is formed which confers many of the solubility characteristics of the lipid on the lipid–SRS-A complex.[3, 4]

SRS-A is formed in sensitized tissue after that tissue is challenged with antigen. If the tissue is perfused, the activity is found in the effluent very shortly after challenge; if the tissue is chopped, the activity leaks rather slowly into the bathing fluid. None is found prior to challenge. Kellaway and Trethewie who first reported the occurrence of SRS during anaphylaxis (1940), were unable to study the SRS present in the perfusate from lung because this activity was overshadowed by the histamine inevitably present in the solution. The findings I shall mention were made since 1952 by myself and by the group working under Professor Uvnäs.

The amount of SRS-A formed in a tissue varies with the severity of the anaphylactic reaction, as does the release of histamine.[3, 5] The time-course of the washing-out of the two substances from perfused lung, is somewhat different as shown in Fig. 1. As you see, the two substances are detectable initially about 20 sec after the antigen reaches the tissue, but whereas the histamine (which is released) pours out suddenly, the SRS-A (which has to be formed) takes over 2 min to reach a peak rate of outflow. It is also seen that SRS-A continues to be detectable after the flood of histamine is over.

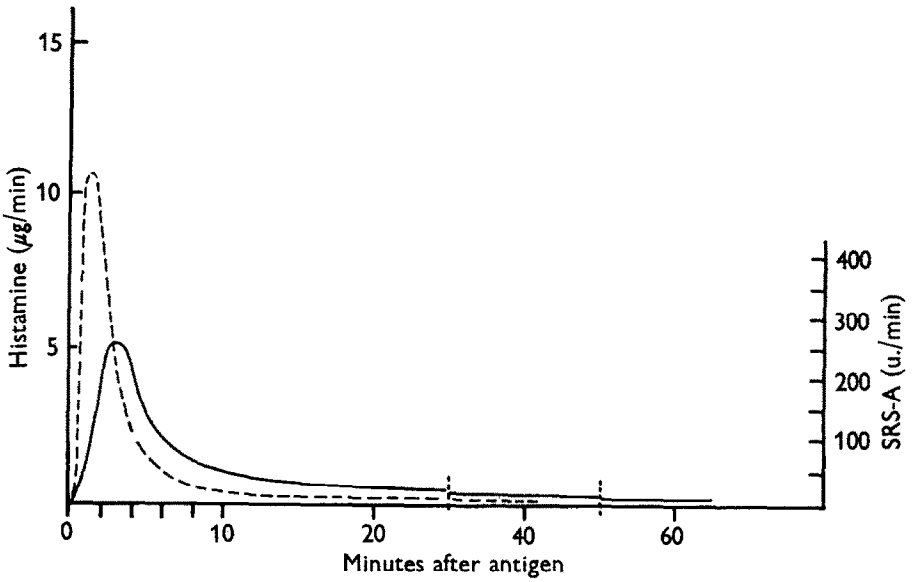


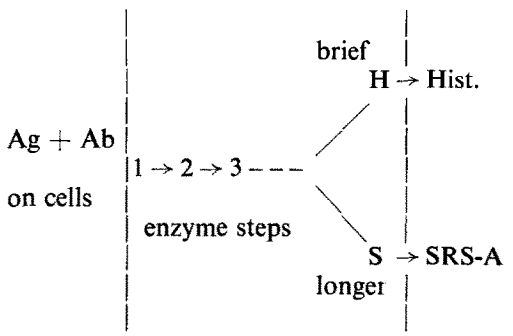
FIG. 1. Time course of the outflow of histamine - - - and SRS-A —— from the perfused whole lung of a guinea pig. (From Brocklehurst.[5])

TABLE 1. SUBSTANCES WHICH INTERFERE WITH THE ANAPHYLACTIC RELEASE OF HISTAMINE*[6-21]

(a) Antagonists	
iodoacetate	phenylbutazone
N-ethyl maleimide	3-hydroxy-2-phenyl cinchoninic acid
p-chloromercuribenzoate	antipyrine
allicin	phenazone
indole	N-acetyl-L-tryptophan ethyl ester
skatole	N-acetyl-L-phenylalanine ethyl ester
phenol	L-tryptophan ethyl ester
ε-amino caproic acid	L-phenylalanine ethyl ester
β-phenyl propionic acid	
DFP	
nicotinic acid	C_6 to C_{12} fatty acids, e.g. decanoic
nicotinamide	hip seed polysaccharide
diethyl nicotinamide	polyphloretin phosphate
cyanide	EDTA
azide	oxalate
zinc, lead, nickel	
anoxia	
dinitrophenol	
(b) Enhancers	
succinic acid	low ionic strength
α-keto glutaric acid	
maleic acid	
glucose	

* In many cases the yield of SRS-A has also been determined, and it has always been found to change in a comparable manner.

SRS-A is formed following anaphylaxis in many tissues and several species.[5, 6] The richest sources are the aorta and lung of the guinea pig, and the lung of asthmatic humans. Professor Uvnäs and his group have shown a correlation between mast cell damage, histamine released, and SRS-A formed, and conclude that SRS-A comes from mast cells.[7–9] My view is that these three events are not necessarily correlated except in so far as they are the outcome of tissue damage resulting from hypersensitivity.[4] Nevertheless the events leading to histamine release and the formation of SRS-A must have much in common because the agents which reduce or enhance the yield of histamine, alter the yield of SRS-A in a roughly comparable degree. The inhibitory agents differ greatly in type, as may be seen in Table 1. They include substances which destroy SH-groups, esterase inhibitors such as phenol and indole, fatty acids of C5 or longer, acidic polymers, DFP, and synthetic ester substrates of chymotrypsin. It seems most unlikely that all these agents act on the same enzyme step, and suggests that there are several stages each one essential to and quantitatively involved in the anaphylactic reaction. I visualize it thus:



The only clearly demonstrated pharmacological property of SRS-A is its ability to contract a limited range of smooth muscle preparations.[6, 4, 22] Those more useful to the pharmacologist are the guinea pig ileum for routine assay, and also the rabbit duodenum and the hamster colon for parallel quantitative assay when characterization is needed. SRS-A produces a very prolonged effect with slow relaxation after washing as shown in Fig. 2. There is no tachyphylaxis as is evident from Fig. 3, indeed there is a residual sub-threshold stimulation which persists after washing, and enhances the response to other agents applied shortly after it as shown in Fig. 4. Pharmacological analysis shows that this effect is direct on the muscle; atropine and hexamethonium have no inhibitory effect on the contraction produced in the guinea pig ileum, yet the response is severely reduced when the temperature falls below about 34 °C.[23] The property of particular interest to the clinician is the very marked ability of SRS-A to contract human bronchioles *in vitro*.[2] (Note added in proof: broncho-constriction *in vivo* has recently been demonstrated by Herxheimer.[24])

The effect of human SRS-A on human bronchial rings of 3–5 mm diameter completes the picture of the occurrence and effectiveness of SRS-A in asthma. This bronchoconstriction is produced with doses which are ineffective on comparable guinea pig tissue,[2] thus offering an explanation of the poor effect which antihistamines have in asthma, compared with the marked protection observed in the guinea pig subjected to anaphylactic shock.

Some test preparations on which SRS-A has no detectable action are of interest as a means of differentiation from other substances in the SRS group. They include the rat uterus, and blood pressure of the cat or rabbit. Many of the negative tests are unsatisfactory or inconclusive due to the lack of adequate amounts of relatively pure material. A notable example is the possible effect on vascular permeability in skin.

All the usual smooth muscle relaxants will inhibit the contraction produced by SRS-A,[25] but no specific antagonist of real potency is known. Ca^{2+} can inhibit the contraction and also suppress the contraction already developed, thus showing that this effect is probably at the cell-membrane, and is unlikely to be a simple combination of SRS-A + Ca^{2+} to remove SRS-A from solution. This observation is of interest because Ca^{2+} has been used empirically in antihistamine sprays used to relieve asthma, and it may also provide a clue to the manner in which the SRS-A initiates the contraction.

Chemically SRS-A is an acid[6, 23, 26] and presumably exists as the Na salt in the usual bath media. The salt is very water-soluble and the free acid (at pH 3) is also water-soluble. The actual partition ratio of the free acid between an aqueous and organic phase will depend largely on the nature and amount of lipids present. This became clear during attempts to find a suitable solvent-pair for counter-current separation of SRS-A. When repeatedly extracted with amyl acetate, the aqueous phase at pH 3 retained a variable amount (often as much as 40 per cent) of the SRS-A, which could not be removed by the organic solvent, but if a little lecithin was added to the SRS-A and shaken, the activity could be extracted with the amyl acetate.

The most useful solvent for extraction of SRS-A from an aqueous solution (at pH 3) is peroxide-free diethyl ether, but even this solvent will contain much less SRS-A than the aqueous phase (at equilibrium) unless there is lipid present to increase its solubility in ether. Recovery into aqueous medium is rapid and complete at pH 7, and usually 5% $NaHCO_3$ is used.

Chromatography on paper[6] or silicic acid always results in heavy losses, so does electrophoresis on any support medium such as cellulose, agar or starch.[23] But this does not imply that SRS-A is a mixture of substances because if charcoal-purified material is separated by electrophoresis on a density gradient, all the activity is found in a single rather narrow band migrating anodally at pH 8.[26] Preparation in this way gives good recoveries although the purified material is rather unstable.

Up to 1000 biological units of material purified on an ethanol–water gradient, with ammonium acetate–acetic acid buffer, weight about 0·5 mg, and fail to give any indication of the chemical radicals present when examined by infra-red absorption spectroscopy or for ultra-violet absorption[27]. This test would detect about 10 μg of neuraminic acid derivatives, so that failure to detect 1000 and 200 EDs (in two experiments) casts serious doubt on the identity of SRS-A as methoxy-neuraminic acid,[28] although of course a much more potent derivative might have escaped notice.

The acid nature of SRS-A permits it to be located after chromatography on paper by spraying with indicator.[6] This same region shows neither ultra-violet absorption nor fluorescence.[27]

Thus we know that SRS-A is an acid substance, very water soluble and able to pass cellophane with ease, showing a marked tendency to adsorb or form complexes. It has a narrow range of biological activity but is probably a major factor in asthma.

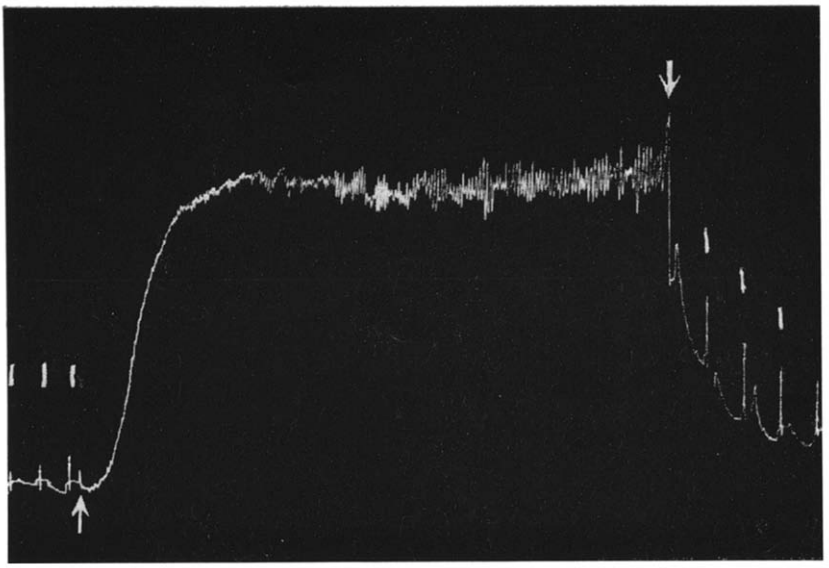


FIG. 2. The response of the guinea pig ileum to SRS-A approximately 2 units/ml applied at ↑ and washed out at ↓ after 5 min contact. Vertical bars denote washing and rest periods each occupying 40 sec. Atropine 10^{-6} M and mepyramine 10^{-6} M present throughout.

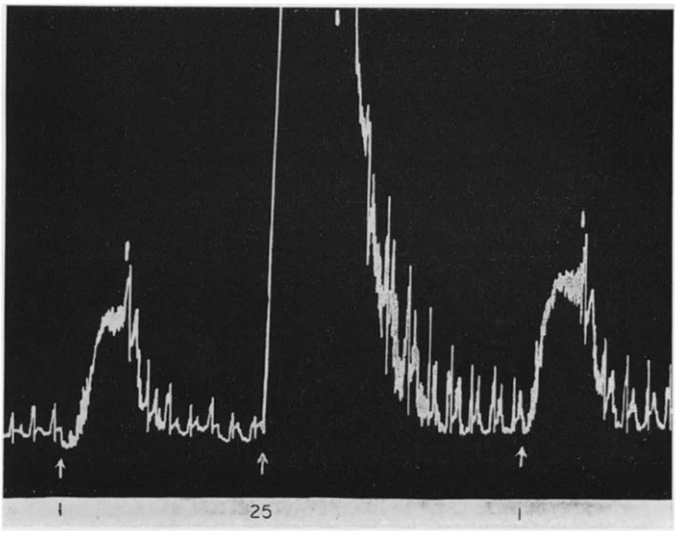


FIG. 3. Contraction of the guinea pig ileum produced by 1 unit, 25 units and 1 unit/ml respectively, of purified guinea pig SRS-A left in contact with the tissue for 2 min between the arrows and the vertical bars. Relaxation to baseline after 25 units, occupied five cycles each of two washes, rest, contact period, taking 7 min altogether. The second dose of 1 unit shows enhancement 12 min after washing out the 25 unit dose.

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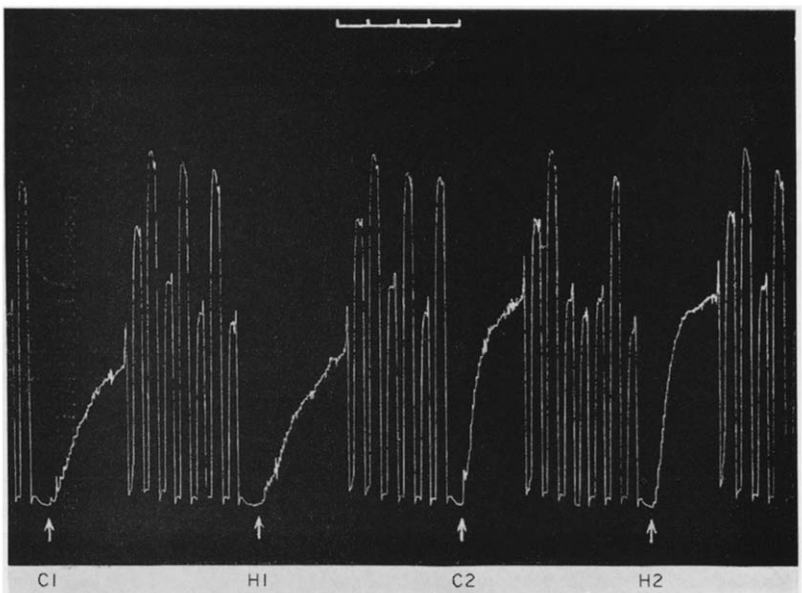

FIG. 4. Responses of the guinea pig ileum to purified SRS-A from guinea pig (C1 and C2) and from man (H1 and H2) in matching doses of approximately 1 and 2 units/ml. Unlabelled contractions were produced by histamine 5 and 10 ng/ml, and show enhancement resulting from the prolonged effect of SRS-A. Atropine 10^{-6} M present. Contact time in minutes. Kymograph stopped during two washes and rest period totalling 50 sec between doses.

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It seems likely that its participation in tissue reactions may not be restricted to immediate type allergic reactions, but we do not as yet know enough of its chemistry to test the belief that it may be formed following insults to tissue by venoms, trauma, chemicals or heat—or the tuberculin type of allergic reaction.

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