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LIPID SPASMOGENS APPEARING IN CONNECTION WITH HISTAMINE LIBERATION

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Abstract—Histamine release was induced from perfused cat paws and isolated rat mast cells with compound 48/80, and from sensitized guinea pig lungs with antigen. Concomitantly occurring biologically active substances were separated by various chromatographic procedures (silicic acid column, silicic acid impregnated paper, etc.). From all the species several spasmogenic principles were obtained. Chemical and biological properties indicated the presence of unsaturated fatty acids, phosphatidyl and phosphatidal choline and an SRS principle. The SRS principles from the three species seemed to be identical or very closely related.

IN SPECIES like the cat, rat and guinea-pig the release of histamine caused by polymer agents such as compound 48/80, principles extracted from *Cyanea* and *Ascaris*, and by antigens (in guinea-pig only by antigens) is accompanied by the appearance of other spasmogenic factors. Among those spasmogenic substances a factor causing a slow contraction of the guinea-pig ileum, "The Slow Reacting Substance" ("SRS") has aroused special attention.[1, 2] It has been suggested to be an acid lipid.

The "SRS" is formed during the release process and the concomitant appearance of histamine and "SRS" suggested that the two agents came from the same source, the mast cells.[3] An "SRS" like factor was also observed to occur when isolated mast cells were exposed to compound 48/80.[4]

Two enzymes, phosphatidase A and chymotrypsin have been reported able to degranulate mast cells,[5] and to release histamine from isolated rat mast cells.[6, 7] The two enzymes seem to trigger the same energy-requiring process[8, 9] as is activated by the polymer agents mentioned above. Enzyme activities reminding of those of phosphatidase A and chymotrypsin have been observed in mast cells.[10, 11] Provided that such enzymes were activated during the histamine release process split products of phospholipids and peptides should appear. Such products might have spasmogenic properties.

This paper will be concerned with some characteristics of smooth muscle stimulating principles that occur on histamine release from tissues as well as isolated mast cells.

METHODS

Histamine release was induced by compound 48/80 in cat paws and isolated rat mast cells and by antigens in sensitized guinea-pig lungs. Biological assay of histamine was made on guinea-pig ileum and of other spasmogenic substances on guinea-pig ileum and rabbit duodenum.

The spasmogenic principles were extracted, separated and purified by a procedure resulting from years of "trial and error" and therefore subjected to many modifications.

The lyophilized material from perfusion and incubation fluids was extracted by organic solvents such as acetone, ethyl alcohol, ether, etc. and then chromatographed on silicic acid. This material was then subjected to paper chromatography, ion exchange chromatography, electrophoresis etc. The details of the purification procedures are under publication.[12]

RESULTS

The release of histamine was invariably accompanied by the appearance in the perfusion or incubation fluid of a principle (or principles) causing a slow contraction of the guinea-pig ileum. Fig. 1 illustrates the appearance of "SRS" from perfused

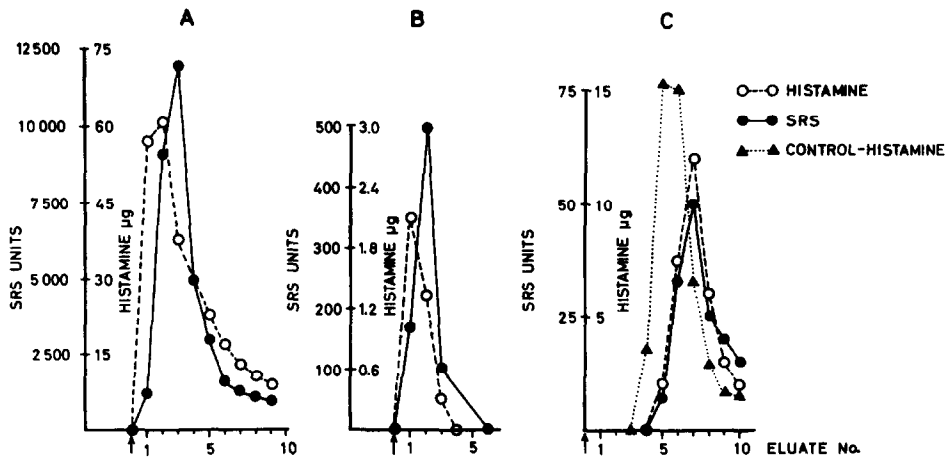

FIG. 1. Histamine and "SRS" in perfusates from cat paw (A), guinea pig lung (B) and isolated rat mast cells (C). Releasing agent in A compound 48/80 50 µg/paw, in B antigen = egg albumin 10 ml/lung, in C compound 48/80 1 µg/ml. O- - - -O histamine, ●——● "SRS"; × . . . . × control infusion of histamine.

cat paw (A), guinea-pig lung (B) and isolated rat mast cells (C). On chromatography on silicic acid column the non-histamine spasmogenic activity was found exclusively (guinea-pig lung and isolated mast cells) or mainly (cat paw) in the chloroform–methanol (5 + 5) fraction. The cat paw delivered spasmogenic material also in the chloroform eluate. The spasmogenic material from incubates of guinea-pig lung and isolated mast cells showed a more composed silicic acid chromatogram. High spasmogenic activity was found both in the chloroform and the chloroform–methanol (5 + 5) eluates. From Fig. 2 emerges the distribution of active material between tissue and incubation fluid. The more lipophil material was evidently retained by the tissue while most of the material eluted with chloroform–methanol (5 + 5) appeared in the incubation fluid. This fact may explain why chromatograms of perfusates exhibited spasmogenic activity only or mainly in the chloroform–methanol (5 + 5) eluate. The incubation time was another influencing factor, since the spasmogenic principles formed were rapidly inactivated. Inactivating factors occurred in the mast cells themselves, in serum, in lung tissue and various other organs.

The fractions separated by silicic acid chromatography differed considerably in their spasmogenic properties. The principle(s) in the chloroform eluates elicited a rather rapid, transient contraction of the guinea-pig ileum. The contraction therefore was not a typical "SRS" effect. Tachyphylaxis occurred after a few contractions.

Unsaturated fatty acids are known to elicit a similar contraction of the guinea-pig ileum and to exhibit tachyphylaxis. They are eluted from silicic acid columns wthi chloroform. The spasmogenic activity in the chloroform eluate might therefore be due to the presence of unsaturated fatty acids.

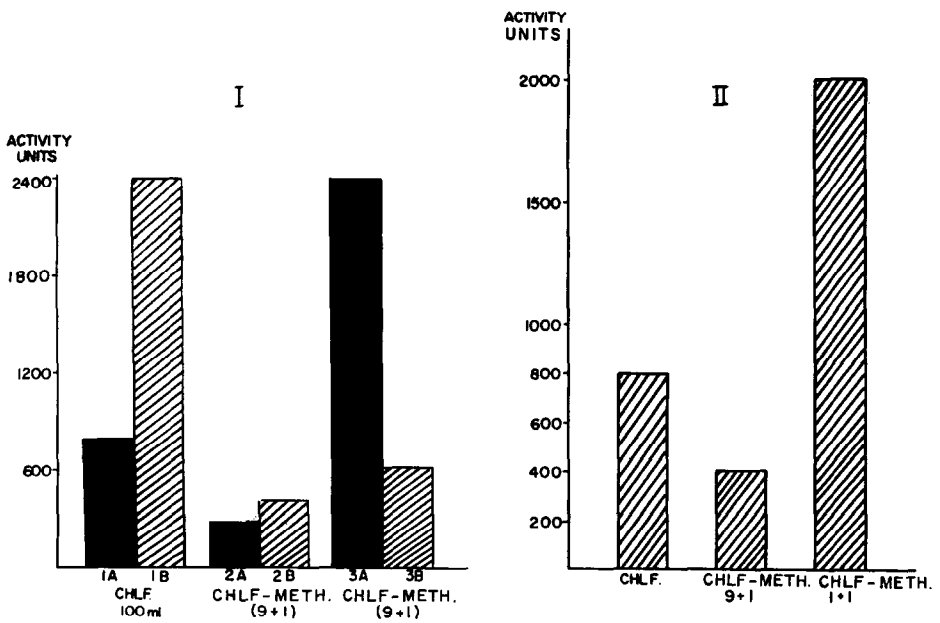

FIG. 2. Separation by silicic acid chromatography of spasmogenic principles from sensitized guinea pig lung (I) and isolated rat mast cells (II).
I. Releaser: egg albumin. A: incubation fluid. B: lung tissue.
II. Releaser: compound 48/80.

However, our main interest was focused on the spasmogenic principle eluted in chloroform–methanol (5 + 5); since this principle elicited a typical "SRS" contraction. The contraction was slow and about 3 min were required for full relaxation. The spasmogenic activity seemed to be rather confined to the guinea-pig ileum. Of purified material from cat paw a concentration in the test bath of 0·001 μg/ml sufficed to elicit a contraction of the guinea-pig ileum, while to stimulate the rabbit duodenum doses 400–600 times as high were required. No tachyphylaxis was observed. No action was observed on the rat small intestine, the oestrogen treated rat uterus and the non-pregnant guinea-pig uterus with doses 100–500 times the threshold dose on guinea-pig ileum. "SRS" from guinea-pig lung showed the same specificity in its spasmogenic action.

The "SRS" materials studied by Brocklehurst, Chakravarty and others were reported to stimulate the rabbit small intestine, fowl rectal caecum and rat colon. These

stimulating effects were probably due to the presence of other active principles in their preparations—e.g. those appearing in our chloroform eluates. Prior to silicic acid chromatography our "SRS" materials from cat paw and guinea-pig lung exhibited a similar wide range of spasmogenic activity, but on purification of "SRS" the actions on other smooth muscle organs than guinea-pig ileum progressively declined.

"SRS" was rechromatographed on silicic acid column. Such material was subjected to paper chromatography (e.g. *n*-propanol–ammonia–water/6:3:1). The exact extension of the area of biological activity was difficult to establish since in order to obtain sufficient material for reliable biological assay the chromatography paper had to be cut into rather broad strips before extraction. However, most of the "SRS" material was extracted from an area between Rf 0·7 and 0·8. (The Rf values varied somewhat with the amount of material, the temperature, the pre-treatment of the paper, etc.) When "SRS" materials from guinea-pig lung and from isolated mast cells were chromatographed against "SRS" from cat paw as "reference material", the three "SRS" materials yielded identical Rf values (Fig. 3).

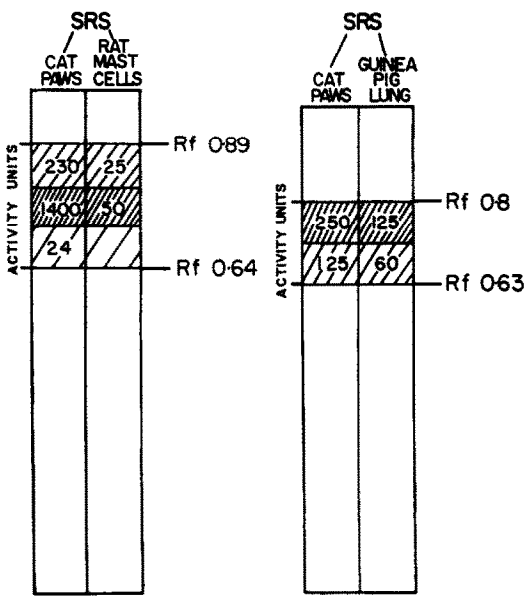

FIG. 3. Paper chromatogram (*n*-propanol; NH_3; H_2O) of "SRS" material from cat, guinea-pig and rat after two silicic acid chromatographies. Note close similarity of Rf values from the three species.

Within the area of "SRS" activity were obtained positive staining reactions for phosphatides, choline, phosphorus, aldehyde, carboxyl groups and acyl ester linkages. When "SRS" was prepared from cats, guinea-pigs or rats given 0·5 ml ^{32}P i.m. 18 hr before perfusion or incubation, the paper chromatogram showed a radioactive spot within the area of biological activity. The ninhydrin, sugar and orcinol tests were negative.

The solubility properties as well as the behaviour on silicic acid columns and on paper led us for some time to believe that "SRS" might be a choline-containing

phosphatide. However, further analysis contradicted this assumption. The phosphatide and the peak of radioactivity usually did not fall within the area of maximal "SRS" activity. Incubation of the "SRS" material with phosphatidase A did not reduce the "SRS" activity in spite of the fact that the phosphatide component was changed into a lysocompound. Further degradation of the lysocompound gave glycero-phosphoric acid, glycero-phosphoryl choline and phosphoryl choline, identified on silicic impregnated and formaldehyde-treated paper.

Since the results indicated that the "SRS" was not a phosphatide but linked to or merely mingled with such material the phosphatides were removed by extraction with chloroform. The "SRS" material was retained in the chloroform insoluble rest, which now became easily soluble in water.

This material was further purified by anion exchange chromatography (Ecteola) and column electrophoresis showing it to have acid properties. The chemistry of this very highly active material is under investigation.

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