THE EFFECTS OF HISTAMINE RELEASE ON THE LIPID CONTENT OF THE ISOLATED PERFUSED LUNGS OF SENSITISED GUINEA-PIGS

BY V. O. MARQUIS AND W. G. SMITH

From the Research Laboratory in Biochemical Pharmacology, School of Pharmacy, Sunderland Technical College, County Durham

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The histamine release induced in isolated perfused sensitised guinea-pig lungs by antigen, trypsin, Russell viper venom, and compound 48/80 has been compared. At equi-active dosage for histamine release, these four substances released varying amounts of slow reacting substances. Neither histamine release nor the release of slow reacting substances appeared to be responsible for the changes in cholesterol, glyceride or lipid phosphorus of the lung tissue observed in these experiments.

THE release of lipid from guinea-pig lungs as a result of an anaphylactic reaction has recently been described by Smith (1962a). These observations have been extended by Goadby and Smith (1962) who have reported that anaphylaxis *in vivo* caused marked changes in the lipid metabolism of guinea-pig lung tissue. Since the loss of lipid from lung tissue that occurs under these conditions is accompanied by the release of histamine and the slow reacting substance of anaphylaxis (SRS-A), a comparative study has now been made of antigen and three other releasers of histamine and slow reacting substances (Russell viper venom, compound 48/80 and crystalline trypsin).

EXPERIMENTAL

Release of Histamine

Guinea-pigs of either sex weighing 200 g. were sensitised by the subcutaneous injection of 100 mg. of commercial egg albumin in 1 ml. of normal saline. They were fed on Diet 18 pellets and received 50 mg. of ascorbic acid each morning in drinking water contained in amber glass bottles. Overnight they were given tap water. Three weeks after the sensitising dose of antigen, the animals were killed and their excised lungs perfused with Tyrode solution through the pulmonary artery as described by Brocklehurst (1960). After injection of the histamine releaser into the blood-free lungs, perfusion was stopped for a 2 min. period and then restarted at the rate of 2 ml./min. The perfusate was collected for 30 min., centrifuged to remove blood cells, and then examined for histamine and slow reacting substances. Increasing doses of each of the four histamine releasers were administered to groups of between five and ten guinea-pigs. The total yield of histamine from each lung was calculated from the result of each histamine assay; and for each dose level of histamine releaser a mean quantity of histamine released and a standard deviation was calculated.

Isolation of Histamine from Perfusate

With all histamine releasers except antigen it was found to be necessary to extract histamine from the perfusate before the biological assay to determine its histamine content. Russell viper venom, compound 48/80, and crystalline trypsin exhibit pharmacological actions on isolated ileum which interfere with the assay of histamine. After examining a number of methods for the extraction of histamine from perfusate, the column chromatographic technique of Roberts and Adams (1950) using Decalso F was selected for use. A short column of the material was prepared in a 50 ml. capacity burette as follows. A small pad of cotton wool was inserted into the burette tube just above the tap. Decalso F (60–80 mesh, L. Light & Co.) without pretreatment was introduced in small quantities and packed down with a glass rod until a column 10 cm. high × 1 cm. diameter containing about 5 g. of Decalso F was obtained. A small pad of cotton wool was placed on the top of the column followed by 0·5 g. of washed white sand. The pH of the perfusate was adjusted to between pH 8·2 and 8·6 by the addition of 0·2N sodium hydroxide. The perfusate was then passed through the column at a flow rate of 1·0 ml./min. The eluate from the column at this stage was freeze-dried if required for assay of slow reacting substance. The adsorbed histamine was eluted from the column using 4·0 ml. of 0·880 ammonia followed by 50 ml. of chloroform saturated with ammonia gas, prepared by bubbling dry ammonia gas through chloroform until gas ceased to dissolve. The collected eluate was taken to dryness under reduced pressure and the dry residue dissolved in 50 ml. of absolute ethanol containing 4 per cent hydrochloric acid. After evaporation under reduced pressure, the final residue was dissolved in 30 ml. Tyrode solution ready for biological assay.

Assay of Histamine

This was made on isolated guinea-pig ileum suspended in 4 ml. of aerated Tyrode solution using the usual four point Latin square design.

Isolation of Slow Reacting Substances from Perfusate

After adsorption of the histamine onto Decalso F, the column eluate was freeze-dried and then extracted with ethanol as described by Chakravarty (1960).

Assay of Slow Reacting Substances

The isolated slow reacting substances were dissolved in Tyrode solution and assayed on isolated guinea-pig ileum in 4 ml. aerated Tyrode solution containing atropine 10^{-7}M and mepyramine 10^{-6}M. The assay design used was the four point Latin square design described by Chakravarty (1960) for the assay of SRS-A. The guinea-pig ileum gives a contraction to 1 unit/ml. of SRS-A similar in size to that seen with 0·01 μg./ml. of histamine (Smith, 1962b). The standard used in these assays was a laboratory standard containing 20 units of SRS-A per ml.

Estimation of the Lipid Content of Perfused Lungs

Immediately after perfusion, the lung lobes were dissected from the bronchi, coarsely chopped and freeze-dried. Each freeze-dried lung was extracted with 200 times its own weight of chloroform: methanol (2 : 1) for 24 hr. Lipid analyses were confined to cholesterol (Hanel and Dam, 1955), glyceride (Van Handel and Zilversmit, 1957), and lipid phosphorus (Bartlett, 1959) after preliminary treatment with silicic acid as described by Goadby and Smith (1962). The final results were calculated as mg./g. of freeze-dried lung tissue.

Materials

Crystalline trypsin was obtained from British Drug Houses Ltd. and compound 48/80 and Russell viper venom were generously given by Burroughs Welcome & Co. Ltd.

RESULTS

The Quantitative Release of Histamine

The amounts of histamine released by increasing doses of the four histamine releasers investigated are shown in Figs. 1 to 4. No two

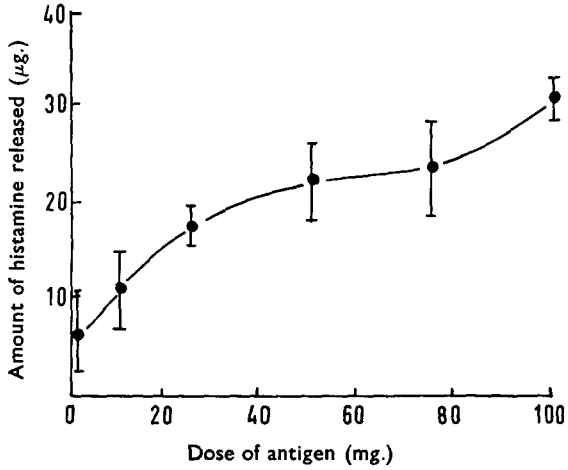

FIG. 1. Histamine released by antigen from sensitised guinea-pig lung.

histamine releasers showed identical dose-effect relationships. The curve relating histamine release to dosage of compound 48/80 had an unusual shape. Doses in excess of 5 mg. appeared to cause less histamine release than some lower doses. These yields of histamine were confirmed in duplicate experiments throughout the whole dose range. Further investigation showed that the anomaly was due to interference by compound 48/80 in the assays of histamine from which the histamine yields were calculated.

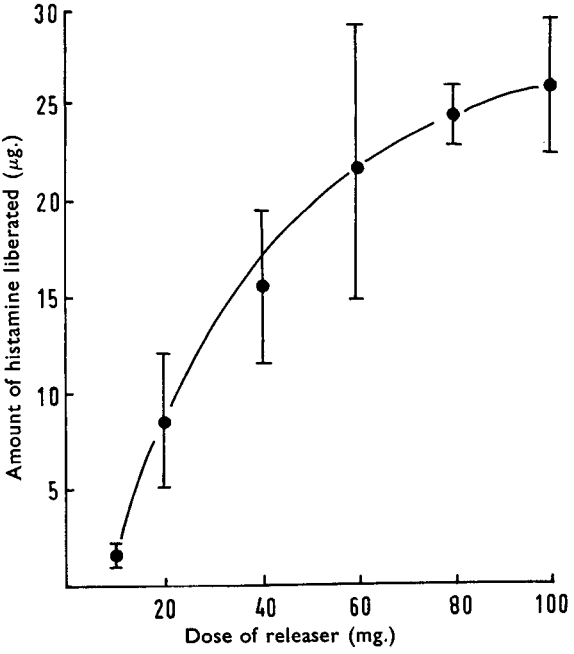

FIG. 2. Histamine released by crystalline trypsin from sensitised guinea-pig lung.

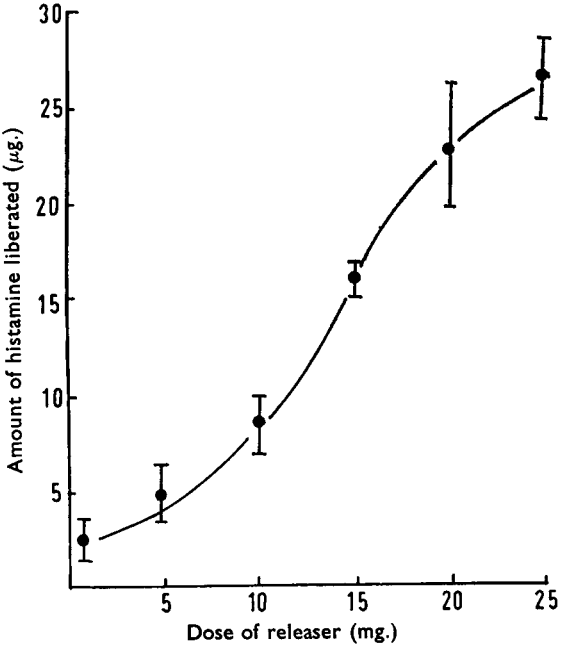

FIG. 3. Histamine released by Russell viper venom from sensitised guinea-pig lung.

At concentrations in excess of 80 μg./ml. in Tyrode solution (the concentration of compound 48/80 in perfusate after a histamine releasing dose of 5 mg.), compound 48/80 was adsorbed onto Decalso F. It could subsequently be eluted, in the manner used for the elution of adsorbed histamine, with ammonia and then chloroform saturated with ammonia.

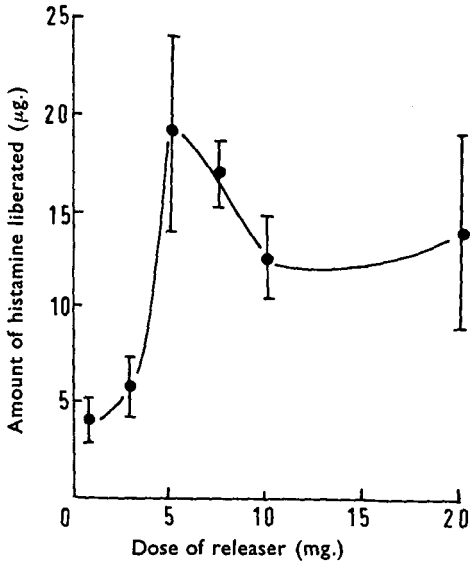

FIG. 4. Histamine released by compound 48/80 from sensitised guinea-pig lung.

When the ammonia-chloroform eluate was taken to dryness and made up in Tyrode solution, the resultant solution was found to reduce the sensitivity of guinea-pig ileum to histamine. It was thus concluded that the histamine released by compound 48/80 in doses in excess of 5 mg. was contaminated with compound 48/80 in a concentration sufficient to reduce the sensitivity of guinea-pig ileum to histamine. During subsequent biological assay, the contamination was confined to the test solutions and thus produced low estimates of the histamine actually released.

Effect of Histamine Release on Lipid Content of Lung Tissue

From the data given in Figs. 1 to 4 it was concluded that the release of 15 μg. of histamine from perfused guinea-pig lungs could be induced by 16 mg. of antigen (egg albumin), 34 mg. of trypsin, 15 mg. of Russell viper venom, or 4 mg. of compound 48/80. These doses were each administered to a group of ten sensitised guinea-pigs and the observed histamine release agreed very closely with that expected ($15{\cdot}0 \pm 1{\cdot}05$ μg.). The mean amounts of slow reacting substance released are shown in Fig. 5 together with the standard deviation of each mean. A similar yield of slow reacting substance was noted with antigen, trypsin and Russell viper venom. The amount released by compound 48/80 was appreciably less than that released by the other three compounds.

The lipid present in the lungs of these animals was compared with that found in an "experimental control" group which had been perfused with Tyrode solution without administration of a histamine releaser. The results are shown in Fig. 6.

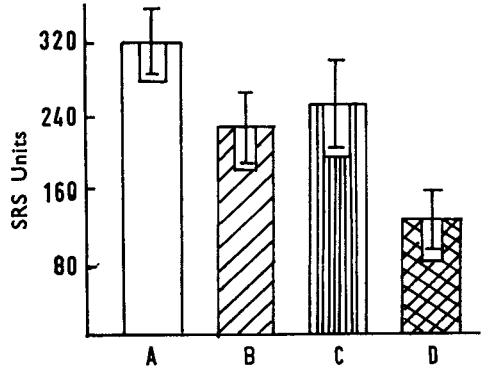

FIG. 5. The slow reacting substances released from sensitised guinea-pig lung by equi-active doses of different histamine releasers. A, Antigen 16 mg. B, Trypsin 34 mg. C, Russell viper venom 15 mg. D, Compound 48/80 4 mg.

The amounts of lipid present in the "experimental control" group were the same as those observed in chopped blood free lungs. Comparative figures are given in Table I.

TABLE I

LIPID CONTENT OF LUNGS

	Controls	Treatment controls
Cholesterol mg./g.	18·90 ± 1·41	18·20 ± 4·03
Glyceride mg./g.	30·40 ± 15·69	30·40 ± 15·27
Lipid phosphorus mg./g. . .	4·86 ± 0·25	4·90 ± 0·30

Fig. 6a shows that whereas antigen, Russell viper venom, and compound 48/80 induced falls in the lipid phosphorus content of the lung, trypsin was without an effect on this lipid fraction. From Fig. 6b, only the Russell viper venom causes a significant rise in the lung cholesterol. Fig. 6c shows that although all four histamine releasers induced an increase in the glyceride content of the lung tissue there was considerable variation in the magnitude of the change.

DISCUSSION

In the event that histamine release in anaphylaxis is due to the activation of a proteolytic enzyme, activation of phospholipase A, or degranulation of mast cells, it is reasonable to expect that the release of histamine and the changes in the lipid content of the lung tissue in anaphylaxis would be closely paralleled by trypsin, Russell viper venom, or compound 48/80 respectively. The results obtained do not support any of these possibilities.

The release of histamine from sensitised guinea-pig lung tissue by antigen is most closely duplicated by trypsin. An accurate comparison on a molar basis is not possible since the molecular weights of these substances are not known with accuracy. Even so, there is a quantitative similarity between the histamine-releasing effects of trypsin and antigen, which is not shared by Russell viper venom and compound 48/80. A comparison confined to the shape of the dose-effect curves might be interpreted as a suggestion that antigen released histamine by a proteolytic action, and that compound 48/80 released histamine by a phospholipase effect.

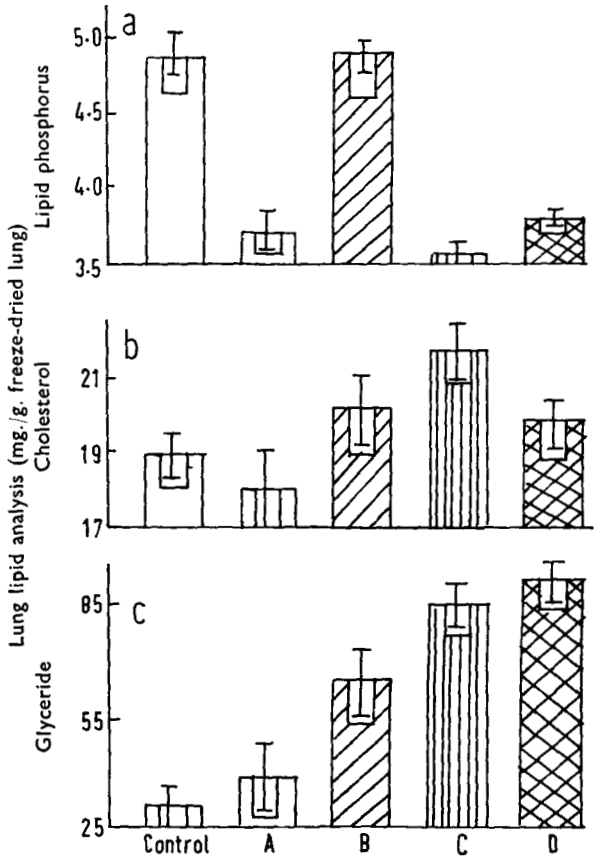

FIG. 6. The lipid content of sensitised guinea-pig lungs after treatment with equi-active doses of different histamine releasers. See FIG. 5 for Key.

The similarity between the histamine-releasing effects of antigen and trypsin is not supported by the changes induced in the lipid content of the lungs. Trypsin did not produce a fall in lipid phosphorus content of the tissue. The effects of antigen on the lung lipids reported here differ slightly from those reported earlier by Smith (1962a). On that occasion loss of glyceride was noted after antigen administration, whereas in the

present study there was no significant change. These two studies agree, however, in that they both show a loss of lipid phosphorus and to a lesser degree of cholesterol.

Since the amount of histamine release was constant in all four groups of lungs whose lipid content was compared, the marked differences in the lipid fractions of the respective groups indicates that histamine release itself is not the primary cause of the changes in the lung lipids. The amounts of slow reacting substance liberated varied from one histamine liberator to another, but the amounts liberated also show no obvious relationship to the lipid changes. The liberated slow reacting substances are not identical in all four cases; the slow reacting substance released by Russell viper venom differs from that liberated by antigen (Smith, 1962b; Schutz and Vogt, 1961).

It is thus concluded that the release of histamine or the release of slow reacting substance during anaphylaxis in guinea-pig lung are not themselves responsible for the changes in lipid metabolism induced by that condition. Such changes are presumably due to alterations in intermediary metabolism induced in sensitised tissue when antigen combines with antibody bound within it, and are manifestations of effects on the tissue metabolism which occur in parallel with effects due to the release of chemical mediators of anaphylaxis.

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