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Release of 5-Hydroxytryptamine from Rat Mast Cells *in vitro*

HISTAMINE is released from mast cells following the injection into animals of histamine liberators. Coincident with the release of histamine other substances have been detected in the plasma capable of causing a slow contraction of isolated smooth muscle¹⁻⁴. Paton⁴ was able to demonstrate the presence of a 'slow-contracting' substance in addition to histamine in the plasma of cats and dogs following an intravenous injection of the potent histamine release agent, compound 48/80. Preliminary experiments have shown that a similar substance appears in plasma collected from rats 5 min. after an intravenous injection of compound 48/80 (1 mgm./kgm.). The origin of the slow-contracting substance in rat plasma was investigated by means of an *in vitro* system consisting of a solution of compound 48/80, rat plasma, mast cells from the peritoneal cavity of the rat, and an isolated rat uterus preparation.

Mast cells were obtained from the rat by a method previously described⁵. The rat uterus preparation was set up according to the method of Sollero *et al.*⁶. Compound 48/80 was dissolved in physiological saline solution to a strength of 10 µgm./ml. Blood was collected from the jugular vein of rats into a syringe containing heparin (5 units/ml. blood). The blood was transferred to a centrifuge tube and plasma free of platelets obtained by centrifugation. All glassware, including syringes and the organ bath, were siliconed with 'Dri-Film' (General Electric Co.) before use.

A mixture was prepared consisting of 0.1 ml. each of the solution of compound 48/80, fresh heparinized plasma and mast cell suspension (containing approximately 1 million mast cells per ml.). When added to the organ bath the mixture was found to cause a contraction of the rat uterus. A similar contraction was produced in the absence of plasma when 0.2 ml. of a mixture consisting of 0.1 ml. vol. of compound 48/80 solution and mast cell suspension was added. 0.1 ml. of the solution of compound 48/80 alone, or when mixed with an equal volume of plasma and added to the bath, failed to stimulate the rat uterus. Similarly intact mast cells alone or with an equal volume of plasma did not cause a contraction of the isolated uterus. Prior treatment of the isolated uterus for 5 min. with dibenamine (1 µgm./ml.), 2-brom-D-lysergic acid diethylamide, known as BOL 148 (1 µgm./ml.), 1-methyl-D-lysergic acid butanolamide, known as UML 491 (0.2 µgm./ml.), or excess 48/80 (1 mgm./ml.) was found to prevent a contraction of the uterus on the addition to the bath of the mixture containing compound 48/80 and mast cells.

These substances are known to inhibit the action of 5-hydroxytryptamine but are not specific. No response of the treated uterus was obtained on the addition to the bath of plasma obtained from rats 5 min. after an intravenous injection of compound 48/80.

This work suggests that a substance capable of causing a contraction of the isolated rat uterus is released from mast cells disrupted by compound 48/80. Histamine is found in mast cells in a concentration of 40 µgm. per million cells, but it does not cause contraction of the rat uterus. The 5-hydroxytryptamine concentration in rat mast cells is approximately 1/20 the concentration of histamine⁷. 5-Hydroxytryptamine was added to the bath in amounts equivalent to the calculated 5-hydroxytryptamine content of 0.1 ml. of the mast cell suspensions, and the resulting uterine contraction was similar to that produced by the mixture containing 0.1 ml. of mast cells and 0.1 ml. of compound 48/80.

The smooth muscle stimulant was released also from mast cells disrupted by a number of procedures shown previously to cause the release of histamine from mast cells⁸⁻¹⁰ including (a) mechanical disruption; (b) distilled water; (c) an extract obtained from rat splenic tissue; (d) dextran, when added to serum obtained from rats following adrenalectomy; (e) addition of human red cells to fresh rat antiserum at 37° C. and pH 7. In experiments (d) and (e) with rat serum it was necessary first to decrease 5-hydroxytryptamine content of the serum by an intraperitoneal injection of reserpine (5 mgm./kgm.) at least 8 hr. before collection of the blood.

It may be concluded that 5-hydroxytryptamine or a similar substance is released together with histamine when rat mast cells are disrupted *in vitro*. It seems likely that this substance is the 'slow-contracting' substance found in rat plasma following the intravenous injection of compound 48/80.

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Interaction of Acetylcholine, Calcium and Depolarization in the Contraction of Smooth Muscle

SMOOTH muscle which has been depolarized by external application of potassium still contracts in response to acetylcholine¹. This response requires calcium (ref. 2, and personal communication from D. H. Jenkinson). Calcium ions alone cause the depolarized smooth muscle to contract. If an isolated