Polylysine as histamine releaser

SIR,—L-Polylysine, a synthetic polymer of L-lysine, is known to affect the such membrane of different cells. Thus it liberates a variety of small molecules, as adenylic acid and amino-acids, from Ehrlich ascites tumour cells (Kornguth & Stahmann, 1961); it also causes haemolysis (de Vries, Stein & others, 1954). I have now tested its capacity to release histamine.

Mast cells obtained from the rat peritoneum by a method described previously (Bergmann, Chaimovitz & others, 1962), were centrifuged, washed and re-suspended in saline, buffered to pH 7·4, and incubated with 50 μg/ml L-polylysine [molecular weight 3300 (Yeda Research and Development Co., Rehovoth, Israel)]. Samples were withdrawn after 15, 30 and 60 min and centrifuged; the supernatant was then assayed on the guinea-pig ileum. Controls were incubated with saline only and centrifuged simultaneously with the experimental samples.

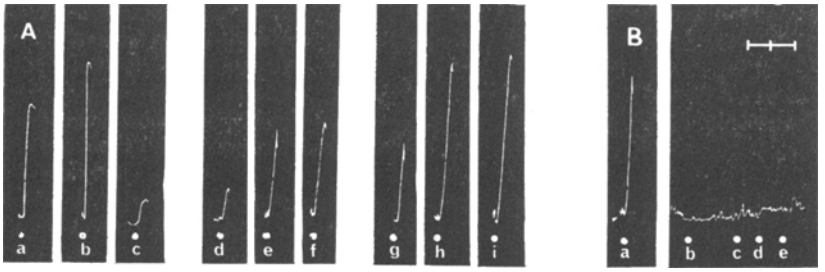

FIG. 1A. Release of smooth muscle stimulant from rat mast cells under the influence of polylysine. A loop of fresh guinea-pig ileum (3 cm length) was suspended at 37° in a 25 ml organ bath, containing Tyrode solution. The contractions of the gut were recorded with an isotonic lever, using a 10-fold magnification: a, histamine, 20 ng/ml, and b, 30 ng/ml. c, polylysine, 2 μg/ml. d–f, 0·3 ml of supernatant obtained by incubating mast cells with saline for 15, 30 and 60 min, respectively, at 37° and centrifuging. g–i, 0·15 ml of supernatant remaining after centrifugation of mast cell suspension; g, after 15 min, h, after 30 min and i, after 60 min incubation at 37° with polylysine, 50 μg/ml. Note that the final concentration of the peptide in the organ bath in experiments g–i was only 0·3 μg/ml.

B. Antagonism between diphenhydramine and the smooth muscle stimulant, released from rat mast cells. Conditions as in A. a, histamine, 30 ng/ml. b, diphenhydramine, 40 ng/ml; 2 min later, at c, without washing, addition of histamine (concentration as in a). d, 1 ml of supernatant, obtained after 30 min incubation of mast cells in saline at 37°; e, 0·5 ml of supernatant, obtained after 30 min incubation of mast cells with 50 μg/ml polylysine. Note that the volumes added in d and e are equiactive with the amount of histamine in a, when diphenhydramine is absent. Time scale 2 min.

It is evident from Fig. 1A that polylysine is a histamine releaser. Under the conditions used, a maximal action was obtained in about 30 min. The contraction was completely inhibited by diphenhydramine (Fig. 1B).

It is known that polyamines liberate histamine from tissue cells (MacIntosh & Paton, 1949); however, these compounds may—at least in part—act by penetration into the cytoplasm where they replace the histamine bound to anionic sites (Åborg, Novotny & Uvnäs, 1967). In contrast, polylysine probably attaches itself to the cell membrane, as was found to be the case with human erythrocytes (Nevo, de Vries & Katchalsky, 1955).

It now appears possible that the increased capillary permeability, seen after the intradermal injection of polylysine in rabbits (Frimmer & Schischke, 1965),

may be, in part, an indirect effect of the liberation of histamine from tissue cells.

Acknowledgement. The author is grateful to Mr. R. Knafo for technical help and for preparation of the records.

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December 25, 1967

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The influences of drugs on the uptake of 5-hydroxytryptamine by nerve-ending particles of rabbit brain stem

SIR,—Recent progress in the technique of isolation of nerve-ending particles (synaptosomes) and synaptic vesicles from brain (Whittaker, 1959; DeRobertis, Rodriguez de Lores Arnaiz & Pellegrino de Iraldi, 1962) has made it possible to investigate the detailed mechanisms of uptake, binding and release of biogenic amines at the subcellular level. Maynert & Kuriyama (1964) found that nerve-ending particles or synaptic vesicles of brain, when incubated in a medium containing noradrenaline or 5-hydroxytryptamine (5-HT), can take up these amines from the medium against the concentration gradient, and they suggested that both nerve ending particles and synaptic vesicles possessed a transport system for noradrenaline and 5-HT. Furthermore, they found that reserpine inhibited the uptake of these amines. Independently, Robinson, Anderson & Green (1965) showed that nerve ending particles and microsomes of brain can take up 5-HT and histamine *in vitro*. Little is known about the kinetics of such uptake so far.

Reserpine, cocaine, desipramine and prenylamine have been reported to inhibit the catecholamine uptake-concentrating mechanism of adrenergic neurons (Hillarp & Malmfors, 1964; Lundmar & Muscholl, 1964; Carlsson & Waldeck, 1965; Malmfors, 1965), and the present study was undertaken to investigate the influences of these drugs on the uptake of 5-HT by nerve-ending particles *in vitro*.

Male rabbits, weighing about 2·5 kg were used. Two brain stems (*ca* 5 g) were homogenized in ice-cold 0·32 M sucrose with a Teflon pestle and made up to about 50 ml. The P_1-fraction was separated by centrifuging the homogenate at 900 *g* for 10 min. This P_1-fraction was washed twice with 0·32 M sucrose and the washings were added to the supernatant fluid from the P_1 preparation. The P_2-fraction, a crude mitochondrial fraction, was prepared by centrifuging the supernatant at 11,500 *g* for 20 min. The method of subsequent sub-fractionation of the P_2-fraction was similar to that described by Gray & Whittaker (1962). The P_2-fraction was resuspended in 0·32 M sucrose (2 ml/g of original tissue) and 5 ml of this suspension was laid on the top of a discontinuous density gradient consisting of 12 ml each of 0·8 M and 1·2 M sucrose per tube, and centrifuged at 53,500 *g* for 2 hr. This resulted in the subfractions A, B and C which contained predominantly myelin, nerve ending particles and mitochondria