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THE RELEASE OF HISTAMINE BY POLYMYXIN B AND POLYMYXIN E

BY

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Some of the side-effects of polymyxins B and E in man and experimental animals might be due to the liberation of histamine by these antibiotics. Such a liberation could account for the "triple response" on injection of the histamine-free antibiotics in man (Swift and Bushby, 1953), and the close similarity in the dog between the depressor action of massive doses of polymyxins B and E (Brownlee, Bushby, and Short, 1952) and that of the histamine liberator 48/80 (Baltzly, Buck, de Beer, and Webb, 1949; Paton, 1951). It would also provide a rational explanation for the subjective side-effects of polymyxin B in man being relieved by antihistamine drugs (Kagan, Krevsky, Milzer, and Locke, 1951). Evidence that the polymyxins do in fact release histamine in the dog and the rat and have other properties in common with known histamine liberators is presented here.

METHODS

The polymyxins used in these experiments were therapeutic samples prepared for parenteral injection and were free from histamine (less than 0.1 μg. histamine/mg.). The quantities are expressed in mg. on the basis that pure polymyxin B and E contain 10.000 u./mg. The polymyxin E had a potency of 8,000 u./mg. and the polymyxin B 7,000 u./mg.

Rats.—Rats weighing 80–150 g. from an inbred Wistar colony were used in all experiments. To determine effects on skin histamine, rats were killed by a blow on the head at various intervals after dorsal subcutaneous injection of the polymyxins or 48/80; areas of skin of approximately 2 cm.^{2} were removed from the ventral abdomen and transferred to trichloracetic acid as rapidly as possible. The histamine was extracted in the manner used by Haas (1940) and assayed on isolated guinea-pig ileum. To assess oedema formation, groups of five rats were given water by stomach tube (5% of the body-weight) immediately before subcutaneous injection of polymyxin or 48/80 and the diameters of the feet measured with micrometer callipers. When antihistamines were used, they were injected subcutaneously 10 min. before polymyxin or 48/80.

Intradermal Effects.—The increase in capillary permeability around the site of the intradermal injection of polymyxin B, E, or 48/80 (in 0.1 ml.), in groups of at least six guinea-pigs of 350–400 g., was determined by the procedure described by Miles and Miles (1952), except that the Evans Blue (0.5 ml. of a 1% soln.) was injected directly into the heart instead of intravenously. The area of blueness on the external surface of the skin was measured.

Dogs.—The effect of intravenous polymyxin or 48/80 on the carotid blood pressure, the packed cell volume, and the histamine content of heparinized blood (or plasma) drawn from the femoral artery, were determined in dogs anaesthetized with pentobarbitone sodium. Packed red cell volumes were measured after centrifuging at 1,500 *G.* for 30 min. in a haematocrit. The histamine equivalent of the plasma itself or of extracts of whole blood was determined on the carotid blood pressure of cats under pentobarbitone sodium, or on isolated guinea-pig ileum, or both. The method of Code (1937) was used to extract histamine from blood and was adapted for extraction of skin as by Haas (1940). The skin histamine concentration after polymyxin was compared with that of similar pieces of skin from the opposite flank removed before giving the polymyxin.

Cats.—The depressor action of the polymyxins and 48/80 in cats was also determined under pentobarbitone anaesthesia.

RESULTS

Effects in Rats

Polymyxins B and E and also 48/80 caused vasodilatation, and oedema of the muzzle and limbs in the rat. These effects were associated with the depletion of skin histamine.

Comparison of the histamine concentrations of the skin taken remotely from the injection site at various intervals after subcutaneous injection has shown that polymyxin B is a more powerful histamine liberator in the rat than is 48/80 (Fig. 1). After 5 mg./kg. of either substance the skin histamine was reduced by about 80% in four hours and did not return to normal levels until about 10

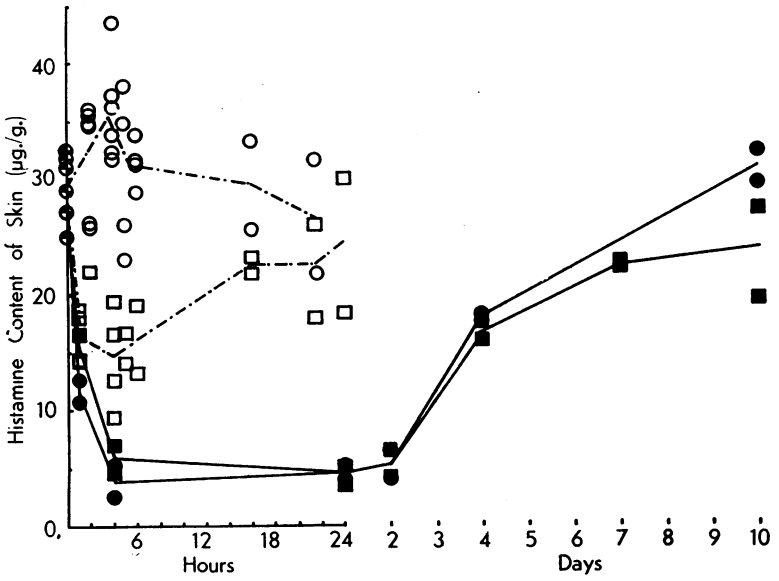

FIG. 1.—The histamine content of the skin of rats at intervals after the subcutaneous injection of polymyxin B or 48/80. Open squares, polymyxin B, 1 mg./kg.; filled squares, polymyxin B, 5 mg./kg.; open circles, 48/80, 1 mg./kg.; filled circles, 48/80, 5 mg./kg.; half-filled circles, controls.

days later. Whereas 1 mg./kg. polymyxin B temporarily reduced the skin histamine by about 50%, the same dose of 48/80 appeared to increase the histamine content of the skin—extracts of the skin removed 4 hr. after 1 mg./kg. 48/80 contained significantly more histamine than the controls ($P=0.01$–0.02). (An increase followed by a decrease in extractable skin histamine after local application of mustard oil in rats has been described by Haas (1940).) Polymyxin E also depleted the skin histamine, but was not so active as polymyxin B or 48/80. The skin histamine concentration 24 hr. after 5 mg./kg. polymyxin E was about 30% below normal (3 rats), and at 4 and 24 hr. after 10 mg./kg. polymyxin E it was reduced by 74% (2 rats) and 58% (3 rats) respectively. Although it is a more powerful histamine liberator, polymyxin B (LD50 approx. 50 mg./kg.) is no more toxic than 48/80 when given subcutaneously to rats.

Fawcett (1954) found that the intraperitoneal injection of 48/80 in a large volume of Tyrode caused a release of histamine into the fluid in the peritoneum and, as previously described by Mota, Beraldo, and Junqueira (1953), a loss of granules from the mast cells of the mesentery. When injected in the same way, 5 mg./kg. polymyxin B in 20 ml. affected the mast cells in the same manner as did 5 mg./kg. 48/80; in two rats given polymyxin the histamine content of the peritoneal fluid reached higher concentrations (3.3 and 3.4 μg./ml.) than it did in two others given 48/80 (1.7 and 2.0 μg./ml.). Loss of granules from mast cells of the mesentery also occurred after subcutaneous injection of 5 mg./kg. of either polymyxin or 48/80. Norton and de Beer (1955) have shown that polymyxin B causes fragmentation of rat mast cells *in vitro*.

Oedema in hydrated rats caused by either 5 mg./kg. polymyxin B or 10 mg./kg. 48/80 was not reduced even by massive doses of antihistamines (chlorcyclizine 100 mg./kg. or α-1-(4-methylphenyl) - 1 - (2 - pyridyl) - 3 - pyrrolidino prop-1-ene monohydrochloride [" Actidil "] 20 mg./kg.).

Intradermal Response

Swift and Bushby (1953) noted that the intradermal injection of polymyxin E produced a typical histamine " triple response." In guinea-pigs injected with Evans Blue, polymyxin B or E caused a leakage of the dye similar to that produced by somewhat smaller concentrations of 48/80. The mean size of lesions with 0.5, 2, and 8 μg. polymyxin were 2.1, 4.7, and 7.2 mm. respectively, and with 0.25, 1, and 2 μg. 48/80 were 3.5, 5.3, and 7.3 mm. respectively; the mean value for saline was 2.8 mm.

Effects in Dogs

General.—The fall in blood pressure caused by large intravenous doses of polymyxin B or E in anaesthetized dogs is delayed, and resembles that with 48/80. With 19 injections of polymyxin E the average delay was 13.9 ± 4.1 sec., and with 8 injections of 48/80 the average delay was 10.9 ± 4.3 sec. A typical fall in a dog given 5 mg./kg. polymyxin B is illustrated in Fig. 2. Such falls are associated with a rise in the concentration of circulating histamine, but the concentrations reached are not as high as after 48/80. Histamine was estimated either by testing the plasma itself or by testing an extract of whole blood prepared by Code's method. Both the untreated plasma and the extracts of blood obtained shortly after inject-

ing large doses of polymyxin B or E had a depressor action in the anaesthetized cat and a spasmogenic action on isolated guinea-pig ileum greater than that of samples taken before injection of polymyxin. Parallel quantitative assays with histamine showed fair agreement. The samples were as sensitive to the antihistamine "Actidil," and as resistant to atropine, as was histamine, although a slight effect of the plasma samples persisted after the antihistamine was given.

The rise in circulating histamine was associated with a slight fall in the skin histamine of two dogs. In the experiment illustrated in Fig. 2 the skin histamine fell from 8.6 μg./g. to 3.5 μg./g. 2 hr. after injection of the polymyxin.

Comparison with 48/80.—Polymyxins B and E are much less powerful histamine-liberators in the dog than is 48/80, and polymyxin B seemed to be slightly less active than polymyxin E.

Polymyxin B at 5 mg./kg. lowered the blood pressure by 85–135 mm. Hg in each of four dogs. The concentration of circulating histamine did not increase sufficiently in two of these animals for its estimation by direct testing of the plasma itself, but in the two other dogs extracts of the blood showed a small increase in circulating histamine after injection of the polymyxin B (Fig. 2). In another dog 10 mg./kg. polymyxin B raised the concentration of plasma histamine from less than 0.05 μg./ml. to 0.1–0.15 μg./ml. within 1 min., the plasma histamine falling to 0.08 μg./ml. within 4 min. and to below 0.05 μg./ml. within 10 min. Polymyxin E at 5 mg./kg. lowered the blood pressure by 90–130 mm. Hg in 9 out of 10 dogs, one animal being refractory. The plasma histamine concentration generally rose within 1 min. to between 0.1 and 0.3 μg./ml., and then declined rapidly. The packed red cell volume increased from initial values of 38–41% to 39–49% in 3 min. and to 40–52% in 10 min. In two dogs, 2 mg./kg. polymyxin E caused depressions of 70 and 120 mm. Hg respectively and increased the plasma histamine from less than 0.05 μg./ml. to approximately 0.1 μg./ml.

Both the duration of the depressor action of polymyxin and the increase in plasma histamine are small compared with that caused by 48/80. This at 0.2 mg./kg. commonly increases the plasma histamine to 1 μg./ml. or more. Confirmation that polymyxin E is considerably less than one-tenth as active as 48/80 was obtained by injecting both compounds in the same dog. Polymyxin E, 2 mg./kg., produced a fall from 166 to 46 mm. Hg and 6 min. later the blood pressure was 116 mm. Hg; the highest plasma histamine concentration found was 0.1 μg./ml. One hr. later, when the blood pressure had returned to 146 mm. Hg, 0.2 mg./kg. 48/80 lowered the blood pressure to 44 mm. Hg, and after 6 min. the blood pressure was only 60 mm. Hg: the plasma histamine reached a value of 1 μg./ml.

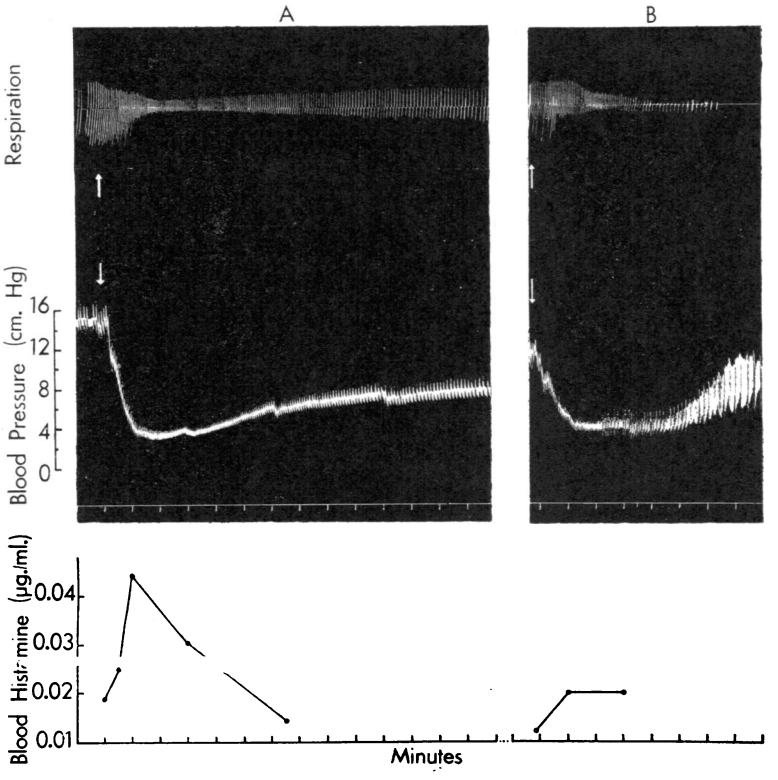

FIG. 2.—The effect of intravenous polymyxin B on respiration, blood pressure, and blood histamine concn. in an anaesthetized dog. A, a first dose of 5 mg./kg. polymyxin B; B, a similar dose 2½ hr. later. Time 1 min.

Tachyphylaxis.—Brownlee *et al.* (1952) noted tachyphylaxis to the depressor action of polymyxin. We have found that the tachyphylaxis is associated with a smaller

liberation of histamine with the second than with the first injection. This is illustrated for polymyxin B in Fig. 2. In another animal 5 mg./kg. polymyxin E lowered the blood pressure by 122 mm. Hg and produced a plasma histamine level of 0.2 μg./ml.; one hr. later a second dose of 5 mg./kg. caused a fall of similar magnitude but of much shorter duration, and the plasma histamine did not exceed 0.05 μg./ml.

Other Effects.—The very large doses of polymyxins B and E (5 and 10 mg./kg.) used in the foregoing experiments approximate to lethal doses. The toxic effects consist of severe vascular engorgement, particularly of the liver, and a resulting fall in blood pressure and respiratory depression. Following intravenous polymyxin B or E, after brief apnoea, the breathing was often temporarily increased in rate and depth, but after 1 to 2 min. the tidal volume nearly always decreased, and then breathing became less frequent and sometimes stopped. At this stage most but not all dogs could be saved by artificial respiration. The effects of polymyxin on respiration are cumulative, even when a period of 2½ hr. elapsed between doses (Fig. 1) and this contrasts with the tachyphylaxis to the depressor effect. Bronchoconstriction is probably largely responsible for the decrease in tidal volume, and was shown in one deeply anaesthetized dog by the method of Konzett and Rössler, (1940).

Prior administration of massive doses of "Actidil" (10 mg./kg.) intravenously to each of four dogs reduced the rate of the fall in blood pressure caused by 5 mg./kg. polymyxin B or E, without significantly altering its ultimate extent or decreasing the toxic respiratory effects of the polymyxin.

Effects in Cats

Given intravenously to cats polymyxin B and E caused a delayed depressor response resembling that with 48/80. The effect was negligible (less than 20 mm. Hg) with 2 mg./kg. polymyxin B in 6 of 7 cats and in one amounted to only 26 mm. Hg; depressions of 30–85 mm. Hg were caused in 4 cats given 5 mg./kg. polymyxin B. Since as little as 0.1 mg./kg. 48/80 causes a profound fall in blood pressure (Paton, 1951) polymyxin B appears to be a much less powerful histamine liberator than 48/80 in the cat.

Bronchial tone, measured by the method of Konzett and Rössler (1940) in a cat under deep chloralose anaesthesia, was slightly increased by 5 mg./kg. polymyxin B and very greatly increased for over an hour by 12 mg./kg.

Discussion

The polymyxins B and E release tissue histamine, their potency in this respect varying relative to that of 48/80 under different conditions. Subcutaneously in rats they are about as active as 48/80 in releasing histamine from skin remote from the site of injection, and in causing degranulation in the mast cells of the mesentery. Intravenously in dogs their action is feeble, but the histamine liberated is sufficient to account for at least part of their delayed depressor action. In these animals the rise in circulating histamine and the skin depletion are small compared with those caused by 48/80, even when the blood pressure falls to very low levels. As these investigations were made on material containing 7,000–8,000 u./mg. the action may be due to an impurity; this is unlikely, however, because Brownlee *et al.* (1952) found that the depressor action was produced by even the purest material (10,000 u./mg.).

Although antihistamines do not abolish the effects of the polymyxin, the rate of fall in blood pressure caused by polymyxin B or E was slower after an antihistamine, which suggests that the effects are at least modified. The lessening of some of the subjective side-effects by the concomitant use of antihistamines reported by Kagan *et al.* (1951), together with our finding that polymyxins liberate histamine in other species, suggests that some of the side-effects of polymyxin in man may be due to a slight histamine release.

Summary

1. Polymyxin B and polymyxin E injected subcutaneously in rats are about as active as 48/80 in releasing skin histamine remote from the site of injection and causing degranulation of the mast cells in the mesentery.

2. In contrast, when injected intravenously in dogs and cats, their histamine-releasing activity is not powerful.

3. In dogs they cause a small rise in circulating histamine, and this is associated with their depressor and bronchoconstrictor effects in this species.

4. These observations may be related to the finding that some of the subjective side-effects of polymyxin in man are relieved by antihistamine drugs.

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