## Histamine release by *OO'*-diethylbebeerine in the isolated rat diaphragm

SIR,—Histamine release by (+)-tubocurarine in the rat isolated diaphragm was found by Rocha e Silva & Schild (1949) to be enzymatic in character. Moussatché & Prouvost-Danon (1958), Yamasaki, Muraoka & Endo (1960), Rothschild, Vugman & Rocha e Silva (1961), Uvnäs & Diamant (1961) among others, have shown that tissue responsiveness to histamine-releasing stimuli requires metabolic cellular activity. The present note shows that the same is true for *OO'*-diethylbebeerine, a semisynthetic curarizing drug, having about 10 times more histamine-releasing potency than (+)-tubocurarine in the rat (Rothschild & Corrado, 1963).

TABLE 1. EFFECT OF 2,4-DINITROPHENOL (DNP) ON HISTAMINE RELEASE FROM THE ISOLATED RAT DIAPHRAGM BY *OO'*-DIETHYLBEBEERINE

| Treatment of the tissue during pre-incubation* | Histamine released (% of total) | Inhibition (%) |
|---|---|---|
| — | 15·6 ± 2·8 | — |
| Glucose 4·5 mM | 16·5 ± 2·6 | — |
| DNP, 0·3 mM | 2·4 ± 1·2 | 85 |
| Glucose + DNP | 13·3 ± 3·8 | 19 |

* 20 min at 37° in Krebs–Ringer phosphate buffer. After preincubation, 200 μg/ml of *OO'*-diethylbebeerine was added to all tubes and incubations continued for another 20 min. Released and residual (tissue bound) histamine was estimated by bioassay on the guinea-pig isolated ileum by standard techniques. Each result represents the average of 4 experiments from which the contribution of spontaneously released histamine has been deducted.

Table 1 shows the effect of *OO'*-diethylbebeerine on histamine release from the rat isolated diaphragm. The effect takes place both in complete and in glucose-free media, but is extensively inhibited by 2,4-dinitrophenol in the glucose-free medium. In the presence of glucose the inhibitor is ineffective. Dinitrophenol is an uncoupler of aerobic high-energy phosphate bond synthesis which, however, does not block the initial anaerobic steps of glucose consumption by mammalian cells. It is probable therefore, that the metabolites required for the histamine-releasing response of rat mast cells to *OO'*-diethylbebeerine can arise through the anaerobic metabolism of glucose.

A dependence on cell metabolism similar to that shown here for the histamine-releasing activity of a curarizing drug, has been previously demonstrated for certain other basic "chemical" histamine releasing compounds like 48/80 and its analogues, (+)-tubocurarine (Rothschild, 1966) and sinomenine (Yamasaki & Saeki, 1966). Such a dependence however, does not seem to be a requirement for the action of all simple basic histamine releasers active on rat tissues, since it could be shown (Rothschild, 1962, 1966), that histamine release from isolated peritoneal fluid rat mast cells by ring chlorinated analogues of catecholamines (dichloroisoprenaline, dichloroadrenaline and dichloronoradrenaline) proceeds unhindered in cells treated with 2,4-dinitrophenol in a glucose-free medium.

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**Enlargement of liver in rats after chronic administration of flumedroxone acetate**

SIR,—The synthetic steroid flumedroxone acetate (17-acetoxy-6α-trifluoromethylpregn-4-ene-3,20-dione; WG537; Demigran) has been used as a prophylactic treatment for migraine (summarized in Lundberg, 1966). The experiments reported here followed the observation that 100 mg/kg of flumedroxone acetate produced in the laboratory rat, after chronic intraperitoneal treatment, a liver weight increase. This steroid drug, together with 17-acetoxy-3β(β-carboxypropionyloxy)-6-trifluoromethylpregn-5-ene-20-one (VD682) was synthesized and provided by Leo Pharmaceutical Products Ltd., Ballerup, Denmark.

Female albino Porton rats weighing between 100–200 g, maintained on standard laboratory chow with water *ad libitum*, were used. Each steroid was suspended in water (1 ml) using compound tragacanth powder and introduced by gastric intubation. Control and treated animals were killed 24 hr after the last treatment and body and liver weights determined.

Table 1 shows that rats treated with flumedroxone acetate and VD682 have an increased liver weight when compared to control animals, or animals treated

TABLE 1. LIVER WEIGHT OF RATS AFTER TREATMENT WITH FLUMEDROXONE AND VD682

| Drug | Dose mg/kg (No. days) | Animal weight range g | Mean body weight g (No. animals) | Liver weight g/100 body weight (range, g) |
|---|---|---|---|---|
| Flumedroxone acetate | 10 (7) | 100–149 | 130·4 (3) | 5·7 (6·9–8·3) |
| | 20 (7) | | 145·2 (2) | 6·3 (7·6–8·9) |
| | 50 (7) | | 141·5 (4) | 6·3 (7·9–9·6) |
| | 100 (7) | | 145·2 (4) | 7·3 (9·4–11·8) |
| Flumedroxone acetate | 100 (3) | 100–200 | 152·5 (3) | 5·7 (8·2–9·2) |
| | 100 (5) | | 150·4 (2) | 6·0 (8·8–10·6) |
| | 100 (7) | | 145·2 (4) | 7·3 (9·4–11·8) |
| | 100 (14) | | 195·0 (2) | 8·2 (15·0–16·7) |
| VD682 | 50 (7) | 100–149 | 137·2 (3) | 7·1 (8·4–10·8) |
| | 100 (14) | 150–200 | 185·4 (4) | 10·5 (18·6–20·1) |
| Compound tragacanth powder | 700 (14) | 100–149 | 137·2 (6) | 3·9 (4·3–6·0) |
| | 700 (14) | 150–200 | 161·4 (5) | 4·1 (4·9–7·9) |
| None | — | 100–149 | 138·1 (8) | 3·8 (4·4–7·5) |
| | — | 150–200 | 176·2 (9) | 3·9 (5·3–8·2) |

TABLE 2. LIVER WEIGHT OF RATS AFTER TREATMENT WITH STEROID COMPOUNDS FOR 9 DAYS

| Drug | Dose mg/kg | Mean body weight g (No. of rats) | Liver weight g/100g body weight (range, g) |
|---|---|---|---|
| Progesterone .. .. .. | 25 | 160·2 (5) | 3·8 (5·4–6·8) |
| | 50 | 152·5 (6) | 5·0 (7·2–7·9) |
| | 100 | 163·3 (4) | 5·2 (7·2–8·1) |
| Flumedroxone acetate .. .. | 50 | 138·2 (4) | 7·2 (8·7–10·6) |
| VD682 .. .. .. .. | 50 | 136·7 (4) | 7·8 (9·4–12·1) |
| None .. .. .. .. | — | 164·1 (10) | 3·8 (4·2–7·1) |