5. G. H. MASSIEU, B. G. ORTEGA, A. SYRQUIN and M. TUENA, *J. Neurochem.* **9**, 143 (1962).
6. L. NAFTALIN, *Nature, Lond.* **161**, 763 (1948).
7. S. P. BESSMAN, J. ROSSEN and E. LAYNE, *J. biol. Chem.*, **201**, 385 (1953).
8. C. F. BAXTER and E. ROBERTS, *J. biol. Chem.* **233**, 1135 (1958).
9. E. ROBERTS and S. FRANKEL, *J. biol. Chem.* **190**, 505 (1951).
10. G. RINDI, V. PERRI and U. VENTURA, *Nature, Lond.* **183**, 1126 (1959).
11. J. AWAPARA and B. SEALE, *J. biol. Chem.* **194**, 497 (1952).
12. C. F. BAXTER and E. ROBERTS, *J. biol. Chem.* **236**, 3287 (1961).

Effect of catecholamines and their chloro-analogs on the *in vitro* release of histamine from cells of rat peritoneal fluid

(*Received* 30 *July* 1962; *accepted* 1 *August* 1962)

THE HISTAMINE-RELEASING activity of dichloroisoproterenol (DCI), a powerful inhibitor of the effects of epinephrine on β-receptors,[1, 2] as well as that of dichloroepinephrine and dichloroarterenol, has not been previously reported. Epinephrine has been studied in this regard, but conflicting results about its activity have been presented by Koch and Szerb[3] who found it active in the perfused rat lung whereas Mongar and Whelan,[4] could not demonstrate its activity on a variety of rat tissues *in vitro*. The results presented below indicate that dichloroepinephrine, dichloroarterenol and DCI are comparatively potent releasers of histamine from cells of rat peritoneal fluid. In contrast, the corresponding catecholamines were found to be devoid of this activity.

METHODS

Adult Wistar rats were exsanguinated and their peritoneal fluid cells collected by washing the peritoneal cavity with Krebs–Ringer phosphate buffer, pH 7·3, containing 0·1% glucose. These cells contain histamine which is predominantly bound to the mast cell fraction of the cellular population. After centrifugation the cells were resuspended in buffer and representative samples placed in the incubation flasks. After the addition of the amines, the flasks were kept at 37 °C for 15 min with continuous, gentle agitation. Blanks contained cells in buffer only. After incubation the cell suspensions were centrifuged and washed twice with fresh buffer. Histamine was estimated by bioassay on the guinea-pig ileum. Since all the amines tested inhibited the response of this preparation to histamine, residual rather than liberated histamine was measured. For this the washed cells were heated to boiling with 0·1 N HCl for 5 min to liberate residual bound histamine. Blanks were similarly extracted, and their residual histamine content, to which a value of 100 per cent was assigned, furnished the basis of reference for the estimation of the release occurring in the treated samples. The spontaneous release occurring during incubation of the blanks was always less than 10 per cent.

Drugs. L-Adrenaline, British Drug Houses; dichloroepinephrine (DL-β-hydroxy-N-methyl-3,4-dichlorophenylethylamine), dichloroarterenol (DL-β-hydroxy-3,4-dichlorophenylethylamine), and dichloroisoproterenol (DL-β-hydroxy-N-isopropyl-3,4-dichlorophenylethylamine), Lilly Research Laboratories; DL-arterenol and DL-isoproterenol, Sterling-Winthrop Laboratories. With the exception of adrenaline all drugs were in the form of the hydrochlorides.

The results shown in Table 1 indicate that epinephrine, arterenol ,and isoproterenol were unable to release significant amounts of histamine when tested at 3- or 10-mM levels; in contrast, their chloro-analogues were highly effective at 3 Mm, and practically ineffective at 1-mM concentrations. Pretreatment with iproniazid *in vivo* (50 mg/kg i.v. 2 hr before removal of the peritoneal cells), or *in vitro* (preincubation with 3 mM iproniazid), did not alter these results, indicating that intracellular destruction of the catecholamines by the amine oxidase, said to exist in mast cells,[5] could not be the reason for their ineffectiveness. Results similar to those shown in Table 1 were obtained when the *in vitro* effects of DCI and isoproterenol on the morphology of mast cells of rat mesentery were

TABLE 1. EFFECT OF CATECHOLAMINES AND THEIR CHLORO-ANALOGS ON THE *in vitro* RELEASE OF HISTAMINE FROM CELLS OF RAT PERITONEAL FLUID

Amine	Histamine retained (%) Mean ±s.e.
Epinephrine, 10 mM	100 ± 0 (3)*
Epinephrine, 3 mM	109 ± 11 (4)
Dichloroepinephrine, 3 mM	8 ± 2 (4)
Dichloroepinephrine, 1 mM	107 ± 12 (3)
Arterenol, 10 mM	90 ± 27 (3)
Arterenol, 3 mM	89 ± 8 (5)
Dichloroarterenol, 3 mM	10 ± 5 (4)
Dichloroarterenol, 1 mM	77 ± 15 (3)
Isoproterenol, 10 mM	93 ± 13 (2)
Isoproterenol, 3 mM	94 ± 16 (3)
Dichloroisoproterenol, 3 mM	3 ± 2 (4)
Dichloroisoproterenol, 1 mM	101 ± 12 (3)

* Number of experiments performed.

examined. Microscopically visible degranulation was found only after DCI treatment. At the 3-mM level this compound induced partial shedding of granules in 81 per cent of the cells, whereas 34 per cent of the cells were affected at the 1-mM level. In contrast, after treatment with 3 mM isoproterenol, the percentage of cells showing morphological alterations was not different from that found in blanks incubated in the absence of the amine (9·5 and 10 per cent respectively).

In a review of the work of several authors, who have attempted to correlate structural changes of basic organic compounds with variations in histamine-releasing activity, Paton[6] concluded that increases in bulk or chain length enhanced this activity, possibly by increasing lipophilic character, thereby allowing for a greater rate of penetration of the releasing agent into the mast cell. Mayer[7] has noted that DCI crosses the blood-brain barrier much more rapidly than do the catecholamines. He attributed this to the greater liposolubility of the chloro-derivative. Differences in liposolubility in membrane components of the mast cell could explain the variations between the histamine-releasing and mast cell-degranulating capacities of the catecholamines and those of their chloro-analogs.

Acknowledgements—Adrenaline and isoproterenol were kindly supplied by Dr. S. B. Henriques, S. Paulo, Brazil; dichloroepinephrine and dichloroarterenol by Dr. B. Levy, Atlanta, Ga., U.S.A. The expert technical assistance of A. Castania is acknowledged.

Department of Pharmacology,
Faculty of Medicine,
Ribeirão Preto, S.P., Brazil

A. M. ROTHSCHILD

REFERENCES

1. C. E. POWELL and I. H. SLATER, *J. Pharmacol. exp. Ther.* **122**, 480 (1958).
2. N. C. MORAN and M. E. PERKINS, *J. Pharmacol. exp. Ther.* **124**, 223 (1958).
3. J. KOCH and J. SZERB, *Arch. int. Pharmacodyn.* **81**, 91 (1950).
4. J. L. MONGAR and R. F. WHELAN, *J. Physiol., Lond.* **120**, 146 (1953).
5. P. HAGEN, R. J. BARNETT and F. L. LEE, *J. Pharmacol. exp. Ther.* **126**, 91 (1959).
6. W. D. M. PATON, *Pharmacol. Rev.* **9**, 269 (1957).
7. S. E. MAYER, *J. Pharmacol. exp. Ther.* **135**, 204 (1962).