A NOTE ON THE INFLUENCE OF CHLORPROMAZINE AND DIETHAZINE ON THE STORES OF CATECHOLAMINE IN THE ADRENAL GLANDS AND AORTIC WALLS OF RATS

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Stores of catecholamines accumulated in the adrenal glands and in the walls of the aortae of rats during treatment with diethazine or chlorpromazine. Diethazine failed to antagonise the release by reserpine of tissue stores of catecholamines and increased tissue sensitivity to the pressor effects of tyramine, probably because release of catecholamines from tissue stores by tyramine is enhanced. Chlorpromazine antagonised the release of adrenal medullary amines by reserpine and delayed the restoration of these stores after depletion.

THE factors controlling the magnitude of the stores of catecholamine in the walls of blood vessels (Schmiterlow, 1948) and the part these stores may play in postganglionic sympathetic activity are little understood. For this reason we were interested to compare the effect of two amino-derivatives of phenothiazine on the catecholamine stores in the adrenal glands and aortae of rats. The two compounds, chlorpromazine and diethazine, were selected because of known differences in their actions within the autonomic nervous system. Chlorpromazine depresses vasomotor reflexes operative through the medulla and hypothalamus (Dasgupta and Werner, 1954) and has peripheral anti-adrenaline action (Courvoisier, Fournel, Ducrot, Kolsky and Koetschet, 1953) but exerts no blocking action in sympathetic ganglia (Reuse, 1954; Holzbauer and Vogt, 1954). By contrast, diethazine does not block cardiovascular reflexes in the medulla and has no anti-adrenaline action: it causes peripheral vasodilation by depression of ganglionic transmission and suppresses cardiac inhibitory reflexes by means of an atropine-like effect (Heymans, 1949).

METHODS

Female wistar rats, 150 to 200 g. in weight, fed diet 41 b of Stein with water, were divided at random into groups which were separately housed under similar conditions of heating, lighting and humidity. Different daily treatments were assigned to each group of rats. Thus: saline only; chlorpromazine 5 mg./kg. or diethazine 10 mg./kg. once daily or mecamylamine 7·25 mg./kg. twice daily, each in 0·2 ml. 0·9 per cent NaCl w/v, by stomach tube. Treatments were given on four consecutive days; experiments were made on the fifth day.

Preparation of Extracts

Adrenal glands and aortae, removed under deep ether anaesthesia, were rapidly weighed, sliced and ground in ice cold 0·1N HCl with a knife point of silver sand. Each extract was transferred with washings (total volume, adrenals 5 ml.; aorta, 2·5 ml.) to a centrifuge tube, which was heated in a boiling water bath for 2·5 min., rapidly cooled and spun.

Supernatants were stored for 3 to 7 hr. at −7° and were thawed and brought to pH 6·7 with 0·5N NaOH and to a known volume just before bioassay. Assays of total catecholamine in extracts of adrenal glands were made either on the rat colon (Gaddum, Peart and Vogt, 1949) or on the mean arterial pressure of the spinal cat, using 4 × 4 Latin Square designs and (−)-adrenaline as standard. The adrenaline present in extracts of aortae was assayed on the rat uterus (Gaddum, Peart and Vogt, 1949) against (−)-adrenaline; since the quantities of amine in these extracts were small it was rarely possible to complete more than three lines of a 4 × 4 block design. Assays of tyramine in terms of (−)-noradrenaline were made on the mean arterial pressure of treated and control rats under urethane anaesthesia.

TABLE I

THE EFFECT OF DRUGS ON THE TOTAL CATECHOLAMINE IN THE ADRENAL GLANDS OF RATS. CHLORPROMAZINE 5 MG./KG., DIETHAZINE 10 MG./KG. ONCE DAILY, MECAMYLAMINE 7·5 MG./KG. TWICE DAILY, ORALLY, FOR 4 DAYS. RESERPINE 5 MG./KG., I.P. ONCE ON THE FOURTH DAY

Drug used	Body weight, g.	Assay method	Total catecholamine in adrenal glands as (−)-adrenaline, µg.
Chlorpromazine	166 ± 5·2 (5)	Cat B.P.	76·4 ± 6·7 (5)**
Diethazine	166 ± 6·8 (5)		99·0 ± 9·2 (5)**
Mecamylamine	160 ± 5·3 (5)		55·0 ± 6·7 (5)*
None	172 ± 4·7 (5)		42·6 ± 4·0 (5)
Chlorpromazine	178 ± 4·4 (8)	Rat colon	88·9 ± 13·4 (8)**
Diethazine	181 ± 3·6 (8)		82·4 ± 7·8 (8)**
None	183 ± 6·3 (8)		36·6 ± 5·6 (8)
Chlorpromazine	170 ± 5·5 (6)	Rat colon	66·6 ± 7·4 (6)*
Reserpine	189 ± 5·8 (6)		23·7 ± 4·2 (6)*
Chlorpromazine, Reserpine	168 ± 5·3 (6)		59·7 ± 8·1 (6)
Diethazine	173 ± 6·2 (6)		83·3 ● 6·7 (6)*
Diethazine, Reserpine	175 ± 5·7 (6)		41·6 ± 5·9 (6)††
None	191 ± 6·4 (6)		46·8 ± 5·7 (6)

The significance of drug effects has been examined by '*t*' test and is indicated by asterisks for the effect of a single drug and by † for the action of a second drug: one, P = <0·05; two, P = <0·01.
The values shown are means ± standard errors of the means followed by the numbers of rats within brackets.

RESULTS

Daily oral treatment of rats with either chlorpromazine, 5 mg./kg., or diethazine 10 mg./kg. for 4 days had, by the fifth day increased the total pressor catecholamine in the adrenal glands (Table I) and the adrenaline extractable from the aortic walls (Table II). Whereas reserpine

TABLE II

THE EFFECT OF DRUGS ON THE STORES OF ADRENALINE IN THE WALLS OF RAT AORTAE. TREATMENTS AS IN TABLE I

Drug used	Body weight, g.	Assay method	ng. (−)-adrenaline/100 mg. aorta
Chlorpromazine	174 ± 7·2 (5)	Rat uterus	36 ± 5·2 (5)**
Diethazine	181 ± 6·2 (6)		52 ± 9·0 (6)**
None	177 ± 5·8 (6)		14 ± 2·3 (6)
Chlorpromazine	170 ± 5·5 (6)	Rat uterus	28 ± 4·7 (6)*
Reserpine	189 ± 5·8 (6)		10 ± 4·3 (6)
Chlorpromazine, reserpine	168 ± 5·3 (6)		26 ± 4·9 (6)
Diethazine	173 ± 6·2 (6)		49 ± 5·4 (6)*
Diethazine, reserpine	175 ± 5·7 (6)		27 ± 4·3 (6)†
None	191 ± 6·4 (6)		15 ± 3·4 (6)

Tests for the significance of drug effects as for Table I.

5 mg./kg. i.p., given on the fourth day, depleted greatly or reduced the catecholamine stored in the adrenal glands of normal rats and of rats under treatment with diethazine, reserpine was ineffective in this respect in the rats pretreated with chlorpromazine (Table I). Significant reduction in the adrenaline extractable from the aortic walls was demonstrable, after reserpine only, in those rats which had been pretreated with diethazine (Table II). Mecamylamine, 7·5 mg./kg. orally twice daily for 4 days, also increased the total catecholamine in the adrenal glands (Table I).

TABLE III

THE EFFECT OF DRUGS ON THE RESTORATION OF CATECHOLAMINE IN RAT ADRENAL GLANDS AFTER DEPLETION BY RESERPINE. RESERPINE 5 MG./KG. I.M. TO ALL ANIMALS AT ZERO TIME. THEREAFTER, CHLORPROMAZINE 5 MG./KG. OR DIETHAZINE 10 MG./KG. ORALLY AT 6 HR., THEN 12 HRLY. EXPERIMENTS AT 40 TO 46 HR.

Drugs used	Body weight, g.	Assay method	Total catecholamine as μg. (−)-adrenaline
Reserpine alone	193 ± 6·9 (6)	Rat colon	35·6 ± 6·8 (6)
Reserpine, chlorpromazine ..	190 ± 6·5 (6)		20·7 ± 4·2 (6)*
Reserpine, diethazine	189 ± 7·8 (6)		51·3 ± 5·4 (6)*
Reserpine alone	175 ± 4·7 (6)	Rat colon	44·6 ± 8·1 (6)
Reserpine, chlorpromazine ..	178 ± 6·2 (6)		33·3 ± 6·4 (6)
Reserpine, diethazine	177 ± 5·9 (6)		53·8 ± 7·4 (6)

Test for significance as in Table I.

Daily oral treatment of rats for 4 days with diethazine 10 mg./kg. increased the pressor effect of tyramine relative to (−)-noradrenaline. Chlorpromazine was ineffective in this respect (Table IV).

TABLE IV

THE EFFECT OF DRUGS ON RELATIVE SENSITIVITY OF RATS UNDER URETHANE ANAESTHESIA TO THE PRESSOR EFFECTS ACTIONS OF (−)-NORADRENALINE BITARTRATE AND TYRAMINE HYDROCHLORIDE. TREATMENTS AS IN TABLE I

Drug used	μg. tyramine HCl equivalent to 1 μg. (−)-noradrenaline bitartrate
None	141 ± 21·6 (6)
Chlorpromazine ..	125 ± 23·4 (6)
Diethazine	73 ± 12·4 (6)*

Tests for significance as in Table I.

DISCUSSION

The results of experiments summarised in Tables I and II clearly demonstrate the accumulation of stores of catecholamine in the adrenal glands and in the walls of the aortae of rats during treatment with either diethazine or chlorpromazine. Similar accumulation of adrenal medullary stores results from the prolonged administration of small quantities of the ganglion blocking agents hexamethonium, tetraethylammonium or pentolinium ions (Mawji and Lockett, 1962) and from treatment with mecamylamine (Table I). Diethazine resembles the ganglion blocking drugs (Mawji and Lockett, 1962) in failing to antagonise the action of reserpine on tissue stores (Tables I and II) but differs from the ganglion

blocking drugs in increasing sensitivity to the pressor effects of tyramine (Table IV). This potentiation of the pressor effects of tyramine is almost certainly attributable to enhancement of the release of catecholamine from tissue stores by tyramine (Burn and Rand, 1958, 1959; Lockett and Eakins, 1960; Euler and Lishajko, 1960) since the action of reserpine on tissue stores appears to be increased by treatment with diethazine (Table II). Chlorpromazine, like cocaine (Mawji and Lockett, 1962) antagonised the release of adrenal medullary amines by reserpine (Table I) and delayed the restoration of these stores after their depletion (Table III) probably by reduction in the rate of resynthesis of catecholamine.

REFERENCES

Burn, J. H. and Rand, M. J. (1958). *J. Physiol.*, **144**, 314–336.
Burn, J. H. and Rand, M. J. (1959). *Ibid.*, **147**, 135–143.
Courvoisier, S., Fournel, J., Ducrot, R., Kolsky, M. and Koetschet, P. (1953). *Arch. int. Pharmacodyn.*, **92**, 305–361.
Dasgupta, S. R. and Werner, G. (1954). *Brit. J. Pharmacol.*, **9**, 389–391.
Euler, U. S. von and Lishajko, F. (1960). *Experientia*, **16**, 376.
Gaddum, J. H., Peart and Vogt, M. (1949). *J. Physiol.*, **108**, 467–481.
Holtzbauer, M. and Vogt, M. (1954). *Brit. J. Pharmacol.*, **9**, 402–407.
Heymans, C. (1949). *Arch. int. Pharmacodyn*, **79**, 123–128.
Lockett, M. F. and Eakins, K. E. (1960). *J. Pharm. Pharmacol.*, **12**, 513–517; 720–725.
Mawji, S. and Lockett, M. F. (1962). *Ibid.*, **14**, in the press.
Reuse, J. J. (1954). *C.R. Soc. Biol. Paris*, **148**, 192–193.
Schmiterlow, C. G. (1948). *Acta physiol. scand.*, **16**, suppl. 56–113.