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ROLE OF ADRENAL HORMONES IN MAINTAINING TISSUE STORES OF NORADRENALINE DURING INCREASED SYMPATHETIC ACTIVITY

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It is known that adrenergic nerves do not lose their transmitter in the splanchnotomized, the adrenal-demedullated, or the adrenalectomized organism; even the isolated perfused heart synthesizes noradrenaline from tyrosine at a rate comparable to that calculated *in vivo* (Spector, Sjoerdsma, Zaltzman-Nirenberg, Levitt & Udenfriend, 1963). Since, however, adrenergic fibres are capable of taking up catecholamines from the circulation, there might be conditions when the adrenal medulla could contribute to the maintenance of the transmitter in adrenergic nerves or terminals.

By infusing into rats ^{14}C-labelled noradrenaline and measuring its specific activity in urine and tissue, Kopin & Gordon (1963) calculated that 80% of the heart noradrenaline was synthesized in the tissue while 20% was taken up from circulating amine, some of which might have been of adrenal origin. In reserpine-treated rats, demedullation of the adrenal glands retarded the restoration of cardiac catecholamines (Bhagat & Shideman, 1964).

In the present investigation the efficiency of heart tissue in maintaining its stores of noradrenaline without support from the adrenal medulla was tested under conditions of vigorous sympathetic activity produced by drugs which do not, like reserpine, interfere with storage mechanisms of amines. The effect of these drugs, β-tetrahydronaphthylamine and histamine, was compared in normal, adrenalectomized and adrenal-demedullated rats.

METHODS

Adrenalectomies

Male rats weighing approximately 140 g were adrenalectomized under ether anaesthesia. They were maintained by replacing the drinking water with 0.9% sodium chloride solution, and were used for the experiment 6 to 14 days after adrenalectomy. Nearly 20% of the rats died between the third and fifth day, but the surviving animals looked vigorous. Unoperated controls were of the same age.

Adrenal demedullations

Male rats of a mean weight of 90 g were anaesthetized with ether, their adrenals exposed, cut in half with scissors and the adrenal tissue squeezed out of the slit in the capsule. The rats were given 0.9% sodium chloride solution to drink for one week only. They were used seven to

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eight weeks after the operation when they weighed about 250 g. There were no casualties. Controls were of the same age as the operated rats and grew up with them.

Drugs

β-Tetrahydronaphthylamine hydrochloride, 30 mg/kg, was injected subcutaneously, histamine hydrogen phosphate (10 or 20 mg/kg of the base) intraperitoneally. The hearts were excised 4 hr after injection of the drug.

Noradrenaline estimations

The rats were decapitated and the heart was excised. The aorta and left ventricle were dissected away and discarded, and the auricles together with the remaining part of the right ventricle pulverized after freezing in liquid nitrogen. The powder was homogenized in twice its weight of 0.1N HCl. One or two hearts were used for a single estimation; the homogenate of two hearts was made up to 16 ml., of which 4 ml. were used for the estimation, 4 ml. in order to check for recovery by the addition of 250 ng noradrenaline, and the remainder was kept in reserve. Recoveries were first done for every estimation, but when they had proved consistent, only for each group of four estimations. All figures in the Tables are corrected for recoveries. Protein precipitation by perchloric acid, neutralization with K_2CO_3, adsorption on a column of Dowex 50 × 8 (Bertler, Carlsson, Rosengren & Waldeck, 1958), elution with 8 ml. 0.33N HCl and fluorimetry after formation of a trihydroxyindole derivative (von Euler & Lishajko, 1961) have been described in detail (Sharman, Vanov & Vogt, 1962). A Locarte filter fluorimeter was used with Chance OX1 as primary and Ilford Bright Spectrum 625 as secondary filter. When eluates had to be kept overnight, they were brought to *p*H 3 to 4 and stored in the cold. Recoveries averaged 75%.

RESULTS

Table 1 summarizes the observations on intact and adrenalectomized rats. Adrenalectomy as such did not change the noradrenaline content of the heart. The two drugs used lowered its content in normal as well as in operated animals. Histamine was given in two doses, 10 and 20 mg/kg; these doses were equally well tolerated by the adrenalectomized and the normal rats, and lowered the noradrenaline content of the heart by approximately 30%. The loss did not appear to be dose-dependent, and therefore the results are grouped together in Table 1.

TABLE 1

NORADRENALINE CONTENT (MEAN AND RANGE) OF HEART* AND VAS DEFERENS IN MALE RATS. EFFECT OF ADRENALECTOMY, AND INJECTION OF DRUGS

Estimations (no.)	Mean body weight at death (g)	Drug injected (mg/kg)	Operation	Noradrenaline content of tissue: Heart (μg/g)	Fall caused by drug (%)	vas deferens (μg/g)
5	146	None	None	2·2 (1·9–2·4)	—	21·5 (15–26)
8	162	β-Tetra†, 30	None	1·7 (1·2–1·9)	23	20 (15–24)
5	188	Histamine, 10 or 20	None	1·5 (1·1–2·1)	32	—
5	154	None	Adrenalectomy 5–8 days previously	2·1 (2·0–2·3)	—	23·5 (18–29)
9	164	β-Tetra, 30	Adrenalectomy 5–8 days previously	1·4 (1·2–1·6)	33	20 (16–24)‡
5	183	Histamine, 10 or 20	Adrenalectomy 9–14 days previously	1·5 (1·2–2·1)	29	—

* Auricles and anterior wall of right ventricle only. † β-Tetrahydronaphthylamine hydrochloride. ‡ 5 estimations only.

β-Tetrahydronaphthylamine caused a much milder degree of restlessness and exophthalmos in the adrenalectomized rats, obviously because there was no release of medullary amines to contribute to the toxic signs. In the intact rats, cardiac noradrenaline fell by 23% ; in the adrenalectomized rats, by 33%. This difference is small, but statistically significant ; since the rats which were less affected by the drug lost more noradrenaline, the results suggest some impairment of transmitter synthesis in the heart due to the adrenalectomy.

The noradrenaline content of the vas deferens was not affected by the injection of β-tetrahydronaphthylamine, whether the rats were intact or adrenalectomized. Table 1 shows that the scatter is larger than it is in heart tissue.

In order to ascertain whether the lack of cortical or medullary hormones accounted for the greater loss of cardiac noradrenaline found in adrenalectomized rats given β-tetrahydronaphthylamine, an experiment was carried out on adrenal-demedullated rats.

TABLE 2

NORADRENALINE CONTENT (MEAN AND RANGE) OF HEART* IN MALE RATS. EFFECT OF ADRENAL DEMEDULLATION† AND OF SUBCUTANEOUS INJECTION OF β-TETRAHYDRONAPHTHYLAMINE

(30 mg/kg of the hydrochloride)

	Estimations (no.)	Mean body weight at death (g)	Mean adrenal weight (g)	Whether drug injected	Noradrenaline content (μg/g)
Intact	3	272	42	No	1·6 (1·4–1·8)
	3	262	50	Yes	1·2 (1·1–1·4)
Demedullated	8	258	30	No	1·4 (0·9–2·1)
	9	256	30	Yes	1·4 (1·1–1·8)

* Auricles and anterior wall of right ventricle only. † Performed 7–8 weeks before the experiment.

The rats were allowed nearly two months for a satisfactory regeneration of the adrenal cortex ; this, however, meant that they were older than those of the first series at the time of the experiment, and this led (Table 2) to lower figures for heart noradrenaline in the unoperated controls. Nevertheless, in these controls, β-tetrahydronophthylamine caused the same percentage fall in cardiac noradrenaline (25%) as in the younger rats of Table 1. The demedullated rats, however, showed no fall at all, in contrast to the adrenalectomized rats in which the loss had been 33%. When the results for the demedullated rats were calculated not in concentrations, but in absolute amounts of noradrenaline, the picture was just the same: a mean of 336 ng per heart was present in the untreated rats, and of 334 ng in the injected animals. It was strange that the uninjected demedullated rats showed a greater scatter in noradrenaline content than normal rats. The reason for this was not apparent: there was no correlation between cardiac noradrenaline concentration and size of the regenerated adrenals which ranged from 18 to 42 mg. The signs of sympathetic stimulation in demedullated rats were similar to those in the adrenalectomized, and milder than those in normal rats.

DISCUSSION

Both adrenalectomized and adrenal-demedullated rats showed less exophthalmos, less restlessness, and often no change in temperature after the injection of β-tetrahydronaphthylamine ; this indicates that the release of catecholamines from the adrenal medulla plays an important part in the toxic signs elicited by this drug. Nevertheless, adrenalectomized and demedullated rats differed in the effect produced on the noradrenaline content of the heart. The rats without adrenals lost one third of their noradrenaline as a result of drug action, the rats without medulla showed no such loss. It would therefore appear that in the presence of the adrenal cortex resynthesis of noradrenaline in the heart takes place more efficiently.

It may then seem strange that intact rats, given β-tetrahydronaphthylamine, lose some noradrenaline from the heart tissue whereas adrenal-demedullated rats do not. The explanation may lie in the fact that the toxicity of the drug is so much greater when there is an adrenal medulla present to be stimulated ; the greater functional disturbance caused by the drug may then account for either increased release or impaired resynthesis of cardiac noradrenaline.

These experiments have confirmed that the adrenal medulla does not contribute to the maintenance of normal concentrations of cardiac noradrenaline under resting conditions ; furthermore, they have not provided any evidence that such a role can be played by the adrenal medulla when serious demands are made on noradrenaline resynthesis by drugs causing increased sympathetic activity.

SUMMARY

1. In adult male rats, injected with β-tetrahydronaphthylamine hydrochloride 30 mg/kg, the noradrenaline content of the heart fell by 23% in 4 hr but that of the vas deferens was unaffected.

2. After adrenalectomy the loss of cardiac noradrenaline was slightly, but significantly, increased to 33%, in spite of the fact that signs of toxicity were much reduced.

3. Adrenal-demedullated rats showed a larger scatter in the noradrenaline content of the heart than unoperated animals. There was no fall in cardiac noradrenaline after injection of β-tetrahydronaphthylamine ; it therefore appears likely that lack of cortical hormones was responsible for the loss seen in adrenalectomized rats.

4. Injection of histamine (10 or 20 mg/kg) into intact and adrenalectomized rats lowered the noradrenaline content of the heart by about 30%.

5. It is concluded that even under conditions of overactivity of the sympathetic system the adrenal medullary hormones cannot be shown to play an appreciable part in maintaining or replenishing the tissue stores of noradrenaline.

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