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Noradrenaline content of the heart of the adrenal-demedullated rat

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Summary

1. The noradrenaline (NA) concentration in the "heart" (atria and right ventricle) of male rats was estimated at different periods following adrenal demedullation. For 1–3 weeks after the operation there was, in all rats, a reduction in NA content of the tissue, whereas, after somewhat longer intervals, the concentrations had returned to normal in some but not in all animals, so that the range of values was very wide.

2. In order to be able to test the effect of 2-aminotetralin on the cardiac NA of animals deprived of their medulla but having normal initial NA concentrations, an interval of 6–8 months was allowed to elapse between operation and injection of the drug; at this time the effect of the drug on cardiac NA was the same in intact and demedullated animals. There is thus no reason to attribute to the adrenal medulla any supporting role in the resynthesis of cardiac NA during periods of increased sympathetic activity.

3. The time course of the pronounced fall in cardiac NA after adrenal demedullation resembles the time course of sodium retention shown by Gaunt, Renzi, Gisoldi & Howie (1967) to follow this operation. It is therefore suggested that it is the change in electrolyte metabolism which is responsible for the abnormality of NA storage; both phenomena occur in demedullated but not in adrenalectomized animals.

Introduction

An earlier paper (Avakian & Vogt, 1966) investigated the question whether the adrenal medulla contributed to the upkeep of tissue stores of noradrenaline (NA) in conditions of enhanced sympathetic activity. It made use of an injection of 2-aminotetralin (β-tetrahydronaphthylamine hydrochloride) to stimulate sympathetic discharge and compared the loss in heart NA in normal and adrenal-demedullated rats. Whereas intact rats lost 23% of their cardiac NA, adrenal-demedullated rats lost none; because completely adrenalectomized rats behaved similarly to intact and not to demedullated rats, it appeared that the demedullated rats showed some peculiarity which was not related to the absence of the medulla and which was worth following up. A large scatter found in the NA content of the heart of demedullated rats not given 2-aminotetralin was an additional fact requiring an explanation.

Methods

All rats used were males and came from the same supplier. Adrenalectomies and adrenal demedullations were performed under ether. For the purpose of demedullation the adrenal capsule was incised and the contents squeezed out

through the incision. 1% sodium chloride solution replaced the drinking water for the whole survival period after adrenalectomy and for 1 week after demedullation. The experiments were performed 10 days after adrenalectomy and at intervals ranging from 1 week to 8 months after demedullation. Immediately before killing some of the animals, the blood pressure was measured under pentobarbitone (55 mg/kg intraperitoneally) by a pressure transducer connected to a cannulated carotid artery.

Drugs

2-Aminotetralin hydrochloride (β-tetrahydronaphthylamine hydrochloride) 30 mg/kg was injected intraperitoneally in one group of rats which was killed 4 hr after the injection; it was given subcutaneously to another group which was killed 2 hr after the injection.

Noradrenaline estimations

The auricles and anterior wall of the right ventricle were dissected away from the left ventricle, which was discarded. The estimations were carried out essentially as described earlier (Avakian & Vogt, 1966), but instead of pulverizing the frozen tissue it was ground up in 2 ml. cold 0·1 N HCl (containing about 20 mg ascorbic acid) in a glass homogenizer. The reagents for developing the fluorescence were modified to 25% NaOH (instead of 20%) and 1% ascorbic acid (replacing 2% ascorbic acid).

Results

Age dependence of cardiac noradrenaline

This was investigated because the adrenalectomized rats used previously (mean weight 160 g) had been 3 weeks younger than the demedullated rats (mean weight 250 g). The NA concentration in the heart of rats from the same source was found to drop slightly with age, but there was no difference within the range of weights previously used.

TABLE 1. *Effect of 2-aminotetralin on the NA concentration in the heart of intact, adrenalectomized and adrenal-demedullated rats. All rats had reached the same age on the day of the experiment*

Expt. No.	Condition of rats	Interval between operation and exp. (weeks)	No. of rats	Body weight at death, g (mean±S.E.)	Adrenal weight, mg. (mean±S.E.)	Dose of 2-amino-tetralin (mg/kg)*	NA in heart, μg/g† (mean±S.E.)
1	Intact		13	277±11	45±2	0	1·26±0·04
			12	296±11	49±3	30	0·80±0·05
	Adrenalectomized	1·5	12	244±12	—	0	1·16±0·04
			10	255±8	—	30	0·87±0·05
	Demedullated	6	10	245±12	26±1	0	0·99±0·08
			9	261±10	32±1	30	1·02±0·04
2	Intact		7	255±11	40±1	0	1·28±0·08
			6	259±10	45±3	30	0·88±0·09
	Demedullated	3	7	251±11	29±6	0	0·68±0·05
			6	257±11	34±4	30	0·62±0·04

* Rats were killed 4 hr after intraperitoneal injection of the drug. † Left ventricle discarded; figures corrected for losses (mean recovery 76%).

Administration of 2-aminotetralin

A first experiment using 2-aminotetralin was planned in the same way as that performed earlier, but it was so arranged that all rats were of the same age at the day of the final experiment, and that the interval between demedullation and experiment was reduced to 6 weeks. The findings (Table 1) are very similar to those obtained earlier: the fall in heart NA after 2-aminotetralin was approximately the same in normal and adrenalectomized rats, but demedullated rats showed no fall after the drug. In this experiment, however, untreated demedullated rats had a significantly ($P<0{\cdot}01$) lower heart NA concentration than normal or adrenalectomized rats. This was even more pronounced (experiment 2, Table 1) when the interval between demedullation and experiment was reduced to 3 weeks.

These results suggested that the NA content of the heart of the demedullated rat, in contrast to that of the adrenalectomized animal, was low in the early postoperative phase. Furthermore, it was possible that the apparent lack of effect of 2-aminotetralin after demedullation was a result of the low initial NA value. These possibilities were examined in the experiments summarized in Tables 2 and 3.

In experiment 1 (Table 2) periods up to 8 months were allowed to elapse between demedullation and estimation of cardiac NA. There was no significant difference in NA concentration between operated rats and their controls. Neither was there more than a slight tendency to smaller NA concentrations in experiment 2, in which the interval had been 3·5 weeks in one half and 5·5 weeks in the other half of the group. In contrast, Table 1 showed an experiment in which, after an interval of 3 weeks, the difference between cardiac NA in operated and normal rats had been 47% and highly significant. Experiments 3 and 4 (Table 2), in which postoperative intervals of 1 and 1·5 weeks were used, showed equally significant reductions (by about one-third) in cardiac NA after demedullation. It follows that early after demedullation, and for no longer than 3 weeks, cardiac NA is consistently low; as the interval is prolonged, the mean concentration approaches normal figures, but individual values may remain low and account for the large scatter found in previous work.

In order to avoid the early postoperative effects on initial NA concentrations, the action of 2-aminotetralin was therefore compared in normal rats and in rats in which a long interval (6–8 months) had elapsed since demedullation (Table 3). After the

TABLE 2. *Effect of time elapsed since demedullation on cardiac concentration of NA in male rats*

Exp. No.	Condition of rats	Interval between operation and exp. (weeks)	No. of rats	Mean body weight (g)	Mean adrenal weight (mg)	Blood pressure mm Hg (mean ± S.E.)	NA in heart, μg/g* (mean ± S.E.)	% reduction in cardiac NA after demedullation
1	Intact		14	468	—	146±6	1·46±0·06	
	Demedullated	20 or 34	12	487	—	134±5	1·37±0·06	6
2	Intact		11	214	36·4	122±5	1·26±0·08	
	Demedullated	3·5 or 5·5	12	232	25·3	133±4	1·15±0·08	9
3	Intact		12	134	26·4	—	1·71±0·05	
	Demedullated	1·0	12	130	13·3	—	1·25±0·02†	27
4	Intact		9	113	27·8	120±5	1·75±0·08	
	Demedullated	1·5	10	106	18·6	104±3†	1·18±0·07†	33

All demedullations were carried out on 3·5 weeks old rats; intact rats born on the same day as operated rats served as controls.
* Left ventricle discarded; figures corrected for losses. † Differences significant.

drug, both groups had the same mean NA concentration in the heart ; the difference from the mean of controls which had not been injected was small, and only significant in the unoperated animals. Yet, a look at the range of NA concentrations and the state of the rats in individual experiments left no doubt that operated and intact rats reacted in the same way. Because the groups injected on any one day were always made up of equal numbers of operated and control rats, it became obvious that much of the scatter was related to variations in the day-to-day action of the drug on both demedullated and intact rats. One factor deciding the outcome was the ambient temperature. If this was in the range of 18°–20° C, the rectal temperature of the rats usually fell after the injection, and cardiac NA was sometimes, but not consistently, lowered. If the room temperature rose to 25° C, hyperthermia, occasional convulsions, and a high incidence of low cardiac NA concentrations were seen. There was no difference between intact and operated rats in the incidence of the more serious toxic effects of the drug, and the total number of rats in which the cardiac NA fell to or below 1·0 μg/g was six in either group.

These experiments confirm the earlier conclusion that release and resynthesis of NA in the heart during the enhanced sympathetic activity elicited by 2-aminotetralin are not modified by the absence of the adrenal medulla.

It was conceivable that the rats referred to in Table 3 which had been demedullated when 24 days old and kept for many months had produced a compensatory hypertrophy of any pre-aortic chromaffin tissue which might have still existed at the time of operation. In two rats the pre-aortic tissue was searched for chromaffin cells in serial sections, but none was found.

Discussion

The most striking finding of this work is that of a low cardiac NA concentration in the first 3 weeks after adrenal demedullation ; this does not occur after removal of the whole adrenal gland. After either operation the rats had 0·9% sodium chloride to drink for a week, and neither group showed any overt signs of ill-health.

It is known that adrenal demedullation may lead to a phenomenon known as " adrenal regeneration hypertension " (Skelton, 1955). Furthermore, several workers have found that hypertension, due to a variety of causes, is accompanied by a fall in the NA content of many tissues, including the heart, and explained this by a fault in the storage mechanism for amines (de Champlain, Krakoff & Axelrod, 1967 ; Mogil, Itskovitz, Russell & Murphy, 1969 ; Willard & Fuller, 1969). Yet Table 2 shows that the demedullated rats were not hypertensive ; in fact, the recently operated animal had a significantly lower blood pressure than the intact controls. This rules out the possibility that the fall in cardiac NA is directly related to any hypertension

TABLE 3. *Effect on injection of 2-aminotetralin (30 mg/kg subcutaneously) on the cardiac NA of rats demedullated 26–34 weeks previously*

Condition of rats	No. of rats	Body weight, g (mean±S.E.)	Adrenal weight, mg (mean±S.E.)	NA in heart, μg/g*	
				Mean±S.E.	Range
Normal	14	473±12	48±2	1·11±0·14	0·40–2·08
			(controls	1·46±0·06	1·14–1·86)
Demedullated	16	476±11	32±2	1·17±0·12	0·42–2·11
			(controls	1·37±0·06	0·99–1·76)

* Values corrected for losses. Demedullation at the age of 3·5 weeks. Rats killed 2 hr after injection of 2-aminotetralin; uninjected controls in brackets.

which may follow adrenal regeneration, but other findings suggest that it may be caused by changes in electrolyte metabolism which precede and later accompany hypertension. Quite severe sodium retention (in contrast to the sodium loss after adrenalectomy) has been shown (Gaunt *et al.*, 1967) to occur in demedullated male rats given a light sodium load. This retention is most pronounced during the first postoperative week and then subsides gradually. Its time course thus resembles that of low cardiac NA concentrations. Hypertension is not present during this phase of salt retention, even if encouraged by additional measures such as unilateral nephrectomy, or salt supplements to the diet (Skelton, 1955 ; Oelsner & Skelton, 1961). During this first week after demedullation, sodium appetite is reduced (Gaunt, Gisoldi, Herkner, Howie & Renzi, 1968). Sodium retention is abolished by hypophysectomy and restored by ACTH (Gaunt, Gisoldi, Smith & Giannina, 1969) ; it can be counteracted by injecting corticosterone, and it is explained by assuming that the regenerating adrenal produces a sodium retaining steroid the effect of which is not balanced by the production of normal amounts of corticosterone. One of the steroids which has been incriminated is 18-hydroxydesoxycorticosterone (Birmingham, Rochefort & Traikov, 1965), but the evidence is still incomplete. The widely held view that the formation of excessive amounts of desoxycorticosterone is responsible does not explain the phenomena in the first weeks of demedullation (Gaunt *et al.*, 1968).

De Champlain, Krakoff & Axelrod (1968) gave rats one week's treatment with desoxycorticosterone acetate and salt and found storage of NA reduced before hypertension had developed. They therefore consider that the disturbed sodium balance is the factor underlying the changes in tissue NA which later also accompany hypertension. Tobian & Redleaf (1957) have examined the electrolytes in the aorta of rats administered desoxycorticosterone and salt and found a rise in the sodium content of the vessel wall even in those rats which did not become hypertensive. The conclusion suggested by these facts is that the short-lived sodium retention described in demedullated rats by Gaunt *et al.* (1967) is responsible for the temporary reduction in heart noradrenaline observed in this work.

In contrast to demedullated rats, adrenalectomized rats lose sodium, and it is therefore not surprising that their cardiac NA concentration remains normal. In both demedullated and adrenalectomized rats synthesis of cardiac NA is accelerated (Landsberg & Axelrod, 1968 ; Westfall & Osada, 1969 ; Neff, Ngai, Wang & Costa, 1969).

The experiments on the effect of 2-aminotetralin on cardiac NA of demedullated rats were repeated on rats which had been demedullated several months before the experiment, so as to be able to start from a normal baseline of NA concentrations. In these conditions intact and operated rats responded in the same way: when serious signs of toxicity developed, particularly on warm days, severe falls in cardiac NA ensued, but when the effects were restricted to exophthalmos and some fall in rectal temperature, the falls in NA were small or absent. Thus resynthesis of NA under conditions of increased sympathetic activity did not appear to be affected by the lack of medullary tissue.

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