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The effect of acute adrenalectomy on the blood pressure responses to noradrenaline and to preganglionic nerve stimulation

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The quantitative contribution of the adrenal medulla to the pressor response resulting from stimulation of the entire preganglionic sympathetic spinal outflow of the rat, has been estimated. Adrenalectomy reduced the pressor responses not only to preganglionic nerve stimulation but also to injected noradrenaline. This reduction in cardiovascular sensitivity occurred within about 10 min of adrenalectomy and continued throughout the course of each experiment. Intramuscular injection of cortisone (20 mg/kg) restored cardiovascular sensitivity to injected noradrenaline thus allowing analysis of the adrenal medullary component of the overall response to spinal nerve stimulation.

The contribution of the adrenal medulla to the pressor response elicited by stimulation of the whole spinal sympathetic vasomotor outflow was considered by Gillespie & Muir (1967) to be small and variable. Since it was desired to use this preparation to examine ganglionic and adrenal medullary stimulants, an estimate of the contribution of the adrenal medulla to the total pressor response has been undertaken.

EXPERIMENTAL

C.S.E. male or female rats, 200–300 g, were pithed (Gillespie & Muir, 1967) under ether–halothane anaesthesia, and artificially respired using a Palmer miniature ideal pump at a stroke volume of 1 ml/100 g and at a rate of 50 strokes/min. Total bilateral adrenalectomy, via two lateral incisions was made using the same anaesthetic. "Sham adrenalectomized" rats were prepared by leaving the adrenal glands untouched after the incisions had been made.

The pithing rods, made from 16 gauge stainless steel wire, were dipped in Shellac varnish and, when dry, the area which was eventually to lie in the thoracico-lumbar region of the spinal cord was scraped clear of varnish. This length of rod had been previously determined in an animal of comparable size. An area of rod lying outside the body was also cleared of insulating varnish for attachment of an electrode. The indifferent electrode (a flattened serum needle) was placed subcutaneously in the dorsum.

Blood pressure was recorded from the left common carotid artery, using a Bell and Howell pressure transducer (Type 4-326-L212) and displayed on a Devices two channel pen recorder.

Heparin, 2000 units/kg was injected intravenously at the beginning of the experiment. Drugs were injected into the left femoral vein in a volume not exceeding 0·15 ml and were washed in with 0·2 ml saline.

Parameters of stimulation

Square wave pulses of 0·03 ms duration were delivered from a Multitone stimulator at a rate of 3 Hz for 15 s at intervals of 5 min. To eliminate any blood pressure change resulting from a change in heart rate, a stimulation strength of 20 V was used because, although of submaximal stimulation strength, previous examination using a heart rate recording system (Drew, Fozard & others, 1970) indicated that little or no change in heart rate occurred during stimulation at 20 V or less. Gillespie, MacLaren & Pollock (1969) have shown that heart rate and blood pressure changes can occur independently in this type of preparation.

The parameters used ensured that each pressor response showed no evidence of any residual effects from the preceding treatment.

Violent muscle contractions which occurred throughout the period of stimulation were prevented by the intravenous administration of 1 mg/kg (+)-tubocurarine.

Drugs

The drugs used were cortisone acetate (Cortistab—Boots Pure Drug Co. Ltd.), hydrocortisone sodium succinate (Glaxo), deoxycortone acetate (Organon), adrenaline acid tartrate (B.D.H.), tubocurarine chloride (Tubarine—Burroughs Wellcome & Co.) and noradrenaline acid tartrate (Hoechst). Apart from deoxycortone acetate, all drugs were injected in 0·9% sodium chloride solution, and doses calculated as the salt except in the cases of noradrenaline and adrenaline which were made up from stock solutions, stored in 0·01N hydrochloric acid, of 1 mg/ml calculated as the base.

Deoxycortone acetate was made up as 2 mg/ml suspension in propylene glycol.

RESULTS

Intact rats

Responses to injected noradrenaline

The characteristic response to an intravenous injection of noradrenaline, 500 ng/kg, was a rapid rise in blood pressure commencing 2 or 3 s after injection, and reaching a peak after about 5 s, whereupon a characteristic lowering of the diastolic pressure occurred. The diastolic pressure subsequently recovered over the next 2 or 3 s, and occasionally exceeded its initial peak (Fig. 1a).

The rises in both systolic and diastolic pressures were well maintained and did not start to wane until about 30 s after the injection.

Responses to preganglionic nerve stimulation

The typical pressor response evoked by stimulation of the entire spinal sympathetic preganglionic outflow consisted of a rapid rise in blood pressure lasting some 10–15 s (Fig. 2a). Occasionally this rise occurred in two distinct phases (Fig. 2b) and was seen as an initial phase, which reached a plateau about 5 s after stimulation had begun, and a secondary phase which followed after 2 or 3 s. After having attained its maximum, the response often began to decline before stimulation had ended. The decline of the overall pressor response after cessation of stimulation was variable; at stimulation voltages above 20 V a definite inflexion in the recovery phase occurred about 20–30 s after the end of stimulation, and coincided with the response described by Gillespie & Muir (1967) as the adrenal medullary component of the response.

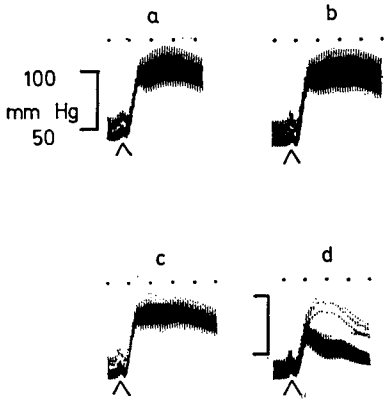

FIG. 1. The pressor responses evoked from an intravenous injection of noradrenaline 500 ng/kg i.v. a. The response in normal rats. b. The response in rats sham adrenalectomized 2 h previously. c. The response in rats adrenalectomized and treated with cortisone 20 mg/kg, i.m. 2 h previously. d. The response in rats adrenalectomized 2 h previously but with no replacement therapy. (NOTE different calibration). The slight response before each pressor response is the injection artifact and indicates the point of injection of noradrenaline. Time dots above traces are at 10 s intervals.

At 20 V stimulation strength, or less, this effect was less noticeable and appeared instead as a reduction in the overall rate of decline of the pressor response, frequently accompanied by an increase in pulse pressure (Fig. 2a, c).

The effect of acute adrenalectomy

The removal of, or ligating of the blood vessels of, the adrenal gland during the course of an experiment reduced the responses to preganglionic nerve stimulation. Such an effect was expected as a consequence of removal of the adrenal medulla, but the responsiveness to injected noradrenaline was also decreased. These effects

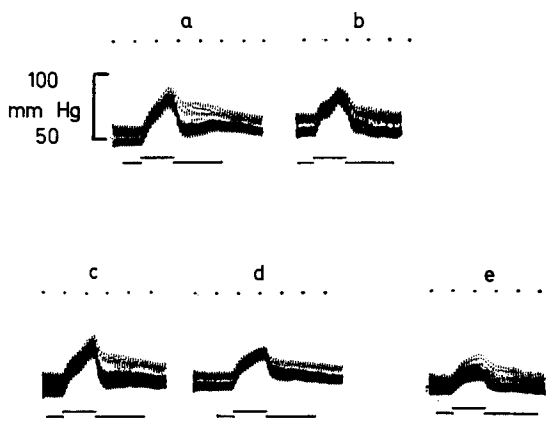

FIG. 2. The pressor responses evoked by stimulation of the entire spinal sympathetic preganglionic outflow at 20 V 0·03 ms pulse duration 3 Hz for 15 s. a + b. The response in normal rats. c. The response in rats, sham adrenalectomized 2 h previously. d. The response in rats adrenalectomized and treated with cortisone 20 mg/kg i.m. 2 h previously. e. The response in rats adrenalectomized 2 h previously, but with no replacement therapy. (NOTE: Different calibration.) Time bar below traces indicates period of stimulation. Time dots above traces are at 10 s intervals.

were first observable within 10 min of adrenalectomy and a continual decline in both types of response progressed throughout the course of the experiment. A progressive fall in the resting blood pressure usually accompanied these events, despite the fact that surgical procedures caused little or no haemorrhage.

The infusion of adrenaline

The infusion of adrenaline at the rate of 200–250 ng/kg min^{-1} over periods of up to 20 min raised the resting blood pressure by about 10 mm; pulse pressure and heart rate were also increased, but the responsiveness to either injected noradrenaline or preganglionic nerve stimulation was not restored to normal levels.

Adrenalectomized rats

Chronic experiments

In an attempt to overcome the difficulties arising from acute adrenalectomy, rats were adrenalectomized and chronically treated with a variety of replacement therapies. These involved saline substitution for drinking water and daily injections of deoxycortone acetate (1 mg/kg, s.c.). In some cases, hydrocortisone (2·5 mg/kg, s.c.) was also given. Such treatment lasted 3 to 4 weeks after adrenalectomy.

None of the replacement therapies restored cardiovascular sensitivity to catecholamines.

Hydrocortisone infusion

The infusion of hydrocortisone at 4–5 μg/kg min^{-1} for 15–20 min, into chronically adrenalectomized animals, maintained on deoxycortone acetate and saline only, failed to influence the decreased sensitivity to injected noradrenaline or nervous stimulation. However, it was observed that a single injection of cortisone, administered intramuscularly a short while before experimentation with these chronically adrenalectomized animals did result in some restoration of cardiovascular sensitivity towards catecholamines. Accordingly a more comprehensive study of the action of cortisone was undertaken.

The effects of cortisone administration

The effect of cortisone, administered at the time of adrenalectomy was examined 2–4 h after the operation. For comparison, adrenalectomized untreated rats and sham adrenalectomized rats were also examined. Table 1 compares the responsiveness to noradrenaline injection and preganglionic nerve stimulation in these preparations, and also in intact rats.

It is clear from these results that sham adrenalectomized and intact rats showed similar responsiveness to the test procedures, but that adrenalectomized rats differed, in that their resting blood pressure, after pithing, was 10–15 mm lower, and the pressor responses to preganglionic nerve stimulation and noradrenaline injection were markedly reduced.

Cortisone, 20 mg/kg intramuscularly, restored the resting blood pressure, after the animals had been pithed, to a level comparable with that observed in intact and sham adrenalectomized rats. The pressor responses to injected noradrenaline were also restored but the responses to preganglionic nerve stimulation was only partially restored.

Table 1. *The resting blood pressure, and the pressor responses to noradrenaline injection and total sympathetic preganglionic outflow stimulation in normal, sham adrenalectomized, adrenalectomized and adrenalectomized cortisone-treated pithed rats*

	Rise in systolic and diastolic pressures after i.v. injection of 500 ng/kg NA (in mm of Hg)	Diastolic pressure rise following stimulation of the total preganglionic sympathetic outflow (in mm of Hg)	Resting systolic and diastolic pressures after pithing (in mm of Hg)
Intact rats (11)	63·3 ± 2·8 55·0 ± 3·0	37·5 ± 2·5	66·3 ± 2·4 49·1 ± 2·0
Rats sham adrenalectomized, 2 h before experiment (10)	63·1 ± 1·9 49·6 ± 2·5	36·5 ± 2·5	65·6 ± 2·7 46·6 ± 1·8
Rats adrenalectomized and treated with cortisone 20 mg/kg i.m. 2 h before experiment (11)	62·1 ± 1·5 47·6 ± 2·8	27·9 ± 2·7*	67·7 ± 2·4 49·3 ± 1·6
Rats adrenalectomized 2 h before examination. No replacement therapy (10) ..	50·3 ± 2·4* 31·1 ± 1·0*	20·4 ± 2·5*	55·0 ± 0·7* 41·3 ± 1·1*

All values are given as the mean, ± the standard error of mean.
( ) denotes the number of animals in the group.
* $P < 0.05$ compared with normal rats.

Cortisone, 20 mg/kg intramuscularly, also prevented the occurrence of some gross changes in adrenalectomized animals. Shortly after adrenalectomy the animals' activity was reduced, respiration became slow and laboured and, characteristically, marked piloerection occurred.

Cortisone, 10 mg/kg intramuscularly, was only partially successful restoring cardiovascular sensitivity to catecholamines, and deoxycortone acetate, 5 mg/kg, by the same route was unsuccessful.

Apart from the quantitative aspects, described in Table 1, certain qualitative changes can also be discerned.

Noradrenaline injection

In adrenalectomized animals, the pressor response to injected noradrenaline was seen as a slowly occurring rise in pressure which was not well maintained, and declined immediately after having attained its peak. Since the diastolic pressure declined more rapidly than the systolic pressure a large increase in pulse pressure was apparent (Fig. 1d). Cortisone restored noradrenaline responsiveness to that observed in intact and sham adrenalectomized animals (Fig. 1a, b, c).

Preganglionic nerve stimulation

The responses to stimulation of the entire preganglionic sympathetic spinal outflow were similar in intact and sham adrenalectomized animals (Fig. 2a, b, c), but in adrenalectomized animals it was much reduced, and no secondary rise was ever apparent.

Systolic and diastolic pressures were equally increased (Fig. 2e). Cortisone pretreatment increased the size of the pressor response, but at no time did the secondary phase, apparent in intact and sham adrenalectomized preparations, return (Fig. 2d). Neither did the pressor response begin to decline until stimulation was ended, and the pulse pressure was generally reduced throughout the period of stimulation.

Consequently, since cortisone (20 mg/kg, i.m.) restored both the resting blood pressure and the responsiveness to noradrenaline injection, of adrenalectomized rats, to levels comparable with those observed in intact rats, it is suggested that the reduction in magnitude and change in shape of the response to preganglionic nerve stimulation was a consequence of the removal of the adrenal medullary component of the response. Thus it is estimated that the adrenal medullary component of the response, in intact animals, was about 24%.

Adrenal demedullation

Adrenal demedullation instead of total adrenalectomy, did not prevent the onset of reduced cardiovascular sensitivity when examined 2 h after demedullation. This was probably due to destruction of the functional integrity of the remaining adrenal cortex.

Fig. 3 indicates that 18 h after adrenalectomy there is a further reduction in cardiovascular sensitivity.

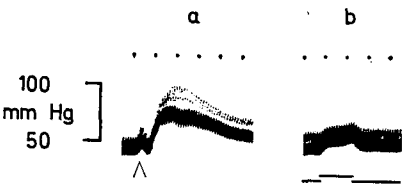

FIG. 3. The responses to injection of noradrenaline 500 ng/kg, i.v. (a) and to preganglionic stimulation at 20 V 0·03 ms pulse duration. 3 Hz for 15 s (b) in a rat adrenalectomized, and again no replacement therapy, 18 h previously. Time dots above tracing are at 10 s intervals.

DISCUSSION

Changes in cardiovascular function which occur as a result of adrenocortical insufficiency have been described by many authors (Ramey & Goldstein, 1957; Bush, 1962; Hofmann & Sobel, 1964). Typical symptoms are a reduction in pulse pressure, blood volume and peripheral resistance, and an inability of the heart to respond normally to changes in venous return or to increases in systemic arterial pressure. The condition is accompanied by a varying degree of hypotension.

Changes in cardiovascular sensitivity after adrenalectomy in the rat have been described by Zweifach, Shorr & Black (1953) using the rat mesoappendix preparation. This preparation showed a progressive decline in sensitivity to repeated injections of noradrenaline.

Imms & Jones (1968) have observed a decreased arterial pressure and decreased responsiveness to catecholamines in adrenalectomized rats, whilst Cartoni & Carpi (1968) demonstrated reduced responsiveness to endogenously released catecholamines after nerve stimulation.

These consequences of adrenalectomy probably account for a number of observations reported in this present work, namely:

(1) The inability of adrenalectomized rats to maintain the diastolic pressure rise following injection of noradrenaline.

(2) The failure of the diastolic pressure rise to exceed that of the systolic pressure, during preganglionic nerve stimulation in adrenalectomized animals only.

(3) The lowered resting systolic and diastolic pressures observed in adrenalectomized animals.

Furthermore, the piloerection observed in adrenalectomized rats probably reflects the reflexly induced hyperactivity of the sympathetic nervous system which occurs after adrenalectomy (Imms & Jones, 1968).

Carpi & Cartoni (1968) and Cartoni & Carpi (1968) have demonstrated that replacement therapy with both mineralocorticoids and glucocorticoids is necessary for the complete restoration of cardiovascular sensitivity. As in our results, they noted that deoxycortone acetate and saline administration were insufficient to maintain reactivity. That additional chronic (and acute) administration of hydrocortisone failed to restore sensitivity to catecholamines is in agreement with the findings of Rosenfeld, Sevy & Ohler (1959) and Ross (1961), who have shown that even large doses exert no effect on catecholamine sensitivity *in vivo*.

The ineffectiveness of adrenaline infusion to restore cardiovascular sensitivity indicates that a reduction in the background circulatory level of adrenal medullary hormones is not responsible for the changes following adrenalectomy, as reported by Harakal, Sevy & others (1968).

Acute administration of deoxycortone acetate was also without effect as observed by Ramey, Goldstein & Levine (1951).

However, acute administration of cortisone (Fritz & Levine, 1951; Zweifach, Shorr & Black, 1953) or corticosterone (Imms & Jones, 1967) proved useful in restoring cardiovascular responsiveness. The present work and that of Imms & Jones suggests that acute administration of corticosteroids of mixed activity may reverse the more immediate effects of adrenalectomy and that the more advanced changes which occur some time later may account for the conflict with the effects of chronic treatment demonstrated by Carpi & Cartoni. This may be a consequence of changes in ionic balance which occur, but no determination of these factors has been attempted in the experiments reported in this paper.

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