Post-adrenalectomy hypotension in rats; absence of baroreflex resetting or effect of naloxone

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Summary

1. Male Wistar rats were either bilaterally adrenalectomized or sham-operated, and given 1% sodium chloride solution instead of tap water to drink. Seven days later, arterial blood pressures were recorded directly from conscious freely moving rats.

2. Systolic and diastolic blood pressures were significantly lower in the adrenalectomized rats, whereas heart rates were significantly higher than in sham-operated animals. The tachycardia was due to a combination of sympathetic hyperactivity and reduced vagal tone, which may have been reflex responses to a reduction in effective blood volume.

3. Baroreflex control of the sinus node was assessed from the pulse interval responses to rises (induced by methoxamine) or falls (induced by glyceryl trinitrate or sodium nitroprusside) in systemic arterial blood pressure. The relation between pulse interval and systolic blood pressure was described by the same curve in sham-operated and adrenalectomized rats, indicating that, in the latter, there was no change in baroreflex setting or sensitivity.

4. Intravenous administration of naloxone (2mg/kg) had no effect on systemic arterial blood pressure in adrenalectomized rats, suggesting that endogenous opiates were not contributing to the hypotension.

Key words: adrenalectomy, baroreflexes, hypotension, naloxone.

Introduction

If rats are bilaterally adrenalectomized and given 1% sodium chloride solution to drink, they show a transient systemic arterial hypotension. The time course of blood-pressure recovery varies in different reports [1–3], but in our experience [3] salt-maintained adrenalectomized rats are hypotensive 7 days after operation, and blood pressure gradually returns to normotensive levels within the next 3 weeks. Moses [4] showed that, in salt-maintained adrenalectomized rats, body fluid volumes were abnormal 7 days after operation—interstitial volume being increased and intracellular and plasma volumes being decreased. After adrenalectomy there is also an impaired myocardial contractility [5] and reduced vascular sensitivity to noradrenaline [6]; thus it is possible that the post-adrenalectomy hypotension is due to a combination of these effects. However, there are at least two other factors which could contribute to the problem. With elevation of systemic arterial blood pressure, the baroreflex control of pulse interval becomes less sensitive [7]. It is therefore feasible that baroreflex control of the sinoatrial node adapts to the low blood pressure following adrenalectomy and hence does not effectively oppose the hypotension. To explore this possibility we have studied the reflex pulse interval responses to pharmacologically induced changes in arterial blood pressure and the effects of autonomic blockade on heart rate in conscious rats which had been adrenalectomized 7 days previously.

Adrenalectomy causes an increase in plasma and pituitary levels of β-endorphin [8, 9]. Since this endogenous opiate has been demonstrated to exert powerful depressor effects in rats [10], it is...
possible that elevated β-endorphin levels might contribute to the hypotension which follows adrenalectomy. We have therefore also studied the effects of the opiate antagonist naloxone on blood pressure in hypotensive adrenalectomized rats.

A preliminary account of some of this work has been given [11].

Methods

Animals

Male Wistar rats weighing between 260 and 290 g were bilaterally adrenalectomized (n = 21) or sham-operated (n = 24) by the dorsal approach under halothane anaesthesia. Sham-operation involved exteriorization, but not manipulation, of the adrenal glands. From the time of operation onwards all animals were given 1% sodium chloride solution instead of tap water to drink and had free access to food (Heygates diet 41B). The experiments were performed 7 days after operation and the animals were divided into four groups.

Groups I–III were anaesthetized with sodium methohexitone (Brietal, Lilly; 60 mg/kg intraperitoneally) and catheters, filled with sodium chloride solution (154 mmol/l: saline) containing heparin (12.5 units/ml), were implanted in the right jugular vein (for drug administrations) and abdominal aorta (via the caudal artery) for blood pressure recording. The catheters were fed subcutaneously through a hollow needle, exteriorized at the back of the neck, and then led through a fine wire spring (attached to a harness worn by the rat) and finally out of the cage. The design of catheter and recording system, which has a frequency response adequate to permit the measurement of systolic and diastolic blood pressures, is described in detail elsewhere [12].

Heart rate was recorded on a rate meter (Devices) triggered by the arterial pulse. The animals were allowed to recover from the anaesthetic for at least 5 h before any measurements were made.

Resting values

Control recordings of arterial blood pressures and heart rates were made for 30 min whilst the animals were undisturbed, and the average values during that period were used as the baseline measurements.

Baroreflex control of pulse interval

Group I (eight adrenalectomized rats; seven sham-operated rats). Arterial blood pressure was increased by infusion of methoxamine (0.4 mg/ml; 0.2 ml/min); the slopes and intercepts of the lines relating systolic blood pressure to the pulse interval of the succeeding beat were obtained by linear-regression analysis and used as an index of baroreflex sensitivity [13].

Group II (seven adrenalectomized rats; six sham-operated rats). Arterial blood pressure was reduced by infusion of glyceryl trinitrate (0.8 mg/ml; 0.2 ml/min). The systolic blood pressure was related to the pulse interval of the succeeding beat, but in the case of the adrenalectomized rats there was no linear relationship between the two variables (see the Results section) and hence linear-regression analysis was not performed.

Group III (six sham-operated rats). Sodium nitroprusside (120 μg/ml; 0.2 ml/min) was infused into a group of sham-operated rats to decrease the blood pressure to a similar level as that seen in the adrenalectomized rats given glyceryl trinitrate (see the Results section); as before, the pulse interval and systolic blood pressure were measured.

Autonomic control of heart rate

The heart-rate responses to intravenous injection of atropine (1 mg/kg) followed by propranolol (1 mg/kg) were measured. This experiment was performed on rats in group I (see above) 30 min after the baroreflex responses had been tested.

Plasma electrolytes

Six adrenalectomized rats and five sham-operated rats (group IV) were anaesthetized with diethyl ether and exsanguinated by cardiac puncture. Blood was centrifuged at 3000 rev./min (MSE centrifuge, radius 16.3 cm) and the sodium and potassium concentrations in the plasma were measured by flame photometry (EEL model 150).

Effects of naloxone

Naloxone was administered as a bolus injection (2 mg/kg) and arterial blood pressures were recorded for the following 30 min. This experiment was performed on rats in group I (see above) 90 min before the baroreceptor reflexes were tested.

Drugs

The following drugs were used: naloxone hydrochloride (Endo Laboratories Inc.); methox-
amine hydrochloride (Vasoxyl; Burroughs, Wellcome and Company); glyceryl trinitrate (Macarthys); sodium nitroprusside (Sigma); propranolol hydrochloride (Inderal; Imperial Chemical Industries Ltd); atropine sulphate (Sigma). All drugs were dissolved in sterile saline and administered in a volume of 0.1 ml, flushed in with a further 0.1 ml of saline; intravenous administration of 0.2 ml of saline alone had no effect on arterial blood pressure or heart rate.

Statistics

Values are expressed as the mean ± 1 SEM; n is the number of animals. Differences were tested for statistical significance using Student’s paired or unpaired t-test.

The slopes and intercepts of the lines relating systolic blood pressure and pulse interval were obtained by regression analysis. In each case the probability value derived from the correlation coefficient and appropriate sample size was less than 0.01.

Results

Resting values

In the resting state, arterial blood pressures in the adrenalectomized rats (140 ± 3/86 ± 3 mmHg systolic/diastolic; n = 15) were significantly (P < 0.001) lower than in the sham-operated rats (169 ± 2/107 ± 2 mmHg; n = 19). Adrenalectomized rats also had significantly (P < 0.001) higher resting heart rates (431 ± 7 beats/min) than the sham-operated rats (378 ± 4 beats/min). In addition to being higher, the heart rates of the adrenalectomized rats were noticeably less variable.

Baroreflex control of pulse interval

There were no significant differences between either the slopes (sham-operated = 1.38 ± 0.10 ms/mmHg; adrenalectomized = 1.40 ± 0.18 ms/mmHg) or the intercepts (sham-operated −74 ± 20 ms; adrenalectomized −66 ± 23 ms) of the regression lines from the two groups relating systolic blood pressure to pulse interval during methoxamine infusion (Fig. 1). When glyceryl trinitrate was used to lower arterial pressure, there was a linearly related shortening of the pulse interval in sham-operated rats (Fig. 2). This relationship had a slope (1.39 ± 0.07 ms/mmHg) and intercept (−70 ± 10 ms) which were not significantly different from those for the response to methoxamine infusion (see above). In adrenalectomized rats, infusion of glyceryl trinitrate caused a marked reduction in arterial blood pressure, but when systolic blood pressure fell below about 130 mmHg there was no further shortening of pulse interval (Fig. 2). This occurred at a pressure below that reached with glyceryl trinitrate infusion in sham-operated animals; indeed, in these animals such low pressures were unachievable with this drug, whatever dose was used. For this reason, a separate group of sham-operated animals were infused with sodium nitroprusside. Under these conditions sham-operated animals showed the same ‘flattening’ of the relationship between pulse-interval and systolic blood pressure when the latter was below
about 130 mmHg (Fig. 3). Above this pressure the relation between pulse interval and systolic arterial blood pressure was the same as for glyceryl trinitrate infusion.

Combining these data it can be seen that the relation between pulse interval and arterial blood pressure was the same in sham-operated and adrenalectomized rats (Fig. 4). Under resting conditions, results for sham-operated and adrenalectomized rats lay at different points on the same curve, i.e. the tachycardia seen in the former could be a baroreflex response to the low pressure.

![Graph showing relationship between systolic blood pressure and pulse interval](image1)

**Fig. 3.** Relation between systolic blood pressure and pulse interval during a reduction in blood pressure induced by infusion of sodium nitroprusside in sham-operated rats (*n* = 6). There was a linear relationship between the two variables until systolic blood pressure fell below about 130 mmHg, after which there was no further shortening of the pulse interval.

![Graph showing systolic blood pressure and pulse intervals](image2)

**Fig. 4.** Systolic blood pressures and the pulse intervals of the succeeding beats during manipulations in arterial pressure induced by methoxamine (circles), glyceryl trinitrate (triangles) and sodium nitroprusside (squares) in sham-operated rats (filled-in symbols) and adrenalectomized rats (open symbols). The data are a combination of those used in Figs. 1-3. There is no evidence for resetting or a change in sensitivity in the baroreflex control of pulse interval in the hypotensive adrenalectomized rats.

**Autonomic control of heart rate**

The resting heart rate in adrenalectomized rats (433 ± 10 beats/min; *n* = 8) was significantly higher than in sham-operated rats (380 ± 5 beats/min; *n* = 7; *P* < 0.001). Adrenalectomized rats showed a smaller heart rate response to atropine (+12 ± 1 beats/min) than sham-operated rats (+75 ± 4 beats/min); arterial blood pressures did not change in either group. Subsequent administration of propranolol caused a larger bradycardia in adrenalectomized rats (−111 ± 7 beats/min) than sham-operated rats (−80 ± 7 beats/min), and there was a small reduction in systolic blood pressure in the adrenalectomized rats. After atropine and propranolol, the heart rate of adrenalectomized rats was lower (317 ± 12 beats/min) than that of sham-operated rats (358 ± 3 beats/min; 0.01 > *P* > 0.001).

**Plasma electrolytes**

Seven days after adrenalectomy, plasma sodium (144 ± 1 mmol/l; *n* = 6) and potassium (5.6 ± 0.1 mmol/l) concentrations were significantly different from those of sham-operated rats (sodium = 153 ± 2 mmol/l; 0.01 > *P* > 0.001; potassium = 5.0 ± 0.1; *P* < 0.001; *n* = 5).

**Effects of naloxone**

Intravenous administration of naloxone did not affect arterial blood pressures in either sham-operated rats or adrenalectomized rats. Before naloxone, arterial blood pressures were 166 ± 3/105 ± 3 mmHg (systolic/diastolic) in the sham-operated rats (*n* = 7) and 137 ± 5/82 ± 4 mmHg in the adrenalectomized rats (*n* = 8); after naloxone they were 163 ± 3/102 ± 3 mmHg and 139 ± 6/81 ± 4 mmHg respectively.

**Discussion**

The present results have confirmed our previous observation [3] that 7 days after operation, adrenalectomized rats which had been drinking 1% sodium chloride solution are, nonetheless, hypotensive. The hypotension was accompanied by a tachycardia, which was due to a combination of sympathetic hyperactivity and reduced vagal tone. The tachycardia was not an inherent property of the myocardium since, in the presence of propranolol and atropine, the 'intrinsic' heart rate was lower in the adrenalectomized rats than in the controls. This was presumably a manifestation of the post-adrenalectomy myocardial depression reported by others [5].
Interestingly, there was no evidence that the baroreflex control of pulse interval was reset to the low pressure. Baroreflex resetting in hypertension is well-documented [7, 14], but there are fewer studies on baroreflex function in hypertension. Salgado & Krieger [15] showed a reduction in the threshold and saturation pressures for aortic baroreceptor discharges after 6 h of hypotension induced by haemorrhage and after 48 h of hypotension induced by reserpine in rats. Furthermore, long-term (45–50 days) exposure to hypotension of one carotid sinus in rats was accompanied by an increase in potassium concentration which influences baroreceptor discharge in the opposite direction [19]. On balance, we do not believe there are any strong reasons for predicting that baroreflex control of pulse interval should be reset in hypotensive, adrenalectomized rats.

To our knowledge, the only report of the effects of hypotension on the baroreflex control of pulse interval is that of Chen et al. [17] in anaesthetized man. In that study it was shown that, when blood pressure was returned to normotensive levels after several hours of sodium nitroprusside-induced hypotension, the sensitivity of the response to a hypotensive stimulus was enhanced and the set-point was lowered [17]. Once again, it is difficult to extrapolate from those experiments to the present situation in which the reflex was tested in conscious adrenalectomized rats which were still hypotensive and had been for several days.

The hypotension which follows adrenalectomy is accompanied by changes in body-fluid and electrolyte balance [4]; this may be relevant to the present discussion, since there is evidence that baroreceptor discharge is sensitive to changes in extracellular fluid electrolyte concentration, through an effect on membrane excitability [18, 19]. Lowering the sodium concentration in the fluid perfusing the aortic arch increases the threshold pressure for baroreceptor discharge and reduces the sensitivity; raising the potassium concentration has the opposite effects [19]. Relatively large changes (25–50%) in ion concentrations are required to influence individual baroreceptor fibre discharges, but a reduction in the extracellular fluid sodium concentration of only 5% is sufficient to elicit a reflex increase in blood pressure and urine flow [18].

In the present study there was a 4% reduction in plasma sodium concentration and an 8% increase in plasma potassium concentration 7 days after adrenalectomy. Although the change in sodium concentration was of the same order of magnitude as in the experiments of Kunze et al. [18], two details should be noted. Firstly, in the experiments of Kunze et al. [18], extracellular sodium and potassium levels were changed acutely, whereas the electrolyte disturbances in adrenalectomized animals develop slowly; in these two situations the effects of electrolyte imbalance on membrane excitability are likely to differ [20]. Secondly, the reduction in plasma sodium concentration in the adrenalectomized rats was accompanied by an increase in potassium concentration which influences baroreceptor discharge in the opposite direction [19].

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References


