Effect of adrenalectomy on the development of isolation-induced hypertension in rats

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Summary

1. Male Wistar rats develop systolic arterial hypertension when housed in glass metabolism cages. The present experiments were designed to investigate the involvement of the adrenal glands in this form of hypertension.

2. Rats were bilaterally adrenalectomized and maintained by either salt supplementation (1% sodium chloride solution instead of tap water to drink) or steroid replacement (corticosterone solution in the drinking water).

3. Adrenalectomized rats treated as above did not develop hypertension in response to isolation, whereas sham-operated rats (drinking either 1% saline or tap water) did.

4. Hypertension in the sham-operated rats was not accompanied by a renal retention of sodium and water.

5. It is concluded that increased adrenal activity is involved in the development of isolation-induced hypertension, but not by causing a fluid retention and hence volume expansion. The relative contributions of adrenal medullary and cortical activity remain to be determined.

Key words: adrenalectomy, hypertension, isolation.

Introduction

We have shown that housing male Wistar rats individually in glass metabolism cages causes systolic arterial hypertension [1]. The development of the hypertension can be prevented by treatment with β-adrenoceptor antagonists [2] and, when established, can be reversed by the latter or by 6-hydroxydopamine [2, 3]. For these reasons we have previously suggested that hyperactivity of the sympathetic nervous system might contribute to the development of the hypertension [2]. However, we have also observed increased corticosteroid levels and altered cardiac tissue sensitivity to noradrenaline in our isolated rats [4]; this led us to suggest that elevated corticosterone activity in plasma might also contribute to the development and maintenance of the hypertension [4]. The present work was designed to answer this question.

Unfortunately, it is not possible to do this simply by removing the adrenal glands, since adrenalectomized rats, given neither steroid nor salt supplements, usually die within 2 weeks of the operation, probably due to circulatory failure [5]. However, it is claimed that adrenalectomized rats given 0-9% saline to drink survive well and have a normal mean arterial blood pressure by day 7 after operation [5]. Similarly, arterial blood pressure is said to be normal in adrenalectomized rats given maintenance doses of glucocorticoid [6, 7].

The present work describes the effects of adrenalectomy, with either salt or steroid supplementation, on the development of isolation-induced hypertension.

Methods

Male Wistar rats weighing between 250 and 300 g were used in this study. Room temperature was maintained at 20 ± 2°C and lights were on from

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07.00 to 19.00 hours. All measurements were made between 07.00 and 09.00 hours.

Expt. 1. Effects of isolation on adrenalectomized rats drinking 1% saline

Rats were bilaterally adrenalectomized (*n* = 8) or sham-operated (*n* = 12) by the dorsal approach under halothane anaesthesia. Sham-operation involved exteriorization, but not manipulation, of the adrenal glands. In the text, the days have been numbered, day 0 being the day of operation. The animals were then left for 7 days to recover while housed in groups of six and allowed free access to food (Pilsbury’s diet 41B) and 1% saline to drink.

Blood pressures. Systolic blood pressure was measured in conscious rats by the tail-cuff method on day 7 after operation and daily thereafter for 5 days. Rats were then transferred to the metabolic cages (‘Metabowls’, Jencons) and left undisturbed for 5 days; in the text this is referred to as continuous isolation. For the remaining 11 days, the animals were removed from the metabolic cages for a period of about 1 h daily and grouped for the measurement of blood pressure; this is referred to as intermittent isolation. The procedure of blood pressure recording involves heating the animals at 35°C for 20 min and causes a significant weight loss. The animals were therefore weighed before and after each heating period and the weight change was taken as body fluid loss and included in the balance data.

Balance studies. Food intake (+0.1 g), 1% saline intake (+1.0 ml), urine output (+0.1 ml) and faecal output (+0.01 g) by each animal was measured daily on the first 6 days of isolation and on the final 2 days of the experiment.

Urine analysis. The sodium and potassium concentrations were measured by flame photometry (EEL, model 150).

Faecal analysis. Weighed samples of faeces were dried in an oven at 80°C for 72 h and the water content was determined from the weight loss. Further weighed samples were dissolved in fuming nitric acid and centrifuged at 3000 rev./min for 10 min and the sodium and potassium concentrations of the supernatant were measured by flame photometry.

Food analysis. Water and electrolyte contents of the food were measured by the same procedure as outlined above for the faeces. The water content of the food was 9.3%; the sodium content was 0.14 mmol/g; the potassium content was 0.19 mmol/g. The sodium concentration in the saline was also measured by flame photometry (176 mmol/l).

Plasma composition. Animals were killed at the end of the experiment and blood was taken from the heart into heparinized syringes.

Packed cell volume (PCV). A small sample of blood was transferred to a heparinized micro-capillary tube, centrifuged for 5 min (micro-haematocrit centrifuge, Hawksley, U.K.) and PCV was determined.

The remainder of the blood sample was centrifuged at 3000 rev./min for 10 min and the plasma was separated for analysis.

Osmolality. The osmolality of a 2 ml sample was determined twice by freezing-point depression and the mean value recorded.

Electrolytes. The sodium and potassium concentrations were measured by flame photometry.

Completeness of adrenalectomy was checked by macroscopic examination; data from animals with any signs of adrenal tissue were discarded from the experiment.

The results of this first experiment raised three questions: (1) what is the effect on blood pressure of drinking saline?, (2) do intact rats left undisturbed for 5 days, but in groups, show an increase in blood pressure? and (3) what changes in blood pressure occur in adrenalectomized rats postoperatively when they are housed in groups?

Expt. 2. Effects of salt supplementation on blood pressure in intact and adrenalectomized rats

Systolic blood pressure was measured by the tail-cuff method in conscious rats daily for 7 days before operation, while the animals were drinking tap-water, and the recordings made on the last 5 days were noted. One group (*n* = 6) was left as unoperated controls, one group (*n* = 6) was bilaterally adrenalectomized and one group (*n* = 6) was sham-operated. As before, operations were performed by the dorsal approach under halothane anaesthesia, and rats were left for 7 days to recover from the operation. In the unoperated control rats blood pressures were measured daily during this period. From day 7 after operation measurements of blood pressure were made over the same time course as in the first experiment, but the animals were housed in groups of six. All animals had free access to food and 1% saline to drink.

At the end of the experiment, the animals were killed and PCV and plasma electrolytes were measured as before.

Expt. 3. Effects of steroid replacement and isolation on blood pressure in adrenalectomized rats

Systolic blood pressure was measured for a week before operation while the animals were
drinking tap water. Rats were then either bilaterally adrenalectomized ($n = 6$) or sham-operated ($n = 6$) under halothane anaesthesia and allowed 7 days to recover. Immediately after operation the adrenalectomized rats were given corticosterone (Sigma) in the drinking water (160 µg/ml containing 1% ethanol). Corticosterone intake was monitored daily for the group. Sham-operated rats were given 1% ethanol in the drinking water.

Systolic blood pressure was measured on day 7 after operation and daily thereafter. There was a significant increase in the systolic blood pressure of the adrenalectomized rats on day 9 after operation (see the Results section). The concentration of corticosterone was therefore adjusted until the systolic blood pressure was not significantly different from the level before operation. At this stage (day 19 after operation) rats were transferred to individual glass metabolic cages and left undisturbed for 5 days, still with a corticosterone solution substituted for the drinking water. Systolic blood pressure was measured on the fifth day after isolation and daily for a further 5 days.

**Statistical analysis**

Results are expressed as means ±1 SEM. Differences were tested for statistical significance by Student’s paired or unpaired $t$-test.

**Results**

**Expt. 1. Effects of isolation on adrenalectomized rats drinking 1% saline**

**Blood pressures.** (In the text hypertension means having a systolic blood pressure greater than 150 mmHg.) Before isolation, systolic blood pressure was significantly ($P < 0.01$) lower in adrenalectomized rats than in sham-operated animals (Fig. 1). After 5 days of continuous isolation there was a significant ($P < 0.001$) increase in the systolic blood pressure of sham-operated rats, but not of adrenalectomized rats (Fig. 1). During the remaining 11 days of intermittent isolation, all the sham-operated animals remained hypertensive. Systolic blood pressure gradually increased in the adrenalectomized rats to reach values which were significantly higher than the pre-isolation level on and after isolation.

![Fig. 1. Systolic blood pressure (means ± SEM) of sham-operated rats (○; $n = 12$) and bilaterally adrenalectomized rats (●; $n = 8$) before and after 5 days of continuous isolation in glass metabolic cages (■); all animals were given 1% saline to drink throughout the experiment. Systolic blood pressure was significantly lower in adrenalectomized rats compared with sham-operated rats 7 days after operation ($P < 0.001$ on each day). After 5 days of continuous isolation there was a significant increase in the systolic blood pressure of sham-operated rats ($P < 0.001$), but no significant change in the blood pressure of adrenalectomized rats. During the remaining period of intermittent isolation (□) systolic blood pressure remained high in the sham-operated rats and gradually increased in the adrenalectomized animals to become significantly higher than the pre-isolation level on day 19 and thereafter ($P < 0.05$).](image-url)
FIG. 2. Water intakes and total measured water outputs by (a) adrenalectomized (n = 8) and (b) sham-operated (n = 12) rats during the first 6 days of isolation and on the last 2 days of the experiment. The difference between the total intake (●) and the water intake from the food (○) represents the volume drunk. The total measured output is represented by the total block of the histogram, which comprises: mean (±SEM) urinary output (□); mean (±SEM) faecal water loss (□); mean (±SEM) water loss during the heating period before blood pressure measurement (□). The distance between the intake (●) and the total block gives a measure of retention. Note that the marked difference in the total intake is due to the greater volume drunk by the adrenalectomized rats.

Body weight. There was no significant difference between the body weights of the two groups of animals at the start of the experiment. Five days of continuous isolation caused no change in the body weight of sham-operated rats (before isolation = 269 ± 5 g; after 5 days of isolation = 269 ± 4 g) and a slight, but insignificant fall in the body weight of adrenalectomized rats (before isolation = 256 ± 10 g; after 5 days of isolation = 246 ± 9 g). Adrenalectomized rats gained significantly more weight (+28 ± 5 g) than sham-operated rats (+10 ± 2 g; 0.01 > P > 0.001) during the subsequent 12 days of intermittent isolation.

Balance studies

Intakes and outputs of fluid. Fig. 2 shows the intakes and measured outputs of fluid by sham-operated rats and adrenalectomized rats; respiratory losses were not assessed. The fluid intakes and urine outputs of the adrenalectomized rats were significantly higher than those of sham-operated rats on day 16, 17, 26 and 27 (P < 0.05 on each day). However, the cumulative retention of water for the first 6 days of isolation was not significantly different in adrenalectomized rats (43.6 ± 3.9 ml) compared with sham-operated rats (46.7 ± 1.9 ml). Adrenalectomized rats were in slightly more positive water balance at the end of the experiment, although the difference was only significant on day 26 (adrenalectomized rats = 12.5 ± 1 ml; sham-operated rats 9.2 ± 0.6 ml; 0.01 > P > 0.001).

Intakes and outputs of sodium. The sodium intake from the saline solution and the sodium output in the urine was significantly higher in the adrenalectomized rats on days 16, 17, 26 and 27 (P < 0.05 on each day) compared with sham-operated animals (Fig. 3). However, there was no significant difference between the overall sodium balances of the two groups at any stage in the experiment (Fig. 3; cumulative retention after 6 days; adrenalectomized rats = 7.1 ± 1.4 mmol, sham-operated rats = 7.7 ± 0.8 mmol).

Intakes and outputs of potassium. There were no significant differences between the intakes or measured outputs of potassium by adrenalectomized rats compared with sham-operated rats (Fig. 4).

Plasma composition

PCV. Isolated, adrenalectomized rats had a significantly lower PCV than sham-operated animals housed under similar conditions (adrenalectomized = 38.2 ± 0.2%; sham-operated = 43.9 ± 0.5%; P < 0.001).

Plasma electrolytes. The sodium concentration in the plasma of the sham-operated rats (150.3 ± 1.5 mmol/l) was significantly higher than that of the adrenalectomized rats (142.8 ± 0.9 mmol/l; 0.01 > P > 0.001) in isolation.
Isolation in adrenalectomized rats

FIG. 3. Sodium intakes and measured sodium outputs by (a) adrenalectomized \((n = 8)\) and (b) sham-operated \((n = 12)\) rats during the first 6 days of isolation and on the last 2 days of the experiment. The difference between the sodium intake from the food \((\circ)\) and the total sodium intake \((\bullet)\) represents sodium intake from the saline which the animals drank. Total measured output of sodium is represented by the total block of the histogram which comprises: mean \((\pm SEM)\) urinary sodium output \((\square)\); mean \((\pm SEM)\) faecal sodium loss \((\square)\). The distance between the total intake \((\bullet)\) and the total block gives a measure of retention.

FIG. 4. Potassium intake \((\bigcirc)\) and measured potassium outputs by (a) adrenalectomized \((n = 8)\) and (b) sham-operated \((n = 12)\) rats during the first 6 days of isolation and on the last 2 days of the experiment. The total measured potassium output is represented by the total block of the histogram which comprises: mean \((\pm SEM)\) urinary potassium output \((\square)\); mean \((\pm SEM)\) faecal potassium loss \((\square)\). The distance between the intake and the total block gives a measure of retention.

There was no significant difference between the plasma potassium concentration in adrenalectomized rats \((5.0 \pm 0.3 \text{ mmol/l})\) and sham-operated rats \((4.3 \pm 0.2 \text{ mmol/l})\) in isolation.

Expt. 2. Effects of salt supplementation on blood pressure in intact and adrenalectomized rats

The systolic arterial blood pressures of the three groups before operation were not different (Fig. 5). Replacing the drinking water with 1% saline did not affect the systolic blood pressure of the control rats (Fig. 5). There was no change in the systolic blood pressure of sham-operated animals drinking saline 1 week after operation. Furthermore there was no change in systolic blood pressure after the 5 day period when the animals were left undisturbed. However, systolic blood pressure was significantly \((0.01 > P >\)
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FIG. 5. Systolic blood pressure (means ± SEM) in group-housed rats. Blood pressure was measured for a week before either sham-operation (○; n = 6) or adrenalectomy (△; n = 6); a third group of rats was left as unoperated controls (▲; n = 6). Operated animals were given 1% saline to drink immediately after surgery (day 0); unoperated control rats were given saline at the same time. There was no significant change in the systolic blood pressure of sham-operated or control animals throughout the experiment. Systolic blood pressure was significantly reduced in the adrenalectomized rats 7 days after operation (0.01 > P > 0.001), but gradually returned to a level not significantly different from the pre-operation level by day 20 (P > 0.05 on each day thereafter). Leaving the animals undisturbed, but group-housed, for 5 days (between days 11 and 16) had no significant effect on systolic blood pressure.

Expt. 3. Effects of steroid replacement and isolation on blood pressure in adrenalectomized rats

Before operation, there was no difference between the systolic blood pressures of the two groups. One week after operation [during which time the adrenalectomized rats were given corticosterone (160 µg/ml) in the drinking water], the systolic blood pressure of the adrenalectomized rats was significantly higher than that of the sham-operated group (Fig. 6) and continued to rise for the next 2 days. The concentration of steroid was halved on day 9 after operation, but systolic blood pressure remained high. The concentration of steroid was further reduced on day 14 (40 µg/ml) and this resulted in a prompt

0.001) reduced 1 week after adrenalectomy (Fig. 5), and gradually returned to the pre-operation level by day 20 after operation (Fig. 5) (a pattern of change similar to that shown by the adrenalectomized rats in Expt. 1); thus the increase in blood pressure in the latter cannot be attributed to isolation.

Plasma composition

PCV. Group-housed adrenalectomized rats had a lower PCV (37.7 ± 0.6%) than sham-operated (43.2 ± 0.7%; P < 0.001) or control rats (42.7 ± 0.7%; P < 0.001) housed under similar conditions.

Plasma electrolytes. The concentrations of sodium in the plasma of group-housed adrenalectomized rats (142.6 ± 2.2 mmol/l), sham-operated rats (141.6 ± 1.8 mmol/l) and control rats (139.7 ± 0.5 mmol/l) were not significantly different.

Adrenalectomized rats had a higher plasma potassium concentration (4.8 ± 0.4 mmol/l) than either sham-operated rats (3.6 ± 0.1 mmol/l; 0.02 > P > 0.01) or the control rats (3.5 ± 0.1 mmol/l; 0.01 > P > 0.001).
Isolation in adrenalectomized rats

FIG. 6. Systolic blood pressure (means ± SEM) in sham-operated rats (○; n = 6) and adrenalectomized rats given corticosterone (□; n = 6). A corticosterone solution of known concentration was given in the drinking fluid and intake (and hence dose) was monitored. The concentration of corticosterone (160 µg/ml) was initially too high and caused an increase in systolic blood pressure. The concentration was therefore adjusted on days 9 (80 µg/ml) and 14 (40 µg/ml) until the blood pressure returned to resting levels. After 5 days of continuous isolation (■) there was a significant increase in the systolic blood pressure of sham-operated rats, but no significant change in the systolic blood pressure of adrenalectomized rats. This difference persisted for the remaining 4 days of intermittent isolation (□).

fall in systolic blood pressure (Fig. 6). With this concentration of steroid, the daily intake of corticosterone was approximately 1 mg per rat (Fig. 6).

On day 19, when the rats were transferred to the metabolic cages, the systolic blood pressure of the two groups was not significantly different. After 5 days of continuous isolation there was a significant (P < 0.001) increase in the systolic blood pressure of sham-operated rats, but not of adrenalectomized rats (Fig. 6); this difference persisted until the end of the experiment.

Discussion

In the present study we have shown that adrenalectomized rats given either steroid or salt supplementation do not develop hypertension in response to short-term isolation. This finding appears to differ from reports by previous workers using other models of hypertension. Thus, in spontaneously hypertensive rats [6], in rats made hypertensive by sound deprivation [8] and in salt-induced hypertension [9] it has been shown that maintenance doses of adrenal steroids were sufficient to permit the hypertension to develop in the absence of the adrenal glands. But, in all those studies, salt was given in addition to the steroid and so it is difficult to make any direct comparisons with the present work. Indeed, in our experiments, those animals which showed signs of adrenal tissue at autopsy and which had been drinking 1% saline did develop hypertension; this may be analogous to the adrenal-regeneration hypertension described by Skelton [10].

There is a large variation in the maintenance doses of glucocorticoids used by different workers. The initial dose regimen used by us was chosen on the basis of work by Buckingham & Hodges [11] and File et al. [12], both of whom gave rats a solution of 160 µg of corticosterone/
ml to drink and found that this produced normal circulating plasma levels. But our finding that adrenalectomized rats developed hypertension with this level of steroid intake supports the earlier report of Knowlton et al. [13]. They observed that adrenalectomized rats given 2-5 mg of cortisol/day, subcutaneously, developed systolic arterial hypertension; in the present study the average intake was 4 mg/day with the initial concentration and blood pressure did not return to normotensive levels until the intake was reduced to approximately 1 mg/day. This low intake is comparable with the dose used by Louis & Spector [6], which produced normal levels of blood pressure in adrenalectomized Wistar rats and which permitted the development of genetic hypertension.

In the present study we found no differences between the cumulative retentions of fluid and electrolytes by adrenalectomized rats and sham-operated rats during the first 6 days of isolation when the sham-operated rats developed hypertension. Thus it is unlikely that the involvement of the adrenal glands in the development of the hypertension was through mineralocorticoid activity causing a renal retention of salt and water, which is consistent with our earlier findings [14].

In addition to investigating the role of the adrenal glands in the development of isolation-induced hypertension, the design of the present experiments has enabled us to study several aspects of renal and cardiovascular physiology in adrenalectomized rats. We have shown that adrenalectomized rats maintained on 1% saline show an initial hypotension, with blood pressure returning to resting levels within 3 weeks after operation; this pattern of change was similar, irrespective of whether the animals were housed individually or in groups. A transient hypotension in salt-maintained adrenalectomized rats has been reported previously, although the time course of recovery varies [5, 15].

The mechanisms responsible for the restoration of blood pressure, after adrenalectomy, are unclear. Imms & Maisey [16] and Berns et al. [17] suggest that activation of the renin–angiotensin system plays some part in the recovery of normal blood pressure after adrenalectomy. If circulating levels of angiotensin II were high, one might have expected to observe increased drinking (see review by Fitzsimons [18]). At the end of our experiment fluid intake was higher in the adrenalectomized rats but, without further information, it is not possible to determine whether this was due to increased circulating angiotensin II or was merely a reflection of an increased salt appetite in adrenalectomized rats [19]. The finding that the increased fluid consumption was not associated with an increased food intake might indicate that the drinking was in response to a dipsogenic stimulus.

It is possible that circulating levels of anti-diuretic hormone (ADH) are high in adrenalectomized animals [20–22]. ADH may help to restore normal blood pressure either by causing a fluid retention, and thereby expanding extracellular fluid volume, or by exerting a pressor effect on the peripheral vasculature. At the end of our experiment the water balance was more positive in adrenalectomized rats than in sham-operated rats. Although the difference was not always significant on a daily basis, it is possible that, cumulatively, the excess retention of 2–3 ml/day by the adrenalectomized rats produced a significant volume expansion. This suggestion is consistent with our finding of a greater body weight gain and a reduced PCV in the adrenalectomized rats. However, Imms & Neame [5] found that 7 days after adrenalectomy and salt supplementation, when mean arterial blood pressure was normal, there was no indication of a blood or plasma volume expansion. But Wright [23] found that 28 days after adrenalectomy and salt supplementation (a time more in keeping with the present work) PCV and serum protein values were decreased, indicating that plasma volume may have been increased.

Without further information it is not possible to determine the cause of the recovery of normal blood pressure levels in adrenalectomized rats.

In conclusion, we have shown that the adrenal glands play an important part in the development of isolation-induced hypertension, since adrenalectomized rats given either salt supplementation or corticosteroid replacement do not develop hypertension in response to short-term isolation. However, the role of the adrenal glands in this form of hypertension is not through mineralocorticoid activity causing a renal retention of salt and hence water, thereby expanding effective circulating volume; the relative contributions of adrenal medullary and cortical activity [24] remain to be determined.

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References
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