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The effect of adrenal demedullation on cardiovascular responses to environmental stimulation in conscious rats

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1 Circulating plasma adrenaline has been implicated in the facilitation of neurogenic pressor responses and development of hypertension.

2 Bilateral adrenal demedullation in rats did not affect body weight, urine output, urinary electrolyte (Na^+, K^+ and Cl^-) excretion, nor plasma corticosterone concentration, indicating the selective nature of the demedullation procedure.

3 Adrenal demedullation did induce significant reductions in adrenal catecholamine content, plasma adrenaline levels, resting blood pressure and heart rate in conscious rats, but did not affect alerting-induced increases in blood pressure.

4 The adrenal medulla and circulating plasma adrenaline appear to contribute to the maintenance of resting cardiovascular parameters, but would not appear to be involved in nor facilitate the cardiovascular responses to environmental stimulation.

Introduction

Adrenaline has been shown to facilitate sympathetic neurotransmitter release (Adler-Graschinsky & Langer, 1975) and neurogenic pressor responses (Borkowski & Quinn, 1983; 1984; Quinn *et al.*, 1985) and has been implicated in the development of a raised blood pressure (Majewski *et al.*,1981; Borkowski & Quinn, 1983; 1985). Stress may play a role in the development and maintenance of hypertension and marked elevations in plasma catecholamines, particularly adrenaline, have been observed in animals stressed by handling and restraint (Buhler *et al.*, 1978). Indeed Rosecrans *et al.* (1966) induced a sustained elevation in blood pressure in rats, alerted repeatedly by exposure to an environmental stimulus (flashing lights, loud noise and cage vibration), over a 16 week period.

The present study indicates that adrenal catecholamines appear to be involved in the maintenance of resting blood presssure and heart rate, but not in alerting-induced increases in blood pressure.

Methods

Male, normotensive Alderley Park Wistar (APW) rats, weighing 250–275 g, were used throughout. One group of 6 rats underwent bilateral adrenal demedullation, under Saffan (alphaxalone/alphadolone; 12 mg kg^{-1}, i.v.) anaesthesia, the other group of 6 rats was sham-operated. One week later, the left common carotid artery was catheterised in each animal under Saffan anaesthesia to facilitate the direct recording of blood pressure. Twenty-four hours after catheterization, the rats were placed in individual holding tubes (6 cm diameter, 30 cm long), allowed to settle for a period of 2 h (during which time the urine was collected for subsequent determination of electrolyte content) and alerted, by exposure to an environmental stimulus consisting of a flashing light (0.25 s ON/0.25 s OFF), 10 kHz buzzer at 100 dB (0.25 s ON/0.25 s OFF) and vibration of the holders at 2 Hz, in three 10 s bursts separated by 1 min intervals at time 0, 30, 60, 120 and 240 min. Blood pressure was recorded via Bell and Howell 4-422 pressure transducers and displayed on a Lectromed MX6 recorder. Heart rate was derived from the blood pressure pulse-wave by cardiotachometers. Fifteen minutes after the final stimulation period, blood samples were obtained via the arterial catheters by free flow and plasma catecholamines and corticosterone were assayed, by high pressure liquid chromatography with electrochemical detection and a fluorimetric method respectively, as previously described (Borkowski & Quinn, 1985). The animals were

then killed by cervical dislocation, the adrenal glands removed and assayed for catecholamine content. Urinary Na^+ and K^+ concentrations were measured by flame photometry and Cl^- concentration by a chloride meter (Corning Chloride Meter 920).

Statistical analysis was performed using Student's unpaired t test and significance accepted when $P<0.05$. Values are given as the mean ± s.e. (standard error) and n = number of observations.

Results

The resting mean arterial blood pressure (121 ± 2 mmHg, $n = 6$) and heart rate (448 ± 26 beats min^{-1}, $n = 6$) in sham-operated rats was significantly ($P<0.05$) higher than the blood pressure (106 ± 6 mmHg, $n = 6$) and heart rate (338 ± 26 beats min^{-1}, $n = 6$) in adrenal demedullated animals. This difference was maintained throughout most of the subsequent recording period (Figure 1). The alerting-induced increases in blood pressure were slightly lower, but not significantly different except at the 60 min time-point, in adrenal demedullated as compared to sham-operated animals (Figure 1). The heart rate responses during alerting were very variable and not amenable to any meaningful analysis. Plasma adrenaline levels were significantly ($P<0.001$) lower in demedullated (22 ± 10 pg ml^{-1}, $n = 6$) than in sham-operated (277 ± 16 pg ml^{-1}, $n = 6$) rats, while plasma noradrenaline levels remained largely unaffected, being 278 ± 32 pg ml^{-1} ($n = 6$) and 308 ± 40 pg ml^{-1} ($n = 6$) respectively. Adrenal gland catecholamine content was reduced by more than 99% ($P<0.001$) in demedullated animals (noradrenaline (NA) = 95 ± 8 ng and adrenaline (Ad) = 83 ± 26 ng per pair of adrenals, $n = 6$) compared to sham-operated rats (NA = 9.8 ± 0.5 μg and Ad = 28.8 ± 1.0 μg per pair, $n = 6$).

Plasma corticosterone levels were not significantly different in adrenal demedullated (10.6 ± 1.9 μg 100 ml^{-1}, $n = 6$) compared to sham-operated (12.1 ± 1.6 μg 100 ml^{-1}, $n = 6$) rats, indicating that little cortical damage had occurred during demedullation and that the adrenal cortex remained both intact and functional.

The selective nature of the demedullation procedure is further evidenced by the ability of the rats to maintain their salt/water balance. The urine volume produced by the adrenal demedullated rats, during the 2 h pre-stimulation period, was not significantly different from that produced by the sham-operated animals (4.3 ± 0.3 ml, $n = 6$ compared to 4.7 ± 0.4 ml, $n = 6$). In addition, there was no significant difference between the urinary cation content of adrenal demedullated rats (Na^+ = 155 ± 7 mEq l^{-1}, an excretion rate of 0.24 ± 0.01 mEq 100 g^{-1} 2 h^{-1}, K^+ = 33 ± 3 mEq l^{-1} or 0.05 ± 0.004 mEq 100 g^{-1} 2 h^{-1}, $n = 6$) and the sham-operated controls (Na^+ = 135 ± 6 mEq l^{-1} or 0.24 ± 0.01 mEq 100 g^{-1} 2 h^{-1}, K^+ = 25 ± 3 mEq l^{-1} or 0.04 ± 0.005 mEq 100 g^{-1} 2 h^{-1}, $n = 6$). Although the urinary Cl^- content of

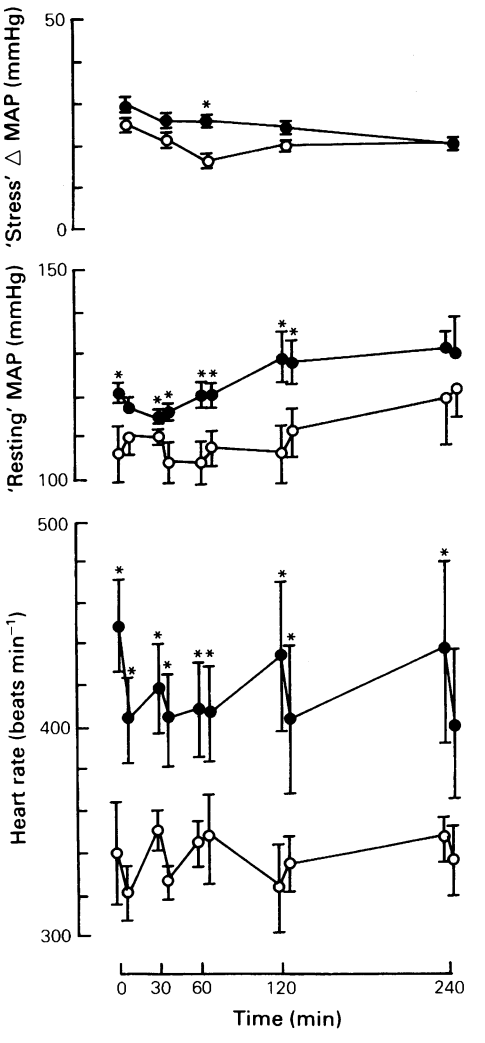

Figure 1 Resting mean arterial blood pressure (MAP) and heart rate, immediately before and 5 min after environmental stimulation, and the increases in mean arterial pressure (ΔMAP) induced by environmental stimulation in sham-operated (●) and adrenal demedullated (○) conscious rats at time, 0, 30, 60, 120 and 240 min. Each point represents the mean of 6 observations and standard errors are indicated by the vertical line. *$P<0.05$, denotes significant differences between values in sham-operated and adrenal demedullated animals at the same time point.

adrenal demedullated rats (134 ± 5 mEq l^{-1}, $n = 6$) was significantly ($P < 0.05$) greater than that of the sham-operated animals (115 ± 5 mEq l^{-1}, $n = 6$), this difference became negligible when expressed in terms of Cl^- excretion (0.21 ± 0.008 mEq $100\,g^{-1}\ 2\,h^{-1}$, compared to 0.20 ± 0.009 mEq $100\,g^{-1}\ 2\,h^{-1}$, $n = 6$).

The body weight of the adrenal demedullated rats (273 ± 2 g, $n = 6$) was not significantly different from that of the sham-operated animals (268 ± 2 g, $n = 6$), giving further evidence of the ability of adrenal demedullated rats to survive without further post-operative intervention, unlike adrenalectomized rats which require salt supplementation.

Discussion

The full profile of the alerting (exposure to flashing-lights, audiogenic stimulation and cage vibration)-induced haemodynamic changes is, as yet, unknown. Nevertheless, exposure to this environmental stimulus increases blood pressure acutely and has been shown to elevate plasma adrenaline levels and lead to the development of hypertension in rats (Rosecrans *et al.*, 1966). Since adrenaline has been widely implicated in the facilitation of sympathetic activity (for review see: Majewski, 1983), it was of interest to ascertain the effects of adrenaline depletion on cardiovascular parameters and responses to alerting.

The heart rate responses to environmental alerting were very variable and not amenable to any meaningful analysis. However, resting heart rates were significantly higher in sham-operated compared to adrenal demedullated rats. Whilst adrenal catecholamines are released during alerting (Rosecrans *et al.*, 1966), it is likely that plasma adrenaline levels were already elevated due to the stress of restraining the rats in individual holders. In adrenal demedullated rats this would not have been the case (indeed, adrenal catecholamines were reduced by $> 99\%$ and plasma adrenaline levels by 92% in these animals), but in the sham-operated rats, the increased plasma adrenaline levels may have been exerting a direct effect on the heart or have been facilitating cardio-accelerator drive, as has been shown in man during the infusion of subpressor doses of adrenaline (Brown & Dollery, 1984). The increased heart rate, direct effects of the circulating plasma adrenaline upon vascular smooth muscle and facilitation of sympathetic tone may all account for the higher resting blood pressures observed in the sham-operated rats.

Despite the much higher plasma adrenaline levels in sham-operated rats, the alerting-induced increases in blood pressure were not significantly greater than those observed in adrenal demedullated rats (particularly when the increases were compared as percentage changes from the resting blood pressures), indicating that circulating plasma adrenaline does not appear to be involved in nor facilitate alerting-induced increases in blood pressure. Nevertheless, this observation is not at variance with an involvement of circulating adrenaline, of adrenal origin, in the facilitation of sympathetic activity and in the development and maintenance of hypertension, nor does it preclude an involvement of alerting-induced increases in blood pressure with the development of a sustained elevation in blood pressure. However, no evidence of an adrenaline-induced facilitation of alerting-induced pressor responses, nor indeed any involvement of adrenaline in these acute responses, was found.

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