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# CORTICOSTEROID INVOLVEMENT IN THE CHANGES IN NORADRENERGIC RESPONSIVENESS OF TISSUES FROM RATS MADE HYPERTENSIVE BY SHORT-TERM ISOLATION

T. BENNETT & SHEILA M. GARDINER

Department of Physiology and Pharmacology, Queen's Medical Centre, Clifton Boulevard, Nottingham, NG7 2UH

**1** The responses to noradrenaline and to noradrenergic nerve stimulation of spontaneously beating right atria, electrically driven left atria and vasa deferentia taken from rats made hypertensive by short-term isolation have been compared with the responses of tissues from normotensive, group-housed animals.

**2** Adrenocortical activity of isolated animals was assessed by plasma corticosterone determinations and measurement of adrenal weights.

**3** The hearts of the isolated animals were weighed and the myocardial contents of water, sodium, potassium and calcium were measured.

**4** Spontaneously beating right atria from isolated animals showed a lower resting rate, no difference in the response to nerve stimulation but a greater sensitivity to noradrenaline compared to atria from group-housed animals.

**5** Vasa deferentia from isolated animals showed a decreased maximal response to noradrenaline, but no change in noradrenaline sensitivity or in the response to transmural stimulation.

**6** There were indications of hyperactivity of the adrenals throughout a 5 week period of isolation, manifest as elevated plasma corticosteroid levels and increased adrenal weights.

**7** Myocardial levels of sodium and calcium were elevated at the same time as the tissue level of potassium was reduced, but heart weights did not significantly change.

**8** It is possible that adrenal steroid action caused the changes in tissue ionic balance. These ionic disturbances may have been responsible for some of the changes in tissue sensitivity found in the isolated hypertensive animals.

## Introduction

In many forms of experimentally-induced hypertension there is a change in the responsiveness of various tissues to a number of agonists (see e.g. Hinke, 1965, Haeusler & Haefely, 1970, Hallbäck, Lundgren & Weiss, 1971, Greenberg & Bohr, 1975, Caulfield, Paterson & Wayyes, 1976, Le Lorier, Hedtke & Shideman, 1976, Altman, Da Ponte & Worcel, 1977a, Bhattacharya, Dadkar & Dohadwalla, 1977). Studies of this phenomenon were originally confined to vascular tissues, and the changes seen were attributed to increased arterial wall thickness (resulting from the elevated pressure load; Haeusler & Haefely, 1970, Hallbäck *et al.*, 1971). However, the validity of this hypothesis has been questioned since more recent investigations have shown an altered reactivity of gastrointestinal smooth muscle from spontaneously hypertensive rats (SHR, Altman *et al.*, 1977a) and of vasa deferentia from SHRs (Caulfield *et al.*, 1976) and DOCA-saline hypertensive rats (Le Lorier *et al.*, 1976). Hinke (1965) had previously suggested that adrenal steroids may have caused some of the changes in tissue reactivity of DOCA-saline hypertensive animals by influencing the cellular ionic balance, whereas other workers (Caulfield *et al.*, 1976, Bhattacharya *et al.*, 1977) have suggested that genetic influences may be responsible for the changes found in SHRs. Thus, the question of whether the changes are the cause or result of the hypertension still remains to be answered.

Previous work in this laboratory (Gardiner & Bennett, 1977) demonstrated a form of hypertension, induced by short-term isolation of rats, with charac-

teristics similar to those of the stress-induced hypertension reported by Rosencrans, Watzman & Buckley (1966); the hypertension in the latter case is associated with increased sympathetic nerve activity and elevated plasma corticosteroid levels.

This paper describes a comparison of the responses to noradrenaline (NA) and to noradrenergic nerve stimulation, of atria and vasa deferentia from normotensive rats housed in groups and from rats made hypertensive by short-term isolation. Differences were found in some of the responses of tissues taken from the two groups of animals. The possible involvement of adrenal hyperactivity was investigated in groups of animals which were killed after different periods of isolation; adrenals were weighed, and plasma corticosterone levels, plasma electrolyte concentrations and the myocardial contents of water, sodium ($Na^+$), potassium ($K^+$) and calcium ($Ca^{2+}$) were measured.

## Methods

Male Wistar rats weighing between 200 and 250 g were used. Control animals were housed in groups of 4 in standard laboratory cages; systolic blood pressure (BP) was measured daily by the tail-cuff method (Bûnag, 1973).

Systolic arterial hypertension was induced in rats by 5 days of continuous isolation in metabolic cages ('Metabowl', Jencons; for experimental conditions see Gardiner & Bennett, 1977). In the text, 'hypertension' means a systolic BP greater than 145 mmHg. After the initial 5 day period of continuous isolation, systolic BP was measured daily; during this time the animals were housed individually except for 1 h daily when they were grouped and handled for the measurement of BP (in the text this is referred to as intermittent isolation).

### *Organ bath studies*

Systolic BP was measured in grouped, normotensive animals ($n = 9$) and isolated, hypertensive animals ($n = 11$) for 7 days before they were killed. Immediately after death, right and left atria and one vas deferens were taken from each animal and the tissues were placed in separate jacketed organ baths (atria in 20 ml baths; vasa in 60 ml baths). The baths were filled with Krebs physiological saline solution of the following composition (mM); NaCl 119, KCl 4.7, CaCl 2.5, $MgSO_4.7H_2O$ 1.2, $NaHCO_3$ 25, $NaH_2PO_4.2H_2O$ 0.9 and glucose 11.1, kept at 37°C and constantly gassed with 95% $O_2$ and 5% $CO_2$ to maintain the pH at 7.4. A reservoir containing the gassed solution at 37°C was attached to each of the baths so that the bathing fluid could readily be changed. Atropine sulphate ($5 \times 10^{-6}$M; BDH) was present in the baths containing the atria to abolish cholinergic responses. Tissues were allowed 30 min to equilibrate before any measurements were made.

*Right atria.* Spontaneously beating right atria under 1 g tension were suspended between parallel platinum wire electrodes; this tension was maintained throughout the experiment. Contractions were recorded through Glass force-displacement transducers (FT 10C) attached to a Grass polygraph (model 79D). The frequency of contraction was recorded from the transducer by means of a tachograph (model 7PF4, Grass Instrument Co., Quincy, Mass). Due to the influence of contractile frequency on the force of contraction (Koch-Weser & Blinks, 1963) only the changes in rate of the spontaneously beating right atria were measured.

The positive chronotropic responses to noradrenergic nerve stimulation (pulse width 2 ms, strength 80 V for 10 s) were measured over a range of frequencies (0.1 to 10 Hz) and log frequency-response curves were constructed.

Log concentration-response curves to NA ((−)-noradrenaline bitartrate, Sigma) were obtained by measurement of the positive chronotropic response to increasing concentrations of the drug ($1 \times 10^{-10}$ to $1 \times 10^{-5}$ M). Each concentration of NA was added to the bath in a constant volume (0.2 ml) and left in contact with the tissue until the response was maximal. The bath was then rinsed twice and the tissue was allowed 4 min to recover before the next concentration of NA was added.

*Left atria.* Quiescent left atria were suspended under a tension of 1 g between parallel platinum wire electrodes, and electrically driven by field stimulation. The stimulus parameters used (4 Hz, 2 ms duration, 8 to 10 V strength) had little or no effect on intramural nerves. Contractile force was measured with a Grass force-displacement transducer as described above. Intramural noradrenergic nerves were stimulated by increasing the stimulus strength to 100 V for 10 s (Blinks, 1966) in the presence of atropine ($5 \times 10^{-6}$M). Log concentration-response curves to NA were obtained as described above for the right atria.

*Vasa deferentia.* Vasa deferentia were suspended under 1 g tension between parallel platinum wire electrodes and attached to an isotonic transducer (SRI); contractions were recorded on a flat bed pen recorder (Servoscribe). The response to transmural stimulation (10 Hz, 0.2 ms duration; 140 V every 4 min) was measured. Log concentration-response curves to NA were established in the manner described above. The drug was added in a volume of 0.6 ml over a concentration range of $1 \times 10^{-7}$ to $1 \times 10^{-3}$ M.

*Data analysis.* Values are given as the mean ± 1 standard error of the mean (s.e. mean); $n$ is the number of animals. Drug concentrations refer to the final bath concentration. Log frequency-response curves to noradrenergic nerve stimulation and log concentration-response curves to NA were tested for parallelism and horizontal shift by regression analysis (95% confidence limits). The heights of the maximal responses were measured and differences were tested for statistical significance by Student's unpaired $t$ test.

*Biochemical analyses*

Groups of animals were killed after different periods of isolation and their tissues were taken for biochemical analysis. One group of experimental animals ($n = 6$) was killed after 24 h isolation and blood was taken for plasma corticosterone determination (see below). Further groups of animals were killed after 5 days of continuous isolation ($n = 6$), 5 days of continuous isolation followed by 7 days of intermittent isolation ($n = 6$; a time schedule corresponding to that of the animals used in the organ bath studies), and after 5 days of continuous isolation followed by 5 weeks of intermittent isolation ($n = 6$). Control animals were killed after a 7 day period of BP measurement.

*Plasma corticosterone.* Blood was collected from the heart in the presence of heparin, and centrifuged at 4000 $g$ for 15 min at 4°C; 2 ml aliquots of plasma were removed and stored frozen for not longer than 2 weeks before analysis. Plasma corticosterone concentration was measured by a method based on that described by Mattingly (1962). All glassware was cleaned in chromic acid followed by sodium metabisulphite and distilled water, and all pipettes were plugged with cotton wool before use. The methylene dichloride which was used was a purified form (BDH—FDPC) and thus the extraction procedure described by Mattingly (1962) was unnecessary. Corticosterone acetate (Sigma; 12.5 μg/100 ml) was used as the standard. Fluorescence activity was measured with an Aminco Bowman fluorimeter (excitation 450 nm; analyser 520 nm).

*Plasma electrolytes.* The concentrations of $Na^+$ and $K^+$ were measured in the remainder of the plasma samples by flame photometry (EEL Model 150).

*Myocardial water and electrolyte content.* Hearts were excised, cleaned, blotted dry and weighed. The tissues were then dried at 80°C for 72 h and reweighed to determine the water content by difference. A weighed portion of the dried sample was then treated with a mixture of equal parts glacial acetic acid and trichloroacetic acid (Sparrow & Johnstone, 1964) and the calcium concentration of the solution was measured by atomic absorption (Unicam SP90 Atomic absorption spectrophotometer). Lanthanum oxide (1%) was present in all standards and samples to prevent phosphate ions from interfering with calcium absorption. Calcium carbonate solutions of various concentrations were used to construct the standard curve.

The remainder of the dried tissue was dissolved in fuming nitric acid, centrifuged at 4000 $g$ for 15 min and the $Na^+$ and $K^+$ concentrations of the supernatant were measured by flame photometry.

The adrenals were dissected, cleaned, blotted dry and weighed.

*Data analysis.* Results were tested for statistical significance by Student's unpaired $t$ test. Values are given as the mean ± 1 standard error of the mean (s.e. mean); $n$ is the number of animals.

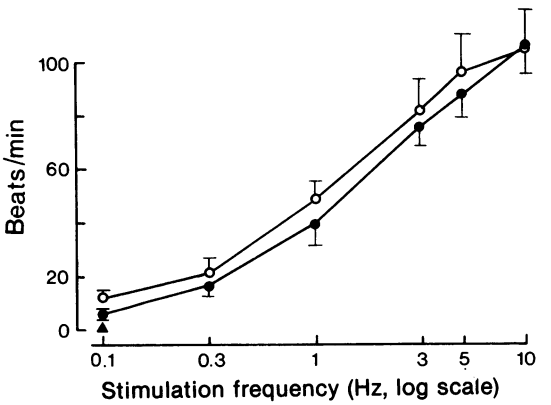


**Figure 1** Chronotropic responses of spontaneously beating right atria to graded increases in frequency of noradrenergic nerve stimulation. There was no significant difference between the slopes of the log frequency-response curves obtained in atria from group-housed (●; 53.1 ± 1.3 beats $min^{-1}$ log $Hz^{-1}$; $n = 9$) or isolated (○; 51.4 ± 5.87 beats $min^{-1}$ log $Hz^{-1}$; $n = 11$) rats. The horizontal position of the curves (calculated from the frequency required to give a response of 20 beats/min) was not significantly different (grouped = − 0.56 ± 0.064 log Hz; isolated = − 0.69 ± 0.093 log Hz).

## Results

Five days of continuous isolation in metabolic cages caused a significant systolic arterial hypertension in all groups of animals; this change persisted throughout the following 5 week period during which some of the animals remained in isolation.

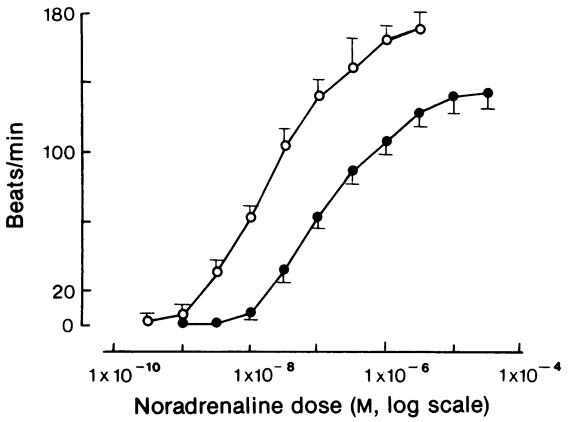


**Figure 2** Log concentration-response curves for the chronotropic effects of noradrenaline (NA) on spontaneously beating right atria. There was no significant difference between the slopes of the log concentration-response curves obtained in atria from grouped (●; 47.4 ± 2.5 beats $min^{-1}$ log $M^{-1}$; $n = 9$) or isolated (○; 54.6 ± 3.4 beats $min^{-1}$ log $M^{-1}$; $n = 11$) rats. Atria from isolated rats showed a significant leftward shift of the curve, calculated from the dose required to evoke a response of 50 beats/min (grouped = −7.2 ± 0.047 log M; isolated = −8.29 ± 0.071 log M; $0.01 > P > 0.001$). The maximum response to NA was significantly greater in atria from isolated rats (grouped = 137.78 beats/min; isolated = 173 ± 7.5 beats/min; $0.01 > P > 0.001$).

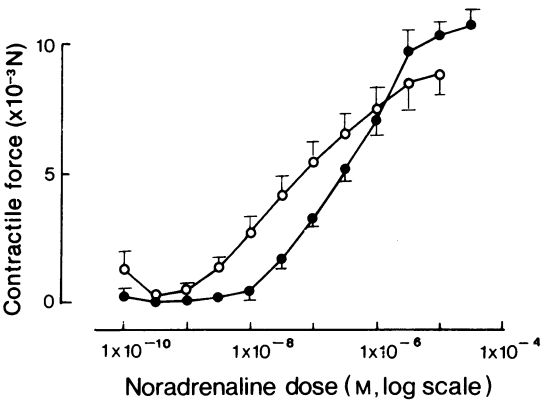


**Figure 3** Log concentration-response curves for the inotropic effect of noradrenaline (NA) on electrically driven left-atria from grouped (●; $n = 9$) and isolated (○; $n = 11$) rats. The slope of the curve obtained from isolated animals ($3.21 \pm 0.4 \times 10^{-3}$ newtons (N)/log M) was significantly less than that from grouped animals ($5.51 \pm 0.44 \times 10^{-3}$ N/log M; $0.02 > P > 0.01$). There was no significant difference between the maximum inotropic responses in atria from the two groups of animals (grouped = $10.8 \pm 1.6 \times 10^{-3}$ N; isolated = $8.4 \pm 0.9 \times 10^{-3}$ N).

*Organ bath studies*

*Right atria.* The resting rate of spontaneously beating right atria from the isolated animals (232 ± 8 beats/min) was significantly less than that of atria from group-housed animals (254 ± 6 beats/min: $0.05 > P > 0.02$).

There was no significant difference between the positive chronotropic responses to noradrenergic nerve stimulation of atria from isolated or group-housed animals (Figure 1) but the NA sensitivity was significantly greater in right atria from isolated animals than in atria from group-housed animals (Figure 2).

*Left atria.* The resting contractile force of electrically driven left atria from isolated animals was $5.5 \pm 0.6 \times 10^{-3}$ newtons (N) whereas it was $9.7 \pm 1.3 \times 10^{-3}$ N in atria from group-housed animals ($0.01 > P > 0.001$).

The positive inotropic response to noradrenergic nerve stimulation was 32% smaller in atria from isolated animals (grouped = $7.4 \pm 0.85 \times 10^{-3}$ N; isolated = $5.1 \pm 0.2 \times 10^{-3}$ N; $0.02 > P > 0.01$).

The slope of the log concentration-response curve to NA obtained from the atria of isolated animals was significantly less than that from atria of group-housed animals (Figure 3). Thus, although a leftward shift was apparent in the early portions of the curve from isolated animals it was not evident at the higher concentrations of NA.

*Vasa deferentia.* The contractile responses to transmural stimulation of vasa from isolated and group-housed animals were not significantly different.

The gradient of the log concentration-response curve to NA was 27.53 ± 3 mm/log M in vasa from isolated animals whereas it was 47.6 ± 7.16 mm/log M in vasa from group-housed animals, but this difference was not significant ($0.1 > P > 0.05$). However, the maximum response to NA was significantly less in vasa from isolated animals (3.74 ± 0.54 mm) than in the tissues from group-housed animals (6 ± 1.2 mm; $0.05 > P > 0.02$).

*Biochemical analyses*

*Plasma corticosterone.* Of the 6 animals isolated for 24 h, 5 became hypertensive; plasma from these 5 animals only was used for plasma corticosterone estimation.

In all groups of animals, plasma corticosterone levels were significantly elevated (Table 1). There was also a significant increase in the fresh weight of the adrenals from the fifth day of isolation onwards (Table 1).

*Plasma electrolytes.* The electrolyte concentration of extracellular fluid can be estimated from the plasma electrolyte concentration using the conversion factor 0.992 (White & Rolf, 1955; Audia, 1959) where:—

Extracellular $Na^+/K^+$ (mmol/kg extracellular water) = Plasma $Na^+/K^+$ (mmol/l) × 0.992.

Extracellular $K^+$ concentration was significantly reduced in rats killed after 5 days of continuous isolation whereas the $Na^+$ concentration in this group was unchanged (Table 2). After 40 days of isolation, both extracellular $Na^+$ and $K^+$ concentrations were significantly reduced (Table 2).

*Myocardial water and electrolyte content.* There was no significant difference between the heart wet weights of any group of isolated animals compared to control. After 40 days of isolation the heart wet weight was increased (grouped = 3.31 ± 0.05 g/kg body weight; isolated = 3.47 ± 0.04 g/kg body weight; $0.1 > P > 0.05$) but this change was not significant. Likewise, the water content of the hearts did not significantly change.

There was a significant increase in the $Ca^{2+}$ and $Na^+$ content of the myocardium in animals killed after 5 or 12 days of isolation but not in animals killed after 40 days of isolation (Figure 4a & b). The

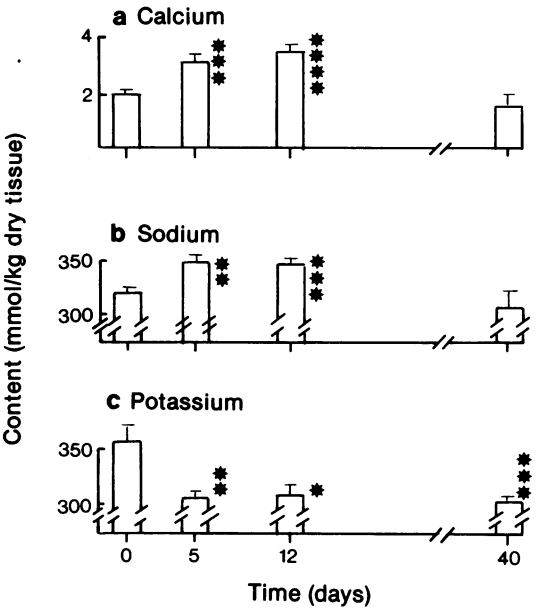


**Figure 4** Mean (±s.e. mean) values for the calcium (a), sodium (b) and potassium (c) contents of cardiac tissue taken from rats ($n = 6$ in each case) after different periods of isolation. *$0.05 > P > 0.02$; **$0.02 > P > 0.01$; ***$0.01 > P > 0.001$; ****$P < 0.001$ by Student's unpaired $t$ test.

**Table 1** Mean (±s.e. mean) values for the adrenal weights and plasma corticosterone levels of groups of rats killed after different periods of isolation

| *Period of isolation* | n | *Adrenal weight (paired; fresh) (g/kg body wt.)* | *Plasma corticosterone (μg/100 ml)* |
|---|---|---|---|
| Control | 9 | 0.127 ± 0.002 | 7.1 ± 0.75 |
| 24 h continuous | 5 | 0.121 ± 0.004 | 39.7 ± 4.65*** |
| 5 days continuous | 9 | 0.14 ± 0.004* | 14.6 ± 0.97*** |
| 5 days continuous + 7 days intermittent | 5 | 0.147 ± 0.005* | 32.2 ± 2.05*** |
| 5 days continuous + 5 weeks intermittent | 5 | 0.154 ± 0.007** | 14.5 ± 1.71** |

*** $P < 0.001$; ** $0.02 > P > 0.01$; * $0.05 > P > 0.02$ by Student's unpaired $t$ test.

$K^+$ content was significantly reduced in all three groups (Figure 4c).

## Discussion

The present results demonstrate that the noradrenergic responsiveness of atria and vasa deferentia taken from animals made hypertensive by short-term isolation is different from that of tissues taken from rats which have been housed in groups. Some of the differences between the two groups were characterized by changes in the slope of the concentration-response curves and maximal responses to NA, alterations consistent with changes in the energetics of the actin-myosin interaction as a result of a change in the availability of, for example, $Ca^{2+}$ (Kalsner, 1974). Other differences were in the form of a leftward shift of the concentration-response curve which generally arises as a result of impaired NA uptake (Trendelenburg, 1966).

The hypertension induced by short-term isolation of rats is similar in many ways to the hypertension seen with chronic exposure to environmental stress (Rosencrans *et al.*, 1969; Gardiner & Bennett, 1977). Environmental stress can cause an enhanced release of glucocorticoids together with adrenocortical hyperactivity (Raab, 1966) and such changes were found in the present study

One property of corticosteroids is to block the extraneuronal uptake of NA by tissues (Iversen & Salt, 1970; Nicol & Rae, 1972) and this could affect tissue NA sensitivity (see above). Bassett & Cairncross (1976a) elevated the endogenous release of corticosteroids in rats by signalled foot shock and found that the NA sensitivity of the left atrium was increased. Further work (Bassett & Cairncross, 1976b & c) indicated that this phenomenon was due to the uptake blocking activity of the steroids. Corticosteroids can also increase the intracellular $Na^+:K^+$ ratio (although to a lesser extent than mineralocorticoids) and the intracellular $Na^+$ concentration is closely linked with $Ca^{2+}$ fluxes (Glitsch, Reuter & Scholz, 1970, Reuter, Blaustein & Haeusler, 1973, Blaustein, 1974; 1977). The present findings of an increased $Na^+$ and $Ca^{2+}$ content of the myocardium at the same time as a decreased $K^+$ content may therefore have been the result of steroid action. It is possible that some of the changes in tissue responsiveness herein reported were the result of this ionic imbalance. The administration of exogenous corticosteroids to animals, mimicking the endogenous release during stress, alters the NA responsiveness of vascular (Schömig, Lüth, Dietz & Gross, 1976) and non-vascular (Gibson & Pollock, 1976) tissue. The sensitivity changes brought about by this procedure are characterized by a leftward shift and a flattened slope of the concentration-response curve (Schömig *et al*, 1976) and an increased maximum response (Gibson & Pollock, 1976); these changes have been attributed to increased $Ca^{2+}$ availability (Gibson & Pollock, 1976) rather than uptake blockade.

Thus it is likely that one or both of these properties of corticosteroids contributed to the changes in tissue sensitivity shown in isolated, hypertensive rats. Interestingly, a lowered intracellular $K^+$ concentration has been found in vascular smooth muscle of SHRs (Jones 1973; 1974; Hermsmeyer, 1976a & b, Altman, Garay, Papadimitriou & Worcel, 1977) and DOCA-saline hypertensive rats (Jones, Sander & Kampschmidt,

**Table 2** Mean ( ±s.e. mean) values for the extracellular $Na^+$ and $K^+$ concentrations (derived from plasma concentrations, see text) in rats killed after different periods of isolation

| *Period of isolation* | n | *Extracellular sodium (mmol/kg extracellular water)* | *Extracellular potassium (mmol/kg extracellular water)* |
|---|---|---|---|
| Control | 6 | 145.2 ± 1.95 | 6.26 ± 0.16 |
| 5 days continuous | 6 | 140 ± 2.42 | 5.53 ± 0.16** |
| 5 days continuous + 7 days intermittent | 7 | 149 ± 1.03 | 6.52 ± 0.09 |
| 5 days continuous + 5 weeks intermittent | 6 | 132.8 ± 3.45** | 5.43 ± 0.26* |

* $0.05 > P > 0.02$; ** $0.02 > P > 0.01$ by Student's unpaired *t* test.

1977). Also, an increased $Ca^{2+}$ content has been shown in the myocardium of rats made hypertensive by renal clipping (Tobian & Duke, 1964), DOCA-saline administration and genetic breeding (Furuta, 1977). It is possible, therefore, that ionic changes could have been responsible for the enhanced responsiveness to NA found in the tissues of these various hypertensive models (Hinke, 1965; Hermsmeyer, 1976a & b; Altman *et al.*, 1977a). A decreased maximum response to NA of the vas deferens with no change in NA sensitivity and no change in the response to transmural stimulation (as reported here) has also been found in vasa taken from SHRs (Caulfield *et al.*, 1976). Caulfield *et al.* (1976) attributed these findings to genetic influences, but we would suggest that they may have been due to hormonal disturbances.

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