Dependence of deoxycorticosterone/salt hypertension in the rat on the activity of adrenergic cardiac nerves

C. BELL and ELSPETH M. MCLACHLAN

Department of Physiology, University of Melbourne, and Department of Physiology, Monash University, Victoria, Australia

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Summary

1. Chronic hypertension was induced in Wistar rats with intact kidneys by subcutaneous implantation of 50 mg of deoxycorticosterone acetate (DOCA) in wax and addition of sodium chloride (9 g/l) to the drinking water.

2. The development of DOCA/salt hypertension, as monitored by tail-cuff plethysmography, was prevented by: (a) destruction of the peripheral adrenergic nerves with neonatal administration of guanethidine (80 mg/kg subcutaneously for the first 14 days postnatally); (b) bilateral stellate ganglionectomy; (c) oral administration of the β-adrenoreceptor antagonists propranolol or atenolol (1 mg day⁻¹ kg⁻¹) during the period of DOCA/salt treatment.

3. The dose of DOCA used was sufficient to inhibit the atrial Uptake pathway completely: this process appears to participate in termination of action of neurally released noradrenaline in the heart.

4. It is suggested that this model of DOCA/salt hypertension is due to adrenergic enhancement of cardiac output in the presence of an increased sodium load. The enhancement may be partly due to deficient myocardial inactivation of noradrenaline.

Key words: adrenergic fibres, β-adrenoreceptor blockade, catecholamine metabolism, chemically-induced hypertension, deoxycorticosterone.

Abbreviations: DOCA, deoxycorticosterone acetate.

Introduction

Many patients with essential hypertension have been found to produce abnormally large amounts of mineralocorticoid hormones (Brown, Fraser, Love, Ferriss, Lever, Robertson & Wilson, 1972; Genest, Nowaczynski, Kuchel & Sasaki, 1972; Luetscher, Beckerhoff, Dowdy & Wilkinson, 1972; Melby, Dale, Grekin, Gaunt & Wilson, 1972; Sennett, Brown, Island, Yarbro, Watson, Slaton, Hollifield & Liddle, 1975). There is also abundant epidemiological evidence to suggest an association between essential hypertension and high sodium intake (Kirkendall, Connor, Abboud, Rastogi, Anderson & Fry, 1972; Page, Damon & Moeller-Ing, 1974; Freis, 1976). Furthermore, chronic administration of the mineralocorticoid DOCA in the presence of a high sodium intake is known to produce hypertension in a variety of animal species (Selye, Hall & Rowley, 1943; Friedman & Friedman, 1949; Kalsner, Ayitey-Smith & Ling, 1971; Terris, Bereck, Cohen, Stanley, Whitehouse & Bohr, 1976; Conway & Hatton, 1977). Clarification of the mechanism underlying the development of this type of experimental hypertension is therefore likely to aid our understanding of essential hypertension in man.

Most animal studies of DOCA/salt hypertension have utilized the rat. In this species, the development of hypertension is characterized by an increased cardiac output (Chrysant, Walsh & Frohlich, 1978); cardiac turnover and plasma catecholamines are also augmented (de Champlain,
Mueller & Axelrod, 1969; Nakamura, Gerold & Thoenen, 1971; Reid, Zivin & Kopin, 1975; de Champlain, Farley, Cousineau & Ameringen, 1976), suggesting excessive sympathetic activity. However, attempts to produce DOCA/salt hypertension in the absence of the sympathetic nerves have proved inconclusive (Ayitey-Smith & Varma, 1970; Finch & Leach, 1970; Clarke, Smookler & Barry, 1970; de Champlain & Ameringen, 1972). The variability in results obtained after chemical or immunosympathectomy might be attributed to the difficulty of producing adequate sympathectomy with either technique: the effect of 6-hydroxydopamine is relatively short-lived compared with the time course of development of hypertension (de Champlain & Ameringen, 1972; Finch, Haeusler & Thoenen, 1973), and neither technique uniformly destroys axons to all vascular beds (Berkowitz, Spector & Tower, 1972; Finch et al., 1973).

Johnson, Cantor & Douglas (1975) have reported that neonatal administration of guanethidine produced a peripheral sympathectomy more complete and permanent than those achieved with either 6-hydroxydopamine or anti-nerve growth factor. We have therefore used this technique of sympathectomy to re-examine the role of the adrenergic nervous system in the production of DOCA/salt hypertension. The effects observed have been compared with those of surgical denervation of the heart and of pharmacological blockade of β-adrenoreceptors. In addition, as mineralocorticoids are known to inhibit tissue uptake of catecholamines in vitro (Iversen & Salt, 1970), the effect of chronic DOCA treatment on cardiac inactivation of neurally released noradrenaline has been assessed.

**Methods**

**Animals**

All rats used in these experiments were nonmotensive inbred Wistar rats, of either sex, fed on a diet of Barastoc GR2 rat cubes (quoted sodium content 3 mg/g) having initially free access to tap water.

**Blood pressure recording**

Systolic blood pressures were recorded indirectly with an occlusive tail-cuff (Bryom & Wilson, 1938) and a photoelectric pulse detector (Grass PTT1) connected to a Grass 7P8 preamplifier and a Grass 7D polygraph. For each rat, two or three pressure readings were obtained under ether anaesthesia and again at the time of reappearance of vibrissal movements. The means of these two sets of values seldom differed by more than 5 mmHg and there was no consistent variation in either direction between the values obtained under anaesthesia and those obtained at reawakening. The pressures cited represent the average of the means of the two sets of measurements for each animal. All readings were made between 09.00 and 12.00 hours, so as to minimize any diurnal fluctuations in pressure (Hall, Nasseth & Hall, 1977), by a technician who had no knowledge of the experimental designs.

**Induction of hypertension**

Moulded pellets containing 50 mg (1.3 × 10⁻⁴ mol) of DOCA (Percorten; Ciba–Geigy) in 200 mg of paraffin wax were implanted subcutaneously on one flank under ether anaesthesia. Subsequent to the operation, drinking water was substituted by sodium chloride in tap water (9 g/l). Nephrectomy was not performed. In experiments concerned with the effect of chemical sympathectomy on development of hypertension, implantation was performed at 7 weeks of age. In all subsequent experiments, the animals were 15–19 weeks of age at the time of implantation.

**Fluorescence histochemistry**

Tissue samples were frozen in liquid propane, cooled with liquid nitrogen and freeze-dried. Catecholamines bound in adrenergic axons were rendered visible in paraffin sections by fluorescence histochemistry after condensation with formaldehyde vapour (Falck & Owman, 1965).

**Chemical sympathectomy**

Guanethidine sulphate (Ismelin; Ciba–Geigy) dissolved in sterile, pyrogen-free distilled water was administered daily to newborn rats in doses of 50–100 mg (1–2 × 10⁻⁴ mol)/kg subcutaneously and injection volumes of not more than 0.02 ml/10 g body weight.

**Surgical cardiac sympathectomy**

Under pentobarbitone sodium anaesthesia, the stellate ganglia were located retropleurally between the first and second ribs after antero-lateral reflection of the scapulae. As much of each ganglion as was accessible without rib resection
was excised. Sham operations were performed with the same procedure, except that no attempt was made to expose the ganglia after exposure of the ribs. The wounds were closed with Michel clips and broad-spectrum topical antibiotic cover was given. At least 10 days were left for recovery from operation before DOCA implantation.

β-Adrenoreceptor blockade

Propranolol (Inderal; I.C.I. Ltd) or atenolol (Tenormin; I.C.I. Ltd) were dissolved in either drinking water or saline so as to give a daily ingestion of approximately 1 mg (3.3 \times 10^{-6} \text{ mol})/kg. The pH of the drinking medium was adjusted to pH 5-0 with dilute HCl and the containers were shielded from light with aluminium-foil wrapping. New drug solutions were prepared every second day.

Assessment of Uptake₂ activity

Isolated, paired atria from normal rats or from weight- and sex-matched rats implanted with a DOCA pellet 1–2 weeks previously were mounted in physiological saline containing 0·1 µg (3 \times 10^{-10} \text{ mol}) of hyoscine/ml and 0·6 µg (2 \times 10^{-9} \text{ mol}) of desmethylimipramine/ml, as described previously (Bell & Grabsch, 1976). Time courses of chronotropic responses to intramural nerve stimulation (0·5 ms, supramaximal voltage, 5 Hz for 5 s) were compared before and after inhibition of Uptake₂ with 0·9 µg (4 \times 10^{-9} \text{ mol}) of metanephrine/ml.

Statistical analysis

Statistical significance of differences of means was assessed with a two-tailed Student’s t-test for unpaired data.

Results

The systolic blood pressures of normal rats of the strain used rose gradually with age from a mean of about 105 mmHg at 30 days to a mean of about 120 mmHg at 90 days. Subsequent to this, little further increase occurred over the age range covered in these experiments. No appreciable difference between blood pressure of males and females was noted, and data from both sexes have therefore been pooled. At no age did any normal animals have blood pressures in excess of 135 mmHg. For all adult animals used, the mean blood pressure was 122 mmHg (SEM 1·1, n = 51).

At all ages, administration of DOCA and salt produced a progressive rise in blood pressure relative to that of age-matched control animals. This hypertension became apparent 7–10 days after commencing DOCA/salt treatment and reached a plateau level after 3–4 weeks approximately 20 mmHg above that of the control animals. Unless a second DOCA implant was given, blood pressures began to decline once again after 5–6 weeks and had in almost all animals returned to control levels 9–10 weeks after commencement of treatment. All comparisons of blood pressures during DOCA/salt administration have therefore been made by using 28 days of treatment. In all groups studied the rise in blood pressure during DOCA/salt treatment was significant at the 0.01% level. Neither implantation of DOCA alone nor addition of salt to the drinking water alone had any significant hypertensive effect.

Effect of chemical sympathectomy on induction of hypertension

The efficacies of sympathectomy produced by neonatal treatment with guanethidine, at 50, 80 and 100 mg day⁻¹ kg⁻¹, were compared in groups of at least eight animals. The lowest dose had little effect on the density of adrenergic axons in atria or mesenteric arteries in the majority of animals. Both high doses produced extensive destruction of axons, but with doses of 100 mg day⁻¹ kg⁻¹ mortality was extremely high (80–90%). The dose of 80 mg day⁻¹ kg⁻¹ (mortality 20–30%) was therefore selected for use. The atria and mesenteric arteries of 13 out of 15 animals so treated and killed at 13 weeks of age contained very few or no visible adrenergic axons, whereas those of the remaining two animals contained substantial numbers of axons. Several other animals treated with guanethidine at 80 mg day⁻¹ kg⁻¹ were kept until they were 150–170 days old and the degree of destruction of their vasomotor nerves was assessed by stimulation of the autonomic spinal outflow after pithing (Gillespie & Muir, 1967). Spinal stimulation (0·5 ms pulses, supramaximal voltage, 2–5 Hz for 5 s), which produced pressor responses of 40–50 mmHg in age-matched controls (see also Bell & Kushinsky, 1978), produced responses of less than 5 mmHg in all guanethidine-treated animals. It was therefore concluded that the guanethidine regimen used caused extensive cardiovascular sympathectomy and that appreciable regrowth of adrenergic axons did not occur at least up to about 150 days of age. However, in order to
minimize the possibility of axonal regrowth we chose to examine the interaction of guanethidine-induced sympathectomy with DOCA/salt treatment in rather younger animals. In a control group of 12 normal rats 50 days old, treatment for the subsequent 28 days with DOCA/salt produced a rise in systolic blood pressure from 106 ± 2.4 mmHg to 124 ± 3.0 mmHg, while over the same period the blood pressure of an age-matched, untreated group of 12 rats rose from 107 ± 2.4 mmHg only to 112 ± 1.4 mmHg (Fig. 1). The blood pressures of 10 control guanethidine-treated rats were slightly lower than those of normal animals at all ages, but showed a slight rise from 101 ± 2.3 mmHg at 50 days to 103 ± 2.0 mmHg at 78 days. However, by comparison with the normal animals, in 10 age-matched guanethidine-treated animals DOCA/salt administration had no hypertensive effect, the blood pressure before implantation being 91 ± 2.9 mmHg and after 28 days' treatment 96 ± 1.4 mmHg (P > 0.6) (Fig. 1).

**Effect of surgical cardiac sympathectomy on induction of hypertension**

Blood pressures of both sham-operated rats (127 ± 2.0 mmHg, n = 9) and rats from which the stellate ganglia had been removed (122 ± 3.2 mmHg, n = 6) were similar to those of age-matched controls (126 ± 2.1 mmHg, n = 15) 10–14 days after operation. In the sham-operated animals, treatment for 28 days with DOCA/salt had a hypertensive effect similar in magnitude to that produced in normal animals, blood pressures being 147 ± 3.0 mmHg for the sham-operated animals and 147 ± 2.8 mmHg for the control group. By contrast, no change in blood pressure

![Graph 1](image1.png)  
**Fig. 1.** Changes in blood pressure (B.P.) in groups of normal and guanethidine-treated rats between the ages of 50 days (open columns) and 78 days (solid columns), and after treatment with DOCA/salt over the same period. *Significantly elevated pressures of the normal, DOCA/salt-treated animals relative to the pressures of the normal, untreated animals at the same age (P < 0.001). The vertical bars are 1 SEM.

![Graph 2](image2.png)  
**Fig. 2.** Blood pressures of age-matched groups of (a) sham-operated and (b) stellate-ganglionectomized rats before (open columns) and after 28 days treatment with DOCA/salt (solid columns). Over this period there was no change in blood pressure (B.P.) of age-matched, untreated animals. *Significant elevation of pressures in the sham-operated DOCA/salt-treated rats relative to their pressures at the start of treatment (P < 0.001). The vertical bars are 1 SEM.

![Graph 3](image3.png)  
**Fig. 3.** Blood pressures of age-matched groups of normal rats and of rats given propranolol or atenolol (1 mg day⁻¹ kg⁻¹) before (open columns) and after 28 days treatment with DOCA/salt (solid columns). Over this period there was no change in blood pressure (B.P.) of age-matched untreated rats. *Significant elevation of pressures in the normal DOCA/salt-treated rats relative to their pressures at the start of treatment (P < 0.001). The vertical bars are 1 SEM.
was seen over the same period in six sympathectomized animals, the blood pressure after 28 days of DOCA/salt treatment being 120 ± 1·8 mmHg (Fig. 2). Histochemical examination of the atria from these animals showed complete adrenergic denervation of the right atrium in four animals, with partial denervation in one other and little effect in the last. There was substantial reduction in density of left atrial adrenergic axons in only two of the six animals: however, decentralization of the residual adrenergic neurons was suggested by the presence of ptosis in all but one instance. Ptosis was not seen in any of the sham-operated animals.

**Effect of β-adrenoreceptor blockade on induction of hypertension**

Administration of propranolol or atenolol in the drinking water so as to approximate a daily dose of 1 mg (2 × 10⁻⁶ mol)/kg had no effect over 1 week on the blood pressures of normal rats (control: 119 ± 2·6 mmHg, n = 14; propranolol: 120 ± 2·1 mmHg, n = 13; atenolol: 123 ± 3·0 mmHg, n = 9). When they were given concurrently with DOCA/salt, however, to the same groups, both drugs prevented the hypertensive effect of DOCA/salt exhibited in age-matched control animals (Fig. 3). The mean pressures recorded after 28 days' treatment were control: 143 ± 1·5 mmHg; propranolol: 126 ± 3·2 mmHg; atenolol: 124 ± 2·0 mmHg.

**FIG. 4.** Time courses of recovery (half-decay times) of chronotropic responses to intramural adrenergic nerve stimulation of atria from normal rats and from age- and sex-matched rats implanted with DOCA, before (hatched columns) and after (open columns) inhibition of Uptake₂ with metanephrine. The vertical bars represent 1 SEM. Significantly different values from the control value before Uptake₂ inhibition: *P < 0·02; **P < 0·01.

**Effect of DOCA treatment on tissue amine uptake**

The time courses of chronotropic responses to intramural adrenergic nerve stimulation were compared in atria from 12 normal rats and 12 rats that had received DOCA implants 7 days earlier (Fig. 4). In atria from normal animals the half-decay time of responses under control conditions was 163 ± 8·6 s, and, inhibition of Uptake₂ with metanephrine increased this to 217 ± 5·2 s (P < 0·01). In atria from DOCA-treated animals, by contrast, the half-decay time of responses before Uptake₂ inhibition was as great as that seen after Uptake₂ inhibition in the controls (227 ± 22·4 s), and metanephrine produced no further prolongation (224 ± 14·6 s). The time courses of both these sets of responses were significantly (P < 0·02) greater than those of the control group before Uptake₂ inhibition.

**Discussion**

Administration of DOCA and salt to rats for several months produces hypertension, which persists after cessation of the treatment, presumably due to baroreceptor resetting and to structural vascular changes (Friedman & Friedman, 1949). By contrast, in our experiments implantation of a single DOCA pellet (50 mg) together with salt administration produced hypertension which remitted spontaneously after 2–3 months unless a second DOCA implant was given. Thus the hypertension which we studied can be regarded as being actively maintained by the treatment given. As neither DOCA nor salt alone had an equivalent effect, both the steroid and an increased sodium load must be necessary to produce a rise in blood pressure.

Neonatal treatment of rats with guanethidine produced in most individuals extensive destruction of adrenergic axons associated with the cardiovascular system, as judged by fluorescence histochemistry and by lack of pressor responses to spinal cord stimulation. In these animals, DOCA/salt treatment had no effect on blood pressure, suggesting that the development of hypertension in normal DOCA/salt-treated rats was dependent on the presence of the adrenergic nervous system.

The prevention of hypertension by guanethidine-induced sympathectomy could have been due to destruction of vasmotor and of cardiac axons. However, stellate ganglionectomy similarly prevented development of hypertension during
DOCA/salt treatment, and sham-operated animals responded with a rise in blood pressure equivalent to that seen in normal animals which had not been subjected to surgical stress. Although the histochemical findings indicated that in some animals ganglionic ablation was incomplete, it seems likely that the preganglionic outflow to the cardiac nerves was disrupted in most cases. These results suggest that it is the activity of the adrenergic nerves to the heart, rather than those to the vasculature, which are essential to establishment of the hypertensive state.

The involvement of the cardiac adrenergic nerves was further confirmed by the observations that oral treatment with low doses of the $\beta$-adrenoreceptor antagonists propranolol or atenolol during the period of DOCA/salt administration also prevented development of hypertension. An anti-hypertensive action of propranolol could be related to its effect on central $\beta$-adrenoreceptors (Day & Roach, 1973; Dollery, Lewis, Myers & Reid, 1973) or to its local anesthetic properties (Howe & Shanks, 1966; Hermansen, 1969). On the other hand, atenolol has been reported not to cross the blood–brain barrier (Barrett, 1977) and is devoid of local anesthetic effects (Barrett, Carter, Fitzgerald, Hull & LeCount, 1973; Harry, Knapp & Linden, 1974). As atenolol was at least as effective as propranolol in preventing DOCA/salt hypertension, it appears therefore that the effects of both drugs were due to blockade of cardiac $\beta$-adrenoreceptors.

Various results have been obtained by previous workers regarding the importance of the adrenergic nervous system in development of hypertension during DOCA/salt treatment. Thus, although immunosympathectomy was reported in one study to prevent hypertension (Ayitey-Smith & Varma, 1970), both this procedure and chemical sympathectomy with 6-hydroxydopamine failed to prevent a rise of blood pressure in several other studies (Finch & Leach, 1970; Mueller & Thoenen, 1970; Clarke et al., 1970; de Champlain & Ameringen, 1972). Similarly, chronic $\beta$-adrenoreceptor blockade over the period of DOCA/salt treatment has been variously reported to have no effect on (Takeda, Sakurai & Imai, 1975; Conway & Hatton, 1977), or to produce a slight (Day & Peters, 1975; Buña, 1977) or a profound (Conway, Darwin, Hilditch, Loveday & Reeves, 1975) decrease in, the degree of hypertension developed. The ineffectiveness of sympathectomy in previous studies might have been due to survival or regrowth of the axons. It is also possible that some difference exists in the nature of the hypertension produced between our model and others recently used, as most workers currently preclude DOCA/salt treatment by unilateral nephrectomy in order to produce a more fulminating hypertension (Friedman & Friedman, 1949). Such a procedure may lead to effects additional to those produced by the DOCA/salt regimen alone, and for this reason was omitted in our experiments.

The rate of absorption of DOCA from the implants which we used was sufficient to produce complete chronic blockade of the atrial uptake pathway. As uptake is responsible for uptake of catecholamines into cardiac muscle cells (Iversen, 1965; Clarke, Jones & Linley, 1969), this result explains the well-documented reduction in cardiac retention of exogenous noradrenaline seen in DOCA/salt-treated rats (de Champlain, Krakoff & Axelrod, 1967; Kazada, Pohlóva, Birb & Kockova, 1969; Louis, Krauss, Kopin & Sjoerdsma, 1970). It is of interest that deficiencies in cardiac uptake processes for catecholamines have also been reported in other hypertensive rat models related to electrolyte imbalance (LeLorier, Hedtke & Shideman, 1976) and in two different strains of rat with genetic hypertension (Salt & Iversen, 1973; Bell & Kushinsky, 1978).

As reported previously (Bell & Grabsch, 1976), and confirmed in the present study, blockade of uptake in isolated atria prolongs the biological activity of transmitter released by stimulation of the intramural adrenergic axons. The frequency of nerve stimulation used was of the same order as the natural firing frequency of adrenergic vasomotor axons (Grosse & Jänig, 1975) and that which produces physiological levels of alteration in adrenergic vasomotor tone (Mellander, 1960). Thus our results are compatible with the view that potentiation of cardiac responses to sympathetic activation because of inactivation of uptake might be the mechanism by which cardiac output and blood pressure are increased by DOCA treatment in the presence of concomitant sodium loading. It is not clear, however, whether synchronous excitation of all postganglionic axons to an autonomic effector, as occurs during experiments in vitro, is equivalent in terms of postsynaptic activation to the same average frequency of axonal firing occurring asynchronously in vivo. The physiological importance of uptake in the heart therefore remains uncertain. An alternative interpretation of our results is that increased cardiac output and blood pressure may result directly from the increased sympathetic cardiac
drive consequent upon increased sodium load and blood volume.

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References


