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Role of Peripheral Adrenergic Responsiveness in the Development of DOCA/NaCl Hypertension in Rats

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Abstract: Alterations in α- and β-adrenergic responsiveness were investigated prior to and during the development of hypertension in rats treated with desoxycorticosterone acetate and NaCl (DOCA/NaCl). The DOCA/NaCl rats became noticeably hypertensive (> 150 mm Hg) six weeks after the initiation of treatment. Prior to the development of hypertension, a reduced *in vivo* and *in vitro* β- and an enhanced α-adrenergic responsiveness of the DOCA/NaCl group resulted. At 2 and 12 weeks of the study, the dipsogenic response to isoproterenol was significantly attenuated in the DOCA/NaCl rats, whereas no difference in the dipsogenic response to 24 hour water deprivation was observed between control and DOCA/NaCl rats. Isoproterenol-induced relaxation of aortic smooth muscle from the DOCA/NaCl treated rats was significantly reduced at 4 weeks and further attenuated at 12 weeks of the study. However, aortic smooth muscle sensitivity to norepinephrine stimulation was significantly increased at 4 and 12 weeks of the study. These results suggest that alterations in both *in vivo* and *in vitro* α- and β-adrenergic responsiveness occur prior to establishment of hypertension of the DOCA/NaCl rats and that these alterations may have a role in the early stages of the development of this form of hypertension.

Elevation of blood pressure in both intact and uninephrectomized rats accompanies chronic administration of the mineralocorticoid hormone desoxycorticosterone (DOC) (1, 2). Suggested mechanisms responsible for DOC-induced hypertension include: an enhanced vascular reactivity (1, 3, 4); sodium retention and expansion of extracellular volume (5, 6); enhanced sympathetic nervous activity (7); alterations in the concentrations of electrolytes in the cell wall (2) and increased concentrations of catecholamines in the blood (8, 7).

Regardless of the ultimate pathogenic mechanism, the presence of sodium and the participation of the sympathetic nervous system in the development and initiation of DOC-induced hypertension have been supported by several experimental findings (2–4, 7, 9). An increase in *in vitro* α-adrenergic responsiveness (3, 4) and a decrease in *in vivo* β-adrenergic responsiveness in DOC/NaCl treated rats (10) have been reported. Additionally, an increase in peripheral sympathetic outflow and plasma catecholamines; a reduction in the number of β-adrenergic receptors (11, 12); and an increase in the number of α-adrenergic receptors (13) have been reported in hypertensive rats.

It has been suggested that a primary pathogenic factor in the development of this form of hypertension could be enhanced vascular reactivity observed in the DOCA/NaCl rat, since the increased responsiveness to α-adrenergic agonists occurs prior to the development of hypertension (3). Sodium deficiency in the DOC-treated rat prevented the occurrence of the enhanced vascular reactivity to norepinephrine (10). This and other reports (14) support the importance of salt in the change in vascular responsiveness. Rats treated with DOC-tap water (15) as well as thyroidectomized DOC/NaCl-treated rats (16) exhibit enhanced vascular reactivity without any change in blood pressure.

The purpose of the present study was to investigate if the alteration in both α- and β-adrenergic responsiveness occurs prior to as well as during the hypertensive state in the DOC/NaCl rats using both *in vivo* and *in vitro* techniques.

Methods

Sixty male Sprague Dawley rats (Blue Spruce Farms) were housed in groups of 2, in hanging stainless steel cages, in a room illuminated from 0700 to 1900 h and maintained at 24 ± 1°C. Food (Purina laboratory chow) was provided *ad libitum*.

Thirty of the rats were lightly anesthetized with ether and subcutaneously implanted with pre-weighed pellet of desoxycorticosterone acetate (DOCA: Sigma Chemical Co.). These pellets were made with an F. J. Stokes tablet maker. A 1% NaCl solution was provided *ad libitum*. The remaining thirty rats were sham treated, and tap water was provided *ad libitum*. Indirect systolic blood pressures (SBP) were measured every other week by means of a Narco-Bio systems transducer and physiograph, using the standard tail cuff technique.

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The dipsogenic response to administration of the β-adrenergic agonist, isoproterenol, was assessed in 6 control and 6 DOCA/NaCl-treated rats 2 and 12 weeks after initiation of treatment. The protocol for all the dipsogenic experiments began between 8:30 and 9:20 a.m., at which time each animal was weighed, placed into individual stainless steel cages without food, and equipped with a 100 ml graduated spillproof water bottle (Bio Serv, Inc.). One hour after the animals were placed in the cages, isoproterenol (10 μg/kg body, wt., *s.c.*) was administered, and the volume of water in the drinking tubes was recorded. Water intake was determined by calculating the volume difference during the 1st and 2nd hour after administration of isproterenol.

The dipsogenic response to 24 hour dehydration was also determined in the same animals at 8 weeks of the study. Animals were deprived of their normal drinking solution for 24 hours, then placed in separate cages and water intake determined as described above.

At both the 4th and 12th week of study, 8 control and 8 DOCA/NaCl rats were decapitated. Two 4 mm rings of the thoracic aorta, cleaned and rid of adherent fat and connective tissue, were obtained and placed in an aerated Kerbs solution at 26°C. Each ring was suspended between two stainless steel hooks inserted through the lumen of the vessel to record circular smooth muscle contraction. The isolated tissue was mounted in a 20 ml temperature controlled (water jacketed) muscle bath and equilibrated for 2 hours under 8 grams of preload tension (23). Using an F-50 microdisplacement myograph transducer with a model DMP-4B Narco Bio-Systems Physiograph recorder, isometric contractions were recorded. The Krebs solution bathing the aortic rings was maintained at 37°C and aerated with an O_2:CO_2 mixture (95:5) to maintain the pH of the solution at 7.3 ± 0.1. The bath solutions were changed every 15 minutes during the equilibration period and between dose-response determinations.

After the 2 hour equilibration period a KCl dose-response curve (DRC) (8–80 mM) was generated. Following the last addition of KCl the baths were rinsed with fresh Krebs solution and the tissues allowed to relax to baseline tension. The tissues were incubated with phentolamine (10^{-5} M) for 45 minutes in order to block its α-adrenergic activity. The bath solutions were changed ever 15 minutes and phentolamine added each time. Sufficient KCl was added to each bath to produce a half-maximal contraction. After the tension generated reached a plateau, a cumulative isoproterenol (ISO) was added, the bath solutions were changed and the tissues allowed to return to baseline conditions. For 1 hour, the bath solutions were changed every 15 minutes, after which a cumulative norepinephrine (NE) DRC (10^{-10} to 10^{-4} M) was generated. Isoproterenol and norepinephrine were purchased from Sigma Chemical Co. Phentolamine (Regitine®) was donated by Ciba-Geigy Laboratories. At the termination of each experiment, the viability of the aortic tissue was assessed with a KCl DRC.

The procedure for determination of the steroid dosage was as follows; pellets were placed in a dessicator for 72 hours and then weighed on an analytical balance prior to the subcutaneous implantation. At the end of the experiment, the intact pellets were removed, cleaned of any adhering tissue, placed in a dessicator for 72 hours and reweighed on the same balance. The value derived from the difference in pre- and post-experimental weights was then divided by the mean body weight of the animal during the time the pellet was implanted.

All data were analyzed and compared by means of Students t-test with significance set at the 95 % confidence interval.

Results

After 2 weeks of treatment (Table I), systolic blood pressure in the DOCA/NaCl rats was significantly increased over the control group, although the animals did not become hypertensive (> 150 mm Hg) until the 6th week of the study.

The dipsogenic responses to the β-adrenergic agonist isoproterenol and to 24 hour water deprivation are summarized in Table II. One hour water intake following administration of vehicle (distilled water) was 0.3 ± 0.3 ml/kg and 0.9 ± 0.4 ml/kg for control and DOCA/NaCl-treated groups, respectively. Water intake in response to administration of isoproterenol was attenuated significantly in the DOCA/NaCl treated rats during both the 2nd and 12th week of the study. However, the dipsogenic response to 24 hour dehydration was similar in control and DOCA/NaCl treated rats. The major effect on water intake was observed in the first hour after administration of either dipsogenic agent.

At both 4 and 12 weeks of the study no significant difference was observed in the development in active tension by rings of aortic smooth muscle from control and DOCA/NaCl treated rats in response to cumulative addition of KCl (Fig. 1 a and b).

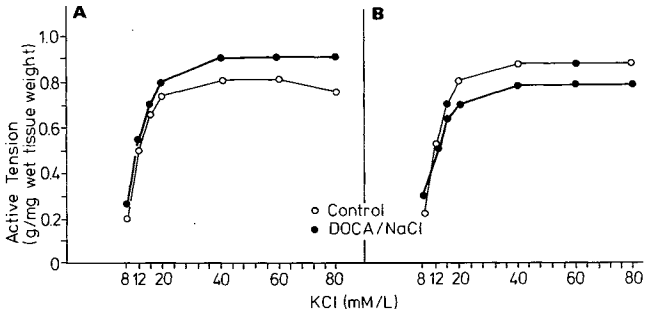

Fig. 1 Dose-response relationship between the concentration of potassium chloride and active tension developed by aortic rings from control and DOCA/NaCl treated rats at 4 weeks (A) and 12 weeks (B). Each point is expressed as the mean and standard error from 6 rats.

Table I. Indirect Systolic Blood Pressures (mm Hg) in Control Rats and Rats Treated with Desoxycorticosterone Acetate (DOCA) and Administered 1 % Sodium Chloride.

Treatment Group	n	Systolic blood pressure during weeks before and after treatment of DOCA/NaCl: −2	0	+2	+4	+6	+8	+10	+12
Control	6	114 ± 3**	112 ± 3	115 ± 2*	115 ± 2*	119 ± 7*	119 ± 4*	107 ± 2*	115 ± 3*
DOCA/NaCl	6	113 ± 3	114 ± 3	127 ± 3	132 ± 3	154 ± 7	149 ± 6	154 ± 5	157 ± 6

**Mean ± SE

*Significantly less than DOCA/NaCl ($p < 0.01$).

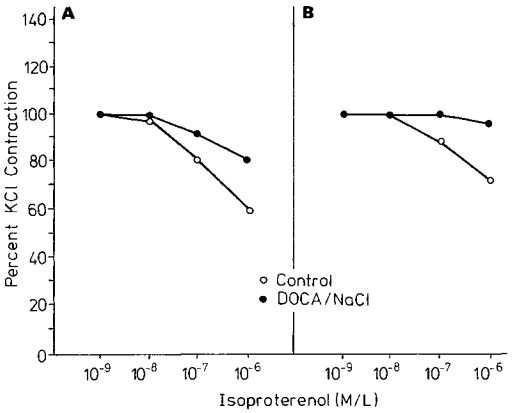

Fig. 2 Dose-response relationship between the concentration of isoproterenol and relaxation of aortic rings from control and DOCA/NaCl treated rats expressed as per cent of KCl concentration at 4 weeks (A) and 12 weeks (B) of treatment. Each point is expressed as the mean and standard error from 6 rats.

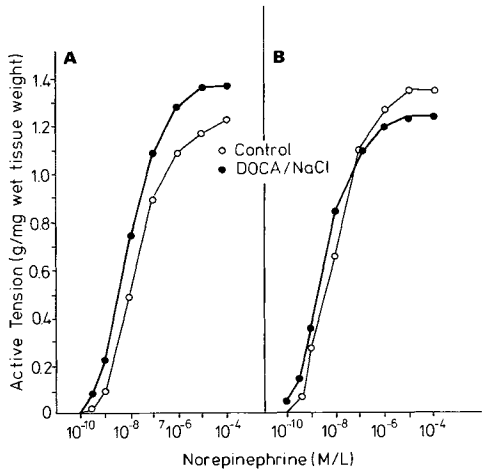

Fig. 3 Dose-response relationship between the concentration of norepinephrine and active tension developed by aortic rings from control and DOCA/NaCl treated rats at 4 weeks (A) and 12 weeks (B) of treatment. Each point is expressed as the mean and standard error from 6 rats.

Table II. Effect of Isoproternol (ISO) Administration and 24 Hour Water Deprivation on 1 and 2 Hour Cumulative Water Intake (mg/kg) in Control and DOCA/NaCl Treated Rats.

Treatment	Week	n	One hour	Two hours
ISO (10 μg/kg b.w., s.c.)	2			
Control		6	6.92 ± 1.15**	6.93 ± 1.17
DOCA/NaCl		6	0.0 ± 0.0*	0.0 ± 0.01
24 hour dehydration	8			
Control		6	24.95 ± 2.61	25.35 ± 2.55
DOCA/NaCl		6	22.92 ± 1.64	26.85 ± 0.77
ISO (10 μg/kg b.w., s.c.)	12			
Control		6	5.03 ± 161	7.32 ± 1.35
DOCA/NaCl		6	0.26 ± 0.26*	0.26 ± 0.26*

**Mean ± SE
*Significantly less than control ($p < 0.001$).

Isoproterenol-induced relaxation of aortic smooth muscle was maximal at a dose of 10^{-6} M for all tissues at both 4 and 12 weeks of the study (Fig. 2 a and b). A significant decrease ($p < 0.01$) in this β-receptor mediated response was seen in aortic rings from the DOCA/NaCl treated rats at 4 weeks (Fig. 2 a), which was further attenuated ($p < 0.001$) in the 12th week of the study, while aortic rings from the control group showed little change (Fig. 2 b).

Aortic rings from the DOCA/NaCl treated rats showed a significant increase ($p < 0.05$) in the sensitivity and contractility to norepinephrine (NE; $10^{-9} - 10^{-4}$ M) stimulation at 4 weeks (Fig. 3 a). A significant increase ($p < 0.01$) in the response to low doses of NE 95 x $10^{-10} - 10^{-8}$ M) was observed also at 12 weeks (Fig. 3 b). However, the contractile response to higher doses of NE at 12 weeks was reduced, although not significantly, when compared to the 4 week study. Significant ($p < 0.05$) differences between the control and DOCA/NaCl group was observed from the calculated ED_{50} obtained from the DRC for norepinephrine. Week 4 ($1.9 \times 10^{-8} \pm 3.1 \times 10^{-9}$ vs $9.7 \pm 2.5 \times 10^{-9}$) and week 12 ($1.3 \times 10^{-8} \pm 3.0 \times 10^{-9}$ vs $4.5 \pm 1.2 \times 10^{-9}$).

The amount of DOCA released from the pellets was calculated to be 1.84 ± 0.14 mg/kg/day for the 4 week DOCA/NaCl group and 1.75 ± 0.10 mg/kg/day for the 12 week DOCA/NaCl group.

Discussion

Hypertension (systolic pressure > 150 mm Hg) resulted after 6 weeks chronic administration of DOCA and NaCl solution to rats. Increase in α-adrenergic and a decrease in β-adrenergic responsiveness was observed prior to the development of hypertension, and maintained during established hypertension. Both *in vivo* and *in vitro* experimental conditions supported these findings. These results suggest that an inverse relationship of the α- and β-adrenergic system is required for the development and maintenance of hypertension in the DOCA/NaCl rat.

An *in vivo* assessment of peripheral adrenergic responsiveness was isoproterenol-induced drinking. This type of thirst may be mediated by way of the renin-angiotensin system (17, 18) or it may be due to a reduction in blood pressure and associated stimulation of vascular or other receptors to initiate a thirst response. Regardless of the mechanism, administration of the non-selective β-adrenergic antagonist propranolol prevents the increase in plasma renin activity, the reduction in blood pressure, as well as the drinking response to administration of isoproterenol (18). Additionally, the β-adrenergic dipsogenic response has been attributed to selective β_2-adrenergic receptors (19). Within two weeks of the DOCA/NaCl treatment, the isoproterenol mediated response was virtually abolished, suggesting a reduced β-adrenergic responsiveness in the DOCA/NaCl-treated group. This attenuated dipsogenic response to isoproterenol is in agreement with previous studies in animals treated either acutely (18) or chronically with desoxycorticosterone. In each of these studies the desoxycorticosterone animals were normotensive at the time of the isoproterenol challenge. The development of hypertension (> 150 mm Hg) did not appreciably alter this attenuated peripheral β-adrenergic response observed in the DOCA/NaCl-treated rats. Taken alone, the reduced dipsogenic response of the DOCA/NaCl-treated rats does not necessarily imply an altered β-adrenergic system, for chronic treatment with DOCA has been shown to reduce plasma renin activity (PRA) as well as the amount of renin released into the circulation when isoproterenol is administered (18). However,

the apparent reduced β-adrenergic responsiveness in the DOCA/NaCl-treated rats can be related to a reduced responsiveness of β-adrenergic receptors that mediate the release of renin in the DOCA/NaCl-treated group as well as the possibility that stored renin was not available for isoproterenol-induced release. Since the drinking response to a 24 h dehydration was similar in both the control and DOCA/NaCl-treated group, it suggests that the attenuated drink response to isoproterenol observed in the DOCA/NaCl-treated group was not a generalized thirst response, but is specific to an altered β-adrenergic responsiveness.

The *in vitro* experiments utilizing rat aortic smooth muscle complement in the *in vivo* studies with respect to the β-adrenergic system while providing additional concurrent information on α-adrenergic responsiveness. A significant decrease in the β-adrenergic receptor mediated relaxation of the aortic smooth muscle was observed in the DOCA/NaCl-treated rat prior to the development of hypertension. This response was further attenuated as blood pressure was elevated. Collectively, this data suggests that DOCA/NaCl treatment reduces the β-adrenergic response at vascular, cardiac and possibly kidney sites and that this reduced responsiveness can be associated with the development and maintenance of hypertension in rats. However, it does not imply any specific alteration in receptor-response mechanism, only that some change has occurred in the overall mechanism, possibly at the level of the receptor or at a level beyond the receptor. A fewer number of β-adrenergic receptors previously reported to occur in both cardiac (21) as well as vascular (22) membranes from DOCA/NaCl-treated hypertensive rats may be implicated. However, it is yet to be evaluated if this reduced number of β-adrenergic receptors occurs before hypertension develops.

At 12 weeks of the study the maximal response to KCl in the DOCA/NaCl-treated rats was decreased slightly suggesting possible structural alterations in the vascular smooth muscle that results in a decrease in contractility because of the increase in blood pressure (17). Higher doses of norepinephrine also demonstrated this decrease. More importantly, though, the increase in sensitivity of the aortic smooth muscle to physiological levels of norepinephrine remained, implicating the increase in α-adrenergic responsiveness as a causative factor in the development of hypertension in the DOCA/NaCl rat.

Numerous studies have supported some participation of the sympathetic nervous system in the initiation of hypertension induced by DOCA/NaCl treatment. Previous emphasis has been devoted to an enhanced α-adrenergic responsiveness as the potential factor in the development of hypertension. However, it has recently been observed that moderate supplementation with NaCl increases α-adrenergic responsiveness without affecting blood pressure or β-adrenergic responsiveness (18). Collectively, the results reported here indicate that by itself the increased α-adrenergic responsiveness is not enough to evaluate blood pressure, but if the increased α-adrenergic responsiveness is coupled with a decreased β-adrenergic response, the net effect could be an increase in blood pressure, possibly by an enhanced peripheral vascular resistance. Additional studies should be undertaken in order to further elucidate the mechanism of this alteration in the peripheral adrenergic system in the DOCA/NaCl model of hypertension, as well as other types of experimentally induced hypertension and essential hypertension.

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