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Cerebral Intraventricular 6-Hydroxydopamine Prevents Vascular Changes in the Mineralocorticoid Hypertensive Rat[1] (42094)

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Abstract. The effect of cerebral intraventricular administration of 6-hydroxydopamine (6-OHDA) on blood pressure and vascular smooth muscle responsiveness in deoxycorticosterone acetate (DOCA)-treated rats was assessed. Rats treated with 6-OHDA and DOCA had significantly lower systolic blood pressures (142 ± 8 mm Hg) than rats treated with DOCA alone (185 ± 5 mm Hg). After 5 weeks of DOCA treatment, femoral arteries and aortae were excised from these rats, cut helically into strips, and placed in a muscle bath to record isometric force. Dose–response curves to serotonin were shifted to the left in femoral arteries from DOCA-treated rats compared to both control and 6-OHDA–DOCA-treated rats (ED_{50}: DOCA = 6.8×10^{-8} *M*, control = 27.9×10^{-8} *M*, 6-OHDA–DOCA = 13.4×10^{-8} *M*). Arachidonic acid, the prostaglandin precursor, produced greater maximal contractions in femoral artery strips of DOCA-treated rats (358 ± 56 mg) than in those from controls (115 ± 31 mg). The maximal response to arachidonic acid in arteries from 6-OHDA–DOCA rats (203 ± 78 mg) was not different from control values. Ouabain produced a greater maximal response in aortic strips from DOCA rats (658 ± 165 mg) compared to those from control (196 ± 72 mg) or 6-OHDA–DOCA (309 ± 87 mg) rats. We conclude that increased vascular responsiveness to serotonin, arachidonic acid, and ouabain in DOCA hypertensive rats is secondary to a central action of the mineralocorticoid.

Mineralocorticoid (i.e., deoxycorticosterone) administration coupled with a high salt intake produces hypertension in several animal species including the rat (1). The exact mechanism through which the mineralocorticoids cause hypertension is not known. Mineralocorticoids, such as deoxycorticosterone acetate (DOCA), act on the kidney to promote sodium and water retention (2–4). The resultant increase in blood volume may trigger a series of reflex changes that could result in elevated arterial pressure (5, 6). Brody and co-workers (7, 8) and others (9) have demonstrated that destruction of periventricular tissue in the preoptic area of the anterior hypothalamus (AV3V) prevents the development of DOCA hypertension. Similarly, depletion of central nervous system catecholamines with 6-hydroxydopamine (6-OHDA) also prevents hypertension in this model (1, 10, 11).

In any model of hypertension, the final effector that maintains the elevated blood pressure is the vasculature. Therefore, it is possible that a "blood pressure regulating center" destroyed by these procedures may be expected to influence vascular reactivity. The purpose of this study was to examine the effect of central catecholamine depletion by 6-OHDA on blood pressure and vascular smooth muscle responsiveness to several agonists in DOCA-treated rats.

Methods. *Treatment of rats.* Sprague–Dawley rats weighing approximately 300 g were used in this study. All animals were maintained on a standard laboratory diet and received drinking water containing 1.0% NaCl and 0.2% KCl *ad libitum.*

All rats had cannulae stereotaxically implanted into the right lateral cerebral ventricle under pentobarbital anesthesia (coordinates from bregma: AP 0.6 mm, ML 1.4 mm, DV 4–5 mm). Following a 5-day recovery period, the rats received either 6-OHDA administered intraventricularly (IVT) as two 250 μg injections 3 days apart or an equal volume (10 μl

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each injection) of 0.1% ascorbate vehicle.[2] Seven days after this treatment, all rats were unilaterally nephrectomized and received either DOCA (200 mg/kg) impregnated in silicone rubber or silicone rubber alone implanted subcutaneously. Thus four groups of rats were used in this study: vehicle–silicone (CONTROL), 6-OHDA–silicone (6-OHDA), vehicle–DOCA (DOCA), and 6-OHDA–DOCA.

Systolic blood pressures were determined by tail cuff measurements in the conscious rat at intervals throughout the experimental period. Isolated vascular strip experiments were performed 5 weeks after the DOCA implantation.

Preparation of arteries. Rats were killed by a blow to the head, and femoral arteries and aortae were excised and stored in physiological salt solution (PSS). The millimolar composition of the PSS was NaCl, 130; KCl, 4.7; KH_2PO_4, 1.18; $MgSO_4{\cdot}7H_2O$, 1.17; $NaHCO_3$, 14.9; dextrose, 5.5; and $CaCl_2{\cdot}2H_2O$, 1.6 (femoral arteries) or 2.5 (aortae); and $CaNa_2$ EDTA, 0.03. Arteries were cut helically into strips (0.8–1.0 × 8–10 mm) under a dissecting microscope and mounted vertically on a glass holder in a muscle bath containing oxygenated PSS (95% O_2, 5% CO_2) at 37°C. The upper ends of the strips were connected to force transducers (Grass FT.03) to record isometric force. A passive force was placed on femoral artery (500 mg) and aortic (1500 mg) strips which allowed a maximum contraction in response to norepinephrine (5.9 × 10^{-6} M). Previous experiments (12, 13) have demonstrated that these levels of passive force are optimal for maximal force generation in response to norepinephrine. Strips were equilibrated for 90–120 min in normal PSS before the initiation of experiments.

Drug protocol. Cumulative concentration–response curves were obtained for norepinephrine, serotonin, and arachidonic acid in femoral artery strips and for ouabain in aortic strips. Responses to norepinephrine and serotonin were normalized to the maximum response to allow assessment of vascular sensitivity.

Drugs. Drugs used were serotonin creatinine sulfate (Sigma Chemical Co.), norepinephrine bitartrate (Sigma Chemical Co.), arachidonic acid (Sigma Chemical Co.), and ouabain (Sigma Chemical Co.).

Statistical analysis. Data are reported as the means ± standard error of the mean (SEM). ED_{50} values (dose of agonist producing a half-maximal response) were determined following logit transformation of dose–response curves. Threshold concentrations were determined from graphical representations of the individual curves. Dose–response curves were analyzed by two-way analysis of variance and between group comparisons were performed using Duncan's multiple range test. An unpaired analysis (Student's t test) was used to compare systolic blood pressures, absolute maximal force responses, ED_{50} values and threshold values between rat groups. Results with $P < 0.05$ were considered statistically significant.

Results. *Blood pressure.* The systolic blood pressures of CONTROL (127 ± 2 mm Hg; $N = 8$) and 6-OHDA groups (128 ± 3 mm Hg; $N = 6$) of rats were not different at the end of the 5-week experimental period. At this time the DOCA group (185 ± 5 mm Hg; $N = 10$) showed a significantly elevated blood pressure compared to the CONTROL group. The 6-OHDA–DOCA group (142 ± 8 mm Hg) of rats had systolic blood pressures that were significantly higher than the CONTROL group of rats, but significantly lower than DOCA-treated rats. Systolic blood pressures of the 6-OHDA group were not statistically different from the 6-OHDA–DOCA group.

Vascular responses. Cumulative addition of agonists caused a dose-dependent contraction in all strips. The sensitivity or half-maximal effective dose (ED_{50}) of norepinephrine was not altered in femoral artery strips from DOCA hypertensive rats compared to the other three groups of rats (Table I). Femoral arteries from DOCA rats were more sensitive to serotonin than arteries from CONTROL rats (Table I, Fig. 1). Vascular sensitivity (ED_{50} value) to serotonin in fem-

[2] This injection volume or a larger volume (15 μl) is commonly used for drug delivery into the lateral cerebral ventricles of rats [see Ref. (1, 10, 14, 15)]. It seems unlikely that this injection procedure was responsible for the experimental observations since all rats (CONTROL and DOCA-treated) received the same injection volume (10 μl of 6-hydroxydopamine in 0.1% ascorbate or 10 μl of 0.1% ascorbate, alone).

TABLE I. ED_{50}, THRESHOLD, AND MAXIMAL RESPONSES OF FEMORAL ARTERY AND AORTIC STRIPS

	ED_{50} (M)	Threshold (M)	Maximal responses (mg)[a]
Norepinephrine			
CONTROL (N = 8)	$5.7\ (\pm 1.1) \times 10^{-8}$	—	618 ± 54
DOCA (N = 10)	$5.1\ (\pm 0.3) \times 10^{-8}$	—	508 ± 61
6-OHDA (N = 6)	$10.9\ (\pm 1.0) \times 10^{-8}$	—	642 ± 86
6-OHDA–DOCA (N = 6)	$8.2\ (\pm 1.0) \times 10^{-8}$	—	656 ± 86
Serotonin			
CONTROL (N = 8)	$27.9\ (\pm 6.8) \times 10^{-8}$	—	671 ± 64
DOCA (N = 10)	$6.8\ (\pm 1.0) \times 10^{-8}$*	—	672 ± 83
6-OHDA (N = 6)	$27.4\ (\pm 6.4) \times 10^{-8}$	—	623 ± 123
6-OHDA–DOCA (N = 6)	$13.4\ (\pm 3.3) \times 10^{-8}$	—	705 ± 82
Arachidonic Acid			
CONTROL (N = 6)	—	$24.9\ (\pm 13.8) \times 10^{-7}$	110 ± 30
DOCA (N = 6)	—	$2.7\ (\pm 1.5) \times 10^{-7}$*	352 ± 70*
6-OHDA (N = 5)	—	$66.9\ (\pm 63.2) \times 10^{-7}$	136 ± 76
6-OHDA–DOCA (N = 5)	—	$13.4\ (\pm 3.3) \times 10^{-7}$	203 ± 78
Ouabain			
CONTROL (N = 7)	—	$3.00\ (\pm 1.30) \times 10^{-4}$	196 ± 72
DOCA (N = 7)	—	$0.68\ (\pm .25) \times 10^{-4}$*	658 ± 165
6-OHDA (N = 5)	—	$3.25\ (\pm 1.84) \times 10^{-4}$	290 ± 104
6-OHDA–DOCA (N = 5)	—	$3.92\ (\pm .50) \times 10^{-4}$	309 ± 87

[a] Asterisks indicate a significant difference from CONTROL at $P < 0.05$.

oral arteries from 6-OHDA–DOCA rats was not different from CONTROL or 6-OHDA rats (Table I). The maximum responses to norepinephrine (5.9×10^{-6} M) and serotonin (5.7×10^{-5} M) were not altered by any of the treatments (Table I).

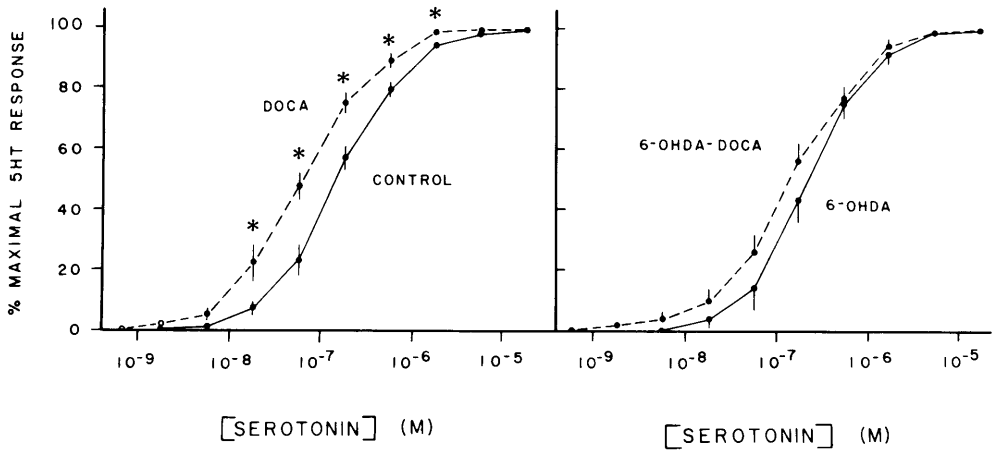

FIG. 1. Effect of serotonin on isometric force of femoral artery strips from CONTROL and DOCA-treated rats (left) and 6-OHDA (IVT) and 6-OHDA–DOCA (IVT)-treated rats (right). Serotonin dose-response curves are normalized to the maximum serotonin response. Data are shown as means ± SEM of 8 and 10 observations for CONTROL and DOCA groups, respectively, and means ± SEM of 6 observations for the 6-OHDA and 6-OHDA–DOCA groups. Asterisks indicate a significant difference from the CONTROL group at $P < 0.05$.

Vascular responsiveness to arachidonic acid was also studied. Femoral arteries from DOCA rats were more responsive to arachidonic acid than arteries from CONTROL rats (Fig. 2). In addition, the threshold concentration, defined as the concentration of arachidonic acid that produced a 50-mg contraction, was significantly lower in strips from DOCA rats (Table I). Intraventricular treatment with 6-OHDA greatly reduced this increased responsiveness to arachidonic acid (Fig. 2). Maximum and threshold responses in vessels from the 6-OHDA–DOCA group of rats were not different from CONTROL or 6-OHDA groups (Table I). The contractile response to arachidonic acid was inhibited by both indomethacin (1.4×10^{-5} M) and acetylsalicylic acid (2.8×10^{-4} M).

The third vascular response examined was the responsiveness of aortic strips to ouabain. Cumulative addition of ouabain to the muscle bath resulted in larger contractile responses in vessels from DOCA hypertensive rats than in those from normotensive CONTROL rats (Fig. 3; Table I). Threshold concentration, defined as the concentration of ouabain that produced a 100 mg contraction, was significantly lower in vessels from DOCA rats compared to CONTROL rats (Table I). Contractile responses to ouabain in the 6-OHDA–DOCA group were not statistically different from the 6-OHDA group (Fig. 3). Threshold concentrations of ouabain in the aortic strips from 6-OHDA–DOCA rats were not different from either CONTROL or 6-OHDA rat group (Table I). Average maximum contractile responses to potassium (130 mM) depolarization were not different in aortic strips from all four groups of rats (680–2200 mg).

Discussion. Central intraventricular injection of 6-OHDA causes a reduction in brain norepinephrine [50–80% reduction in forebrain norepinephrine levels (1, 14)] and prevents the development of DOCA hypertension in rats (1, 10, 11). Previous studies (1, 15) demonstrate that increased vascular sensitivity to norepinephrine and vasopressin does not occur in the renal and mesenteric vasculature of these animals in contrast to sham-treated, DOCA hypertensive rats. The present study confirms these observations on the protective effect of central 6-OHDA treatment on blood pressure and further defines the effects on vascular smooth muscle responsiveness in rats implanted with DOCA.

Vascular sensitivity to serotonin and arachidonic acid in femoral arteries and to ouabain in aortae was increased in rats made hypertensive with DOCA. Sensitivity to these agonists was reduced to control levels in rats treated with intraventricular 6-OHDA prior to implantation of DOCA. Sham-treated rats

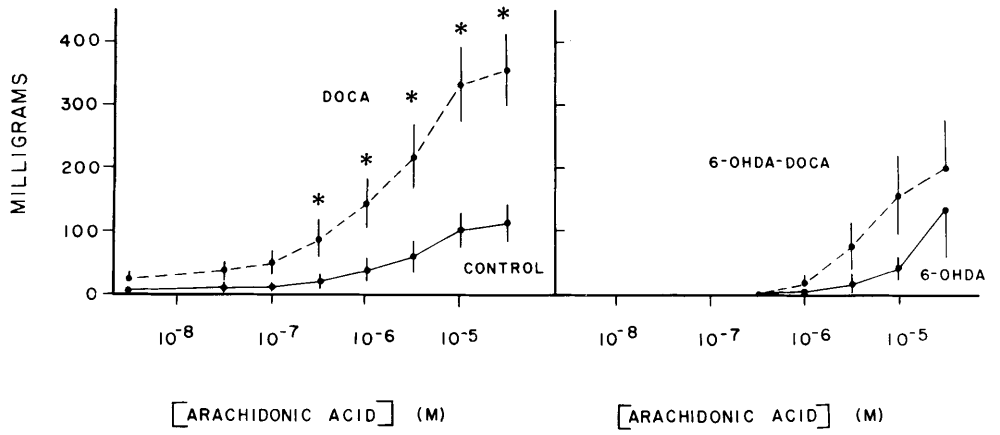

FIG. 2. Effect of arachidonic acid on isometric force of femoral artery strips from CONTROL and DOCA-treated rats (left) and 6-OHDA (IVT) and 6-OHDA–DOCA-treated rats (right). Data are shown as means ± SEM of six observations for CONTROL and DOCA groups, respectively, and means ± SEM of five observations for the 6-OHDA and 6-OHDA–DOCA groups. Asterisks indicate a significant difference from the CONTROL group at $P < 0.05$.

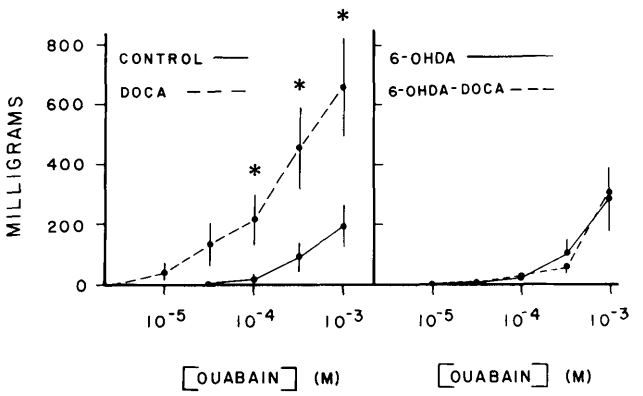

FIG. 3. Effect of ouabain on isometric force of femoral artery strips from CONTROL and DOCA-treated rats (left panel) and 6-OHDA (IVT) and 6-OHDA–DOCA-treated rats (right panel). Data are shown as means ± SEM of seven observations for CONTROL and DOCA groups, respectively, and means ± SEM of five observations for the 6-OHDA and 6-OHDA–DOCA groups. Asterisks indicate a significant difference from the CONTROL group at $P < 0.05$.

showed no changes in vascular sensitivity following intraventricular injection of 6-OHDA. The agonists used in this study are of particular interest since each has been implicated recently as a candidate for maintaining high levels of blood pressure in hypertension. Vascular sensitivity to serotonin is enhanced to a greater extent in hypertension than to several other agonists [norepinephrine, angiotensin II, potassium chloride (16, 17, 18)], and the serotonin antagonist, ketanserin, lowers blood pressure in hypertensive animals and man (19, 20). Arachidonic acid is the precursor of prostaglandins (21) and abnormalities in prostaglandin metabolism may contribute to altered vascular reactivity in hypertension (22, 23). Finally, the contractile response to ouabain is due to the inhibition of the electrogenic sodium pump (24) and alterations in this transport system may play a role in altered vascular reactivity in hypertension (25, 26).

The current study does not indicate whether the observed vascular changes examined are primary or secondary to the development of high blood pressure. It is possible that all the vascular changes observed are the consequence of the elevated blood pressure, and prevention of the rise in blood pressure following DOCA administration by central 6-OHDA treatment therefore eliminates the cause of these changes. However, earlier studies (27, 28) have established that functional changes in vascular smooth muscle occur in DOCA hypertension even if the vessel is protected from the increased wall stress.

The observations of this study suggest that the increased vascular reactivity observed to serotonin, arachidonic acid, and ouabain is not due to a direct action of DOCA and salt on the vascular smooth muscle. The CONTROL animals received sodium in their drinking water and blood vessels from these animals did not demonstrate increased responsiveness to the agents tested [see Ref. (12, 13) for comparisons to rats on tap water]. Furthermore, the DOCA rats treated with 6-OHDA centrally did not develop increased vascular responsiveness, suggesting that direct actions of the mineralocorticoid are not sufficient to produce the vascular changes which characterize this model of hypertension. Experiments performed by Berecek and co-workers (15) demonstrate that DOCA-treated rats do not develop increased vascular sensitivity to norepinephrine and vasopressin when the rats are given a normal dietary intake of sodium. It is possible that DOCA may exacerbate functional vascular changes by a direct action on vascular smooth muscle. Mineralocorticoids act on vascular smooth muscle to cause electrolyte changes (29). However, it appears that these actions of DOCA on vascular smooth muscle are not sufficient to produce hypertension.

In conclusion, the results of this study suggest that central nervous system treatment with 6-OHDA prevents the development and maintenance of DOCA–salt hypertension and can alter the accompanying changes in peripheral vascular responsiveness to several agents (serotonin, arachidonic acid, ouabain).

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