Central and Peripheral Adrenergic Mechanisms in the Development of Deoxycorticosterone-Saline Hypertension in Rats

By **John L. Reid, Justin A. Zivin, and Irwin J. Kopin**

ABSTRACT

The role of the sympathetic nervous system in the development of deoxycorticosterone–sodium chloride (DOCA-saline) hypertension was investigated by measuring plasma levels of norepinephrine, total catecholamines, and dopamine-β-hydroxylase activity at intervals after the initiation of the DOCA-saline regimen. Plasma norepinephrine was significantly higher in DOCA-saline–treated rats at 4 and 7 weeks and in rats treated with saline alone at 4 weeks compared with that in untreated controls. Total plasma catecholamine levels (epinephrine and norepinephrine) and dopamine-β-hydroxylase activity were similar in hypertensive rats, untreated controls, and rats that received either DOCA or saline alone. The increases in plasma norepinephrine levels may have resulted from centrally mediated increases in peripheral sympathetic neuronal activity, since the destruction of central catecholaminergic neurons with intracerebroventricularly administered 6-hydroxydopamine (6-OHDA) prevented both the DOCA-saline–induced rise in blood pressure and the increases in plasma norepinephrine. Rats treated with 6-OHDA consistently drank less water or saline than did vehicle-treated controls. The actions of centrally administered 6-OHDA on blood pressure and plasma norepinephrine levels were not secondary to a reduction in salt intake, however, since intact rats given a similar reduced saline intake became hypertensive and demonstrated elevated plasma norepinephrine concentrations. Chronic salt loading may cause a centrally mediated increase in peripheral sympathetic neuronal activity with raised plasma concentrations of norepinephrine. The increased adrenergic activity in the presence of mineralocorticoid-induced sodium retention leads to the development of hypertension.

■ Increasing evidence suggests that enhanced activity of the peripheral sympathetic nervous system contributes to the development and maintenance of raised arterial blood pressure in several models of hypertension. Turnover of norepinephrine in the heart is increased in renal hypertension (1, 2) or after treatment with the mineralocorticoid deoxycorticosterone and 1% saline for drinking water (DOCA-saline hypertension) in the rat (3, 4) as well as in the neurogenically mediated hypertension following aortic and carotid sinus denervation in the rabbit (5). Urinary excretion of norepinephrine and its metabolites is increased in DOCA-saline hypertension (6), and serum from these hypertensive animals contains a humoral agent that elevates blood pressure (7). Until recently, the absence of sufficiently sensitive, specific assay procedures precluded the direct measurement of norepinephrine and epinephrine in plasma from hypertensive animals.

The central nervous system mechanisms underlying the increases in peripheral adrenergic activity in experimental hypertension have not been clearly defined and may include the modification of sympathetic outflow and changes in the release of hypothalamic trophic hormones. Catecholaminergic neurons in the brain, which utilize norepinephrine as a neurotransmitter, appear to play an important role in the development of hypertension. 6-Hydroxydopamine (6-OHDA), an analogue of dopamine, injected directly into the cerebrospinal fluid of the ventricular system or the cisterna magna causes a long-lasting depletion of brain catecholamines and the degeneration of nerve terminals, particularly those containing norepinephrine (8, 9). Pretreatment with intraventricularly or intracisternally administered 6-OHDA prevents the rise in arterial blood pressure that usually follows renal artery clipping in rats (10) and cellophane perinephritis in rabbits (11). Hypertension following buffer nerve section in rabbits (12) or electrolytic lesions of the nucleus of the solitary tract in rats (13) is similarly abolished. Several groups have reported attenuation of DOCA-saline hypertension in rats pretreated with intracister-

From the Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland 20014.

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Please address reprint requests to Dr. J. L. Reid, Laboratory of Clinical Science/NIMH, Building 10, Room 2D-46, Bethesda, Maryland 20014.

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nally (14) or intraventricularly (10, 15) administered 6-OHDA. Administration of 6-OHDA to the brain also causes a behavioral change resulting in a reduction in water or saline intake (16). It has been suggested that failure to develop hypertension after treatment with DOCA and saline in 6-OHDA-treated rats results from an impairment of saline intake, possibly secondary to diminished fluid excretion, rather than from interference with central pathways regulating peripheral sympathetic activity (16).

In the present study, we used recently developed sensitive radiometric assays (17, 18) to measure plasma levels of total catecholamines and norepinephrine during the development of DOCA-saline hypertension in rats, and we investigated the effects of centrally administered 6-OHDA on saline consumption, plasma catecholamine levels, and arterial blood pressure.

Methods

ANIMALS AND BLOOD PRESSURE RECORDING

Uninephrectomized or sham-operated male Sprague-Dawley rats (100–120 g) were obtained from Zivic Miller Labs, Allison Park, Pennsylvania, and kept under diurnal lighting conditions. The rats were fed a rat pellet diet ad libitum (Wayne Lab Blox Allied Mills, Inc., Chicago, Illinois) and either tap water or 1% (w/v) sodium chloride in water (1% saline to drink).

Systolic blood pressure was measured by a tail cuff plethysmographic method using a pneumatic pulse transducer and a programed electrosphygmomanometer (Narco Biosystems, Houston, Texas, model PE500). Cuff pressure and pulsatile volume changes were recorded simultaneously on a Grass model 5 Polygraph.

Rats were prewarmed in a Perspex cage at 36°C for 10 minutes and habituated to the pressure measurement procedure before the start of the experiment. The tail cuff was inflated at least three times for each rat at each time interval, and the mean systolic blood pressure derived from six measurements (pressures at which pulsations appeared or disappeared) was calculated.

DEOXYCORTICOSTERONE AND SALINE TREATMENT REGIMENS

Hypertension was induced in groups of 8–10 uninephrectomized rats by administering a weekly subcutaneous injection of 25 mg/kg of deoxycorticosterone pivalate (DOCA) (Percorten, Ciba Geigy Corp. Summit, New Jersey) and substituting 1% saline for drinking water (DOCA-saline-treated rats). In studies of the role of circulating catecholamines, three control uninephrectomized groups were examined in parallel. One group received DOCA (25 mg/kg, sc) each week and tap water ad libitum (DOCA group), another was given no DOCA but 1% saline ad libitum for drinking water (saline group), and the final group (controls) drank tap water and received no DOCA. In preliminary experiments, a sham-operated control group that was not given steroid pretreatment and drank tap water ad libitum was kept under identical conditions and compared with the other groups.

CENTRALLY ADMINISTERED 6-HYDROXYDOPAMINE

Fourteen days before the initiation of the DOCA-saline regimen, groups of 7–8 rats were pretreated on two occasions, 24 hours apart, with 200-μg doses (expressed as base) of 6-hydroxydopamine hydrobromide (6-OHDA) (Regis Chemical Co., Chicago, Illinois) by intracerebroventricular injection. These injections were administered using stereotactic coordinates with a small-animal head holder and stereotactic apparatus (D. Kopf Instruments, Tujunga, California) under 1% halothane anesthesia. Control rats received the same volume (20 μliters) of the vehicle (1 mg/ml of ascorbic acid in 0.9% saline).

Two groups of rats given 6-OHDA intracerebroventricularly were examined. One group was treated with DOCA (25 mg/kg, sc) each week and given 1% saline ad libitum to drink (6-OHDA + DOCA-saline group), but the other group did not receive DOCA or saline (6-OHDA group). Three groups treated with intracerebroventricularly administered vehicle were compared. These rats received either DOCA and 1% saline ad libitum (DOCA-saline group), DOCA and a volume of 1% saline equal to the averaged intake of the 6-OHDA + DOCA-saline group in ml/100 g body weight 24 hours^{-1} for the previous day (DOCA-restricted saline group), or tap water ad libitum and no DOCA treatment.

MEASUREMENT OF FLUID CONSUMPTION

Daily fluid intake (water or 1% saline) was measured in all rats in the study in which 6-OHDA was administered intracerebroventricularly and in separate groups of rats receiving DOCA alone or saline alone. These rats were housed in individual cages (15 × 15 × 30 cm) with individual graduated water bottles. Rats were allowed to habituate to the cages for 7–10 days before the experiments were started. Fluid intake was measured daily, and body weight was recorded twice a week. Fluid intake/100 g body weight was calculated using the last recorded weight, and the mean daily intake for each group was derived. As described earlier the mean daily 1% saline intake of the 6-OHDA + DOCA-saline group for the previous day was given to the vehicle + DOCA-restricted saline group so that the saline intake of the two groups was the same. This latter group thus started the DOCA-saline regimen 24 hours later and was maintained 1 day behind the other groups throughout the 5-week study.

TISSUE PREPARATION

In preliminary studies, the levels of total catecholamines and norepinephrine in plasma collected from the lateral tail vein of unanesthetized rats were much lower than those obtained by cardiac puncture or decapitation. This difference was related to the mode of collecting tail vein blood, which involves warming the rats to produce vasodilation (19, 20). Plasma levels of catecholamines are similar in blood obtained from awake rats with an indwelling arterial catheter and blood obtained after decapitation (20). Therefore, experiments were performed using blood obtained after decapitation. The use of anesthetic agents was avoided because of the profound lowering of plasma catecholamine levels in rats following the administration of these agents (20).

At 2, 4, and 7 weeks after the initiation of DOCA-saline regimens, 7–8 rats from treated and control groups were killed by decapitation, and the first 1 ml of blood

from the trunk was collected in an iced heparinized tube through a small glass funnel. The whole blood was centrifuged at 4°C for 5 minutes at 10,000 *g*, and the plasma was harvested. An aliquot of plasma was stored at −20°C for assay of dopamine-β-hydroxylase activity.

For catecholamine assay, protein was precipitated from another aliquot of plasma by adding concentrated perchloric acid to a final concentration of 0.1N, and the mixture was stored at −20°C until assay.

DOPAMINE-β-HYDROXYLASE

Plasma dopamine-β-hydroxylase was measured in duplicate 50-μliter aliquots of plasma by the method of Weinshilboum and Axelrod (21) using 0.03M tyramine as the substrate and optimal copper concentrations (15.2 μM) to overcome endogenous inhibitors. Octopamine internal standards and boiled plasma blanks were run in each assay, and dopamine-β-hydroxylase activity was expressed as nmoles octopamine formed/ml plasma hour^{-1} incubation. Pooled rat plasma samples were also assayed to eliminate variations in the assay due to differences in the activity of the enzyme, phenylethanolamine-N-methyl transferase (PNMT), used in the second stage of the procedure. To exclude changes in endogenous inhibitors in the different treated groups, partially purified bovine adrenal dopamine-β-hydroxylase was added to samples from each assay. No significant change was observed in these dopamine-β-hydroxylase internal standards in plasma from DOCA-saline–treated rats or rats from any of the control groups.

PLASMA CATECHOLAMINES

Plasma total catecholamine levels were measured in 25-μliter aliquots of supernatant fluid from 0.1N perchloric acid–treated plasma by a modification of the method of Coyle and Henry (17) using duplicate samples, 0.1N perchloric acid blanks, and norepinephrine (0.5–1 ng) internal standards. This sensitive radiometric method utilizes a partially purified preparation of catechol-O-methyl transferase (COMT) and ^{3}H-S-adenosyl methionine (^{3}H-S-AME) (specific activity 8.5 mc/μmole) (New England Nuclear Corp., Boston, Massachusetts) and measures both norepinephrine and epinephrine present in plasma. The limit of sensitivity of the assay is 0.02–0.04 ng, which gives values twice those for the perchlorate blanks.

PLASMA NOREPINEPHRINE

Plasma norepinephrine was measured by a specific sensitive radiometric enzymatic method utilizing a partially purified preparation of PNMT (21) and ^{3}H-S-AME (18). The assay, which was a modification of earlier procedures (22, 23), has been described fully elsewhere (18, 19, 24). Aliquots (100 μliters) of the supernatant fluid from 0.1N perchloric acid-treated plasma were incubated in duplicate together with perchloric acid blanks and norepinephrine internal standards (0.5–1 ng). The tritiated product (^{3}H-epinephrine) was absorbed on alumina (Woelm Neutral activity grade A) to separate it from residual ^{3}H-S-AME, and the tritium was assayed by liquid scintillation spectometry. The assay is linear up to 2 ng of norepinephrine, specific, and sensitive (0.03 ng of norepinephrine gives counts twice those for the perchlorate blanks).

BRAIN NOREPINEPHRINE

To confirm the efficacy of the central pretreatment, the norepinephrine concentration was measured in the whole brain of 6-OHDA– and vehicle–treated rats. Brains were homogenized in 0.1 N perchloric acid (1/20, w/v) and centrifuged at 4°C for 10 minutes at 14,000 *g*. Norepinephrine was assayed in 25-μliter aliquots of the supernatant fluid by formation of ^{3}H-methyl-epinephrine using PNMT as described earlier.

STATISTICAL METHODS

Results are presented as the mean ± SE for groups of rats. Differences among groups were tested using one-way or two-way analysis of variance and Newman-Keuls procedures for comparisons of individual means (25) or Student's *t*-test (26).

Results

EFFECT OF UNILATERAL NEPHRECTOMY AND ADRENALECTOMY ON PLASMA CATECHOLAMINE LEVELS

The rats prepared for the DOCA-saline studies were subjected to unilateral nephrectomy 1 week before the DOCA-saline regimen was begun. Since the nephrectomy was accompanied by an ipsilateral adrenalectomy, the norepinephrine and total catecholamine levels in the blood after decapitation were compared with those in uninephrectomized rats and sham-operated controls. Sixteen days after surgery, there was no significant difference in plasma total catecholamine or norepinephrine levels in the two groups of rats (Table 1).

PLASMA NOREPINEPHRINE AND TOTAL CATECHOLAMINE LEVELS IN DOCA-SALINE HYPERTENSION

The blood pressures of the groups of rats examined in the plasma amine study are shown in Figure 1. After only 2 weeks of treatment with DOCA (25 mg/kg) and 1% saline, blood pressure was significantly higher (137.0 ± 2.3 mm Hg) than it was in untreated controls (117.0 ± 4.4 mm Hg) and in both other groups. The rise in blood pressure continued over 7 weeks, but it was most marked between 2 weeks and 4 weeks. The levels of blood

TABLE 1

Effect of Unilateral Nephrectomy and Adrenalectomy on Plasma Norepinephrine and Total Catecholamine Levels

	Norepinephrine (ng/ml)	Total catecholamines (ng/ml)
Unilateral nephrectomy and adrenalectomy	1.08 ± 0.08	6.31 ± 0.58
Sham-operated controls	1.14 ± 0.16	5.86 ± 0.89

All values are means ± SE for groups of eight rats 16 days after either the unilateral nephrectomy and adrenalectomy or the sham-operation. Blood was obtained immediately after decapitation as described in Methods.

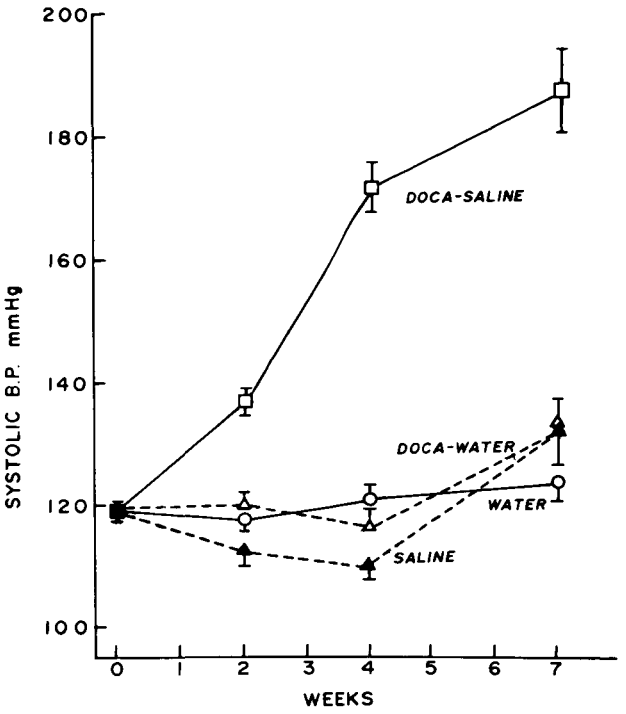

FIGURE 1

Systolic blood pressure (B.P.) (*means* ± SE) *for groups of eight uninephrectomized rats after 2, 4, and 7 weeks of treatment with DOCA (25 mg/kg, sc, weekly) and 1% saline in place of tap water (open squares), DOCA (25 mg/kg) and tap water (open triangles), and 1% saline as drinking water with no DOCA (solid triangles). Control rats (open circles) received tap water for drinking.*

pressure in the groups treated with saline alone or with DOCA alone were not significantly greater than those in the untreated control groups.

After 2, 4, and 7 weeks of treatment with DOCA and 1% saline or with control regimens, plasma norepinephrine was measured by the method using PNMT. Table 2 shows the levels of circulating norepinephrine in these groups of rats. Plasma levels of norepinephrine in the DOCA-saline group were higher than those in the water-fed controls at 2 weeks, but at this time the increase did not achieve significance. However, circulating norepinephrine was significantly elevated in the hypertensive rats at both 4 weeks and 7 weeks compared with that in uninephrectomized controls drinking tap water (Table 2).

Plasma norepinephrine was increased 4 weeks after substitution of 1% saline for drinking water in the absence of mineralocorticoid (saline alone) (Table 2), although at this time the blood pressure of the saline-fed group was actually lower than that of controls (Fig. 1) and the norepinephrine level was not significantly greater than it was in the group given DOCA alone. In rats receiving only saline, plasma norepinephrine returned to control levels at 7 weeks (Table 2). In rats given DOCA and water, plasma levels of norepinephrine were not significantly altered at any time.

Plasma total catecholamines in the various experimental groups did not differ significantly from those in sham-operated controls at any time interval (Table 2). In the DOCA-saline groups at 4 and 7 weeks, in which plasma norepinephrine was significantly elevated, plasma total catecholamine levels were slightly lower than or not different from those in uninephrectomized controls, suggesting that circulating levels of epinephrine were unchanged or slightly reduced in DOCA-saline hypertension. Total catecholamines in plasma were higher at 2 weeks in rats fed saline alone than they were in the other three groups, although the difference did not achieve significance.

The ratio of norepinephrine to total catecholamines is also shown in Table 2. In uninephrectomized controls norepinephrine ranged from 21.5 to 15.7% of total catecholamines, but in the DOCA-saline group norepinephrine represented 32.2, 34.8, and 23.9% of total catecholamines at 2, 4, and 7 weeks, respectively. At 4 weeks, norepinephrine represented a significantly higher percent of total catecholamines than it did in any of the other groups. In the group given only saline, plasma norepinephrine was a similar percent (22.8, 23.5, 14.8%) of total catecholamines as it was in controls.

Although total catecholamine concentrations in plasma did not differ from controls at any time interval, there were indications that the relationship between norepinephrine and epinephrine was altered in DOCA-saline hypertension. The proportion of norepinephrine in plasma was increased in the hypertensive (DOCA-saline) group and unchanged in the normotensive (saline alone) rats.

PLASMA DOPAMINE-β-HYDROXYLASE ACTIVITY IN DOCA-SALINE HYPERTENSION

There were no consistent changes in dopamine-β-hydroxylase activity in plasma in the four groups tested at 2, 4, and 7 weeks. When rats receiving active treatments were compared with uninephrectomized water-fed controls (Table 3), minor differences occurred between the control groups at different times which could be attributed to variations within individual assay days; these differences were eliminated when allowance was made for similar differences in activity in the pool of rat plasma used throughout the study. There was no correlation between plasma, dopamine-β-hydroxylase activity and plasma norepinephrine levels. Changes in circulating norepinephrine were not

TABLE 2

Plasma Norepinephrine and Total Catecholamine Levels in DOCA-Saline Hypertensive Rats and Controls

	Water	Saline	DOCA and water	DOCA and saline
2 Weeks				
NE (ng/ml)	1.14 ± 0.16	1.92 ± 0.28	1.31 ± 0.37	1.95 ± 0.39
CA (ng/ml)	5.86 ± 0.89	9.64 ± 1.58	5.41 ± 0.68	6.57 ± 1.04
NE/CA	0.22 ± 0.04	0.23 ± 0.04	0.22 ± 0.04	0.32 ± 0.05
4 Weeks				
NE (ng/ml)	0.79 ± 0.13	1.40 ± 0.24*	1.29 ± 0.31	1.61 ± 0.20*
CA (ng/ml)	5.75 ± 1.27	5.96 ± 0.81	4.76 ± 1.23	5.61 ± 0.79
NE/CA	0.21 ± 0.04	0.24 ± 0.12	0.29 ± 0.06	0.35 ± 0.08*
7 Weeks				
NE (ng/ml)	0.76 ± 0.04	0.94 ± 0.13	0.95 ± 0.12	1.11 ± 0.13*
CA (ng/ml)	5.67 ± 0.74	8.15 ± 1.53	7.87 ± 1.23	5.08 ± 0.97
NE/CA	0.16 ± 0.02	0.15 ± 0.04	0.17 ± 0.05	0.24 ± 0.05

Plasma norepinephrine (NE) and total catecholamine (CA) levels were measured in DOCA-saline hypertensive rats and three control groups after 2, 4, and 7 weeks of treatment. All values are means ± SE; there were eight rats in each treatment group at each time interval.

* $P < 0.05$ when the data were analyzed by two-way analysis of variance and the means of the treated groups were compared with the respective control water group.

associated with parallel rises in dopamine-β-hydroxylase, nor could the enzyme activity be related to the level of blood pressure.

BODY WEIGHT CHANGES AFTER DOCA-SALINE HYPERTENSION

At the start of the studies body weight ranged from 100 to 120 g. There was no difference between the mean weights of the different groups. Throughout the 7-week treatment period there was no significant difference between control groups and rats treated with DOCA and tap water. However, after 4 weeks and 7 weeks, DOCA-saline groups had a significantly smaller weight gain than did control rats ($P < 0.01$). Rats treated with saline alone had a lower body weight than did control groups, but this difference was not significant. Mean body weights ± SE for the four groups at 7 weeks are shown in Table 4.

INTRACEREBROVENTRICULARLY ADMINISTERED 6-HYDROXYDOPAMINE AND DOCA-SALINE HYPERTENSION

Effect on Fluid Consumption.—The substitution of 1% saline for drinking water led to a considerable increase in daily fluid consumption. The increased fluid consumption occurred in rats given 1% saline alone as well as in those given saline together with DOCA (25 mg/kg, weekly) (Table 5), although intake expressed as ml/100 g body weight day^{-1} was greater in the latter group. DOCA alone did not significantly alter the daily intake of water.

Rats pretreated with intracerebroventricularly administered 6-OHDA 2 weeks before, drank less water than did vehicle-treated controls and this lower daily intake persisted throughout the 5-week study (Fig. 2). However, when 1% saline was substituted for tap water and weekly DOCA injections were begun, 6-OHDA-pretreated rats increased

TABLE 3

Plasma Dopamine-β-Hydroxylase Activity in DOCA-Saline Hypertension

	Water	Saline	DOCA and water	DOCA and saline
2 Weeks	100 ± 6.0	99.12 ± 6.6	90.8 ± 3.3	87.6 ± 5.9
4 Weeks	100 ± 9.6	109.6 ± 9.1	102.1 ± 13.5	118.9 ± 6.7
7 Weeks	100 ± 10.1	105.1 ± 10.4	77.0 ± 9.0	94.0 ± 5.6

Plasma dopamine-β-hydroxylase activity is expressed as the mean (± SE) percent of the activity in uninephrectomized controls at each time interval. The mean dopamine-β-hydroxylase activity in control groups was 11.81 ± 0.66 nmoles octopamine formed/ml plasma hour^{-1} incubation for groups of eight rats on each treatment regimen at each time interval. There were no significant differences between treated and control groups when the data were analyzed by two-way analysis of variance.

TABLE 4

Body Weights of Rats after 7 Weeks

Group	Body weight (g)
Uninephrectomy + water	406 ± 11
Uninephrectomy + water with DOCA	430 ± 12
Uninephrectomy + saline	394 ± 12
Uninephrectomy + saline with DOCA	296 ± 15*

All values are means ± SE determined after 7 weeks of treatment.

* $P < 0.01$ compared with the value for uninephrectomy + water by Student's t-test.

their consumption of saline. At all weekly intervals up to 5 weeks, the average daily saline intake was lower in 6-OHDA–pretreated rats than it was in vehicle-treated rats receiving the DOCA-saline regimen and significantly lower at 2, 3, and 4 weeks (Fig. 2). Although rats pretreated with centrally administered 6-OHDA had a lower daily water intake than did controls, consumption increased 3–4 times in both groups when 1% saline was substituted.

Effect on Blood Pressure.—Blood pressure was measured at weekly intervals in five groups of rats. Two groups (those receiving only 6-OHDA or vehicle intracerebroventricularly) served as controls. Two other groups treated with 6-OHDA or vehicle received DOCA weekly and unrestricted 1% saline; another vehicle-treated group received DOCA weekly, but its saline intake was limited to the volume consumed by the 6-OHDA + DOCA-saline–treated rats 24 hours earlier.

Blood pressure increased in the DOCA-saline–treated intact rats, but there was little change in the control groups (Fig. 2). Rats pretreated with centrally administered 6-OHDA and given DOCA and saline did not show the same pattern of increase in blood pressure as did the intact controls. Only at 5 weeks was the blood pressure in this group significantly higher than that in controls, and at this time it was 50 mm Hg lower than that in vehicle-treated DOCA-saline groups. Although the response of the 6-OHDA–treated rats to the DOCA-saline regimen was profoundly modified, this change was not due to lower saline consumption. When the DOCA-treated intact rats were restricted to the same saline intake as the 6-OHDA–treated rats, they showed a rise in arterial blood pressure almost indistinguishable from that in the unrestricted group (Fig. 2).

Although centrally administered 6-OHDA clearly influences fluid consumption and an adequate saline intake is essential for the development of DOCA-saline hypertension, the interference with the development of DOCA-saline hypertension resulting from central catecholaminergic neuron destruction cannot be accounted for solely by a diminished fluid intake.

Effect of Centrally Administered 6-OHDA on Whole Brain Norepinephrine Levels.—After 5 weeks of DOCA-saline treatment, the norepinephrine concentration in the brains of intact hypertensive rats was 395.2 ± 41.5 ng/g wet weight; in 6-OHDA–pretreated rats, the brain norepinephrine concentration was 145.7 ± 17.6 ng/g, which represents a significant ($P < 0.01$) fall to 36.9 ± 4.4% of the level in vehicle-treated controls.

Effect on Plasma Total Catecholamine and Norepinephrine Levels.—Plasma norepinephrine measured after 5 weeks of DOCA and saline was significantly higher in both unrestricted and restricted saline intake groups compared with that in

TABLE 5

Effect of Deoxycorticosterone (DOCA) and 1% Saline on Daily Fluid Consumption in Uninephrectomized Rats

	Mean daily fluid intake (ml/100 g body wt day^{-1})		
	1–2 weeks	3–4 weeks	4–5 weeks
Uninephrectomy + water	14.7 ± 1.7	13.2 ± 1.8	12.7 ± 0.7
Uninephrectomy + water with DOCA	13.2 ± 1.3	12.1 ± 0.8	12.4 ± 1.2
Uninephrectomy + saline	23.2 ± 4.1	35.4 ± 2.4*	38.0 ± 1.0*
Uninephrectomy + saline with DOCA	30.5 ± 1.0*	41.2 ± 3.1*	44.2 ± 1.4*

Results are expressed as ml of water or 1% saline/100 g body weight day^{-1} at 1–2, 3–4, and 4–5 weeks after initiation of the DOCA-saline regimen and are means ± SE for groups of eight rats. DOCA (25 mg/kg, sc) was administered each week.

* $P < 0.01$ compared with the value for uninephrectomy + water by Student's t-test.

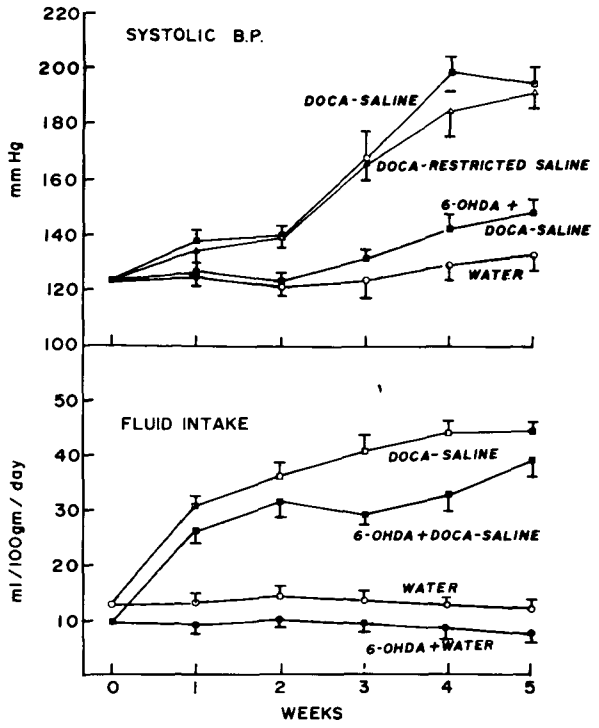

FIGURE 2

Systolic blood pressure (B.P.) *and daily fluid intake* (*means* ± SE) *in groups of 6–8 uninephrectomized rats pretreated with either intracerebroventricularly administered 6-hydroxydopamine (6-OHDA) (200 μg × 2) or ascorbic acid–saline vehicle 2 weeks before beginning weekly DOCA administration and unrestricted 1% saline for drinking water or weekly DOCA administration and 1% saline restricted to the volume consumed by the group of 6-OHDA–pretreated rats the previous day.*

uninephrectomized controls (Table 6). The group pretreated with centrally administered 6-OHDA, in whom blood pressure did not rise following DOCA-saline treatment (Fig. 2), did not have increased levels of norepinephrine in the plasma (Table 6). We have previously observed no significant effect of centrally administered 6-OHDA on plasma norepinephrine levels in water-fed rats (unpublished observations).

Plasma total catecholamine levels did not differ significantly in any treated groups from those in the control group. Thus, the ratio of norepinephrine to total catecholamines was increased in both the DOCA-saline-vehicle-treated groups and similar to that in controls in the 6-OHDA-DOCA-saline-treated rats.

Discussion

Increased ingestion of salt over prolonged periods leads to hypertension in certain strains of rats (27–29). The administration of a mineralocorticoid together with a high salt intake facilitates the development of hypertension (30, 31).

Structural changes in peripheral arterioles appear to participate in the maintenance of hypertension (32, 33); however, they appear to be a later, secondary manifestation. It is unlikely that this factor plays a significant part in the initiation and early development of the raised arterial blood pressure. Neurogenic mechanisms appear to play a role in the development of several experimental hypertensive models including DOCA-saline hypertension. Immunological and chemical sympathectomies have been used to further define the role of postganglionic sympathetic neurons in this model of hypertension, but the results have been conflicting. Immunosympathectomy with antibody to nerve growth factor prevents both DOCA-saline and renal clip hypertension (34). Chemical sympathectomy using intravenously administered 6-OHDA in large doses attenuated the response to

TABLE 6

Effect of Intracerebroventricularly Administered 6-Hydroxydopamine on Plasma Norepinephrine and Total Catecholamine Levels in DOCA-Saline Hypertension

Intracerebroventricular pretreatment	N	Regimen	Norepinephrine (ng/ml)	Total catecholamines (ng/ml)
Vehicle	6	Control (water)	0.85 ± 0.15	9.86 ± 0.98
6-OHDA	6	DOCA + unrestricted saline	0.92 ± 0.22	8.58 ± 2.01
Vehicle	6	DOCA + restricted saline	1.60 ± 0.12*	9.23 ± 0.74
Vehicle	4	DOCA + unrestricted saline	1.30 ± 0.15*	6.83 ± 0.68

All values are means ± SE. Plasma norepinephrine and total catecholamine levels were determined in blood collected following decapitation after 5 weeks of DOCA-saline or control regimens. Intracerebroventricular injections of 6-OHDA (200 μg) or ascorbate-saline vehicle were given twice, 24 hours apart, 7 weeks before death.

* $P < 0.05$ when analyzed by one-way analysis of variance.

DOCA and saline (35) in one study but did not influence the blood pressure in others (36, 37). Neither 6-OHDA treatment nor immunosympathectomy produce a complete and lasting destruction of the sympathetic neurons, particularly not of those in small arterioles of peripheral vascular beds. The adrenal medulla, which is unaffected by intravenously administered 6-OHDA, may compensate for the loss of sympathetic nerve endings by increasing the synthesis of catecholamines (38). de Champlain and van Ameringen (39) have shown that although intravenously administered 6-OHDA or adrenalectomy separately reduces the blood pressure of DOCA-saline hypertensive rats by only a small amount the combination of the two procedures leads to a profound fall in blood pressure.

Extensive studies on peripheral catecholamine metabolism in the DOCA-saline model have revealed increased turnover of norepinephrine in the heart and other peripheral tissues (3, 4) together with reduced tissue levels of the neurotransmitter (3) and an apparent storage defect (40, 41). The urinary excretion of norepinephrine and its metabolites is increased in these hypertensive rats (6).

We observed increases in plasma norepinephrine levels in rats developing DOCA-saline hypertension. The magnitude of the increase was modest compared with the magnitude of that following pharmacological maneuvers such as phenoxybenzamine pretreatment, which causes 4–5-fold increases in plasma levels of norepinephrine (25). However, in DOCA-saline hypertension there is no evidence that the mechanisms of inactivation of norepinephrine are impaired. Neuronal reuptake is unchanged in DOCA-saline hypertension (42) as is the activity of the degradative enzyme, catechol-O-methyl transferase (42). Cardiac activity of monoamine oxidase is increased, but this change probably is a consequence of cardiac hypertrophy (42). Steroids may impair extraneuronal uptake (43) but this change probably does not contribute to the elevated plasma levels of norepinephrine, since total catecholamine levels are not changed and amine levels are not altered in groups treated with DOCA alone. It is most likely that the raised plasma levels of norepinephrine result from increased neurotransmitter release as a result of increased sympathetic neuronal activity. The enhanced neurotransmitter release leads to increases in norepinephrine turnover in nerve endings as previously reported (3, 4) and increases in urinary excretion of the amine and its metabolites (6).

This increased release may reflect increasing maturity of the rats, or it could be related to minor changes in ambient temperature at the time of death. Relatively small changes in environmental temperature can affect plasma norepinephrine levels (19).

Studies on plasma norepinephrine and total catecholamines in intact and bilaterally adrenalectomized rats (20) indicate that circulating norepinephrine is principally derived from transmitter released from adrenergic nerve endings. Epinephrine, which constitutes the remainder of the circulating total catecholamines, derives from adrenal medullary secretion.

The present data suggest that the sympathetic nervous component of the sympathoadrenal system is responsible for the increase in plasma norepinephrine levels observed in DOCA-saline hypertension.

The central adrenergic contribution to the development of DOCA-saline hypertension has been assessed by destruction of these neurons with 6-OHDA administered by intracisternal or lateral cerebroventricular injection. When given by these routes, the catecholamine-depleting and neuronal-destroying actions of 6-OHDA are limited to central noradrenergic and dopaminergic neurons within the brain and the spinal cord (8, 9). There appears to be little effect of 6-OHDA on adrenergic neurons in the central nervous system, since intracisternally administered 6-OHDA does not decrease levels of epinephrine or phenylethanolamine-N-methyl transferase in the central nervous system (unpublished observations). The destructive effects are most marked in the spinal cord where endogenous norepinephrine is reduced to less than 10% of the level in vehicle-treated controls (12, 44). Central 6-OHDA pretreatment prevents the rise in blood pressure when animals are subsequently treated with DOCA and 1% saline (10, 14–16). When 6-OHDA is given up to 3 weeks after the DOCA-saline regimen has been started, hypertension is prevented or reversed (14). The decreased turnover of norepinephrine in the brainstem of DOCA-saline–treated rats (45) may be a reflection of an attempt to decrease noradrenergic activity and compensate for the increased blood pressure, which is more effectively done by destruction of these neurons by 6-OHDA. After 4 weeks of treatment with DOCA and saline, centrally administered 6-OHDA does not influence the level of blood pressure (14). The central neurogenic contribution is thus most prominent during the early stages of the development of the hypertension but not essential once the hypertensive state has been achieved. In the present study, plasma norepineph-

rine levels in rats treated with DOCA-saline for 7 weeks were elevated compared with those in controls but were lower than those in rats treated for 4 weeks. In all groups at 7 weeks, plasma norepinephrine levels were lower than they were at 4 weeks. This fact may also reflect the increasing maturity of the rats or could be related to minor changes in ambient temperature at the time of death, since relatively small changes in environmental temperature may affect plasma norepinephrine (19).

The central nervous system could facilitate the development of DOCA-saline hypertension by hypothalamically mediated changes in fluid consumption in response to the introduction of 1% saline in place of tap water, since centrally administered 6-OHDA can cause profound adipsia and aphagia (46) similar to the reaction following lesions of the lateral hypothalamus (47). Treatment with 6-OHDA does result in a deficit of intake of both water and 1% saline (16 and Fig. 2). However, the lower saline intake could not entirely account for the failure of the blood pressure to rise, since intact DOCA-treated rats fed the same reduced amount of saline still developed hypertension. Lewis et al. (48) in a recent communication have reported similar findings and concluded that a change in saline intake is not the mechanism of action of centrally administered 6-OHDA.

An alternative mechanism by which the central nervous system could participate in DOCA-saline hypertension is through increases in sympathetic outflow. Plasma norepinephrine but not epinephrine is increased in hypertensive rats given DOCA and saline whether saline intake is unrestricted or restricted. However, in rats pretreated with 6-OHDA and given DOCA and saline, plasma norepinephrine is not different from that in untreated controls and blood pressure is not elevated. These data support the hypothesis that the development of DOCA-saline hypertension is dependent on centrally mediated increases in peripheral adrenergic activity.

Plasma dopamine-β-hydroxylase has been proposed as an index of peripheral sympathetic activity (21). Geffen et al. (49) and Schanberg et al. (50) have described correlations between plasma dopamine-β-hydroxylase activity and arterial blood pressure in man. Other reports do not support these observations (51). In the present study, there was no discernible relationship between either plasma norepinephrine or blood pressure and plasma dopamine-β-hydroxylase activity. The data support previous observations (25) that in the rat plasma dopamine-β-hydroxylase activity does not necessarily change with alterations in plasma norepinephrine levels.

Rats receiving 1% saline without DOCA also had modest increases in plasma levels of norepinephrine at 2 and 4 weeks, although their blood pressures were not elevated. Thus, increased plasma catecholamines alone are not sufficient to produce hypertension. This observation has been previously noted in a study of hypertensive subjects and depressed, normotensive patients (52). The substitution of 1% saline for drinking water, with the greatly increased fluid intake which follows, could be a stressful stimulus which leads to increased sympathetic activity. In this light, mineralocorticoid-induced sodium retention with increased total body sodium (53) may result in the expression of the hypertension.

Other factors may participate in the development of raised blood pressure. Vascular smooth muscle from animals with DOCA-saline hypertension has been reported to show increased reactivity to vasoconstrictor stimuli (7, 33, 34, 54). This enhanced reactivity could result from morphological changes in the vessel wall (33) or from alterations in the density or affinity of specific receptors for vasoconstrictor agents. The presence of such changes in the peripheral resistance vessels could potentiate the effects of the increased levels of circulating norepinephrine.

The development and maintenance of hypertension following mineralocorticoid administration and chronic salt loading appear to be related to the interaction of several factors. Early increases in peripheral sympathetic neuronal activity and circulating levels of catecholamines in the presence of sodium retention and increased vascular reactivity lead to an elevation of blood pressure which may later be maintained in the absence of the initial neurogenic trigger.

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