Pharmacology Biochemistry and Behavior, Vol. 2, pp. 537–543. ANKHO International Inc., 1974. Printed in the U.S.A.

Cardiovascular Changes Following DOCA/NaCl or Conditioning in 6-Hydroxydopamine-Treated Rats

JAMES L. HOWARD, RONALD D. SMITH, ROBERT A. MUELLER AND GEORGE R. BREESE

Departments of Psychiatry, Pharmacology and Anesthesiology, Biological Sciences Research Center, Child Development Institute, University of North Carolina School of Medicine Chapel Hill, North Carolina 27514

(Received 27 February 1974)

HOWARD, J. L., R. D. SMITH, R. A. MUELLER AND G. R. BREESE. *Cardiovascular changes following DOCA/NaCl or conditioning in 6-hydroxydopamine-treated rats.* PHARMAC. BIOCHEM. BEHAV. 2(4) 537–543, 1974. – Two intracisternal injections of 200 μg 6-hydroxydopamine reduced brain catecholamine levels 90% and significantly lowered resting heart rate and blood pressure. In a classical aversive conditioning paradigm, 6-hydroxydopamine-treated rats displayed little or no conditioned heart rate response in anticipation of shock, but a potentiated unconditioned response to shock itself. The alteration in heart rate responses may have been due in part to alterations in general activity. Although 6-hydroxydopamine treatment did not abolish the hypertension caused by DOCA/NaCl treatment following uninephrectomy, the increase in blood pressure was significantly less than the blood pressure increase in control rats receiving this treatment. The 6-hydroxydopamine treatment, however, concomitantly reduced the amount of NaCl consumed after DOCA. Since peripheral tyrosine hydroxylase activity and amine levels were not significantly altered by 6-hydroxydopamine treatment, the alterations in cardiovascular responses following 6-hydroxydopamine must result from its central actions. Although 6-hydroxydopamine administration markedly altered the cardiovascular responses to conditioned stimuli, shock, and DOCA/NaCl treatment, it is difficult to ascribe these alterations to ablation of central catecholamine fibers participating directly in cardiovascular control. The link between destruction of catecholamine fibers and changes in cardiovascular responses may be secondary to changes in activity or ingestive behavior.

6-Hydroxydopamine Cardiovascular system Cardiovascular conditioning Experimental hypertension Central catecholamines

DURING experiments examining biochemical physiological, and behavioral effects of 6-hydroxydopamine, alterations in cardiovascular function have been observed [11, 12, 16]. Treatment with 6-hydroxydopamine has been reported to reduce heart rate [4,12] to alter the course of experimental hypertension [13] and to reverse the blood pressure response to footshock [27].

In the present study, the effects of 6-hydroxydopamine administered in combination with pargyline on cardiovascular changes produced by classical aversive conditioning or on the hypertension produced by desoxycorticosterone (DOCA) plus NaCl were examined. The ability of 6-hydroxydopamine to affect a variety of functions other than sympathetic outflow was also evaluated in an effort to understand the cardiovascular effects produced.

METHOD

Male Sprague-Dawley rats supplied by Zivic Miller Laboratories (Allison Park, Pa.) were used in all experiments. Rats initially weighing 180–200 g were given two doses of 6-hydroxydopamine (200 μg) intracisternally, one dose 30 min after pargyline (50 mg/kg) and the second dose one week later without pargyline pretreatment [2,3]. Control animals were injected with pargyline 30 min before intracisternal administration of 25 μl of ascorbic acid vehicle (0.5%). Animals treated in this way usually displayed a severe hypophagia and hypodipsia following the second intracisternal injection of 6-hydroxydopamine [1]. Survival of 80 to 90 percent of the animals were achieved by keeping them warm (group housing) and by giving nutritional supplement for 7 to 10 days as previously described [1]. Animals were allowed at least 2 weeks recovery before being used in any of the experimental procedures. All animals were individually caged and housed in a room with 10 hr light and 14 hr darkness.

After a recovery period of 10 days, baseline blood pressure was determined at weekly intervals by a tail plethysmographic method [21]. In order to produce an experimental hypertension [6] unilateral nephrectomized animals were given 25 mg/kg desoxycorticosterone acetate (DOCA) at weekly intervals and allowed free access to a 1%

saline solution. In some animals, saline consumption was determined at 24 hr intervals.

Other cardiovascular and behavioral measures were recorded with a Beckman dynograph. EKG was recorded from stainless steel safety pins placed subdermally in the thorax. A cardiotachometer converted the R-R intervals to beats per minute. Activity was monitored from a pressure sensitive floor.

Classical conditioning was carried out with the animal placed in a sound attenuated chamber using a method similar to that suggested by Seligman [22] to provide a within subject control for sensitization effects. Two speakers located next to the animal were driven individually by two tone generators which delivered 500 and 1000 Hz tones, respectively, at an intensity clearly audible over background noise. The unconditioned stimulus (UCS) was delivered from a constant current shock source through a tail electrode (26) or through the grid floor at an intensity that produced heart rate acceleration ($\bar{X}$ = 0.4 mA). One control and one 6-hydroxydopamine-treated animal were run simultaneously with the tail shock electrodes or the grid floors in series in an attempt to ensure that both animals would receive identical current intensities. Tone and shock presentations were controlled with solid state circuitry. Two tones, each of 10 sec duration, were presented to the animal. One tone (CS^+) was consistently associated with shock, while the other (CS^-) was randomly distributed and was followed by shock only when it occurred concomitantly with CS^+. The average interval between CS^+ presentations was 70 sec (range 50–90 sec), as was that of CS^-. This resulted in 30 CS^+, 30 CS^- and 5 combined presentations during the 40 min conditioning period.

For analysis of the heart rate conditioning data, CS^- trials were excluded if they occurred within 20 sec of a UCS, to prevent artifactual heart rate changes resulting from the UCS. Trials in which CS^+ and CS^- occurred together were not analyzed. Trials included in the analysis were quantified by averaging heart rate for the 5 sec prior to CS onset (Preperiod) and on a sec-by-sec basis for each second of 17 sec following tone onset. For each trial the difference between the average heart rate value of the preperiod and the average heart rate value for each sec of 17 sec following the tone onset was obtained. Data were analyzed using analysis of variance or Student's *t*-test.

Following completion of the experiments, animals were killed by cervical fracture. Brains were removed and rinsed in cold water and divided into two parts on ice. One brain half was frozen on dry ice and kept at −76° C until analyzed for tyrosine hydroxylase, and the other half was immediately homogenized in 0.4 N perchloric acid and brain catecholamine content determined on the supernatant [2,3]. As required for catecholamine determinations hearts and adrenal (pairs) were removed and homogenized in 0.5 ml 0.24 M sucrose. Tyrosine hydroxylase was isolated from brain tissue [19] and enzyme activity was determined by minor modification of the method of Nagatsu *et al.* [20]. Ganglionic tyrosine hydroxylase activity was assayed according to the method described by Levitt *et al.* [17]. The 3,5H^3-tyrosine (24.7 c/mMole, New England Nuclear Corp.) was purified as described by Mueller *et al.* [18].

The 6-hydroxydopamine HBr was purchased from Regis Chemical Company (Chicago, Ill.), and the desoxycorticosterone trimethylacetate from Ciba (Summit, New Jersey).

TABLE 1

CHRONIC EFFECT OF 6-HYDROXYDOPAMINE TREATMENT ON RESTING BLOOD PRESSURE AND HEART RATE*

Treatment†	Blood Pressure (mm Hg)	Heart Rate (beats/min)
Control	123 ± 4.3	387 ± 9.5
6-Hydroxydopamine	99 ± 2.4 ‡	356 ± 10.9‡

*See Methods. Each value represents the mean ± SEM of at least 8 rats.

†Control rats weighed 477 ± 11 g and 6-hydroxydopamine-treated rats 384 ± 16 g at the time blood pressure and heart rate measurements were made.

‡Different from control values beyond the 0.05 level by two-tailed *t*-test.

RESULTS

Chronic Effect of Intracisternal 6-Hydroxydopamine Treatment on Resting Blood Pressure

Intracisternal administration of 6-hydroxydopamine (200 μg) with pargyline pretreatment (50 mg/kg) followed 7 days later by an additional dose of 6-hydroxydopamine (200 μg) without pargyline produced a drastic reduction of brain norepinephrine and dopamine content (Table 2). Animals treated in this way were found to have a significantly lower resting blood pressure when compared with control 3 weeks after injection (Control = 127 ± 3.5 mmHg; 6-hydroxydopamine = 110 ± 4.4 mmHg; $p<0.01$. Blood pressure remained significantly below normal for as long as 11 weeks after 6-hydroxydopamine treatment suggesting that this effect on blood pressure may be a permanent one (Table 1). A significant reduction of heart rate was also apparent in treated animals (Table 1).

Effect of 6-Hydroxydopamine on Heart Rate Responses in a Classical Aversive Conditioning Paradigm

The 6-hydroxydopamine injection into half of the animals and the use of footshock versus tail shock resulted in 4 treatment groups with 8 animals in each group. The results are displayed in Fig. 1. The two saline-injected groups both displayed conditioned heart rate responses to the CS^+ with heart rate decreasing prior to footshock and increasing prior to tailshock. Neither tailshock nor footshock produced a conditioned response in 6-hydroxydopamine-treated animals. This pattern of results gave a Drug treatment × Shock location × CS type × Seconds interaction, $F(9,252) = 3.03$, $p<0.01$, and subsequent analyses produced CS type × Seconds interactions in the control footshock group, $F(9,63) = 4.36$, $p<0.01$, and control tailshock group, $F(9,63) = 7.18$, $p<0.01$, but not in either 6-hydroxydopamine-treated group. As is apparent, 6-hydroxydopamine treatment did not block but rather enhanced the heart rate increase to shock (Fig. 1). This was substantiated by the drug treatment × Seconds interaction, $F(4,112) = 14.80$, $p<0.01$).

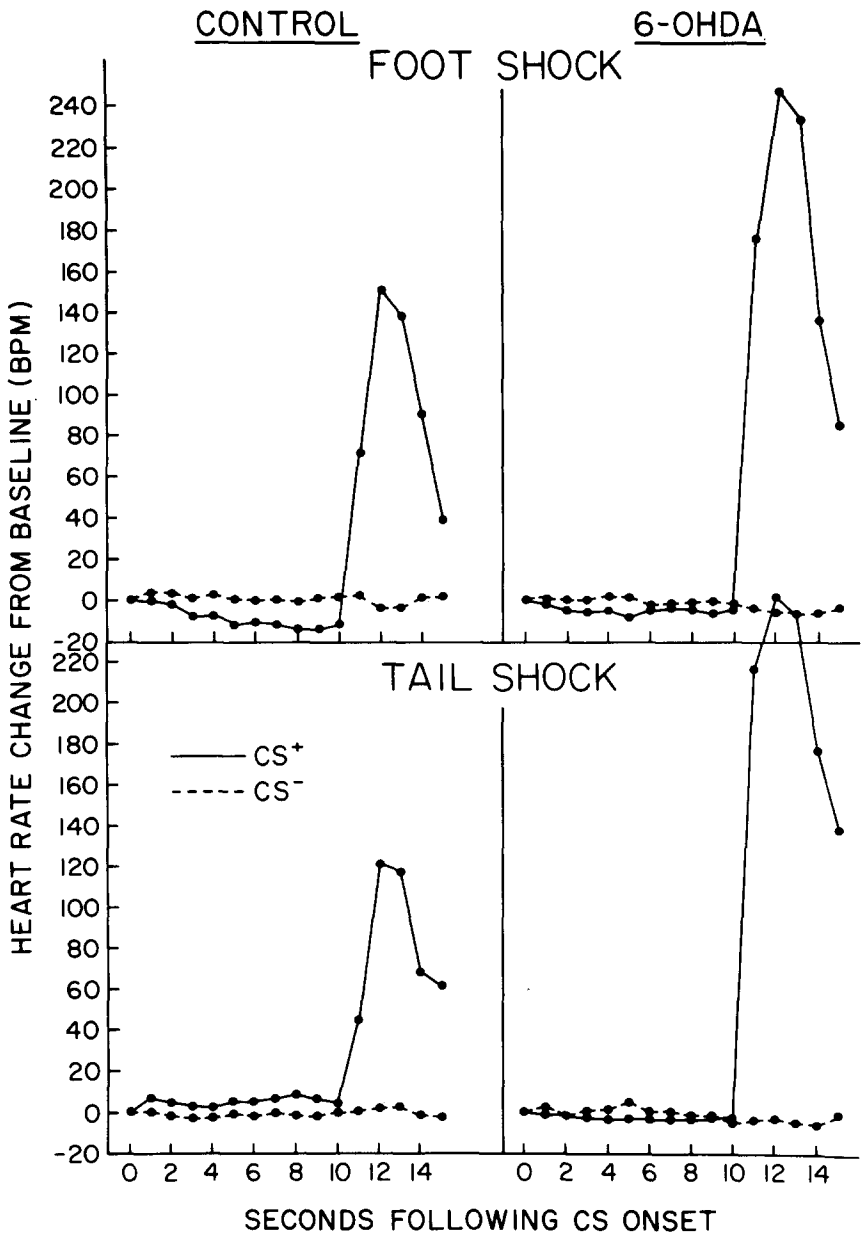

FIG. 1. Heart rate change from baseline following onset of CS^+ or CS^-. Shock was on during Seconds 11 and 12. Each point represents the mean of at least 8 rats. (BPM) = Beats per Minute.

General activity changes which occurred concomitantly with heart rate changes are shown in Fig. 2. A high degree of relationship is apparent between the two measures, and analyses of general activity revealed the same pattern of significant changes as did heart rate.

Effect of 6-Hydroxydopamine on the Development of Hypertension Produced by DOCA/NaCl

Since central catecholamines are thought to play a role in experimental hypertension, 6-hydroxydopamine-treated rats and control rats were uninephrectomized, treated with DOCA and permitted ad lib access to saline. Control rats treated in this manner developed a significant hypertension by the second week of DOCA treatment (Fig. 3). The 6-hydroxydopamine-treated rats also became hypertensive following DOCA administration when compared with rats treated with 6-hydroxydopamine that received no DOCA and were allowed only water to drink (Fig. 3). However, blood pressure did not reach the maximum of 184 ± 6 mm Hg reached by the DOCA-treated control group confirming earlier reports that 6-hydroxydopamine treatment antagonizes this type of hypertension (Fig. 3).

However, during the course of the experiment, it was

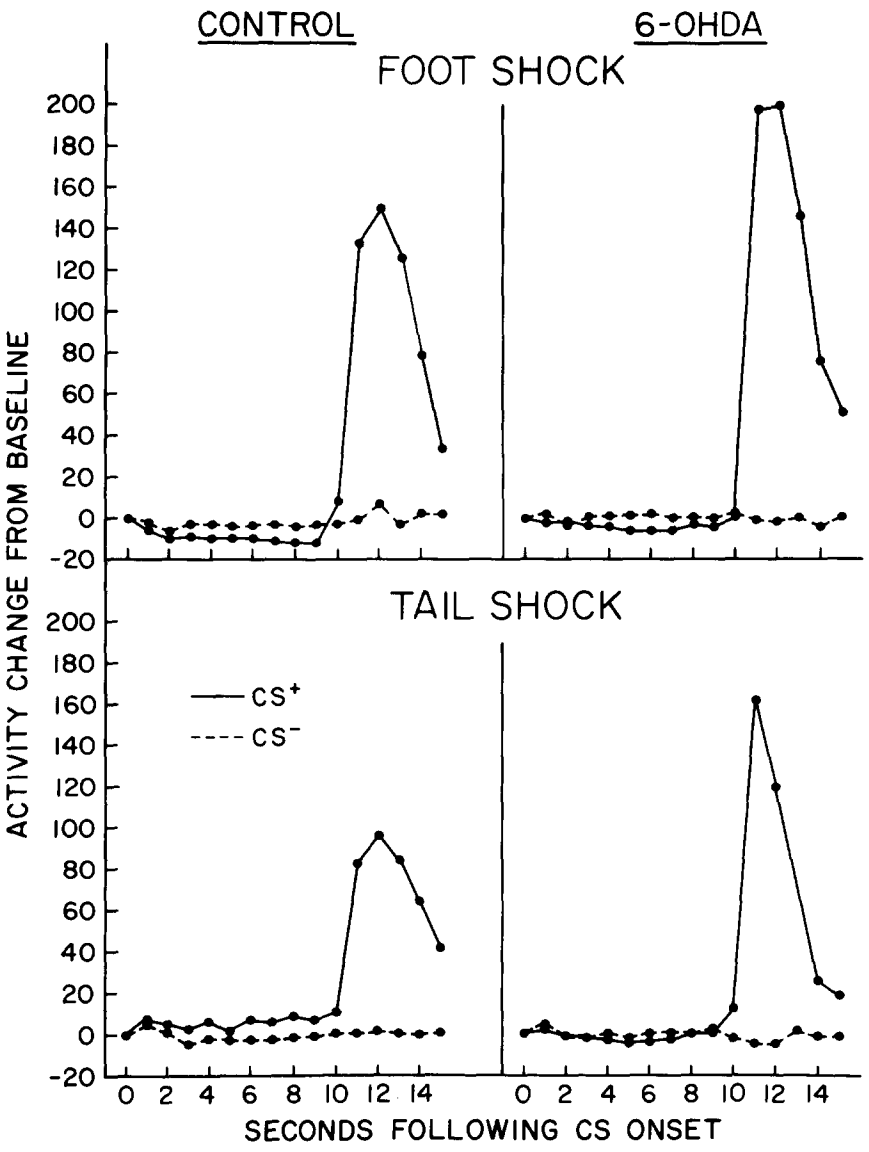

FIG. 2. Activity change from baseline following onset of CS^+ or CS^-. Shock was on during Seconds 11 and 12. Each point represents the mean of at least 8 rats.

observed that rats treated with 6-hydroxydopamine consumed significantly less saline than did DOCA-treated control animals (Fig. 4). Consumption of saline solution by the 6-hydroxydopamine-treated animals seemed to plateau by the fourth week. This plateau was not apparent in control rats. Thus, the reduced intake of saline observed in 6-hydroxydopamine-treated rats might account for the reduced blood pressure response observed in this group.

Effect of 6-Hydroxydopamine Treatment on Central and Peripheral Catecholamine Content and Tyrosine Hydroxylase Activity

Determination of central and peripheral catecholamine levels in animals utilized in the studies described above clearly demonstrates that 6-hydroxydopamine injected intracisternally reduced whole brain norepinephrine and dopamine content without affecting catecholamine content in heart and adrenal (Table 2). Tyrosine hydroxylase activity in brain was also significantly reduced indicating that catecholamine reduction resulted from neuronal destruction (Table 3). While DOCA treatment significantly reduced heart norepinephrine in control animals as described previously [7], this reduction was not evident in 6-hydroxydopamine-treated rats (Table 2). In additional experiments, it was also found that tyrosine hydroxylase activity in superior cervical ganglia was not altered by either treatment (Table 3).

DISCUSSION

In the present study, resting blood pressure and heart rate was found to be consistently lower in 6-hydroxydopamine-treated animals. Since peripheral catecholamines did

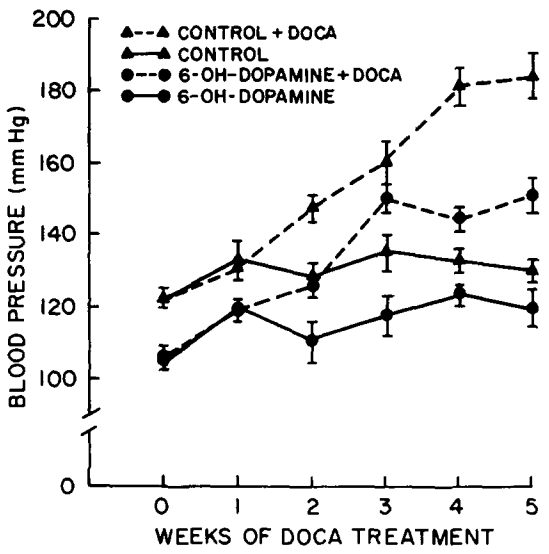

FIG. 3. Development of DOCA/NaCl hypertension in uninephrectomized control and 6-hydroxydopamine-treated rats. Each point represents the means of 13–21 values. Vertical lines denote standard error of the mean. CONTROL + DOCA and 6-OH-DOPAMINE + DOCA groups received 25 mg/kg DOCA s.c. at weekly intervals starting at time zero and were given a 1% NaCl solution to drink. The other two groups received no DOCA and had water to drink.

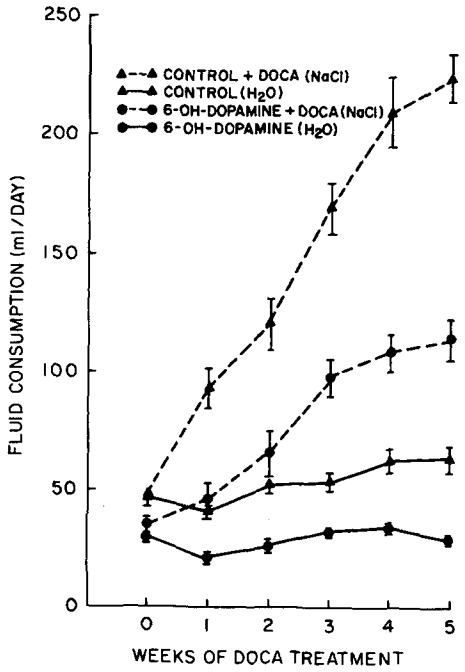

FIG. 4. Water or saline consumption in uninephrectomized control and 6-Hydroxydopamine-treated rats. See Fig. 3 for details. Body weight of DOCA-control and DOCA-6-hydroxydopamine-treated rats was 334 ± 4 g and 290 ± 7 g at zero time and 377 ± 12 g, respectively, at 5 weeks. Body weight of non DOCA-controls and non-DOCA-6-hydroxydopamine-treated animals was 334 ± 10 g and 297 ± 8 g at zero time and 466 ± 18 g and 350 ± 9 g, respectively at 5 weeks.

TABLE 2

CENTRAL AND PERIPHERAL CATECHOLAMINE LEVELS FOLLOWING DESOXYCORTICOSTERONE IN 6-HYDROXYDOPAMINE-TREATED RATS

Treatment*	Brain		Adrenal†	Heart
	Norepinephrine (ng/g)	Dopamine (ng/g)	Catecholamines (μg/adrenal pr)	Norepinephrine (ng/g)
6-hydroxydopamine	33.6 ± 5.5‡	6.0 ± 4.1‡	13.3 ± 0.9	816.9 ± 98.1
Control	418.5 ± 35.1	697.0 ± 119.8	12.9 ± 2.6	711.0 ± 55.5
6-Hydroxydopamine + DOCA	46.0 ± 3.5‡	15.7 ± 7.2‡	14.0 ± 2.0	701.3 ± 56.5
Control + DOCA	490.8 ± 41.4	832.3 ± 90.6	16.6 ± 2.8	478.8 ± 60.3‡

*See Methods. Values represent the mean ± S.E.M. of at least 6 determinations.
†Adrenal catecholamines were assayed including both epinephrine and norepinephrine.
‡Different from control values beyond the 0.05 level by two-tailed *t*-test.

TABLE 3

CENTRAL AND PERIPHERAL TYROSINE HYDROXYLASE ACTIVITY FOLLOWING DESOXYCORTICOSTERONE IN UNINEPHRECTOMIZED 6-HYDROXYDOPAMINE-TREATED RATS

Treatment*	Tyrosine Hydroxylase Activity	
	Whole Brain (mμMoles/g/h)	Superior Cervical Ganglia (cpm/mg protein/h)
6-Hydroxydopamine	0.50 ± 0.12†	0.30 ± 0.06
Control	5.54 ± 0.49	0.33 ± 0.01

*See methods. Values represent the mean ± S.E.M. of at least 6 determinations.
†Different from control values beyond the 0.001 level by two-tailed *t* test.

not appear to be affected by this treatment, this result is probably related to the destruction of central catecholamine containing neurones. However, other experiments [12, 13, 28] have observed no alteration in blood pressure following intraventricular injection of 6-hydroxydopamine even though norepinephrine was reduced to 10 to 50 percent of control in several parts of brain. This apparent lack of effect of 6-hydroxydopamine on resting blood pressure could relate to an asymmetrical amine depletion after intraventricular injection such as that recently described [25], or to insufficient depletion of both norepinephrine and dopamine following 6-hydroxydopamine treatment. The latter possibility seems likely since inhibition of monoamine oxidase prior to injection of 6-hydroxydopamine has been shown to potentiate the actions of intracisternally [3] or intraventricularly [8,29] administered 6-hydroxydopamine on dopaminergic fibers. Further, it is only after virtual total depletion of brain catecholamines that many of the effects of 6-OHDA are observed [5,23].

It has recently been reported [27] that paired shocks to the feet of control rats caused a significant decrease in the blood pressure response, whereas a significant increase in blood pressure was observed in 6-hydroxydopamine-treated rats. These workers attributed their findings to an involvement of CNS catecholaminergic mechanisms in the mediation of the blood pressure response to electrical footshock. In the present study, an exaggerated heart rate response to shock was found in animals with 6-hydroxydopamine treatment, confirming that central catecholamine reduction alters cardiovascular responses to external stressors. However, the fact that general activity also increased to a greater extent in 6-hydroxydopamine-treated rats suggests that the greater heart rate response may be a result of a potentiated somatic reaction to shock. Since sympathetic outflow is known to be closely related to activity [10], results could be interpreted to be dependent upon a behavioral change to shock rather than to a direct effect on sympathetic outflow, although the latter possibility has not been eliminated.

The finding that footshock led to significantly decreased and tailshock to significantly increased heart rates in anticipation of shock in the control animals is not completely in agreement with a previous study [24] comparing the influence of shock location on the formation of conditioned heart rate responses. However, many procedural differences are apparent between the two studies. Regardless, the 6-hydroxydopamine-treated rats did not display significant conditioned heart rate responses in either direction. Rather than relating the lack of a conditioned response to a deficit in efferent cardiac control mechanisms, the lack of formation of a conditioned heart rate response may be due to decrement in the ability to acquire new responses as has been previously reported in several operant paradigms following treatment with 6-hydroxydopamine [5,15]. This is supported by the observation that the general activity measures were in close agreement with the heart rate results.

In another paradigm thought to be related to sympathetic outflow, intracisternal administration of 6-hydroxydopamine reduced, but did not abolish, the hypertensive response to DOCA/NaCl treatment. Such results would be in essential agreement with recent reports which indicated that multiple injections of 6-hydroxydopamine into the lateral ventricle abolished the development of this form of experimental hypertension [9,12]. However, it has previously been reported that the amount of saline consumed correlates with the degree of experimental hypertension developed [6,14]. In the present study, it was clearly demonstrated that 6-hydroxydopamine-treated animals consumed less saline solution following DOCA treatment than did control rats, an observation in accord with the finding that 6-hydroxydopamine reduced preference for saline after DOCA administration [1]. Therefore, while a direct effect of 6-hydroxydopamine treatment on central neural systems responsible for blood pressure control has been proposed [9,27], it is also possible that reduced salt intake may contribute to the lower blood pressure response in 6-hydroxydopamine-treated rats.

If altered sympathetic outflow were responsible for the potentiated cardiovascular response to shock, it would be difficult to explain the reduced blood pressure response in the experimental hypertension paradigm in terms of sympathetic outflow. Thus, the findings in the present study underline the need to consider all physiological and behavioral changes induced by the treatment before ascribing them to a singular mechanism.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Joseph Farmer, Austin Spruill, Susan Hollister, Edna Edwards and Marcine Kinkead for their excellent technical assistance. This work was supported by U.S.P.H.S. grants MH-16522 and HD-03110 and the North Carolina Heart Association (1973-A-11). George R. Breese is a Career Development Awardee (HD-24585) and Robert A. Mueller is a Clinical Pharmacology Faculty Development Awardee, Pharmaceutical Manufacturers Association Foundation.

REFERENCES

1. Breese, G. R., B. R. Cooper, R. D. Smith and L. D. Grant. Alterations in consummatory behavior folllwing intracisternal injection of 6-hydroxydopamine. *Pharmac. Biochem. Behav.* **1**: 319–328, 1973.
2. Breese, G. R. and T. D. Traylor. Effect of 6-hydroxydopamine on brain norepinephrine and dopamine: Evidence for selective degeneration of catecholamine neurons. *J. Pharmac. exp Ther.* **174**: 413–420, 1970.
3. Breese, G. R. and T. D. Traylor. Depletion of brain noradrenaline and dopamine by 6-hydroxydopamine. *Br. J. Pharmac.* **42**: 88–99, 1971.
4. Chalmers, J. P. and J. L. Reid. Participation of central noradrenergic neurons in arterial baroreceptor reflexes in the rabbit. *Circulation Res.* **31**: 789–804, 1972.
5. Cooper, B. R., G. R. Breese, L. D. Grant and J. L. Howard. Effects of 6-hydroxydopamine treatments on active avoidance responding: Evidence for involvement of brain dopamine. *J. Pharmac. exp. Ther.* **185**: 358–370, 1973.
6. DeChamplain, J., L. R. Krakoff and J. Axelrod. Relationship between sodium intake and norepinephrine storage during the development of experimental hypertension. *Circulation Res.* **23**: 479–491, 1968.
7. DeChamplain, J., R. A. Mueller and J. Axelrod. Turnover and synthesis of norepinephrine in experimental hypertension in rats. *Circulation Res.* **25**: 285–291, 1969.
8. Fibiger, H. C., B. Lonsbury, H. P. Cooper and L. D. Lytle. Early behavioral effects of intraventricular administration of 6-hydroxydopamine in rat. *Nature New Biol.* **236**: 209–211, 1972.
9. Finch, L. G., G. Haeusler and H. Thoenen. Failure to induce experimental hypertension in rats after intraventricular injection of 6-hydroxydopamine. *Br. J. Pharmac.* **44**: 356–357, 1972.
10. Gordon, R., S. Spector, A. Sjoerdsma and S. Udenfriend. Increased synthesis of norepinephrine and epinephrine in the intact rat during exercise and exposure to cold. *J. Pharmac. exp. Ther.* **153**: 440–447, 1966.
11. Gupta, P. P., R. C. Srimal and B. N. Dhawan. Central cardiovascular effects of 6-hydroxydopamine. *Eur. J. Pharmac.* **20**: 215–223, 1972.
12. Haeusler, G., L. Finch and H. Thoenen. Central adrenergic neurons and the initiation and development of experimental hypertension. *Experientia* **28**: 1200–1203, 1972.
13. Haeusler, G., M. Gerold and H. Thoenen. Cardiovascular effects of 6-hydroxydopamine injected into a lateral brain ventricle of the rat. *Naunyn-Schmeideberg's Arch. exp. path. Pharmak.* **274**: 211–228, 1972.
14. Hall, C. E., S. Ayachi and O. Hall. Salt hypertension produced by sucrose facilitation of saline consumption in Long-Evans rats. *Tex. Rep. Biol. Med.* **30**: 155–162, 1972.
15. Howard, J. L., L. D. Grant and G. R. Breese. The effects of 6-hydroxydopamine on rat performance in a modified T-maze. *J. comp. physiol. Psychol.* In press, 1974.
16. Howard, J. L., J. P. Leahy and G. R. Breese. Some physiological and behavioral consequences of acute and chronic injection of 6-hydroxydopamine (6-OHDA). *Fedn Proc.* **30**: 541, 1971.
17. Levitt, M. S., S. Spector, A. Sjoerdsma and S. Udenfriend. Elucidation of the rate-limiting step in norepinephrine biosynthesis in the perfused guinea-pig heart. *J. Pharmac. Exp. Ther.* **148**: 1–8, 1965.
18. Mueller, R. A., H. Thoenen and J. Axelrod. Increase in tyrosine hydroxylase activity after reserpine administration. *J. Pharmac. Exp. Ther.* **169**: 74–79, 1969.
19. Musacchio, J. M., L. Julow, S. S. Kety and J. Glowinski. Increase in rat brain tyrosine hydroxylase activity produced by electro convulsive shock. *Proc. natn. Acad. Sci. U.S.A.* **63**: 1117–1119, 1969.
20. Nagatsu, T. M., M. Levitt and S. Udenfriend. A rapid and simple radioassay for tyrosine hydroxylase activity. *Analyt. Biochem.* **9**: 122–126, 1964.
21. Okamota, K. and K. Aoki. Development of a strain of spontaneously hypertensive rats. *Jap. Circul. J.* **27**: 282–293, 1963.
22. Seligman, M. E. P. Control group and conditioning: A comment on operationism. *Psychol. Rev.* **76**: 444–491, 1969.
23. Smith, R. D., B. R. Cooper and G. R. Breese. Growth and behavioral changes in developing rats treated intracisternally with 6-hydroxydopamine: Evidence for involvement of brain dopamine. *J. Pharmac. Exp. Ther.* **185**: 609–619, 1973.
24. Teyler, T. J. Effects of restraint on heart-rate conditioning in rats as a function of US location. *J. comp. physiol. Psychol.* **77**: 31–37, 1971.
25. Vetulani, J., K. Reichenberg and G. Wiszniowska. Asymmetric behavioral and biochemical effects of unilateral injections of 6-hydroxydopamine into the lateral brain ventricle of the rat. *Eur. J. Pharmac.* **19**: 231–238, 1972.
26. Weiss, J. M. A tail electrode for unrestrained rats. *J. Exp. Analysis Behav.* **10**: 85–86, 1967.
27. Williams, R. B., B. Eichelman and L. K. Y. Ng. Depletion of brain amines reverses blood pressure responses to footshock in the rat. *Nature New Biol.* **240**: 276–277, 1972.
28. Yamori, Y., H. Yamke, W. Delong, W. Lovenberg and A. Sjoerdsma. Effect of tissue norepinephrine depletion by 6-hydroxydopamine on blood pressure in spontaneously hypertensive rats. *Eur. J. Pharmac.* **17**: 135–140, 1972.
29. Zigmond, M. J. and E. M. Stricker. Deficits in feeding behavior after intraventricular injection of 6-hydroxydopamine in rats. *Science* **177**: 1211–1214, 1972.