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Neonatal 6-Hydroxydopamine Sympathectomy in Normotensive and Spontaneously Hypertensive Rat[1]

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PAPPAS, B. A., D. A. V. PETERS, M. SAARI, S. K. SOBRIAN AND E. MINCH. *Neonatal 6-hydroxydopamine sympathectomy in normotensive and spontaneously hypertensive rat.* PHARMAC. BIOCHEM. BEHAV. **2**(3) 381–386, 1974. – Neonatal sympathectomy was produced in rats from either a normotensive or spontaneously hypertensive (SHR) strain by repeated s.c. injections of 6-hydroxydopamine (6-OHDA). The SHR rats were considerably more active than the normotensive rats in the open field and activity wheels. Emotionality, defined by the profile of open field activity and defecation scores, was lower in the SHR. Neonatal 6-OHDA had no effect upon emotionality or running wheel activity. Adult endogenous brain norepinephrine was increased in brainstem and decreased in cortex of both strains by 6-OHDA. Tyrosine hydroxylase activity in the brains of normotensive, 6-OHDA injected rats varied directly with altered endogenous NE levels. Preliminary data also indicated increased serotonin synthesis in the brainstem of 6-OHDA injected rats. It was concluded that neonatal injections of 6-OHDA may cause selective degeneration of the descending and dorsal NE brain pathways, and that the behavioral effects of this treatment, while yet obscure, seem to resemble those produced by hippocampal lesions.

6-OHDA Sympathectomy Spontaneous hypertension Activity Emotionality

REPEATED subcutaneous injections of 6-OHDA in the neonatal rat not only produce a widespread and permanent depletion of NE in peripheral sympathetic neurons [1,6] but also significantly alter regional brain contents of NE. Specifically, spinal [6,20], cortical [6, 15, 19, 20], cerebellar [6,20], and hippocampal [6] NE are lowered while brainstem NE is increased [4, 15, 19, 20]. Assay results for the hypothalamus have been conflicting [6, 13, 15, 20]. The following research was carried out to confirm our earlier observations on regional brain NE changes after neonatal 6-OHDA [15] and to assess changes in NE synthesis in the same brain regions, by measurement of the activity of the rate limiting enzyme tyrosine hydroxylase. We also report preliminary data on changes in synthesis of serotonin in the brainstem after the drug treatment.

Secondly, data are reported for open field and running wheel activity of neonatal vehicle and 6-OHDA injected normotensive and hypertensive rats. Previous research has indicated no effect of neonatal 6-OHDA upon various types of behavioral procedures [6,15]. However, this laboratory has recently demonstrated that the drug produces a deficit in the inhibition of water licking in response to foot shock in both normotensive rats and the SHR [17]. This deficit could be due either to reduced emotionality, heightened locomotor activity or to decreased ability to inhibit responding. The test procedures used here permitted assessment of the first two possibilities both in the normotensive rat and in the SHR rat which may be characterized as autonomically hyperactive [14]. We also examined the effect of 6-OHDA sympathectomy on diurnal variations in running wheel activity,

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since intracranial injections of the drug in adult rats not only alters diurnal variations in brain serotonin synthesis but also significantly attenuates light-dark period differences in locomotor activity [10].

METHOD

Animals

Thirty-three male and 32 female offspring of Wistar parents obtained from a local breeder and mated in our laboratory, and 31 male and 27 female offspring of the 26th generation spontaneous hypertensive parents were used. The parent hypertensive rats were of the strain described by Okamoto [14] and were imported to this laboratory from the National Institute of Mental Health, Bethesda, and mated. The rats were weaned at 22 days of age and maintained in colony cages until sacrifice. The colony room illumination consisted of a reversed 12 hr diurnal light-dark cycle.

Injections

Approximately half of the rats were injected subcutaneously on Days 1 through 7 after birth with 50 μg/g, 6-OHDA (2,4,5-trihydroxyphenethylamine, Regis Chemical Co.), dissolved in 0.05 ml of a saline-ascorbic acid solution (1 mg/ml). Injections of 75 μg/g were also administered on Days 10, 15, and 22. The other rats received 0.05 ml vehicle on the same schedule.

Behavioral Tests

At 43 days of age, the rats were quickly weighed and then placed in an open field for 5 min. This apparatus consisted of a 1.03 m square floor divided into 25 equal squares with side walls 0.40 m high. The apparatus was painted white except for black lines delineating the squares. Counts of activity, rearing and number of boli excreted for the 5 min period were recorded. At the termination of this interval, the rats were returned to their individual home cages and left undisturbed until some were sacrificed for endogenous catecholamine assay 2 days later.

At about 120 days of age, 8 rats from each treatment group were placed, for 3 consecutive days, in modified activity wheels (Lafayette Instrument Co.) to which living cages with ad lib food and water were attached. The original relay devices attached to the axle of the wheels were replaced with mercury relays to eliminate the audible click after each whole wheel revolution. These mercury relays were wired to activate counters outside of each sound chamber which recorded wheel revolutions. The wheels were housed in sound attenuated chambers with controlled lighting and temperature (about 22°C) conditions. Sessions began at 8 a.m. for half the rats in each group and at 8 p.m. for the other half. The light-dark conditions within the chambers were the same as those maintained in the colony room (light off at 8 a.m., light on at 8 p.m.), and total revolutions during each of the 12 hr intervals were recorded over the 3 days.

Blood Pressure Measurement

Five naive 6-OHDA and vehicle injected rats about 120 days of age and from both the control Wistar and SHR strains were implanted while under nembutal anesthesia (40 mg/kg i.p.) with a chronic aortic catheter. Four or 5 days later they were again similarly anesthetized. When the rats were no longer overtly responsive to a tail pinch, the catheter was opened and flushed with about 0.10 ml of heparinized saline and connected to a Statham 23Dd transducer and Beckman polygraph for continuous recording of systolic and diastolic blood pressure to assess the peripheral effects of 6-OHDA. Pressures were recorded and scored for 30 min, beginning 5 min after the catheter was flushed. The rats were killed by anesthetic overdose and discarded at the termination of recording.

Biochemical Assays

For our initial assays of endogenous NE, we sacrificed by decapitation 7 vehicle and 7 6-OHDA injected rats from both our control and hypertensive strain. The rats were 45 days old at the time of sacrifice and had been tested for 5 min in an open field apparatus at 43 days of age. The hearts and adrenals were quickly removed and rinsed with saline. The brains were removed concurrently with hearts and adrenals and placed on an ice-cooled, saline-rinsed plate. After dissection of the cerebellum from the whole brains, the remaining brain tissue was sectioned into brainstem, hypothalamus and cortical slices. The brainstem was isolated by coronal cuts at the first spinal nerve and through the pons varolli. The hypothalamus was cleaned as much as possible of optic chiasma and tracts. Cortical slices were systematically sampled from the entire brain, with care taken to exclude underlying white matter. For the amine assays, the tissues were immediately frozen on dry ice and later stored in liquid nitrogen.

At intervals after decapitation which were equal for the normal and 6-OHDA treated groups, the heart, adrenal and brain tissues were fluorometrically assayed for endogenous epinephrine (heart and adrenals only) and norepinephrine (all tissue) by a modification of the trihydroxyindole method [2].

The brain and adrenal tyrosine hydroxylase activity was measured by the method of Peters *et al.* [16] in another group of 11 vehicle and 11 6-OHDA injected rats from our control strain. The rats were about 120 days old at sacrifice and had been administered an open field test at 43 days of age. Brain areas or whole adrenals were homogenized in 5–10 volumes ice cold 0.25 M sucrose. Aliquots of the tissue homogenates were incubated with L-tyrosine-C^{14}(U.L.) in the presence of the dopa decarboxylase inhibitor, N.S.D. 1034 (Smith and Nephew Research Co.). The labelled L-dopa formed was isolated on an alumina column and the radioactivity measured in a Beckman model LS150 liquid scintillation counter. The rate of synthesis of the L-dopa-C^{14} was used as a measure of the tyrosine hydroxylase activity. Assays for endogenous NE were also carried out on the same tissue used for assay of tyrosine hydroxylase activity. Because of technical difficulties, NE levels for the adrenals were not available. Tryptophan 5-hydroxylase activity was assayed on the same tissue homogenates by a previously reported technique [14]. L-tryptophan-3-C^{14} was incubated with brain homogenate in the presence of a monoamine oxidase inhibitor and the 5-hydroxytryptamine-C^{14} formed was isolated on a CG-50 ion exchange resin. The rate of formation of the labelled 5-hydroxytryptamine was used as a measure of the tryptophan hydroxylase activity.

Statistical Analysis

Except where noted, statistical treatments consisted of analyses of variance and post-hoc comparisons (Sheffé test). Data presented in tables consist of sample sizes, means and standard errors.

RESULTS

Body and Organ Weights

Body weights at the time of sacrifice for endogenous NE determinations in the 45 day old rats were significantly lower in the SHR than in the control strain ($p<0.001$). In addition the 6-OHDA injected rats were significantly lighter than vehicle injected rats ($p<0.001$) and females were lighter than males ($p<0.001$). The 6-OHDA injections also caused a greater weight reduction in the SHR than in the control strain ($p<0.05$), while males injected with 6-OHDA were lighter than similarly injected females ($p<0.05$).

The adrenal weights, calculated as a percentage of body weight, of the SHR strain were significantly heavier than those of the control strain ($p<0.001$). Furthermore, while 6-OHDA injections produced a significant over-all reduction in adrenal weight ($p<0.01$), this reduction was of greater magnitude in the SHR than in the control strain ($p<0.05$). Expressed as a percentage of body weight, the hearts of the hypertensive strain were heavier than those of the normotensive strain ($p<0.001$), while 6-OHDA produced a non-significant reduction of heart weight ($p<0.07$).

Blood Pressure

Table 1 shows averaged group blood pressures at 5 and 30 min intervals after recording onset. Analysis of variance of the data revealed significantly higher systolic and diastolic blood pressure in the SHR strain ($p<0.001$) while 6-OHDA significantly lowered diastolic blood pressure in both strains ($p<0.05$). The drug also lowered mean systolic blood pressure in both strains although the effect fell just above statistical reliability ($p<0.06$). In addition to finding an increasing reduction in both systolic and diastolic pressure over time after recording onset for both strains, diastolic pressures of the SHR rats were found to decline more over the 30 min interval than the diastolic pressures of the control strain ($p<0.002$). However, blood pressures of 6-OHDA injected SHR rats still remained significantly elevated above those of the vehicle normotensive rats at all 6 recording intervals.

TABLE 1

SYSTOLIC AND DIASTOLIC BLOOD PRESSURES AT 5 AND 30 MIN AFTER RECORDING ONSET

Group	5 Min	30 Min
WISTAR		
Vehicle	110 ± 6 / 95 ± 6	105 ± 5 / 93 ± 3
6-OHDA	99 ± 4 / 86 ± 4	92 ± 3 / 81 ± 4
SHR		
Vehicle	164 ± 9 / 132 ± 9	149 ± 13 / 113 ± 7
6-OHDA	146 ± 9 / 119 ± 7	134 ± 6 / 107 ± 5

Behavioral Tests

Statistical analysis of the open field data were performed on the mean total squares entered (activity-exploration) during the 5 min test, mean total number of rearings and the proportions of rats within each group who defecated during the test. These data are shown in Table 2.

There was no main effect of the 6-OHDA injections on either of the three open field measures. However, a significant drug by strain interaction on squares entered ($p<0.05$) can be seen from Table 2 to be due to the decreased exploration in the control strain and to the

TABLE 2

SUMMARY OF OPEN FIELD DATA FOR 43 DAY OLD RATS

Strain	WISTAR				SHR			
Drug	VEH		6-OH		VEH		6-OH	
Sex	M	F	M	F	M	F	M	F
N	17	16	16	16	17	14	14	13
Mean Total Squares	56.8 ± 7.4	51.4 ± 3.1	40.1 ± 7.4	55.9 ± 7.8	44.8 ± 5.8	71.6 ± 5.4	51.5 ± 8.1	81.2 ± 5.2
Mean Rears	16.5 ± 2.6	17.4 ± 2.1	14.0 ± 2.3	12.4 ± 3.2	15.2 ± 2.5	22.8 ± 2.1	20.6 ± 1.4	21.2 ± 1.8
Defecation*	8	9	8	6	6	2	6	3

*Number of rats defecating in open field

increased exploration in the SHR strain. Overall, the SHR were considerably more active than the control strain ($p<0.025$), and reared more often ($p<0.025$). In addition, females were more active overall than males ($p<0.01$) and this sex difference was much more pronounced in the SHR strain ($p<0.025$). Table 2 also indicates that the proportion of SHR rats who defecated was less than that of the control strain ($p<0.04$, proportions test) while there was no effect upon defecation frequency of the 6-OHDA injections.

TABLE 3

MEAN TOTAL RUNNING WHEEL REVOLUTIONS. L AND D DENOTE LIGHT AND DARK PERIODS RESPECTIVELY.

GROUP		DAY 1	2	3
WISTAR				
VEH	L	821 ± 34	455 ± 8	302 ± 13
	D	1714 ± 62	985 ± 26	859 ± 30
6-OHDA	L	539 ± 14	367 ± 12	231 ± 6
	D	1275 ± 33	861 ± 31	752 ± 23
SHR				
VEH	L	3055 ± 94	1632 ± 47	1684 ± 33
	D	5055 ± 63	4244 ± 93	5315 ± 84
6-OHDA	L	3181 ± 136	1463 ± 38	1673 ± 45
	D	5830 ± 127	6019 ± 186	6064 ± 145

Table 3 shows the averaged group total activity wheel revolutions for each 12 hr light and dark period plotted for the 3 test days. It can be seen that the SHR rats were considerably more active in the wheel than were the control rats ($p<0.001$). There was no main effect of 6-OHDA upon wheel activity nor was there an interaction of 6-OHDA with strain, although 6-OHDA did tend to reduce activity in the control rats and to increase activity in the SHR rats. All groups showed significantly increased activity during the dark portion of the light-dark cycle ($p<0.001$). However this increase was considerably larger in the SHR rats than in the control rats ($p<0.001$). Overall, activity showed a significant decline across the three test days ($p<0.001$).

Catecholamine Assays

Table 4 shows average NE and epinephrine (E) contents, calculated per gram of tissue weight, for hearts and adrenals, and NE for the 3 brain parts, for 45 day old vehicle and 6-OHDA injected control and hypertensive rats. The strains did not differ in endogenous heart amine contents while the 6-OHDA injections produced reductions ($p<0.001$) of both NE and E contents of the hearts which were the same magnitude for both strains. In contrast to the hearts, both NE and E contents were significantly lower in the adrenals of the SHR strain ($p<0.05$). However, 6-OHDA produced proportional increases of both adrenal amines, which were the same magnitude for the two strains, although only the NE increase was statistically significant ($p<0.05$). In addition to these calculations based on raw data for adrenal NE and E contents, we also calculated for each rat the ratio of adrenal NE to E. Analyses of variance performed on these ratios indicated no effect of either strain or drug treatment on the proportional contents of the two catecholamines. The two strains did not differ in regional brain NE contents. Furthermore, the effect of the 6-OHDA treatment was the same for both groups, namely significantly increased hypothalamic ($p<0.05$) and brainstem NE ($p<0.001$) and significantly decreased NE in the cortex ($p<0.001$).

TABLE 4

SUMMARY OF TISSUE ENDOGENOUS CATECHOLAMINE CONTENTS FOR 45 DAY OLD RATS

Strain	Heart		Adrenal		Cortex	Hypothalamus	Brain Stem
	E	NE	E	NE	NE	NE	NE
Control							
Vehicle	0.16 ± 0.01*	0.63 ± 0.04	199 ± 14	127 ± 5	0.59 ± 0.05	1.60 ± 0.10	0.49 ± 0.05
6-OHDA	0.09 ± 0.01	0.14 ± 0.01	209 ± 18	147 ± 13	0.30 ± 0.07	2.08 ± 0.25	0.86 ± 0.10
SHR							
Vehicle	0.13 ± 0.01	0.72 ± 0.05	162 ± 5	103 ± 8	0.61 ± 0.07	1.64 ± 0.11	0.51 ± 0.04
6-OHDA	0.08 ± 0.01	0.17 ± 0.01	185 ± 8	130 ± 9	0.34 ± 0.07	2.06 ± 0.19	0.85 ± 0.06

*Each value represents the average of tissue sample values from 7 rats. Data are corrected for recovery which averaged about 78% for norepinephrine (NE) and 76% for epinephrine (E) and are expressed as μg/g tissue.

TABLE 5

SUMMARY OF ASSAYS FOR TYROSINE HYDROXYLASE ACTIVITY AND ENDOGENOUS NE FOR 120 DAY OLD NORMOTENSIVE RATS

		Cortex	Hypothalamus	Brain Stem	Adrenals
Sample Size		6	6	5	5
Tyrosine Hydroxylase	Vehicle	2.10 ± 0.10	25.4 ± 1.8	3.80 ± 0.17	848 ± 21
nmole dopa/hr/g tissue	6-OHDA	0.15 ± 0.03*	27.3 ± 1.0	5.09 ± 0.15*	714 ± 68†
	% control	7	107	134	84
Endogenous	Vehicle	0.25 ± 0.09	1.07 ± 0.17	0.46 ± 0.08	–
NE μg/g	6-OHDA	0.10 ± 0.06	1.16 ± 0.17	0.63 ± 0.08	–
	% control	41	108	138	–

*significantly different from vehicle values at $p<0.001$, t-test
†significantly different from vehicle values at $p<0.05$, t-test

Table 5 summarizes the results of assays for tyrosine hydroxylase activity and endogenous NE in brain tissue and adrenal (tyrosine hydroxylase only) for 120 day-old vehicle and 6-OHDA-injected Wistar control rats. The decreased cortical NE level in the 6-OHDA injected rats was accompanied by a significant corresponding reduction in tyrosine hydroxylase activity, while increased brainstem NE levels were matched by increased enzyme activity. The hypothalamus showed slight elevations of both NE and enzyme activity in the 6-OHDA rats while adrenal tyrosine hydroxylase activity was significantly reduced. Endogenous NE levels were not available for the adrenals. However, reference to Table 2 indicates significant elevation of adrenal NE in the 45 day old 6-OHDA group.

In addition to these data for tyrosine hydroxylase activity, we have carried out preliminary assays for tryptophan-5-hydroxylase activity in the brainstems of six 6-OHDA injected and six vehicle injected normotensive rats. Our data show a significant ($p<0.05$, t-test) increase in activity in the brainstems of the 6-OHDA injected rats. Actual values were 17.8 ± 1.4 and 12.2 ± 1.9 nmole 5-HT/g/hr. for the 6-OHDA and for vehicle injected rats respectively, with the mean activity of the 6-OHDA group representing 141% of vehicle control activity.

DISCUSSION

While neonatal 6-OHDA injections were shown here to significantly lower blood pressure in the SHR strain, this decreased blood pressure was still markedly higher than that of the control normotensive rat. In agreement with these data, Clark *et al.* [6] reported that blood pressures of neonatal 6-OHDA injected hypertensive rats from the New Zealand strain were about 13% higher at maturity than those of vehicle-injected normotensive control rats. Since adrenal catecholamine levels were found here to be elevated in the 6-OHDA treated rats, the hypertension may be partially maintained by these amines. Adrenal demedullation [4] and ganglionic blockade [5] have more profound cardiovascular depressing effects in immunosympathectomized than in control rats. Alternately, the effects of 6-OHDA upon brain catecholamine function also reported here may in some as yet unknown manner be responsible for the maintenance of high blood pressure after peripheral sympathectomy. On the other hand, since we did not measure peripheral catecholamine content in rats 120 days of age, the age when blood pressure was recorded, it could be argued that some recovery of sympathetic function may have occurred between 45 days of age and 120 days of age. However, no recovery of endogenous heart NE contents has previously been reported. Rather between 43 and 104 days of age, maturation seemed to increase the relative differences in heart NE content between vehicle and 6-OHDA injected rats [15].

Behaviorally, the SHR rats were considerably more active than our control strain. Furthermore, assuming low defecation and high activity scores in the single open field exposure to reflect low emotionality [8], the SHR rats were also less emotional than our control strain. This agrees with the results of Saari and Pappas [17], who found less shock-induced suppression of water-licking in the SHR than in the same control strain used here.

The failure to observe any simple effect of neonatal 6-OHDA on either our open field measure of emotionality or upon run wheel locomotion generally agrees with the data from this [15] and other laboratories [20]. We have previously found no effect upon one way avoidance [15], but a significant deficit in a shock-induced lick-suppression [17] procedure. Furthermore, two-way avoidance is slightly depressed by neonatal 6-OHDA, but only at a short intertrial interval. (Blouin and Pappas, in preparation). This pattern of behavioral data is reminiscent of that observed after electrolytic-lesioning of the hippocampus [9]. These lesions and neonatal 6-OHDA sympathectomy seem not to affect a fear-emotionality process but rather to impair a response inhibition process. Finally, unlike the effect of intracerebral injections of 6-OHDA in the adult rat [10], neonatal injections of this drug were not found here to have any effect upon diurnal variations in locomotor activity.

Our biochemical data suggest extensive destruction of noradrenergic axons in the cortex. Paradoxically, brainstem neurons show hyperfunction. Furthermore this change may not be specific to catecholaminergic neurons. Rather, our preliminary data indicate considerable enhancement of brainstem serotonin synthesis.

One striking feature of our data is the fact that no consistent effect upon hypothalamic NE is observed. Results from other laboratories have also been inconsistent [12,18]. Decreases in NE content have been reported for spinal cord, cerebellum, hippocampus and cortex while no effect has been observed for the thalamus and corpus striatum [6,20]. This pattern of hyperfunction and depletion suggests axonal destruction in both the descending and dorsal NE pathways as described by Ungerstedt [23], but possibly, sparing of the axons of the ventral NE pathway. Electrolytic lesions in the adult rat, of the locus coeruleus, which seems to be the principal origin of the dorsal NE fibres, causes decreased NE content in the cerebellum, hippocampus and cortex but not in the hypothalamus [21]. These effects are similar to those observed after neonatal 6-OHDA injections. On the other hand electrolytic lesions in the area of pedunculus cerebellus superior, where the axons of the dorsal and ventral systems are adjacent, produce not only regional decreases in NE similar to those observed after lesioning of the locus coeruleus but also significantly reduced hypothalamic NE [21]. If neonatal 6-OHDA produces axonal degeneration only in the spinal and dorsal NE pathways, then the mechanism for this selective destruction has yet to be determined. Perhaps the relatively easier accessibility of these axons [22] or the state of their maturity at time of injection may account for their selective destruction. Alternatively, it has been reported that adult rat brain catecholamine cell bodies show marked regional variation in their susceptibility to the degenerative effects of 6-OHDA [23].

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