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Prevention of Hypertension in the SH Rat: Effects of Differential Central Catecholamine Depletion[1] (39118)

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(Introduced by L. I. Goldberg)

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Central catecholamines have been implicated in the development of hypertension in the Okamoto strain of genetically hypertensive (SH) rats. Six-hydroxydopamine, a neurotoxic agent selective for catecholaminergic neurons, prevents the expression of the hypertensive syndrome when administered to neonatal SH rats (1) or when given intraventricularly to 7-week-old animals (2). In contrast, intraventricular 6-OHDA produces only a transient fall in blood pressure in older SH rats with established hypertension (3). For these reasons it has been suggested that central catecholamines are involved in the "triggering" but not the maintenance of hypertension in the SH rat (3).

Central nervous system lesions combined with fluorescence histochemistry or biochemical analysis have been used to describe the neuronal pathways responsible for the maintenance of normal levels of catecholamines in various regions of the brain (4, 5). Evidence for the involvement of suprabulbar adrenergic systems in blood pressure regulation has been suggested by studies in which the increased blood pressure produced by stimulation of the posterior hypothalamus was blocked by superfusing this region with α adrenergic blocking agents (6). In addition, the blood pressure increase produced by stimulation of the locus coeruleus was prevented by lesions of the posterior hypothalamus, suggesting the involvement of an ascending pathway in this stimulation-induced increase in blood pressure (7). In the present study, we have attempted to explore the role of central noradrenergic mechanisms in the development of hypertension in the SH rat by means of either 6-OHDA administration or selective ablation of ascending noradrenergic pathways. The effect of 6-OHDA administration on the development of blood pressure regulation in normotensive rats of the Kyoto–Wistar strain from which the Okamoto strain is derived (8) was also investigated.

Materials and methods. Male SH rats of the Okamoto strain were obtained from Laboratory Supply Co., Indianapolis, Indiana. Male Kyoto–Wistar rats were furnished by NIH. Animals were housed in group cages containing no more than eight rats per cage. Rats were given access to food and water *ad libitum*. A 6 AM lights-on and 6 PM lights-off cycle of environmental lighting was maintained.

Intraventricular six-hydroxydopamine. Thirty-two-day-old SH or Kyoto–Wistar rats were anesthetized with ether and placed in a Kopf stereotaxic holder. A burr hole was made approximately 1 mm posterior to the bregma and 1.5 mm lateral to the midline. Six-hydroxydopamine (6-OHDA HBr, Sigma, St. Louis, Mo.) (200 μg free base) in 20 μl Merlis solution containing 0.1% ascorbic acid was injected into the right lateral ventricle via a 50 μl Hamilton syringe through a 27 gauge $\frac{1}{2}$-in needle with a stop 4 mm from its tip. Control animals received only the Merlis solution containing ascorbate. The procedure was repeated 6 days later except that the injection was made into the left lateral ventricle.

Lateral tegmental lesions. Bilateral lateral tegmental lesions were placed in 35–40-day-old SH rats that were anesthetized with ether and held in a Kopf stereotaxic instrument with the tooth bar set at zero. A 1.5 mm

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diameter area of skull was removed on either side of the midline at the interaural line. To produce the lesions, a 1 mm stainless steel rod was lowered to the base of the brain 1 mm to either side of the midline at the interaural line. Sham animals were prepared by removing the skull but not lowering the probe. Coordinates for the lesioning procedure and intraventricular injections were according to the atlas of Sherwood and Timiras (9).

Blood pressure measurements were made by the indirect tail-cuff method of Pfeffer *et al.* (10). The animals were warmed for 10–15 min with a 100 W light held approximately 30 cm away. The animals were then placed in a heated restraining device (Narco Bio Systems, Houston, Texas). An occlusion cuff (Buffington Clinical Devices) was placed at the base of the rat's tail proximal to a pressure transducer (Buffington Clinical Devices). Pressure tracings were recorded on a Lumiscribe electrocardiograph (Monotronics Corp., Villa Park, Illinois). Five pressure measurements were recorded for each rat, and the median of these readings was taken as the rat's systolic blood pressure. Pulse rates were obtained from the pressure tracings. The tail-cuff method was validated by comparison of blood pressure obtained with an indwelling catheter in the abdominal aorta in a group of untreated SH rats. Rats were weighed prior to pressure readings. The 6-OHDA treated animals had their blood pressure, heart rate, and body weight monitored biweekly. Animals with tegmental lesions had their pressures monitored approximately every 10 days throughout the development of the hypertensive syndrome (3 months postlesion), and several times during the week prior to sacrifice at 7 months of age.

Animals were killed by decapitation. The brain minus olfactory bulbs was dissected and stored in liquid nitrogen for catecholamine analysis. The brains of 6-OHDA-treated SH and Kyoto–Wistar rats were dissected into the following regions: telencephalon minus septum and corpus striatum, corpus striatum, diencephalon, midbrain, and pons-medulla. The hearts of these animals were removed and stored in liquid nitrogen for subsequent norepinephrine analysis. In addition, the cervical–thoracic spinal cord of the 6-OHDA treated Kyoto–Wistar rats was taken for norepinephrine analysis. The brains of animals with lateral tegmental lesions were dissected into telencephalon and diencephalon. The remainder of the brain was placed in 10% formalin and was subsequently dehydrated, embedded in paraffin, and cut and stained with luxol fast blue and cresyl violet for localization of the lesions. Catecholamines were determined by a modification of the method ot Anton and Sayre (11), using reduced amounts of tissue, reagents, elution, and oxidation volumes.

The data for each experimental group are presented as the mean ± standard error of the mean. Student's two tailed *t* test was used for statistical comparison of sample means.

Results. Effect of intraventricular six-hydroxydopamine on the development of hypertension in the SH rat. Six-hydroxydopamine-treated SH rats had consistently lower blood pressure than vehicle-treated control rats (Fig. 1). At the time of sacrifice (106 days of age) the mean systolic pressure of the 6-OHDA group was 157 ± 8 mm Hg as compared with 202 ± 12 mm Hg ($P < 0.01$) for the control group. The blood pressures of the 6-OHDA-treated rats did not increase significantly over the 6 weeks of blood pressure measurement (148 ± 9 mm Hg at 60 days of age; 157 ± 8 mm Hg at 106 days of age). Vehicle-treated control animals showed elevated blood pressure at 60 days of age (178 ± 5 mm Hg) and remained hypertensive (202 ± 12 mm Hg at 106 days of age). At the time of sacrifice, the mean heart rate of the 6-OHDA-treated group was 379 ± 14 beats per minute as compared with 444 ± 17 beats per minute for the control group ($P < 0.001$). Six-hydroxydopamine treatment may have produced some growth retardation, as seen in Fig. 2. At the time of 6-OHDA administration, treated and control groups weighed the same (6-OHDA, 85 ± 2 g vs control, 86 ± 3 g). Subsequently the 6-OHDA treated group was consistently lighter than control, although at 106 days of age the difference (6-OHDA, 229 ± 9 g; control 240 ± 4 g) was not statistically significant. It was also noted that the 6-OHDA-treated animals did not exhibit clonic convulsions following decapitation.

Table I summarizes the regional brain catecholamine concentrations in the 6-OHDA and control groups. The 6-OHDA-treated animals showed a complete loss of telencephalic (whole telencephalon minus septum and striatum) norepinephrine, a 76% decrease in striatal dopamine, and 64%, 70%, and 52% decreases in norepinephrine respectively in diencephalon, midbrain, and pons-medulla. Mean cardiac norepinephrine concentration in the 6-OHDA rats was higher than control (6-OHDA, 0.72 ± 0.03 μg/g; control, 0.58 ± 0.03 μg/g) ($P < 0.02$), although cardiac norepinephrine content was the same in both groups (6-OHDA, 0.63 ± 0.03 μg; control, 0.57 ± 0.05 μg). The heart weights of the 6-OHDA rats tended to be less than control (6-OHDA, 882 ± 43 mg; control, 972 ± 34 mg) (NS); heart weight expressed per gram of body weight did not differ for treated and control rats.

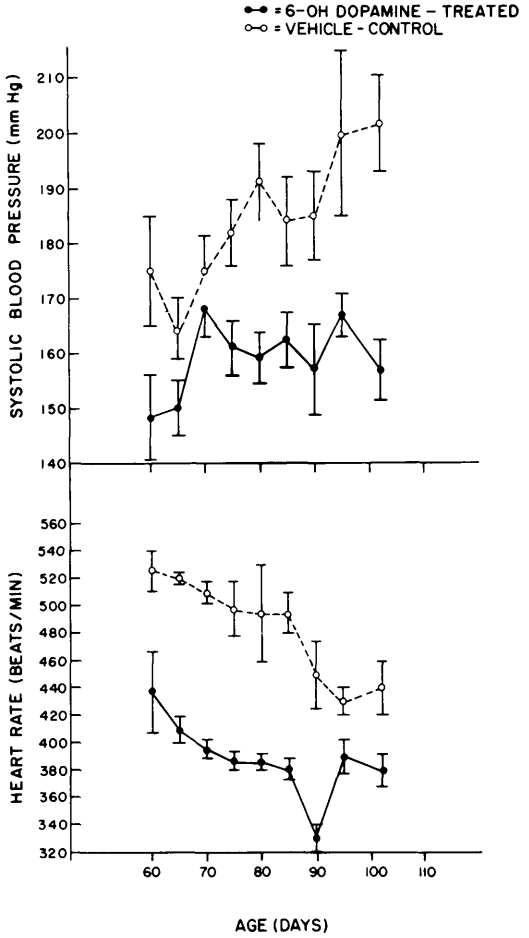

FIG. 1. Top curve: mean systolic blood pressure (mm Hg) of six-hydroxydopamine-treated ●—● and vehicle-control SH rats ○—○ plotted against age. Bars indicate SEM. Bottom curve: mean heart rate of six-hydroxydopamine-treated ●—● and vehicle-control SH rats ○—○ plotted against age. Bars indicate SEM.

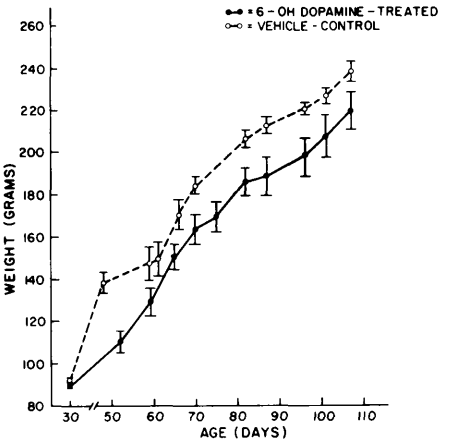

FIG. 2. Growth curves of six-hydroxydopamine-treated ●—● and vehicle-control SH rats ○—○. Weight in grams plotted against age in days. Bars indicate SEM.

Lack of effect of intraventricular 6-OHDA on blood pressure and heart rate in the Kyoto–Wistar rat. The administration of 6-OHDA had no effect on blood pressure or heart rate in Kyoto–Wistar rats. At the time of sacrifice (112 days of age), systolic blood pressure (control, 125 ± 5 mm Hg; 6-OHDA-treated, 125 ± 3) and heart rate (control, 353 ± 56 beats/min; 6-OHDA-treated, 336 ± 17 beats/min) of treated and control animals were not significantly different. As had been seen in 6-OHDA-treated SH rats, the weights of the treated Kyoto–Wistar animals were consistently less than control. At the time of sacrifice, 6-OHDA-treated rats weighed 235 ± 10 g, while vehicle control animals weighed 293 ± 15 g ($P < 0.001$). As was seen with the 6-OHDA-treated SH rats, the treated Kyoto–Wistar did not exhibit clonic convulsions following decapitation.

Table II summarizes the regional brain catecholamine concentrations in the 6-OHDA-treated and control groups of

TABLE I. EFFECT OF 6-OHDA ADMINISTRATION TO SH RATS ON REGIONAL CATECHOLAMINE LEVELS[a]

Region	Telencephalon	Striatum	Diencephalon	Midbrain	Pons-medulla
Catecholamine	Norepinephrine (μg/g)	Dopamine (μg/g)	Norepinephrine (μg/g)	Norepinephrine (μg/g)	Norepinephrine (μg/g)
Control (n = 7)	0.21 ± 0.01	5.22 ± 0.36	0.70 ± 0.04	0.49 ± 0.02	0.40 ± 0.02
6-OHDA (n = 11)	0	1.26 ± 0.26	0.25 ± 0.02	0.15 ± 0.02	0.19 ± 0.03
Decrease from control (%)	−100[b]	−76[b]	−64[b]	−70[b]	−52[b]

[a] Values indicate means ± SEM. n = number of animals.
[b] $P < 0.001$.

TABLE II. EFFECT OF 6-OHDA ADMINISTRATION TO KYOTO–WISTAR RATS ON REGIONAL CATECHOLAMINE LEVELS[a]

Region	Telencephalon	Striatum	Diencephalon	Midbrain	Pons-medulla	Spinal cord
Catecholamine	Norepinephrine (μg/g)	Dopamine (μg/g)	Norepinephrine (μg/g)	Norepinephrine (μg/g)	Norepinephrine (μg/g)	Norepinephrine (μg/g)
Control (n = 4)	0.24 ± 0.01	5.86 ± 0.61	0.39 ± 0.03	0.39 ± 0.02	0.29 ± 0.03	0.14 ± 0.01
6-OHDA (n = 7)	0.01 ± 0.003	2.17 ± 0.22	0.14 ± 0.01	0.18 ± 0.02	0.16 ± 0.03	0.01 ± 0.002
Decrease from control (%)	−96[b]	−63[b]	−64[b]	−54[b]	−45[b]	−93[c]

[a] Values indicate means ± SEM. n = number of animals.
[b] $P < 0.001$.
[c] $P < 0.02$.

Kyoto–Wistar rats. The treated animals showed a 96% decrease in telencephalic (whole telencephalon minus septum and striatum) NE, a 63% decrease in striatal dopamine, and 64%, 54%, and 45% decreases in norepinephrine, respectively, in diencephalon, midbrain, and pons-medulla. The percentage falls in regional brain catecholamine concentration of 6-OHDA-treated Kyoto–Wistar rats as compared with controls are similar to the decreases seen following 6-OHDA treatment of SH rats. Spinal cord norepinephrine (which was not measured in the SH rats) was decreased by 93% in the 6-OHDA-treated Kyoto–Wistar animals. Mean cardiac norepinephrine concentration in the 6-OHDA rats was increased by 32% (6-OHDA, 0.62 ± 0.04 μg/g; control, 0.47 ± 0.02 μg/g, $P < 0.05$), although mean cardiac norepinephrine content was the same in both groups (6-OHDA, 0.45 ± 0.03 μg; control, 0.48 ± 0.03 μg). The heart weights of the 6-OHDA-treated rats (734 ± 40 mg) were 28% less than control (1021 ± 40 mg) ($P < 0.01$), although heart weight expressed per g of body weight was the same in both groups.

Lack of effect of bilateral lateral tegmental lesions in the development of hypertension in the SH rat. Blood pressure during the first 18 weeks of life (3 months postlesion placement) was the same in sham and lesioned animals; both groups developed hypertension over a similar time course. At the time of sacrifice (6 months postlesioning) no difference was seen between the blood pressures of lesioned (190 ± 15 mm Hg) and sham (172 ± 32 mm Hg) groups. There was a significant increase in the heart rate (lesioned 488 ± 22 beats/min vs control 401 ± 22 beats/min, $P < 0.02$) of the animals with lateral tegmental lesions. Although the lesioned rats tended to weigh less than the sham operated animals (273 ± 15 g vs 307 ± 4 g) at the time of sacrifice, this difference was not statistically significant.

Table III summarizes the telencephalic and diencephalic concentrations of norepinephrine in sham and lesioned groups. A 79% reduction was seen in telencephalic

norepinephrine concentration, and a 46% reduction in diencephalic norepinephrine concentration in the lesioned animals.

Histological evaluation showed that the lesions were located in the ventrolateral tegmentum at the level of the caudal inferior colliculus. The lesions were quite similar to those previously described (12), although in general they did not extend posteriorly into the pons. Having been made mechanically rather than electrolytically, the lesions were not cavitary but rather appeared to be bilaterally symmetrical, circumscribed thin lines of destruction (Fig. 3). The lesion tracts destroyed a portion of the lateral central gray before entering the tegmentum. In the tegmentum, the lesions ablated the area between the medial longitudinal fasciculus and the superior cerebellar peduncle. Since the dorsal ascending catecholamine bundle, a major component of the noradrenergic innervation to the forebrain, projects through the region ablated by the lesion, it is probable that a major portion of this pathway is interrupted

TABLE III. EFFECT OF BILATERAL LATERAL TEGMENTAL LESIONS IN THE SH RAT ON FOREBRAIN NOREPINEPHRINE CONTENT

Lesion group	Number of animals	Telencephalic NE (μg/g)	Diencephalic NE (μg/g)
Sham operation	4	0.29 ± 0.01	0.70 ± 0.04
Lateral tegmentum	6	0.06 ± 0.01	0.38 ± 0.02
Difference from sham (%)		−79[a]	−46[a]

[a] $p < 0.001$.

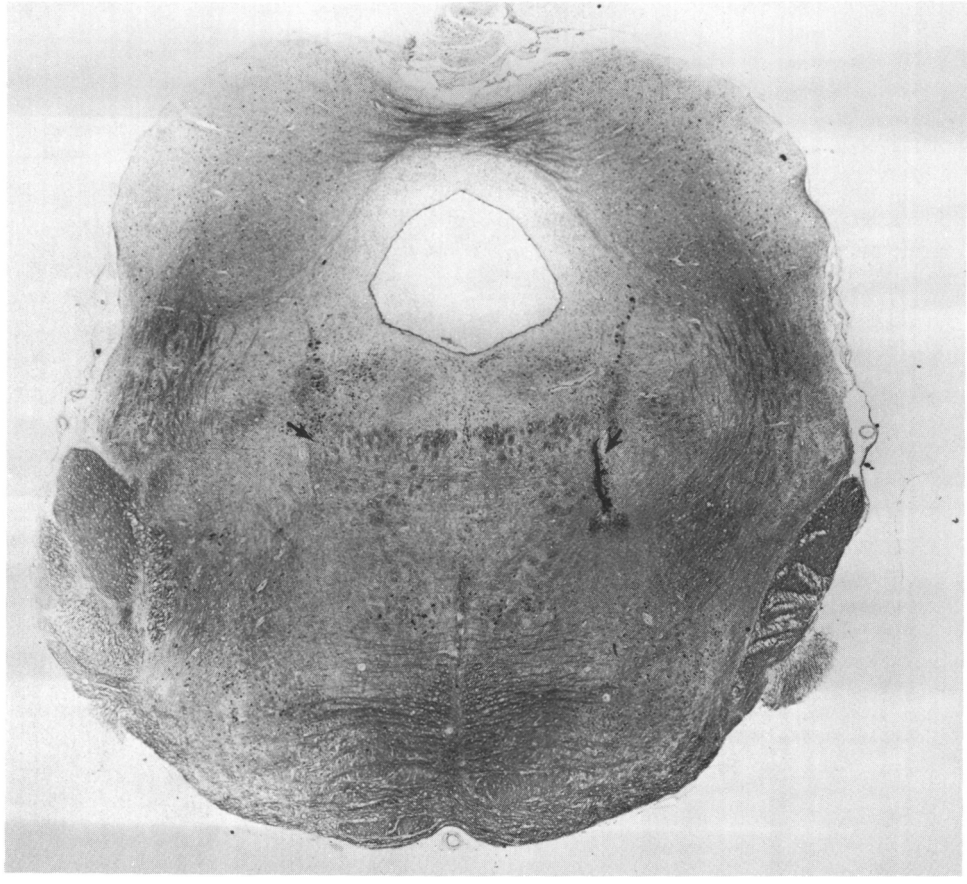

FIG. 3. Histologic section of a representative bilateral lateral tegmental lesion. Arrows indicate the lesion sites, which appear as bilaterally symmetrical thin lines of destruction.

by the lesion. In some cases the lesions extended ventrally to damage other ascending noradrenergic fibers or occasionally the lesions encroached on the anterior locus coeruleus but involvement of the dorsal bundle was common to all lesioned animals.

Discussion. The findings presented, and those of Haeusler *et al.* (2), demonstrate the capacity of 6-OHDA to blunt the expression of the hypertensive syndrome in SH rats of the Okamoto strain. The dosage regimen of 6-OHDA used has no effect on the blood pressure of normotensive Kyoto–Wistar rats despite the fact that the magnitude and regional distribution of the central catecholamine depletions produced by 6-OHDA were similar in both strains of animals. The lack of effect of 6-OHDA treatment on blood pressure in normotensive as contrasted with its effects in genetically hypertensive rats, indicates that central catecholamine depletion does not lead to a nonspecific lowering of blood pressure, but rather interferes selectively with the development of genetically determined hypertension.

In addition to preventing the expression of the hypertensive syndrome, 6-OHDA was found to decrease the heart rate of treated SH animals. Six-OHDA had no effect on the heart rate of Kyoto–Wistar rats. Cardiac norepinephrine content of both SH and Kyoto–Wistar animals was unaffected by intraventricular 6-OHDA. In fact, norepinephrine concentration in the hearts of treated rats was greater than control, indicating that the bradycardia produced in the SH rat by this treatment is not due to a loss of peripheral neurotransmitter and is more likely related to central catecholamine depletion.

Intraventricular administration of 6-OHDA to SH or Kyoto–Wistar rats causes depletion of catecholamines in all regions of the brain (telencephalon, striatum, diencephalon, midbrain, and pons-medulla) and spinal cord. While it is clear that such widespread destruction of central noradrenergic terminals has a marked effect on the development of the hypertensive syndrome in the SH rat, the use of this neurotoxic agent by the intraventricular route does not permit identification of the specific central catecholaminergic systems involved in this process. However, the technique of selective lesion placement can be applied to the study of the participation of specific ascending catecholamine systems in the modulation of the hypertensive syndrome. Three main ascending noradrenergic pathways have been characterized neuroanatomically: the dorsal tegmental bundle, the paraventricular system, and the central tegmental tract (4, 13, 14). These pathways can be individually and selectively interrupted at various levels of brain.

The dorsal tegmental bundle is an ascending fiber tract whose integrity is essential to the maintenance of the noradrenergic innervation to the telencephalon and provides part of the innervation to the diencephalon. Interruption of the dorsal tegmental bundle by means of bilateral lateral tegmental lesions as can be seen in Table III produced a 79% reduction in telencephalic norepinephrine levels as well as a 46% reduction in diencephalic norepinephrine. Despite these decreases, the animals developed the hypertensive syndrome. It seems unlikely therefore that telencephalic norepinephrine is essential for the development of genetically determined hypertension. It is possible, however, that the remaining catecholaminergic innervation to the telencephalon spared by the lesion is still capable of modulating the hypertensive syndrome despite removal of a major portion of the ascending dorsal tegmental bundle. The ability of 6-OHDA to prevent genetically determined hypertension in contrast to tegmental lesions indicates that this drug is effective either because it is removing a critical amount of catecholaminergic nerve endings or because a specific pathway mediating the hypertensive syndrome is among those affected by the drug. The technique of stereotaxic lesion placement should make it possible to determine if such a critical pathway exists.

Summary. Six-hydroxydopamine (6-OHDA) was administered intraventricularly to 6-week-old male spontaneously hypertensive (SH) rats of the Okomoto strain and to normotensive rats of the Kyoto–Wistar strain. In addition, bilateral lateral tegmental lesions were placed in 35–40-day-old SH rats to interrupt ascending noradrenergic pathways. SH rats treated with 6-OHDA did not

develop hypertension and had lower heart rates than control rats. Blood pressure and heart rate of Kyoto–Wistar animals were unaffected by the drug treatment. 6-OHDA produced widespread depletion of norepinephrine throughout the CNS of both SH and Kyoto–Wistar rats. Bilateral lateral tegmental lesions interrupted the dorsal noradrenergic bundle and depleted forebrain norepinephrine. These lesions did not prevent the development of hypertension and led to an increased heart rate. It is concluded that 6-OHDA does not produce its effect through a nonspecific lowering of blood pressure, but rather, that it interferes with the expression of the hypertensive syndrome. The lack of effect seen following depletion of forebrain norepinephrine as the result of interruption of the dorsal noradrenergic bundle indicates that the fibers destroyed by this lesion are not essential for the development of genetically determined hypertension.

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