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Early Behavioral and Catecholaminergic Effects of 6-Hydroxydopamine and Guanethidine in the Neonatal Rat[1]

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PAPPAS, B. A., D. A. V. PETERS, S. K. SOBRIAN, A. BLOUIN AND B. DREW. *Early behavioral and catecholaminergic effects of 6-hydroxydopamine and guanethidine in the neonatal rat.* PHARMAC. BIOCHEM. BEHAV. 3(4) 681–685, 1975. – Newborn rats received 7 consecutive daily injections of 6-hydroxydopamine (6-OHDA) or guanethidine. Locomotor activity, measured at 3 day intervals, was differentially affected by these drugs, although neither drug eliminated a characteristic pattern of ontogeny of locomotor activity. Differing neurochemical effects were also observed. 6-OHDA decreased tyrosine hydroxylase activity in cortex and cerebellum, increased it in the brainstem and had no effect on the hypothalamus. Guanethidine slightly elevated enzyme levels in all four brain regions, with the elevation in brainstem significant at 16 days of age. Regional brain changes in enzyme activity after 4 daily 6-OHDA injections beginning at 1, 5 or 9 days of age indicated that toxic effect of 6-OHDA upon catecholaminergic neurons was age dependent. These data are not consistent with a simple interpretation either in terms of maturational changes in blood brain barrier permeability to 6-OHDA or neuronal uptake of the drug. Further analyses of brainstem areas indicated that the increased brainstem enzyme activity after 6-OHDA was restricted to the pons.

6-OHDA Guanethidine Neonatal Locomotion Catecholamines

PERIPHERAL neonatal injections of 6-hydroxydopamine (6-OHDA) appear to cause selective destruction of the dorsal noradrenergic system [19,23], as well as permanent destruction of the peripheral sympathetic nervous system [15,24]. Recently, Burnstock and his colleagues have demonstrated that chronic administration of guanethidine sulphate in adult rats produces a permanent peripheral sympathectomy [1,8]. Furthermore, injecting guanethidine into the neonatal rat also leads to loss of noradrenergic fluorescence of peripheral sympathetic neurons [6,7]. However, no data were collected on the effects of this treatment upon the central nervous system. Since it has been suggested that at least part of the central effects of neonatal 6-OHDA may be due to the feedback effects upon the brain of the peripheral sympathectomy [17, 18, 19] we compared in this experiment, the early effects of neonatal 6-OHDA and guanethidine upon regional brain activity of tyrosine hydroxylase. In addition, the effect of age of injection of 6-OHDA upon regional brain enzyme activity was examined since recent reports suggest differential age-related development of blood-brain barrier permeation by 6-OHDA [12,21].

Our second major purpose was to compare the effects of these drugs upon the ontogeny of locomotor activity in the rat. Activity increases in the rat until about 15 days of age after which it declines towards adult levels [16]. The increase was interpreted as due to the early maturation of hindbrain noradrenergic circuits which mediate behavioral excitation. However, both brain [14] and peripheral sympathetic [4] catecholamine systems show extensive

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maturational changes during the first two weeks of life. Thus, if guanethidine, unlike 6-OHDA, were shown here to have minimal central effects, then comparison of the effects of these drugs would permit determination of whether the pattern of ontogeny of locomotor arousal reflects central or peripheral maturation of catecholamine neurons.

METHOD

Animals

Multiparous Wistar female rats were mated with males in our laboratory in a 3:2 female–male ratio per colony cage. Ten days after the start of the mating procedure, the females were moved to nesting cages and left undisturbed until parturition. At birth, the litters were culled to 8–12 pups. The pups were weaned at 25 days of age and placed in colony cages in groups of 4–6.

Procedure

Injections. The amount of drug to be administered was calculated from the weight of the entire litter. The drugs were dissolved in 0.9 percent saline vehicle with ascorbic acid added (1 mg/ml) to prevent oxidation. Control rats received only the vehicle, SC in the upper back, while 6-OHDA litters also received 50 mg/kg of the bromide salt (Regis Chemical). Guanethidine litters received 25 or 50 mg/kg of guanethidine sulfate (CIBA Pharmaceutical). Behavioral testing was carried out on pups who received the lower dose – neurochemical assays on those receiving the higher dose. Sympathetic nervous system destruction in the neonatal rat is obtained with a dose of 20 mg/kg while 40 mg/kg produce no enhancement of this effect [6, 7, 17]. These injections were given daily for 8 consecutive days beginning the day of birth. In addition, other rats were injected with either 6-OHDA (50 mg/kg) or vehicle for 4 consecutive days beginning on Days 1, 5 or 9 after birth to determine the effects of age of injection of the drug upon regional brain tryrosine hydroxylase activity.

Infant activity tests. Pups were tested starting at 3 days of age and every third day thereafter until 21 days of age, and at 25 and 30 days. During the period of drug administration (i.e., Days 1–8), the animals were tested prior to injection. On Day 25, testing occurred prior to weaning. Each pup was tested only once and each test point contained pups from several litters.

A 30 minute measure of activity was recorded with a movement sensing device [10] which was sufficiently sensitive to record large torsional body movements. The rats were placed in a cardboard box containing woodshavings which rested on a capacitance sensing coil. The dimensions of the container varied with the age of the animal: Day 3, 6–7.5 cm X 7.5 cm X 5 cm, Day 9, 12 – 7.5 cm X 7.5 cm X 9 cm; Day 15 – 14 cm X 9 cm X 9 cm; Day 18, 21, 25, 30 – 10 cm X 10 cm X 21 cm. Illumination in the testing room was provided by a 25 W red bulb suspended 50 cm above the base of the test apparatus; the temperature of the room was maintained at 85°F. Total duration of the activity test was 30 min. Activity counts were automatically recorded every 5 min by a digital logic array.

Tyrosine hydroxylase assays. Rats who had not undergone activity testing were sacrificed by decapitation. The brains were quickly removed and dissected on an ice-cooled, saline rinsed plate into cerebellum, brainstem (pons, medulla and midbrain), hypothalamus, and cortical slices sampled systematically from the entire cortex. Using a template etched on a glass plate and a fine gauge cutting wire the brainstems of some 25-day old rats were separated into dorso-medial and ventro-lateral pontine sections and a medullary section. These sections were selected so as to contain respectively the locus coeruleus (A6), the A7 noradrenergic nuclei, and finally the A1, A2, A4, and A5 nuclei in the medullary section [25]. After removal of the cerebellum, the brainstem was separated by a coronal cut at the caudal tips of the hemispheres. Coronal cuts were than made 2 mm and 6 mm caudal and parallel to the first. Tissue posterior to the 6 mm cut was discarded thus leaving pontine and medullary tissue blocks. The latter contained the A1, A2, and A4 and A5 nuclei. The pontine tissue block was further subdivided by saggital sections of the lateral quarters. The ventral two-thirds portions of these lateral sections were then removed. Each of these portions contained A7. The remaining medial pontine tissue block was then horizontally sectioned to yield a dorsal half containing A6.

After dissection, the brain areas 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 [19].

RESULTS

Infant Activity

Figure 1 shows mean group activity scores (30 min totals) as a function of age for the 3 groups. The means were calculated from at least 7 scores. Guanethidine injected rats were more active than either vehicle or 6-OHDA injected rats both during the 8 days of drug injections and until 15 days of age, 1 week after cessation of drug injection. The guanethidine rats were significantly more active than 6-OHDA rats by day 6 ($p<0.05$), more active than vehicle controls by Day 9 ($p<0.05$) and showed peak activity at 12 days. Activity began to decline in the guanethidine rats at Day 15 and by Day 18 there were no further differences between them and the vehicle rats.

The 6-OHDA injected rats, on the other hand, were significantly less active in comparison to vehicle controls during drug treatment ($p<0.05$). On the first test day after cessation of drug administration (Day 9), however, the 6-OHDA rats showed a marked increase in activity and were significantly more active than vehicle controls from Days 9 to 21 ($p<0.05$). At 18 days of age, the 6-OHDA group was also significantly ($p<0.05$) more active than the guanethidine group. Peak activity for the 6-OHDA group occurred at Day 18 as it did for the vehicle group. By Days 25 and 30, none of the three groups differed in activity levels, and all three showed a significant increase from Day 25 to Day 30 ($p<0.001$).

Tyrosine Hydroxylase Activity

Eight daily injections of guanethidine and 6-OHDA produced dissimilar effects on regional brain tyrosine hydroxylase activity. As shown in Fig. 2, 6-OHDA reduced

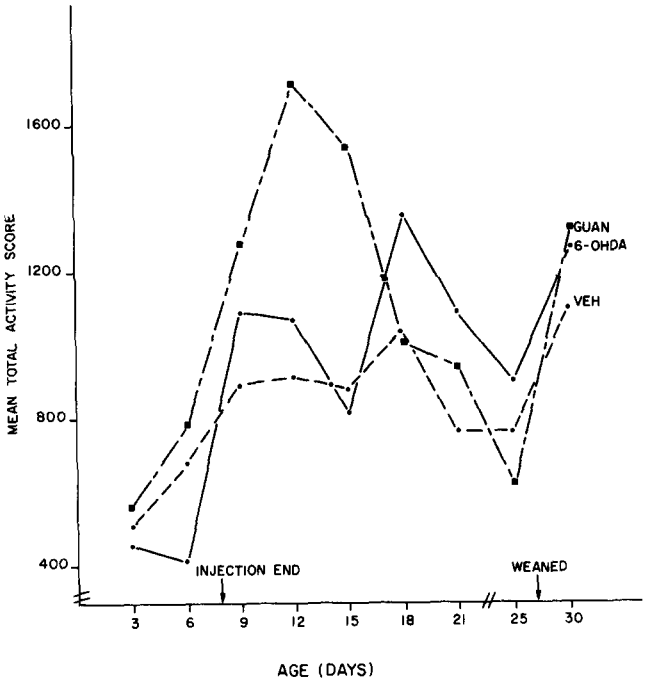

FIG. 1. Mean locomotor activity scores during 30 min test period for rats injected with vehicle, guanethidine or 6-OHDA at Ages 1–8. Each point is the average score of 7–8 rats. Scores for Days 3 and 6 were determined immediately prior to daily drug injections.

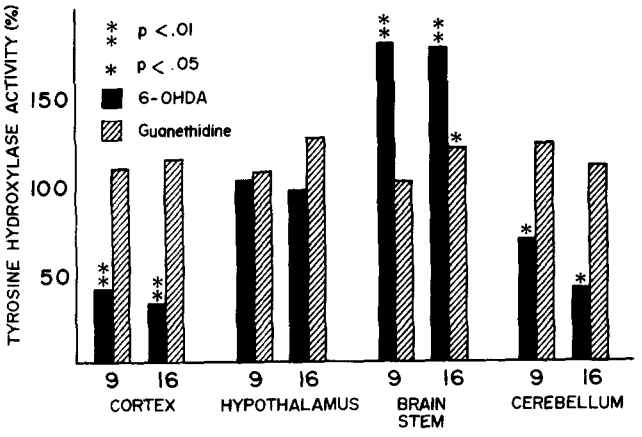

FIG. 2. Tyrosine hydroxylase activity in the 4 brain areas of rats injected with guanethidine or 6-OHDA on Days 1–8. Data are shown for Day 9 and Day 16, and represent average of 6 rats, expressed as a percentage of the value of vehicle controls who were sacrificed and assayed at the same time as each of the drug groups. Asterisks indicate significant differences (t-test) between drug and vehicle control groups.

enzyme activity in the cerebellum and cortex. No change was observed in the hypothalamus while a marked increase was evident in the brainstem. Furthermore, all these effects of 6-OHDA were present at 9 days of age, one day after cessation of injections. In contrast, enzyme activity in all brain regions of guanethidine injected rats was equivalent to

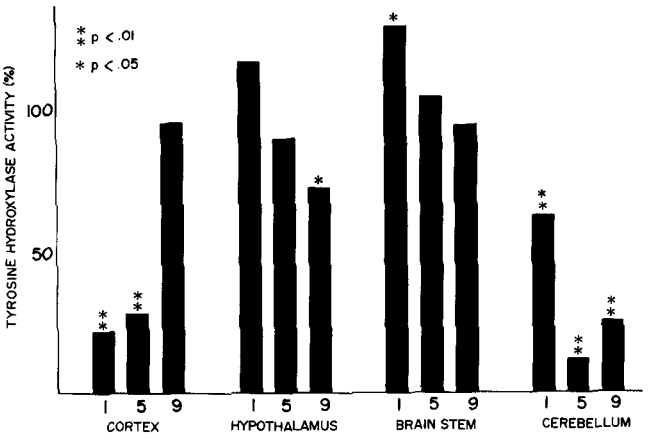

FIG. 3. Mean tyrosine hydroxylase activity in the 4 brain regions (n's = 6) of rats injected on Days 1–4, 5–8, or 9–12 with 50 mg/kg 6-OHDA. Numbers on the abscissa denote the age at which injections commenced. The rats were sacrificed and assayed at 25 days of age along with vehicle control rats and the data are expressed as percentages of these vehicle control values. Asterisks indicate significant differences between 6-OHDA and vehicle values (t-test).

control values both at 9 and 16 days of age, except for a significant increase at 16 days of age in the brainstem.

As shown in Fig. 3, there were also complex and marked maturational changes in the regional susceptibility of brain tissue to the effect of 6-OHDA upon tyrosine hydroxylase activity. Tyrosine hydroxylase activity in the cortex was severely attenuated by 4 days of injections beginning at 9 days. Conversely, hypothalamic enzyme activity was reduced only for rats who received injections beginning at 9 days. Brainstem activity was increased only in rats who were injected beginning on day one, while enzyme activity in the cerebellum was significantly reduced at all injection ages. However, maximal cerebellar reductions were clearly evident when the injections were administered beginning on Day 5 or Day 9.

In rats injected on Days 1–4 with 6-OHDA, and sacrificed at 25 days of age, the increase in brainstem tyrosine hydroxylase activity was not observed in all three brainstem regions. Rather the pontine regions containing the locus coeruleus (A6) and more ventral region containing A7 both showed very significantly increased enzyme activity. As shown in Table 1, the medullary region containing cell groups A1, A2, A4, and A5 showed enzyme activity levels which were not significantly different from vehicle control values.

DISCUSSION

The data on regional brain tyrosine hydroxylase activity indicate that unlike 6-OHDA, guanethidine had no neurotoxic effect upon central catecholamine neurons when injected subcutaneously. These results contrast with those of Luizzi *et al.* [13] who have reported that 5 daily SC injections of guanethidine (50 mg/kg) significantly reduced whole brain dopamine and noradrenaline (Ne) in Swiss Webster mice for up to 30 days after treatment. Since neonatal guanethidine injections permanently reduce Ne levels in peripheral organs [13,17], it seems that the destructive

TABLE 1

TYROSINE HYDROXYLASE ACTIVITY (n MOLE DOPA/HR/g TISSUE) IN BRAINSTEM AREAS OF 6-OHDA AND VEHICLE INJECTED RATS (N = 6). THE RATS HAD BEEN INJECTED ON DAYS 1–4 AND ASSAYED AT 25 DAYS OF AGE. SIGNIFICANCE LEVELS WERE DETERMINED BY *t*-TEST.

	Control	6-OHDA	Percent Control
A6 (locus coeruleus)	14.3 ± 1.4	32.0 ± 2.3	224 ($p<0.001$)
A7	15.0 ± 1.9	31.9 ± 4.7	213 ($p<0.01$)
A1, A2, A4, A5	33.3 ± 1.9	37.0 ± 1.9	111 (N.S.)

effects of 6-OHDA on peripheral sympathetic neurons contribute little to its concomitant central catecholaminergic effects.

In agreement with Liew and Taylor [12] who reported data on regional brain Ne level after several injections at various early ages, our data indicated that cortical catecholaminergic neurons were affected only when 6-OHDA was administered during the first 8 days after birth. Also in agreement, cerebellar enzyme activity was reduced the most by injections during the second week of life while the increase in brainstem activity was elicited only by injections during Days 1–4. Finally, hypothalamic enzyme activity was unaffected by injections throughout Days 1–8, but significantly reduced by injections on Days 9–12. These age related changes in regional brain susceptibility to 6-OHDA are probably not due simply to non-uniform maturational patterns of development of the blood brain barrier as has been suggested [12,21]. This is indicated by the fact that 6-hydroxydopa, a 6-OHDA precursor which has considerably greater access through the blood brain barrier than 6-OHDA, produced neurotoxic effects in the newborn rat similar to those of 6-OHDA [26]. The effects of 6-OHDA on the newborn rat brain may partly depend upon the functional maturity of the catecholaminergic neurons in various brain areas as well as regional blood brain barrier permeability. However, intracisternal injections of 6-OHDA in the one day old rat reduce brainstem and hypothalamic Ne and tyrosine hydroxylase activity [22], suggesting early uptake functioning mechanisms in these areas. Clearly it is not possible to determine from the present findings, the contributions of neuronal functional maturity and blood-brain barrier maturation to the observed effect of neonatal 6-OHDA.

The increased brainstem catecholaminergic activity after neonatal 6-OHDA was found here to be restricted to the region of the locus coeruleus (A6) and the A7 nucleus as described by Ungerstedt [25]. Furthermore, the observed reduction in tyrosine hydroxylase activity in the rest of the brain areas also suggests that the drug's toxic effect, at least when administered during the first 8 days, was restricted to axons emanating from the locus coeruleus. It is not yet clear whether the locus coeruleus is also destroyed by neonatal 6-OHDA, as it is by intraventricular injection in the adult rat [5], with a resulting subsequent axonal hyperproliferation into this region from adjacent nuclei or whether the locus coeruleus itself remains intact and becomes hyperfunctional even though its forebrain and cerebellar axons are permanently destroyed.

Guanethidine and 6-OHDA had differing effects upon infant activity and the locomotor effects of the drugs were restricted to the period of injection and the subsequent 10–12 day period. Campbell and his co-workers [2, 9, 16] have suggested that the ontogeny of locomotor activity in the rat reflects the early maturation of hindbrain, noradrenergic excitatory circuits. In this experiment, our biochemical assay data suggest that neonatal 6-OHDA injections cause early and rapid destruction of the dorsal noradrenergic axons and enhancement of catecholamine synthesis in the brain stem. These neurochemical effects did not alter the normal ontogeny of locomotor activity insofar as 6-OHDA rats showed the same ontogenetic time course of activity changes as did control animals. Guanethidine has peripheral noradrenergic destructive effects similar to those of 6-OHDA [6, 7, 17]. However, as this experiment indicates, it minimally affects brain tyrosine hydroxylase activity. This drug caused hyperactivity, with peak activity in guanethidine injected rats occurring at 12 days of age. Thus, the ontogeny of locomotor activity in the rat does not seem to depend upon the integrity of the dorsal noradrenergic circuit or the peripheral sympathetic system.

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