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EFFECT OF INTRAVENTRICULAR INJECTIONS OF 6-HYDROXYDOPAMINE IN NEONATAL RATS ON THE CATECHOLAMINE LEVELS AND TYROSINE HYDROXYLASE ACTIVITY IN BRAIN REGIONS AT MATURITY

DAVID A. V. PETERS, BRUCE A. PAPPAS, HILLEL TAUB and MATTI SAARI

Department of Pharmacology, University of Ottawa, Ottawa, Ontario, and
Department of Psychology, Carleton University, Ottawa, Ontario, Canada

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Abstract—Intraventricular injections of 6-hydroxydopamine (6-OHDA, 25 μg) on days 1 and 2 after birth produced a marked change in tyrosine hydroxylase activity in only one rat brain region, the corpus striatum, whereas the ability of brain tissue to synthesise catecholamines, as measured by the rate of tyrosine hydroxylation in brain homogenates, was significantly reduced in amygdala, cortex, hippocampus, hypothalamus, septum and thalamus, and greatly increased in both dorsal and ventral pons. Both increases and decreases in the rate of tyrosine hydroxylation in all brain regions with the exception of the corpus striatum and septum were blocked by pretreatment of the newborn rats with desmethylimipramine (DMI) 45 min before the 6-OHDA. Similarly, norepinephrine (NE) levels were reduced in amygdala, cortex, hippocampus, hypothalamus, septum and thalamus, and increased in cerebellum, medulla, midbrain and pons. These 6-OHDA-induced changes in NE were also blocked by DMI pretreatment. The NE and dopamine (DA) levels and the rate of tyrosine hydroxylation were markedly reduced in striatum, and DMI again blocked the depletion of NE but potentiated both the decrease in DA and the decreased rate of tyrosine hydroxylation. It is suggested that the pattern of decreased NE levels in forebrain regions and increased NE levels in hindbrain after neonatal 6-OHDA treatment does not depend on degeneration of forebrain terminals, since at suitably low 6-OHDA levels the NE changes can be shown to occur in the absence of changes in the tyrosine hydroxylase activity.

Systemic administration of 6-hydroxydopamine (6-OHDA) to newborn rats profoundly affects brain catecholamine levels and tyrosine hydroxylase activity in several regions of rat brain at maturity [1–11]. Whereas the depletion of norepinephrine (NE) is severe in most forebrain regions and cerebellum [3–7, 9, 11], the NE level tends to be markedly increased in midbrain and pontine regions [3–6, 9, 11]. A similar pattern of decreases and increases in tyrosine hydroxylase activity has also been reported after neonatal peripheral administration of 6-OHDA [4, 5]. These effects of peripheral injections of 6-OHDA have been attributed to degeneration of forebrain NE terminals arising from the locus coeruleus and partial damage to the latter nucleus followed by regenerative sprouting from it or collateral sprouting from adjacent neurons [12].

Several reports suggest a somewhat different effect of intracerebral 6-OHDA injections in the neonate. Although the same profound reduction in catecholamine levels and tyrosine hydroxylase activity occurs in forebrain [10, 13, 14], the NE level was reported to be unchanged in brain stem [10, 13, 14] and reduced in midbrain [13]. However, Sachs and Jonsson [12] have suggested that regeneration in brain stem NE neurons also occurs after intracerebral 6-OHDA injections provided that low doses of the drug are used (10–50 μg/rat). We now report the effect of small intraventricular injections of 6-OHDA (2 × 25 μg/rat) on both catecholamine levels and tyrosine hydroxylase activity in discrete regions of rat brain.

MATERIALS AND METHODS

Offspring of Wistar rats were given two intraventricular injections of 6-OHDA (25 μg/rat, Regis Chemical Co.), the first within 24 hr after birth and the second 24 hr later, as previously reported [15]. The injections were administered free-hand into alternate ventricles to pups anesthetized by ether. Control animals received the vehicle alone (2.5 μl saline and ascorbic acid, 1 mg/ml). Desmethylimipramine (DMI, 25 mg/kg) was administered subcutaneously 45 min prior to the intraventricular injections in some animals.

The rats were killed by decapitation at approximately 70 days of age, and the brains rapidly removed and dissected into 13 discrete brain regions by the method of Saari and Pappas.* The brain parts were used for the assay of either NE and DA or tyrosine and tyrosine hydroxylase. Tyrosine hydroxylase was assayed by two different procedures both involving the conversion of L-[^{14}C]tyrosine to L-[^{14}C]dopa. In the first method, brain tissue samples were solubilized by homogenation in a hypotonic buffer containing detergent. The solubilized tyrosine hydroxylase was then assayed in the presence of added synthetic cofactor as previously described [16]. Briefly, the brain tissues were homogenized in 0.02 M Tris–acetate

* M. Saari and B. A. Pappas, manuscript in preparation.

buffer, pH 6.0, containing 0.2% Triton X-100, and aliquots were incubated for 30 min at 37° in the presence of a pteridine cofactor (6,7-dimethyl-5,6,7,8-tetrahydropterin stabilized with 2-mercaptoethanol), a dopa decarboxylase inhibitor (NSD 1034) and L-[^{14}C]tyrosine. The L-[^{14}C]dopa produced was isolated on an alumina column [17] and the radioactivity measured in a Nuclear Chicage Mark 1 liquid scintillation system. For the brain regions studied, the radioactivity measured was always at least four times and normally greater than ten times the blank values.

The second method was that reported by McGeer *et al.* [17] and was identical to the first method except that the enzyme was assayed in sucrose homogenates without solubilization or addition of exogenous cofactor. This method involves a more complex system in that the enzyme is located within the intact nerve terminals. In this case, the conversion of L-[^{14}C]tyrosine to L-[^{14}C]dopa is limited not only by the enzyme activity but also by the uptake of substrate into the synaptosomes and by the available endogenous cofactor within the synaptosomes. Since 6-OHDA-induced damage to the nerve terminals might involve the uptake mechanism or the endogenous cofactor system, the assay of McGeer *et al.* [17] was thought to be possibly a better indicator of damage than a measure which involved enzyme activity only. In order to distinguish between the two assays, the term tyrosine hydroxylase is restricted to the solubilized and cofactor supplemented preparation. The data from the McGeer *et al.* [17] assay are referred to as the rate of tyrosine hydroxylation. As for the tyrosine hydroxylase assay, the measured radioactivity from tissue samples was always greater than four times the blank value. After dissection, the brain parts were either immediately homogenized in ice-cold 0.28 M sucrose for the tyrosine hydroxylation assay of McGeer *et al.* [17] or frozen in liquid nitrogen until convenient for the assay of either tyrosine hydroxylase or the catecholamines. Tyrosine levels in the homogenates used for the tyrosine hydroxylase assays were determined by the method of Waalkes and Udenfriend [18]. The NE and DA were assayed spectrofluorometrically by the method of Laverty and Taylor [19] after extraction from an acidified butanol homogenate [20]. The fluorescence readings were at least four times the blank values for all tissues with the exception of the septum for which a tissue to blank ratio of 2–3 was obtained.

RESULTS

Table 1 shows the tyrosine hydroxylase activity in various brain regions of rats receiving two intraventricular injections of 6-OHDA (25 μg) or its vehicle on days 1 and 2 after birth. One group of rats also received a subcutaneous 25 mg/kg DMI injection. The only marked alteration in tyrosine hydroxylase activity was a 66 per cent decrease in corpus striatum. In this experiment, the ability of sucrose homogenates of brain tissue to hydroxylate L-[^{14}C]tyrosine to L-[^{14}C]dopa in the absence of exogenous cofactors (tyrosine-hydroxylating ability) was also studied. Figure 1 shows that the tyrosine-hydroxylating ability was markedly reduced by neonatal 6-OHDA treatment in amygdala, cortex, hippocampus, hypothalamus, septum and thalamus. In contrast, there was a marked increase in activity in both regions of the pons. DMI pretreatment partially or completely blocked both the increase in the rate of tyrosine hydroxylation in pons and the decrease in activity in amygdala, cortex, hippocampus and hypothalamus. Figure 2 shows marked decreases in NE level in cortex, hippocampus, hypothalamus and septum with increases in cerebellum, medulla, midbrain and pons. Both increases and decreases in NE content were blocked by DMI pretreatment. In the same experiment, neonatal 6-OHDA treatment significantly reduced both NE and DA levels as well as the rate of tyrosine hydroxylation in corpus striatum (Fig. 3).

Table 1. Effect of neonatal 6-OHDA and DMI treatment on the tyrosine hydroxylase activity in rat brain regions*

Brain region	Tyrosine hydroxylase (nmoles/g/hr)			
	Vehicle	DMI	6-OHDA	6-OHDA + DMI
Amygdala	31.0 ± 1.6	30.3 ± 1.6	31.0 ± 2.2	29.7 ± 3.1
Cerebellum	11.4 ± 0.5	10.6 ± 0.5	9.6 ± 0.4†	10.2 ± 0.3
Cortex-temporal	18.9 ± 0.7	18.8 ± 0.8	18.1 ± 0.9	19.1 ± 1.6
Cortex-remainder	12.7 ± 0.4	13.2 ± 1.0	11.6 ± 0.8	12.8 ± 0.6
Hippocampus	27.6 ± 0.6	27.0 ± 1.3	26.1 ± 1.4	27.7 ± 1.0
Hypothalamus	79 ± 5	80 ± 5	64 ± 5	70 ± 5
Medulla	21.7 ± 0.8	24.3 ± 2.1	21.2 ± 0.9	24.7 ± 0.8‡
Midbrain	50.4 ± 1.3	46.3 ± 1.3	38.3 ± 1.7†	40.8 ± 3.5
Pons-dorsal	46.6 ± 2.7	54.7 ± 2.1	51.3 ± 3.6	52.1 ± 4.5
Pons-ventral	24.9 ± 1.3	27.4 ± 1.1	22.0 ± 0.8	27.1 ± 2.2‡
Septum	44.5 ± 2.7	50.0 ± 3.0	43.5 ± 4.5	41.8 ± 4.2
Striatum	229 ± 15	230 ± 8	77 ± 11†	64 ± 3§
Thalamus	43.7 ± 2.4	38.9 ± 1.1	39.5 ± 2.4	43.6 ± 3.3

* 6-OHDA (25 μg) was given intraventricularly on days 1 and 2 after birth in alternate lateral ventricles. DMI (25 mg/kg) was given subcutaneously 45 min before each 6-OHDA treatment. The rats were killed at approximately 70 days of age and the total tyrosine hydroxylase activity was assayed in the various brain regions. Results are the mean ± S. E. M. for groups of five rats.

† $P < 0.05$, when compared with the vehicle group.

‡ $P < 0.05$, when compared with the 6-OHDA group.

§ $P < 0.05$, when compared with the DMI group.

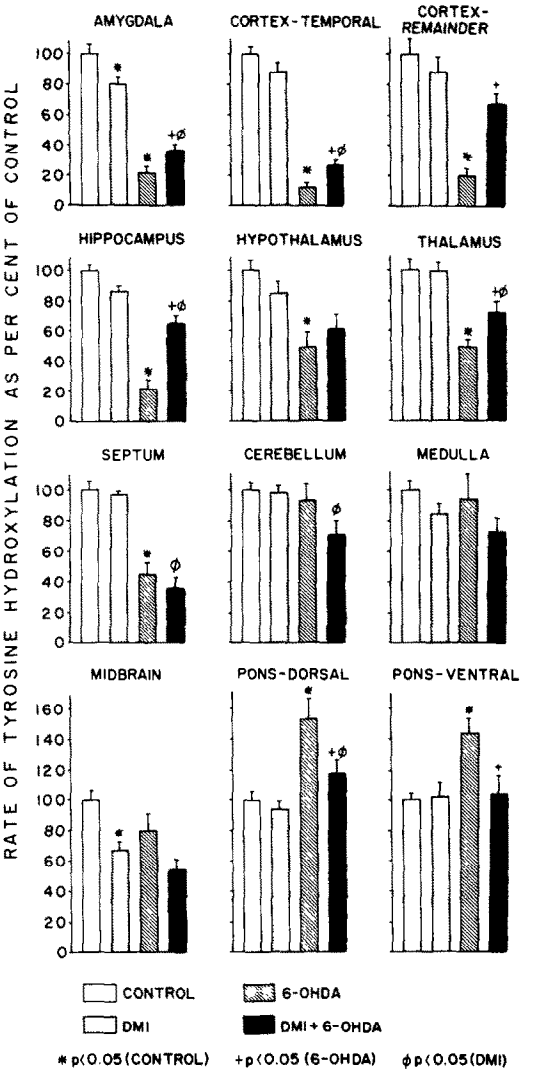

Fig. 1. Effect of 6-OHDA injections to neonatal rats on the rate of tyrosine hydroxylation in brain regions at maturity. Rats were given intraventricular injections of 6-OHDA (25 μg) on days 1 and 2 after birth and were killed at approximately 70 days of age. DMI (25 mg/kg, i.p.) was given to some rats 45 min before the 6-OHDA. The rate of conversion of L-[^{14}C]tyrosine to L-[^{14}C]dopa (tyrosine hydroxylation) was measured in brain homogenates and calculated as a percentage of the control values. Results are the mean ± S.E.M. for goups of five animals.

DMI pretreatment completely blocked the 6-OHDA-induced decrease in NE but significantly potentiated both the decrease in DA and the reduction in the rate of tyrosine hydroxylation in sucrose homogenates.

DISCUSSION

Administration of 6-OHDA to newborn rats appears to produce a permanent destruction of a certain number of central NE nerve terminals without affecting the development of the remaining terminals [21]. The damage to the NE terminals is accompanied by a massive reduction in catecholamine levels, catecholamine uptake and tyrosine hydroxylase activity [3–7, 9, 11, 13, 14]. A single 100 μg intracisternal injection of 6-OHDA to immature rats has been reported to produce an 80 per cent or more reduction

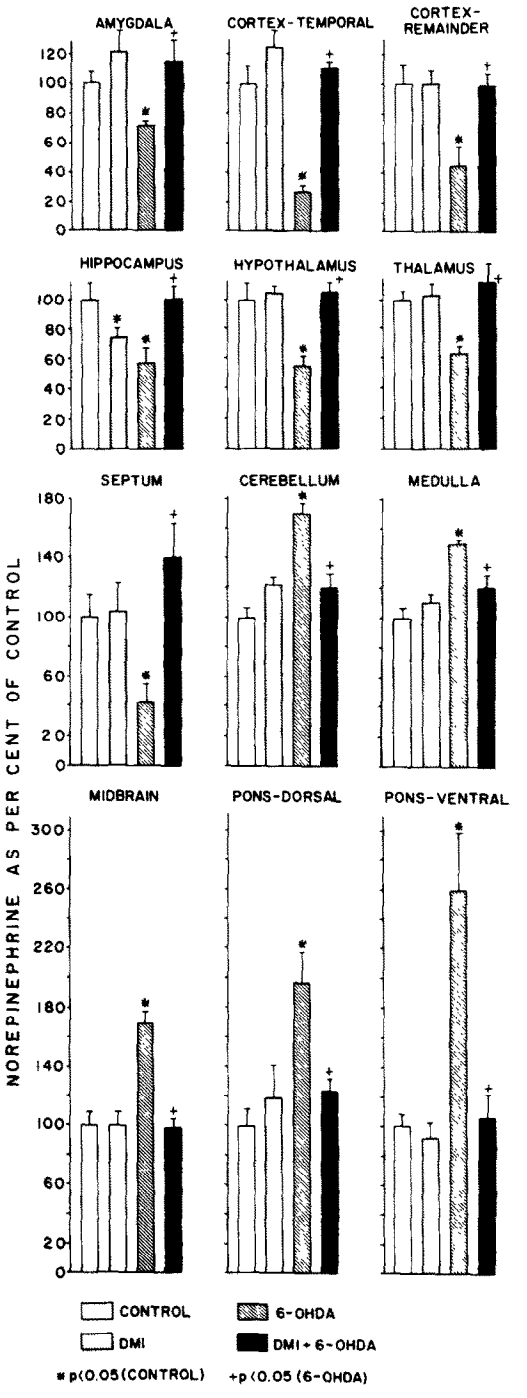

Fig. 2. Effect of 6-OHDA injections to neonatal rats on NE levels in brain regions at maturity. Rats were given intraventricular injections of 6-OHDA (25 μg) on days 1 and 2 after birth and were killed at approximately 70 days of age. DMI (25 mg/kg, i.p.) was given to some rats 45 min before the 6-OHDA. Results are the mean ± S.E.M. for groups of five animals and are expressed as a percentage of control values.

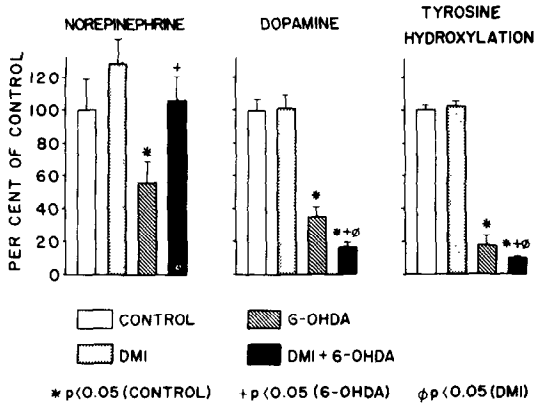

Fig. 3. Effect of 6-OHDA injections to neonatal rats on NE and DA levels and the rate of tyrosine hydroxylation in corpus striatum at maturity. Rats were given intraventricular injections of 6-OHDA (25 μg) on days 1 and 2 after birth and were killed at approximately 70 days of age. DMI (25 mg/kg, i.p.) was given to some rats 45 min before the 6-OHDA. The NE and DA levels and the rate of conversion of L-[^{14}C]tyrosine to L-[^{14}C]dopa in brain homogenates (tyrosine hydroxylation) are given as a percentage of control values. Results are the mean ± S.E.M. for groups of five animals.

in NE and DA levels and tyrosine hydroxylase activity in whole brain [2], whereas smaller doses of 6-OHDA (2 × 25 μg) produced only a moderate depletion of NE and a small decrease in whole brain tyrosine hydroxylase activity without significant change in the DA level [14].

In the present experiments, in which intraventricular rather than intracisternal injections of 6-OHDA were given, the tyrosine hydroxylase activity was markedly lowered in only one brain region, the corpus striatum. In contrast, NE and/or DA levels were greatly reduced in cortex, hippocampus, hypothalamus, septum and corpus striatum. The lack of significant enzyme depletion in all but one region suggests that intraventricular injection of small amounts of 6-OHDA in the neonate does not cause marked destruction of terminals, and therefore the depletion of catecholamines cannot be explained simply on the basis of fewer terminals. The reduction in tyrosine hydroxylase activity in striatum may be due to the close proximity of this region to the site of the injection where it would be expected to be most heavily damaged by 6-OHDA.

In the second experiment, the ability of sucrose homogenates of brain tissue to hydroxylate L-[^{14}C]-tyrosine to L-[^{14}C]dopa in the absence of exogenous cofactor was examined. As discussed in Methods, we considered that it might be a better measure of an altered ability of nerve terminals to synthesize catecholamines than does the tyrosine hydroxylase activity. There was in fact a fairly close correlation between changes in the tyrosine-hydroxylating ability of the sucrose homogenates and alterations in tissue catecholamine levels in marked contrast to the lack of correlation between tyrosine hydroxylase activity and catecholamine levels. The finding of a reduced tyrosine-hydroxylating ability *in vitro* and decreased catecholamine levels *in vivo* suggests that some damage to the nerve terminals has occurred. However, the damage does not seem to be sufficient to cause degeneration of a significant number of processes, since if this were so the tyrosine hydroxylase activity would be expected to be also markedly reduced.

It is now well established that after neonatal 6-OHDA treatment brain stem NE levels and tyrosine hydroxylase activity are increased [3–6, 9, 11, 15]. We now report similar increases in both NE levels and the tyrosine-hydroxylating ability in several brain stem regions without significant changes in tyrosine hydroxylase activity after two 25-μg intraventricular injections of 6-OHDA to neonatal rats. It is of interest that Peterson and Laverty [22] have recently reported a lack of effect of subcutaneous 6-OHDA injections on tyrosine hydroxylase activity in hindbrain regions even though NE levels were markedly altered. The theory has been advanced that destruction of forebrain nerve terminals in the neonatal rats results in anomalous sprouting in the region containing the cell bodies [8, 12] with resulting increases in NE levels and enzyme activity presumably attributable to the increased number of synaptic sites. An alternative theory is that after destruction of forebrain terminals of axons arising from the locus coeruleus both enzyme and storage granules accumulate in the cell bodies [8]. Sachs and Jonsson [23] have reported strong evidence in support of the view that regenerative and/or collateral sprouting occurs after subcutaneous injections of large amounts of 6-OHDA to newborn rats. However, the theory does not seem appropriate to explain our results after intraventricular injections of small amounts of 6-OHDA, since the enzyme activity is not increased in the brain stem regions. Even if sprouting does occur after large doses of 6-OHDA, it does not exclude the possibility that other mechanisms may also be operating. It is conceivable, for example, that damage to certain catecholamine-containing terminals may result in a reduced feedback inhibition at the level of the cell bodies, leading to an enhanced firing rate and compensatory increase in synthesis of noradrenaline in regions containing the cell bodies. The existence of such a feedback inhibition system acting on the locus coeruleus has been proposed to explain the effects of certain α-adrenergic agonistis and antagonists and drugs such as amphetamine and the tricyclic antidepressants on the firing of the locus coeruleus neurons [24]. For example, α-adrenergic receptor antagonists have been shown to increase the firing rate of locus coeruleus neurons whereas α-adrenergic receptor agonists have the reverse effect [24].

DMI has been used to block uptake of 6-OHDA into NE neurons and thereby produce a fairly selective action on DA neurons [25]. In our experiments, DMI reduced or completely blocked both increases and decreases in the NE level produced by 6-OHDA without affecting or even slightly enhancing the DA depletion. Similarly DMI pretreatment blocked or reduced the extent of the 6-OHDA-induced decreases in the tyrosine hydroxylation rates in all brain regions with the exception of striatum and septum. Thus, in those rats receiving both 6-OHDA and DMI the damage was largely confined to the DA-containing neurons, notably in corpus striatum, whereas in rats

treated with 6-OHDA alone both NE- and DA-containing neurons were markedly affected.

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