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BRIEF COMMUNICATION

Hyperactivity in Neonatally Dopamine-Lesioned Rats Requires Residual Activity in Mesolimbic Dopamine Neurons

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LUTHMAN, J., E. LINDQVIST AND S. O. ÖGREN. *Hyperactivity in neonatally dopamine-lesioned rats requires residual activity in mesolimbic dopamine neurons.* PHARMACOL BIOCHEM BEHAV 51(1) 159–163, 1995. – Neonatal destruction of mesencephalic dopamine (DA) neurons in rats through administration of 6-hydroxydopamine (6-OHDA; 75 μg IC) leads to locomotor hyperactivity at adulthood. Treatment with the catecholamine synthesis inhibitor α-methyl-p-tyrosine (H44/68; 250 mg/kg) was shown to reduce the motor activity of neonatally 6-OHDA-lesioned rats to activity levels similar to controls. In both animal groups, DA and metabolite tissue levels decreased after the H44/68 treatment. However, the extent of the H44/68-induced DA decrease was less pronounced in the 6-OHDA-lesioned animals, with no change at all in the dorsal striatum. These results imply that residual activity in mesolimbic DA neurons is required for maintaining the hyperactivity seen after neonatal 6-OHDA lesions, and that this hyperactivity is apparently mediated by postsynaptic alterations.

Dopamine 6-Hydroxydopamine Neonatal Hyperactivity α-Methyl-p-tyrosine

SELECTIVE lesions of mesencephalic dopamine (DA) neurons through administration of the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) in newborn rats leads to locomotor hyperactivity during development that persists into adulthood (5,10,19). This locomotor hyperactivity is in contrast to the akinesia that is seen following lesions of mesencephalic DA neurons by 6-OHDA in adult rats [see (21)].

The contrasting locomotor effects seen after 6-OHDA-induced DA lesions in developing compared to adult rats may be due to several factors. One possibility is that altered activity within residual DA neurons mediates the hyperactivity seen after neonatal DA lesions. Indeed, behavioral and biochemical findings indicate that adaptive changes occur in the DA system after neonatal DA lesions. Neonatally DA-lesioned rats exhibit a greater locomotor response than adult-lesioned rats following DA agonist administration [see (1,8)]. They also demonstrate a compulsive self-mutilatory behavior after D_1 receptor stimulation (1). Consistent with this finding, an upregulation of DA D_1 receptor transduction mechanisms has been shown to occur after neonatal DA lesions (4,12). Also, in contrast to adult-lesioned rats, neonatally DA-lesioned rats do not express any major deficiencies in feeding behavior, an ability that seems to be dependent on remaining DA activity (2,14,16). Moreover, the hyperactivity seen in neonatally DA-lesioned rats can be counteracted by treatment with indirect DA agonists, i.e., *d*-amphetamine and methylphenidate [(18), see also (12)], further indicating specific alterations in DA neurotransmission after neonatal 6-OHDA lesions.

On the other hand, there are data suggesting that nondopaminergic systems may be involved in at least some of the behavioral changes seen after neonatal 6-OHDA lesions. Neonatal 6-OHDA treatment results in alterations in several other

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transmitter systems in addition to the effects seen in the DA system [see (8)]. Most notably, the serotonin (5-HT) levels were found to increase in the striatum (20), due to collateral sprouting of 5-HT fibers (7). Furthermore, treatment with 5-HT antagonists or agonists have been shown to modulate the hyperactivity seen after neonatal 6-OHDA lesions as well as the *d*-amphetamine-induced antagonism of the hyperactivity (6,9).

In the present study, inhibition of catecholamine synthesis in neonatally 6-OHDA-treated rats was induced by the tyrosine hydroxylase (TH) enzyme inhibitor α-methyl-p-tyrosine (H44/68) to directly study whether activity in residual DA neurons may be involved in the hyperactivity seen after early DA lesions.

METHOD

Newborn male rats (Sprague-Dawley, Alab, Sweden) were randomly divided into treated or control groups. On day 3 after birth the pups were anesthetized by hypothermia and 6-OHDA (6-OHDA-Br, Sigma) was injected intracisternally (IC) in a dose of 75 μg (free base) in 10 μl 0.9% NaCl containing 0.2% ascorbic acid. All animals were pretreated with a subcutaneus (SC) injection of the noradrenaline (NA) uptake blocker desipramine hydrochloride (DMI; 25 mg/kg, Pertofran, Ciba-Geigy) 30 min prior to the 6-OHDA administration to protect from lesions of the NA system [see (7)]. Control animals received equal volumes of the vehicle alone following pretreatment with DMI.

At 83–87 days of age, 12 or 10 animals (3 or 2 per group) were simultaneously studied in a computerized multicage motion detection system (Motron activity apparatus) between 0900 and 1600 h. The rats were placed alone in translucent Macrolon III cages (25 × 40 × 30 cm) and motility, locomotion, and rearing were measured simultaneously by means of red-infrared light in combination with horizontal (located in the floor of the apparatus) and vertical arrays of photocell detectors (13). Motility was measured by the low-level grid of horizontal photocell detectors counting all movements of a distance of 4 cm. Locomotion was measured by counting the number of times the rat moved from one-half of the cage to the other (a distance of 32 cm). Rearing was registered by counting the number of times when the rat raised its front legs and/or rested on the haunches, with the upper part of the body breaking the high-level grid of horizontal beams located at a height of 13 cm above the cage floor.

All the rats used were placed in the test cages on each of 5 consecutive days and activity was recorded for a 60-min period the first 4 days. On day 5, the rats were divided into four groups, each consisting of eight rats. The vehicle or 6-OHDA-lesioned animals received an intraperitoneal (IP) injection of either H44/68 (250 mg/kg, 5 ml/kg) or saline (5 ml/kg). The animals were placed in the locomotor cages and motor activity was recorded for a 30-min period, starting 5 min following injection.

The rats were sacrificed by decapitation at 60 min after the H44/68 or saline injections to measure brain monoamine tissue levels. Samples were processed for high-performance liquid chromatography (HPLC), with electrochemical detection as previously described (11). The detection levels were approximately 5 ng/g for the different monoamines measured (3 × noise level).

The data was subjected to analysis of variance (ANOVA) and pair-wise testing between groups was performed with Scheffe's *F*-test.

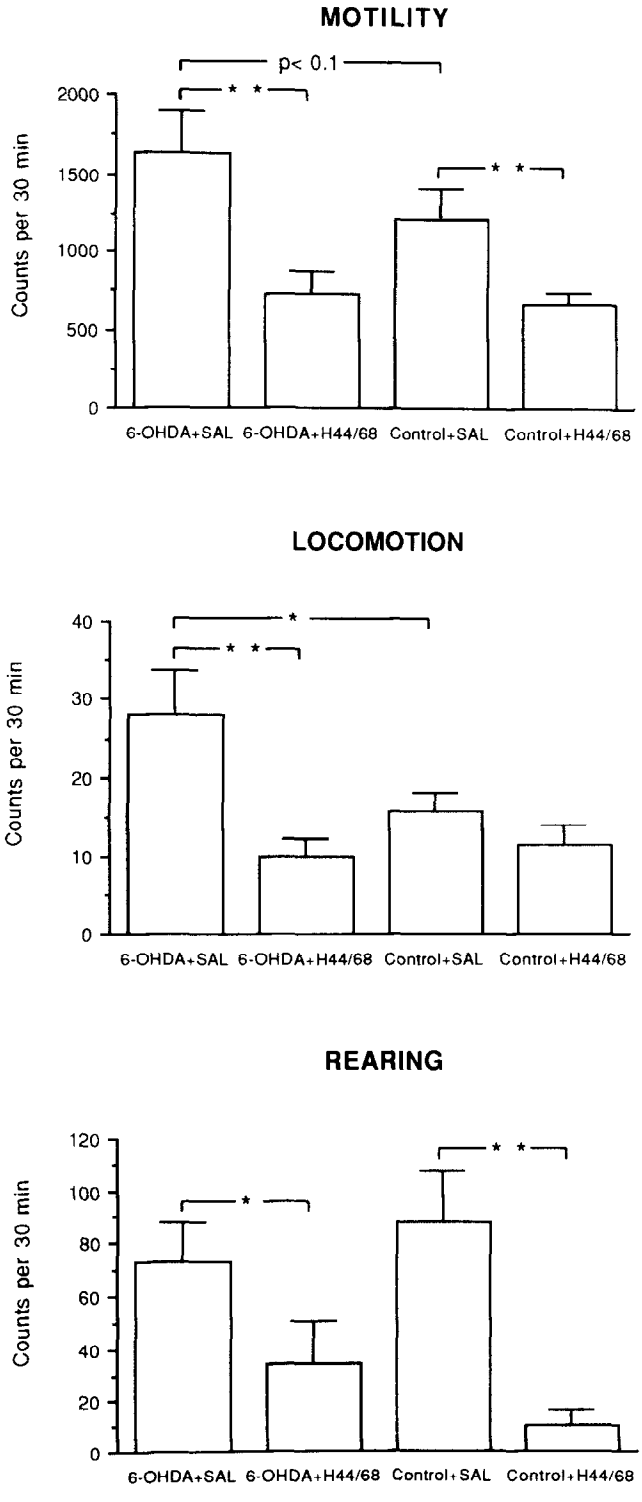

FIG. 1. Motility, locomotion, and rearing counts in adult control rats or dopamine-lesioned rats, neonatally injected with 6-OHDA (75 μg, IC). The motor activity was measured for 30 min, starting 5 min following administration of H44/68 (250 mg/kg IP) or saline (SAL). Values are expressed as means ± SEM of eight rats. $^{*}p < 0.05$; $^{**}p < 0.01$ (analysis of variance followed by pair-wise testing between groups with Scheffe's *F*-test).

RESULTS

Behavioral Findings

There were significant group interactions, $F(3, 28) = 23.3$, $F(3, 28) = 33.7$, $F(3, 28) = 45.4$, for the motility, locomotion, and rearing variables, respectively. Post hoc analysis revealed the following differences: the neonatally 6-OHDA-treated rats demonstrated an increased locomotor activity ($p < 0.05$) as well as a trend for a higher levels of motility ($p < 0.1$) compared to vehicle-treated control rats (Fig. 1). The rearing activity, on the other hand, did not differ between the 6-OHDA rats and control rats (Fig. 1).

The motility decreased significantly in both control ($p < 0.01$) and 6-OHDA-lesioned rats ($p < 0.01$) after administration of H44/68. However, the reduction of the motility was more pronounced in the 6-OHDA-lesioned rats, assuming similar motility as control rats given H44/68 (Fig. 1). Following H44/68 administration, the control rats showed a tendency of reduced locomotion, while the locomotion in the 6-OHDA-treated rats was markedly reduced after H44/68 administration ($p < 0.01$) (Fig. 1). The rearing activity was markedly reduced after H44/68 treatment in both control ($p < 0.01$) and 6-OHDA-lesioned ($p < 0.05$) rats, but less in the control rats (Fig. 1).

Monoamine Levels

The regional tissue levels of monoamines and metabolites are summarized in Table 1. The 6-OHDA treatment induced a 91% decrease in the tissue content of DA in dorsal striatum (caudate-putamen) compared to control rats, $F(3, 28) = 129.0$, $p < 0.001$, while in nucleus accumbens, $F(3, 28) = 53.1$, $p < 0.001$, and olfactory tubercle, $F(3, 28) = 46.0$, $p < 0.001$, DA decreased by 71%. In the substantia nigra, $F(3, 28) = 41$, $p < 0.001$, the DA level decreased by 78% and in ventral tegmental area, $F(3, 28) = 15.2$, $p = 0.003$, by 51% following the neonatal 6-OHDA treatment. Similar changes were seen for the tissue levels of the DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the dorsal striatum, $F(3, 28) = 33.8$, $p < 0.001$, and $F(3, 28) = 83.8$, $p < 0.001$, respectively, nucleus accumbens, $F(3, 28) = 24.0$, $p < 0.001$, and $F(3, 28) = 86.8$, $p < 0.001$, respectively, olfactory tubercle, $F(3, 28) = 15.2$, $p = 0.004$, only DOPAC, and ventral tegmental area, $F(3, 28) = 26.4$, $p < 0.001$, and $F(3, 28) = 12.8$, $p < 0.001$, respectively. A significant decrease of NA was observed in the dorsal striatum, $F(3, 28) = 13.3$, $p < 0.001$, after the neonatal 6-OHDA treatment. The serotonin (5-HT) tissue content in dorsal striatum increased by 107% in the 6-OHDA-treated rats, $F(3, 28) = 13.9$, $p = 0.005$, while the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) increased by 60%, $F(3, 28) = 83.8$, $p = 0.002$. In the olfactory tubercle, 5-HIAA decreased after the 6-OHDA lesion, $F(3, 28) = 16.0$, $p < 0.001$.

In control rats, the H44/68 treatment induced 35–45% depletion of DA and DA metabolites in dorsal striatum and a 50–60% depletion in nucleus accumbens and olfactory tubercle. In the substantia nigra and ventral tegmental area of the control rats, DA was depleted by approximately 50% and DOPAC by 85%. No significant effects were seen on either NA, 5-HT, or 5-HIAA after H44/68 treatment in control rats, apart from a 30% decrease of 5-HIAA in the olfactory tubercle ($p = 0.03$). A tendency of decreased NA in olfactory tubercle and increased 5-HT in the nucleus accumbens was observed. However, ANOVA did not show significant differences in those regions, $F(3, 28) = 0.8$, and $F(3, 28) = 2.1$, respectively.

In 6-OHDA-lesioned rats, the tissue levels of DA and DA metabolites in the dorsal striatum were not affected by the H44/68 treatment. In the nucleus accumbens, olfactory tubercle, substantia nigra, and ventral tegmental area of 6-OHDA-lesioned rats, the DA and DA metabolite levels were reduced by 25–45% (for ANOVA results, see above). No effects were seen on regional tissue levels of NA after the H44/68 treatment in the 6-OHDA-lesioned rats. Although an increase in NA was noted in the nucleus accumbens, ANOVA did not reach significance level, $F(3, 28) = 1.2$. Also, a trend for decreased 5-HT observed in olfactory tubercle, but, again, no significant group interaction was found, $F(3, 26) = 2.8$.

DISCUSSION

These results demonstrate that the enhanced motility and locomotion seen in neonatally 6-OHDA-lesioned rats can be counteracted by treatment with H44/68. The H44/68 treatment induced a reduction in DA and DA metabolite levels in both 6-OHDA-lesioned and control rats, without any major effects on the NA or 5-HT systems. These findings indicate that the hyperactivity seen after neonatal DA lesions requires residual activity within remaining DA neurons.

It is, therefore, possible that an extensive compensatory activity at both pre- or postsynaptic sites occurs in the DA neurotransmission after neonatal DA lesions, leading to overcompensation within DA systems involved in regulation of locomotion. However, in the 6-OHDA-lesioned rats, the DA and DA metabolite levels did not decrease as much as in control rats after H44/68 administration in any of the regions studied. In fact, no significant alterations were seen at all in dorsal striatum. This suggests that the activity in residual DA terminals is actually diminished after the neonatal 6-OHDA lesion. Indeed, it has been shown that remaining DA terminals following neonatal 6-OHDA lesions have a reduced capacity to respond to stimuli that normally enhance extracellular DA, i.e., *d*-amphetamine and potassium (3,8,11). On the other hand, the relative accumulation of *l*-dihydroxyphenylalanin (*l*-DOPA) is enhanced following DOPA decarboxylase inhibition in neonatally 6-OHDA-lesioned rats, while the total levels of *l*-DOPA is substantially reduced (Luthman and Lindquist unpublished observations). Thus, it is possible that after neonatal 6-OHDA lesions, DA and DA metabolites are mainly located in tissue pools that are less sensitive to TH inhibition, e.g., extracellularly as suggested by microdialysis studies (3,8). Consequently, enhanced TH activity in the remaining terminals seems to be important to maintain extracellular DA at levels that can efficiently affect postsynaptic sites rendered supersensitive by the lesion, while still the DA terminals cannot respond sufficiently to challenge. We have, for instance, observed a drastic decrease in motor activity in neonatally DA-lesioned rats following treatment with presynaptic doses of the DA agonist apomorphine, while treatment with higher postsynaptic doses enhanced locomotion much more than in controls (8). Hence, the motor hyperactivity appears to involve increased postsynaptic DA sensitivity, while residual presynaptic DA activity is essential in maintaining this behavior.

In addition, it is also possible that neonatal 6-OHDA lesions cause an imbalance in various parts of the DA system that exert either suppresser or stimulatory effect on locomotor activity [see (8) and (10) for discussion]. The extent of the lesion differs in various DA subsystems, i.e., the substantia

TABLE 1

DETERMINATIONS OF MONOAMINES AND METABOLITES 1 H AFTER H44/68 ADMINISTRATION IN CONTROL RATS AND RATS TREATED WITH 6-OHDA NEONATALLY

Region	DA	DOPAC	HVA	NA	5-HT	5-HIAA
Dorsal striatum						
Control	8621 ± 380	1612 ± 162	957 ± 45	46 ± 3	317 ± 24	353 ± 29
Control + H44/68	5753 ± 232*	1018 ± 104*	549 ± 37*	40 ± 3	300 ± 20	370 ± 9
6-OHDA	807 ± 325*	219 ± 73*	130 ± 42*	30 ± 4*	655 ± 61*	565 ± 62*
6-OHDA + H44/68	928 ± 388	217 ± 109	116 ± 50	21 ± 2	583 ± 69	570 ± 56
Nucleus accumbens						
Control	6222 ± 269	1451 ± 145	1012 ± 34	288 ± 64	937 ± 144	600 ± 40
Control + H44/68	3240 ± 220*	650 ± 41*	388 ± 28*	239 ± 49	1242 ± 67	530 ± 43*
6-OHDA	1786 ± 373*	445 ± 128*	279 ± 54*	211 ± 33	1236 ± 147	571 ± 36*
6-OHDA + H44/68	1375 ± 321†	317 ± 65†	184 ± 39†	411 ± 138	1362 ± 142	602 ± 45
Olfactory tubercle						
Control	4474 ± 224	865 ± 118	266 ± 35	243 ± 40	1301 ± 92	957 ± 38
Control + H44/68	2326 ± 166*	408 ± 43*	97 ± 6*	186 ± 47	1314 ± 111	696 ± 26*
6-OHDA	1321 ± 305*	322 ± 71*	81 ± 18*	146 ± 40	1632 ± 56	592 ± 67*
6-OHDA + H44/68	949 ± 167†	197 ± 43†	57 ± 11	198 ± 52	1377 ± 115	558 ± 27
Substantia nigra						
Control	514 ± 50	112 ± 9	84 ± 6	128 ± 17	1597 ± 67	683 ± 61
Control + H44/68	174 ± 11*	19 ± 3*	n.d.	108 ± 10	1693 ± 135	686 ± 61
6-OHDA	112 ± 20*	26 ± 5*	n.d.	88 ± 9	1541 ± 89	639 ± 52
6-OHDA + H44/68	75 ± 29†	23 ± 8	n.d.	75 ± 18	1567 ± 73	656 ± 25
Ventral tegmental area						
Control	1746 ± 179	544 ± 59	325 ± 27	793 ± 51	2161 ± 114	1655 ± 155
Control + H44/68	665 ± 115*	90 ± 17*	145 ± 18*	759 ± 122	1962 ± 186	1519 ± 87
6-OHDA	860 ± 105*	244 ± 34*	201 ± 17*	770 ± 54	2089 ± 221	1550 ± 113
6-OHDA + H44/68	604 ± 109†	140 ± 43†	147 ± 33†	640 ± 53	1965 ± 161	1540 ± 64

Statistical comparison between the different groups was performed using analysis of variance (ANOVA) followed by pair wise testing between groups with Scheffe's *F*-test

*Statistical difference ($p < 0.05$) compared to the control group; †statistical difference ($p < 0.05$) compared to the 6-OHDA group. n.d. = not detectable.

nigra (A9) system is more severely affected than the ventral tegmental area (A10) system. In the 6-OHDA-lesioned animals, H44/68 treatment affected the DA system in mesolimbic A10 projection regions, while no significant effects were seen in the dorsal striatum. This indicates that remaining neurotransmission in primarily the mesolimbic DA system plays a major role in the hyperactivity. Also, a decrease in cortical DA neurotransmission (10), may further enhance the dopaminergic transmission in the mesolimbic system following neonatal 6-OHDA lesions [see (15)]. Although the present data suggest the importance of the DA neurons in the hyperactivity, other transmitter systems may play a contributory role as well by interacting with DA neurotransmission. Thus, the reduction of the hyperactivity seen after administration of 5-HT_2 antagonists may depend on an altered serotonergic influence over remaining DA neurons (9).

Taken together, the present findings imply an important role of DA neurotransmission, and, in particular, the mesolimbic DA system, in the hyperactivity seen after neonatal 6-OHDA lesions in rats.

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