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NEONATAL 6-HYDROXYDOPAMINE TREATMENT: NORADRENALINE LEVELS AND IN VITRO ^{3}H-CATECHOLAMINE SYNTHESIS IN DISCRETE BRAIN REGIONS OF ADULT RATS

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SUMMARY

Endogenous noradrenaline levels are elevated in medulla oblongata, mesencephalon, pons and thalamus of adult rats which had been treated with 6-hydroxydopamine on days 1, 2, 8 and 15 after birth. Levels in spinal cord, cerebellum, hippocampus/amygdala and cortex are depressed, whereas no significant changes are observed in striatum, hypothalamus and medulla spinalis. The rate at which medulla oblongata synthesizes tritiated noradrenaline and dopamine from tritiated tyrosine in vitro is markedly enhanced. No effect was apparent on catecholamine synthesis in hypothalamus. Tritiated noradrenaline synthesis, but not tritiated dopamine synthesis, in the cortex is depressed. These results support the view that neonatal 6-hydroxydopamine treatment causes a degeneration of noradrenaline nerve terminals in the cortex and induces an increase in noradrenaline terminals in the medulla oblongata.

Chemical sympathectomy by administration of 6-hydroxydopamine (6-OHDA) to newborn animals is a useful tool for the study of the peripheral sympathetic nervous system. The systemic administration of 6-OHDA to newborn rats and mice not only causes a persistent reduction of noradrenaline (NA) levels in the peripheral sympathetic nervous system but also in several regions of the central nervous system, which is most pronounced in the cerebral cortex (1-9). However, this treatment does not lead to an overall depression of NA levels throughout the brain. Marked elevations

have been found in the NA concentrations of the brainstem of rats neonatally treated with 6-OHDA (1-3, 9), with most dramatic increases in the pons-medulla region (4, 6-8).

Subsequently it was shown that, parallel with the changes in endogenous NA levels in the brain following neonatal 6-OHDA treatment, the in vitro uptake of ^{3}H-NA was decreased in synaptosomal fractions from cortex and increased in synaptosomal fractions from the pons-medulla region (5-8). Fluorescence histochemical studies indicated that the increase in endogenous NA level of the pons-medulla and the increased capacity to accumulate ^{3}H-NA by synaptosomal fractions from that region can be ascribed to accumulation of NA in collaterals and an increase in the number of NA terminals (6-8). Recently, Jonsson et al. (8) reported that in adult rats, which had been treated with 6-OHDA at birth, the rate constant of NA loss following inhibition of tyrosine hydroxylase with α-methyl-para-tyrosine (α-mpt) did not differ in forebrain and pons-medulla compared to that in the corresponding regions from vehicle treated controls. This finding was interpreted as indicating a similar turnover of NA (8). However, the in vitro synthesis of ^{3}H-NA and ^{3}H-DA from ^{3}H-tyrosine by homogenates of the pons-medulla region of the treated rats was markedly enhanced (8).

In this communication we present data which further substantiate the differential effects of neonatal 6-OHDA treatment on NA levels and the synthesis of ^{3}H-catecholamines from ^{3}H-tyrosine in vitro in various discrete brain regions of adult rats.

Methods

6-OHDA treatment: Male Wistar rats (TNO, Zeist, The Netherlands), bred in our laboratory, received subcutaneous injections of 100 µg/g 6-hydroxydopamine (6-OHDA, Kistner, A.B. Biotec) on the day

of birth (day 1) and on day 2. Two additional injections of 250 µg/g 6-OHDA were given on days 8 and 15. Littermates treated with vehicle (0.9% NaCl) served as controls. The rats were killed by decapitation at the age of 13 weeks.

Brain dissection and NA assay: Brains were dissected as described by Gispen et al.(10). The brain parts were homogenized in 6 ml 0.4 N $HClO_4$, and the homogenates were centrifuged for 30 min at 2000 g at 4°C. Catecholamines were isolated from the supernatants according to Anton and Sayre (11). NA content of the eluates from Al_2O_3 was assayed following the method of Laverty and Taylor (12). Recoveries for NA were 64%.

In vitro synthesis of ^{3}H-catecholamines: The rate at which brain tissue synthesizes ^{3}H-NA and ^{3}H-DA from ^{3}H-tyrosine in vitro was estimated as described by Friedman et al.(13). Hypothalamus, medulla oblongata and preoptic cortex were chopped in 0.3 mm slices with a McIlwain tissue chopper. The slices were preincubated in 2 ml Krebs-Henseleit-bicarbonate buffer, pH 7.2, containing glucose (2 mg/ml), Na_2EDTA (0.5 mg/ml) and ascorbic acid (0.2 mg/ml) (14) for 10 min at 37°C under an atmosphere of 95% O_2 - 5% CO_2 in 25 ml beakers in a Dubnoff metabolic shaker. 50 µl of a solution containing tyrosine (3 µg) and L-(3,5-^{3}H)-tyrosine (20 µCi, The Radiochemical Centre, Amersham; specific activity 53 Ci/mmol) were added. Incubation was then continued for another 45 min. 4 ml cold Krebs-Henseleit were added to stop the reaction. The contents of the beakers were transferred to centrifuge tubes and centrifuged for 5 min at 10000 g at 4°C. The pellets were resuspended in 4 ml cold Krebs-Henseleit. After a second centrifugation the pellets were homogenized in 4 ml 0.4 N $HClO_4$, containing $Na_2S_2O_5$ (0.5 mg/ml). Carrier NA (0.4 µg) and DA (0.8 µg) were added to the supernatant after centrifugation of the homogenates (20 min, 12000 g).

Tyrosine, ^{3}H-tyrosine, ^{3}H-NA and ^{3}H-DA were purified according to Neff et al.(15), except that ^{3}H-NA was eluted from the Dowex columns with 1.0 N HCl instead of with 0.4 N HCl. Tyrosine was assayed according to Waalkes and Udenfriend (16). Aliquots of the fractions containing ^{3}H-tyrosine, ^{3}H-NA and ^{3}H-DA were counted in a Nuclear Chicago Isocap/300 liquid scintillation counter in 10 ml of a counting solution consisting of toluene and Triton X-100 (2:1 v/v), containing 4 g PPO per l. Recoveries (mean $\pm$ SEM) were 76 $\pm$ 3% for tyrosine, 75 $\pm$ 3% for NA and 61 $\pm$ 2% for DA. The amounts of ^{3}H-NA and ^{3}H-DA formed in 45 min from ^{3}H-tyrosine in mμCi/g tissue were corrected for the specific activity of tyrosine.

Statistical analysis of the data was performed with Student's t-test.

Results

In 13 weeks old rats, which had been treated with 6-OHDA on days 1, 2, 8 and 15 after birth, an increase of 161% was observed in the NA concentration of the medulla oblongata compared to the NA content of the corresponding region from control rats. In adjacent brain areas (pons, mesencephalon and thalamus) NA concentrations were significantly elevated as well. No significant changes were observed in medulla spinalis, hypothalamus and striatum, whereas significant decreases were found in the NA levels of hippocampus/amygdala, cortex, medulla spinalis and spinal cord. These data are summarized in table 1.

Table 2 shows the effects of neonatal 6-OHDA treatment on the rate at which preoptic cortex, hypothalamus and medulla oblongata synthesize ^{3}H-NA and ^{3}H-DA from ^{3}H-tyrosine *in vitro*. ^{3}H-NA synthesis, but not ^{3}H-DA synthesis, was markedly depressed in the preoptic cortex. No effects were observed in the hypothalamus.

Both ^{3}H-NA and ^{3}H-DA synthesis *in vitro* by medulla oblongata were increased. Tyrosine concentrations and specific activities of tyrosine were not different.

TABLE 1

Effects of Neonatal 6-OHDA Treatment on NA Levels in Various Brain Parts 13 Weeks After Birth. Values are given as Means ± SEM of 4-6 Rats

Brain Region	Noradrenaline Content (µg/g) ± SEM		% Change
	Vehicle	6-OHDA	
Spinal Cord	0.13 ± 0.09	0.03 ± 0.01§	-80
Cortex	0.26 ± 0.10	0.06 ± 0.01§	-77
Hippocampus/Amygdala	0.42 ± 0.01	0.16 ± 0.02§	-62
Cerebellum	0.17 ± 0.01	0.10 ± 0.01†	-41
Hypothalamus	1.77 ± 0.13	1.44 ± 0.09	-19
Medulla Spinalis	0.52 ± 0.01	0.52 ± 0.01	0
Striatum	0.53 ± 0.06	0.68 ± 0.04	+26
Thalamus	0.90 ± 0.10	1.29 ± 0.06ϕ	+43
Pons	0.48 ± 0.01	0.68 ± 0.06ϕ	+43
Mesencephalon	0.49 ± 0.01	0.86 ± 0.03§	+76
Medulla Oblongata	0.56 ± 0.04	1.46 ± 0.09§	+161

ϕ $p < 0.05$ compared to vehicle treated control
† $p < 0.01$ compared to vehicle treated control
§ $p < 0.001$ compared to vehicle treated control

Discussion

Our findings corroborate and extend the previous findings that systemic neonatal 6-OHDA treatment affects NA levels in various brain regions differentially (1-9). Increased NA levels were found in thalamus, pons, mesencephalon and, most pronounced, in medulla oblongata. NA levels of hypothalamus, striatum and medulla spinalis were not significantly altered, whereas decreases were observed in the NA levels of the cerebellum, hippocampus/amygdala, spinal cord and cortex. Since the DA concentration of the cortex of these rats is not significantly changed (van Ree

TABLE 2

Effects of Neonatal 6-OHDA Treatment on the Rate of Synthesis of Tritiated NA and DA from Tritiated Tyrosine In Vitro 13 Weeks After Birth. Values are given as Means ± SEM

	n	Tyrosine	S.A. Tyrosine	^{3}H-NA(mμCi/g) / S.A.Tyrosine (% of Contr.)	^{3}H-DA(mμCi/g) / S.A.Tyrosine (% of Contr.)
Vehicle Preoptic Cortex	(7)	13.0±0.5	1.37±0.04	100±14	100±15
6-OHDA	(8)	13.2±0.8	1.52±0.03	36± 4§	104±15
Vehicle Hypothalamus	(7)	21.5±0.8	1.11±0.08	100±12	100± 8
6-OHDA	(7)	23.3±0.9	0.98±0.08	101±10	88± 5
Vehicle Medulla Oblongata	(7)	7.3±0.6	0.84±0.09	100±10	100±10
6-OHDA	(6)	6.8±0.3	0.98±0.07	204±26§	160±21†

† $p < 0.05$ compared to vehicle treated controls
§ $p < 0.005$ compared to vehicle treated controls
S.A. is Specific Activity of Tyrosine in μCi/μg.

and Provoost, unpublished observation), 6-OHDA treatment appears to selectively affect NA terminals in the cortex. This is also indicated by the effects of neonatal 6-OHDA treatment on the rate of synthesis of ^{3}H-NA and ^{3}H-DA from ^{3}H-tyrosine in vitro. Cortical ^{3}H-NA synthesis, but not ^{3}H-DA synthesis, is markedly depressed 13 weeks after birth, again indicating a selective effect on NA terminals in the cortex. Neither ^{3}H-NA synthesis, nor ^{3}H-DA synthesis in the hypothalamus are significantly altered. The rate at which the medulla oblongata synthesizes ^{3}H-NA and ^{3}H-DA from ^{3}H-tyrosine in vitro, however, is significantly increased. The latter finding confirms the observation that ^{3}H-NA and ^{3}H-DA synthesis in vitro by synaptosomal fractions from the pons-medulla region of neonatally 6-OHDA treated rats is increased (8). The enhancement of ^{3}H-DA synthesis may be due to increased input in DA synthesizing neurons as a consequence of the treatment, viz.

by transsynaptical enzyme induction.

Jonsson et al.(8) have reported that NA turnover (=rate constant of amine loss) was similar in pons-medulla and forebrain of control and 6-OHDA treated rats. Turnover rates of NA (= rate constant of amine loss × steady state level (see 17)), calculated on the basis of their results, however, are increased in pons-medulla and decreased in the forebrain, which is in accordance with the parallel changes in the rates of synthesis of ^{3}H-NA of these regions as shown in table 2.

In conclusion, our data further support the concept (7, 8) that systemic administration to newborn animals of 6-OHDA leads to a selective degeneration of NA terminals in the cortex and to an increase in the number of NA terminals in the medulla oblongata region accompanied by an increased NA concentration in this region.

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