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6-HYDROXYDOPAMINE SYMPATHECTOMY IN THE NEONATAL RAT—EFFECTS ON BRAIN SEROTONIN AND HISTAMINE

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Abstract—Treatment of newborn rats with 6-hydroxydopamine resulted in a marked increase in the 5-hydroxyindoleacetic acid level in brainstem and hypothalamus whereas the 5-hydroxytryptamine (serotonin) level in these two tissues was slightly but not significantly decreased. The activity of L-tryptophan-5-hydroxylase was increased in brainstem and hypothalamus but not significantly changed in cerebral cortex. The serotonin turnover appeared to be increased in all three brain areas studied. Treatment of neonatal rats with 6-hydroxydopamine had no apparent effect on the tryptophan level of rat brain or on the activities of other enzymes involved in the metabolism of serotonin. The histamine content of rat hypothalamus was significantly increased, although there was no change in the level in the remainder of the brain. It was concluded that sympathectomy by 6-hydroxydopamine in neonatal rats results in changes in brain serotonin and histamine in addition to the reported changes in norepinephrine.

TREATMENT of adult experimental animals with 6-hydroxydopamine(2,4,5-trihydroxyphenethylamine; 6-OHDA) results in the destruction of peripheral adrenergic nerve terminals, a process often described as chemical sympathectomy,[1,2] although the degeneration of the peripheral nervous system is not complete and the nerve terminals eventually regenerate.[1,3] In order to obtain a central "sympathectomy", 6-OHDA is usually applied locally in the brain,[4,5] since systemic administration in the adult animal has no effect on central catecholamines,[6] presumably due to the inability of 6-OHDA to pass the blood–brain barrier. It has recently been shown, however, that intracerebral injections of 6-OHDA in adult rats produce marked effects other than specific destruction of the noradrenergic neurons, notably nonspecific histopathological changes[7] and altered serotonin (5-HT)[8,9] and dopamine[9–11] levels.

An alternative method of producing a central sympathectomy has recently been used. When administered systemically to newborn rats, 6-OHDA produces a permanent peripheral sympathectomy[12,13]. Moreover, marked reductions in brain norepinephrine (NE) content[12] and uptake[14] were reported, suggesting that 6-OHDA may be able to penetrate into the immature rat brain and produce a permanent central "sympathectomy."

We have recently reported[15]* that in some brain areas of the neonatal rat sympathectomy with 6-OHDA led to significant increases in both NE content and the

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activity of tyrosine hydroxylase, rather than to the marked reductions that would be expected as a result of destruction of central noradrenergic neurons. Pappas and Sobrian[15] therefore suggested that at least some of the central effects of 6-OHDA treatment in neonatal rats could be due to adaptive processes resulting from the peripheral adrenergic nerve destruction. It is evident that if adaptive processes can produce marked changes in central adrenergic neurons, it is possible that nonadrenergic neurons may also be affected. We therefore decided to examine the extent of the central changes after treatment of neonatal rats with 6-OHDA in order to gain a better understanding of the behavioural changes observed after this treatment.

We now report that 6-OHDA treatment of neonatal rats results in marked changes in brain 5-HT metabolism in the adult rat. Although 5-HT levels were almost unchanged, the brain content of the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA), was significantly increased in both hypothalamus and brainstem. The activity of tryptophan-5-hydroxylase, a key enzyme in the synthesis of 5-HT in brain, was similarly increased. In contrast, we were unable to detect any change in the activity of monoamine oxidase or 5-hydroxytryptophan decarboxylase, two other enzymes involved in the metabolism of 5-HT.

Also, the histamine (HA) level in rat hypothalamus was significantly reduced in the 6-OHDA-treated animals. In contrast, the HA content of the whole brain without hypothalamus was unchanged.

It was concluded that 6-OHDA treatment of neonatal rats results in changes in the metabolism of 5-HT and HA, in addition to the previously reported changes in NE metabolism.

MATERIALS AND METHODS

Male Wistar rats were injected subcutaneously on days 1 through 8 after birth with 50 mg/kg of 6-OHDA (2,4,5-trihydroxyphenethylamine, Regis Chemical Co.) dissolved in 0·05 ml of a saline–ascorbic acid soln (1 mg/ml). Control animals received the vehicle alone on the same schedule. This method has been shown to result in marked alterations in the brain NE content and tyrosine hydroxylase activity[15]* in the 6-OHDA-treated animals when measured in the adult animal. The control and experimental rats were killed between 100 and 120 days of age, the brains were quickly removed and the hypothalamus, brainstem and cortex dissected out. For the histamine assay, only hypothalamus was separated, the remaining brain being used without further dissection. Within 5 min of dissection, the samples were weighed and homogenized in a glass tissue grinder fitted with a Teflon plunger (A. H. Thomas & Co.) in either 5–10 vol. of ice-cold 0·25 M sucrose, in 10 ml acidified *n*-butanol[16] or in 5–10 vol. of 0·05 M phosphate buffer, pH 7·2.

Tryptophan-5-hydroxylase was assayed by incubating an aliquot of the sucrose homogenate with L-tryptophan-3-^{14}C (35–45 mCi/m-mole, Amersham-Searle) in the presence of pargyline (Abbott Laboratories) as previously reported.[17] The 5-HT-^{14}C produced was isolated on a CG-50 type 1 (Rohm & Haas) column and the radioactivity assayed in a Beckman LS-150 liquid scintillation spectrometer. Under these conditions, the particle-bound form of the enzyme is measured. The enzyme activity was calculated as nmoles 5-HT formed/hr/g wet wt of tissue, a correction being applied for the small quantity of endogenous substrate present. Monoamine oxidase[18] and

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5-hydroxytryptophan decarboxylase[19] were measured in the same brain homogenates by established radiochemical methods.

Both 5-HT and 5-HIAA were isolated from the acidified butanol extract and assayed fluorimetrically after reaction with *o*-phthaldialdehyde.[16] Fluorometric measurements were made in an Aminco–Bowman spectrophotofluorometer (American Instrument Co.).

An estimate of 5-HT turnover in several brain areas was made by measuring the increase in 5-HT content produced by inhibition of monoamine oxidase with pargyline.[20] Rats were injected i.p. with 30 mg/kg of pargyline (Abbott Laboratories) and killed 2 hr later. Brain areas were assayed for 5-HT as above.

Histamine was assayed in the 0·05 M phosphate homogenate by the single isotope enzyme assay procedure reported by Kobayashi and Maudsley.[21]

RESULTS

The 6-OHDA treatment to neonatal rats did not significantly change the total body wt. As can be seen from Table 1, 5-HT content of brainstem and hypothalamus

TABLE 1. EFFECT OF 6-OHDA TREATMENT OF NEONATAL RATS ON RAT BRAIN 5-HT, 5-HIAA AND TRYPTOPHAN LEVELS*

		Hypothalamus	Brainstem
5-HT	Vehicle	0·920 ± 0·104	0·793 ± 0·039
(μg/g wet wt)	6-OHDA	1·013 ± 0·071	0·848 ± 0·054
	% Control	110	107
5-HIAA	Vehicle	1·10 ± 0·16	0·748 ± 0·116
(μg/g wet wt)	6-OHDA	1·76 ± 0·16†	0·935 ± 0·098†
	% Control	160	125
Tryptophan	Vehicle	3·60 ± 0·38	4·64 ± 0·44
(μg/g wet wt)	6-OHDA	3·66 ± 0·42	4·66 ± 0·48
	%Control	102	100

* Results are given as mean ± S.E.M. for groups of six or more animals.
† Denotes values significantly different from control values at $P < 0·05$.

in the 6-OHDA group was slightly but not significantly greater than control values. However, the level of the 5-HT metabolite, 5-HIAA, was significantly higher in the 6-OHDA-treated animals in both these brain areas. The tryptophan level was not significantly altered by 6-OHDA treatment in any of the three brain areas studied. There was no significant differences in tissue weights, and similar statistical results were obtained when the calculations were made on the basis of total 5-HT, 5-HIAA and tryptophan in the tissues.

Monoamine oxidase and 5-hydroxytryptophan decarboxylase activities in cortex, hypothalamus and brainstem showed no statistically significant differences between experimental and control groups of animals (Table 2). On the other hand, the tryptophan-5-hydroxylase activity was markedly increased as a result of the 6-OHDA treatment in both hypothalamus and brainstem. In cortex the activity was increased, although a statistically significant increase was not reached in this tissue.

Pargyline treatment resulted in the expected marked increase in 5-HT content due to inhibition of 5-HT breakdown. In each brain area, however, the increase in 5-HT

TABLE 2. EFFECT OF 6-OHDA TREATMENT OF NEONATAL RATS ON RAT BRAIN MONOAMINE OXIDASE, 5-HYDROXYTRYPTOPHAN DECARBOXYLASE AND TRYPTOPHAN-5-HYDROXYLASE*

Enzyme	Treatment	Cortex	Hypothalamus	Brainstem
Monoamine oxidase	Vehicle	4·56 ± 0·36	6·06 ± 0·27	7·35 ± 0·42
(μmoles/g/hr)	6-OHDA	4·42 ± 0·25	5·90 ± 0·57	7·71 ± 0·36
	% Control	97	97	105
5-Hydroxytryptophan decarboxylase	Vehicle	0·426 ± 0·042	1·12 ± 0·11	0·442 ± 0·033
(μmoles/g/hr)	6-OHDA	0·448 ± 0·031	1·21 ± 0·07	0·420 ± 0·037
	% Control	105	108	95
Tryptophan-5-hydroxylase	Vehicle	0·63 ± 0·10	5·22 ± 0·29	2·96 ± 0·26
(μmoles/g/hr)	6-OHDA	0·70 ± 0·80	6·79 ± 0·43†	4·28 ± 0·19†
	% Control	110	130	145

* Results are given as mean ± S.E.M. for groups of six or more animals.
† Denotes values significantly different from control values at $P < 0·05$.

was greater in those animals treated during the neonatal period with 6-OHDA than in those receiving the injection vehicles alone (Table 3). These data were consistent with a higher 5-HT turnover rate in the 6-OHDA-treated animals.

The histamine level in hypothalamus was significantly higher in the 6-OHDA-treated animals than in the controls. However, in the remainder of the brain there was no detectable change in the histamine level. The results are summarized in Table 4.

TABLE 3. EFFECT OF PARNATE OR SALINE ON THE 5-HT CONTENT OF BRAIN AREAS IN RATS TREATED DURING THE NEONATAL PERIOD WITH EITHER 6-OHDA OR SALINE*

		Saline		6-OHDA	
Brain area	Treatment	5-HT (μg/g)	Change in 5-HT (μg/g)	5-HT (μg/g)	Change in 5-HT (μg/g)
Cortex	Saline	0·414 ± 0·022		0·486 ± 0·056	
	Parnate	0·494 ± 0·020	+0·080	0·630 ± 0·016	+0·144
Hypothalamus	Saline	0·97 ± 0·04		0·96 ± 0·08	
	Parnate	1·23 ± 0·08	+0·26	1·35 ± 0·08	+0·39
Brainstem	Saline	0·85 ± 0·05		0·79 ± 0·05	
	Parnate	1·51 ± 0·08	+0·66	1·72 ± 0·04	+0·93

* Results are given as mean ± S.E.M. for groups of five rats.

TABLE 4. EFFECT OF 6-OHDA TREATMENT OF NEONATAL RATS ON RAT BRAIN HISTAMINE*

	Histamine			
	Hypothalamus		Remainder of brain	
	(ng)	(ng/g)	(ng)	(ng/g)
Vehicle	21·1 ± 1·3	97 ± 7·7	39·1 ± 3·1	24·2 ± 1·8
6-OHDA	24·5 ± 0·8†	122 ± 5·7†	38·2 ± 3·2	25·8 ± 3·9
% Control	116	126	98	107

* Results are given as mean ± S.E.M. for groups of six or more animals.
† Denotes values significantly different from control values at $P < 0·05$.

DISCUSSION

Subcutaneous injection of neonatal rats with 6-OHDA results in profound changes in the NE content[12]* and tyrosine hydroxylating ability* of several areas of rat brain. It has been suggested that the marked reduction in NE content in brain areas such as cerebellum, hippocampus and cortex is due to a preferential destruction of the noradrenergic neurons by 6-OHDA entering the CNS in early life while the blood–brain barrier is more permeable than in the adult rat.[12–14] A similar reduction in tyrosine hydroxylase activity in brain cortex is consistent with this suggestion, since the bound form of the enzyme is found mainly in the nerve terminals of catecholinergic neurons.[22] The decrease in NE content does not, however, occur in all brain areas; in brainstem, for example, there is a massive increase in both NE content[15]* and tyrosine hydroxylase activity* which is difficult to explain simply on the basis of destruction of central adrenergic neurons. Pappas and Sobrian[15] suggested that the increased NE content was due to feedback effects on the brain resulting from the reduction of peripheral sympathetic function produced by the drug. If these increases are secondary to the destruction of noradrenergic neurons in the periphery or those areas of brain which show marked NE decreases, then a similar activation of non-noradrenergic neurons in brain might be expected.

Sympathectomy with 6-OHDA in neonatal rats has been shown to have no significant effect on the 5-HT content of several brain areas,[23] the DA content of the corpus striatum,[23] the DA-^{3}H uptake in nucleus caudatus[14] or the 5-HT-^{3}H uptake in cerebral cortex.[14] Our results confirm that brain 5-HT levels are practically unchanged by 6-OHDA treatment of neonatal rats, although the 5-HIAA content and tryptophan-5-hydroxylase activity are markedly increased in both hypothalamus and brainstem. The failure of brain 5-HT levels to change after this treatment is not surprising, since 5-HT frequently remains unchanged during significant alterations in 5-HT turnover.[24]

Tryptophan-5-hydroxylase is the enzyme involved in the rate-controlling step of 5-HT biosynthesis from L-tryptophan in brain, although the tissue level of tryptophan may also influence the rate of synthesis, since brain 5-HT levels are affected to some extent by the plasma tryptophan level.[25] An increased ability of brain homogenates to synthesize 5-HT *in vitro* is therefore not conclusive evidence of an enhanced synthesis *in vivo*. Similarly, although an elevated tissue level of 5-HIAA is an indication of increased 5-HT turnover,[24] it is conceivable that, in this case, the increased level is due to a reduced excretion of this metabolite. However, an increase in both the tryptophan hydroxylating activity *in vitro* and the 5-HIAA level *in vivo* strongly suggests an elevated 5-HT turnover.

Additional support for this view was obtained from the experiment in which both 6-OHDA-treated animals and saline-treated controls were injected with a monoamine oxidase inhibitor 2 hr before sacrifice. In each of the brain areas studied, pargyline produced a greater accumulation of 5-HT in the 6-OHDA-treated animals than in the corresponding controls.

Of the three main enzymes involved in the synthesis and breakdown of 5-HT in brain, only tryptophan-5-hydroxylase is affected by 6-OHDA treatment during the neonatal period. Monoamine oxidase and 5-hydroxytryptophan decarboxylase activities were not significantly altered by this drug. These data are in line with many

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reports that brain tyrosine and tryptophan hydroxylases are inducible enzymes. For example, immobilization stress results in a significantly increased hypothalamic tyrosine hydroxylase,[26] and cold exposure produces a marked increase in the same enzyme in the medulla oblongata but not in other brain areas.[27] Chronic treatment with reserpine, which causes massive depletion of catecholamine and 5-HT stores, produced increases in brainstem tryptophan hydroxylase[28] and midbrain tyrosine hydroxylase.[29] It is evident that alterations in brain biosynthetic enzyme activity can occur in the process of adaptation of the central nervous system to a changed environment.

Histamine, a putative central neurotransmitter, may play a role in several brain mechanisms,[30–32] particularly in the hypothalamus which contains the highest concentration of HA in the brain.[33]

These results emphasize that data on the possible involvement of central noradrenergic neurons in behavior obtained by the use of 6-OHDA treatment during the neonatal period should be interpreted with caution. In the first place, destruction of NE-containing nerve endings is far from complete, especially in the lower brain areas. Second, it is probable that compensatory changes in the less damaged neurons have taken place during the period between treatment and the biochemical estimations in the adult animal. This possibility is supported by evidence of an increased NE, HA and 5-HIAA content and enhanced tyrosine and tryptophan hydroxylating ability in hypothalamus and brainstem.

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