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Regional changes in monoamine synthesis in the developing rat brain during hypoxia

T. HEDNER and P. LUNDBORG

Department of Pharmacology, University of Göteborg, Sweden

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4, 14 and 28 days old rats were exposed to a hypoxic environment of 6% O_2–94% N_2 for 30 min. Tyrosine hydroxylase and tryptophan hydroxylase acivity was studied in different brain regions (hemispheres, striatum, midbrain and brainstem) in vivo by measuring the accumulation of dihydroxyphenylalanine (Dopa) and 5-hydroxytryptophan (5-HTP) respectively, after inhibition of aromatic L-amino acid decarboxylase with NSD 1015. Tyrosine and tryptophan levels in the different brain regions were measured simultaneously. The tyrosine and tryptophan levels in the various brain parts were generally not influenced during exposure to hypoxia. Tyrosine hydroxylase activity decreased in most areas in the 4 and 14 days old rats, and all brain areas studied in the 28 days old rats. Tryptophan hydroxylase activity decreased markedly in all brain areas at all ages studied. It is concluded that the enzymes tyrosine hydroxylase as well as tryptophan hydroxylase seem to be equally affected during hypoxia in the different brain regions studied.

Key words: Hypoxia, developing brain, monoamine synthesis

Almost all the various groups of monoamine—containing nerve cell bodies which are present in the adult rat brain are also present at a very early stage during development (Loizou 1969, 1972). From birth on, the levels of dopamine (DA), noradrenaline (NA) and 5-hydroxytryptamine (5-HT) increase with proceeding age and do not reach adult levels until several weeks after birth in the rat, or as in the case of DA, not until adult life (Coyle 1973, Thornburg & Moore 1976). This sequence of events is probably a consequence of the centrifugal outgrowth of axons and terminals from the cell bodies (Loizou 1972).

The activities of the first and rate-limiting enzymes of the catecholamine and indomeamine synthesis pathways, tyrosine hydroxylase and tryptophan hydroxylaste respectively, have been detected during late gestation (Coyle & Axelrod 1972*a*, *b*). Analysis of the regional distribution of tyrosine hydroxylase and tryptophan hydroxylase in the rat brain indicate that the maturation of these enzymes proceed in a caudal to rostral direction (Coyle & Axelrod 1972*b*, Deguchi & Barchas 1972).

Recent studies from these laboratories have demonstrated that in neonatal and developing animals, hypoxia and/or anoxia are accompanied by marked acute alterations in the synthesis of the monoamine neurotransmitters DA, NA and 5-HT (Hedner et al. 1977*a*, Hedner et al. 1977*b*, Hedner et al. 1978). Additionally, we have demonstrated that persistent biochemical and behavioral impairment occur after severe neonatal oxygen deprivation as measured as monoamine synthesis and conditioned avoidance response, respectively (Hedner et al. to be publ.)

As the maturation of the various monoamine neuronal pathways in the brain proceed in an asynchronous way, the present study was undertaken in order to investigate possible differences in sensitivity to hypoxia between different brain regions.

METHODS

Pregnant Sprague-Dawley rats (Anticimex, Stockholm) were housed under regulated dark-light conditions (light period 6 a.m.–6 p.m.) in the department and the time of birth was noted within 12 h. At 4, 14 and 28 days of age,

Table 1. *Tyrosine levels (μg/g wet weight) in various rat brain regions at different ages during normoxia and hypoxia*

Shown are mean ±S.E. Figures within parentheses indicate the number of experiments. Comparisons by *t*-test. n.s.=not significant

Age	Hemispheres	Striatum	Midbrain	Brain stem
4 days				
Control	28.6±1.58 (9) n.s.	32.6±1.93 (9) n.s.	34.4±5.06 (5) n.s.	32.8±2.01 (8) n.s.
Hypoxia	26.7±1.92 (9)	29.5±2.26 (9)	31.9±2.33 (7)	28.1±2.01 (9)
14 days				
Control	28.2±2.13 (10) n.s.	24.2±2.51 (10) n.s.	23.9±1.73 (10) n.s.	30.7±3.46 (10) $P<0.05$
Hypoxia	23.9±2.35 (10)	24.5±1.98 (10)	21.2±1.61 (10)	22.6±1.36 (10)
28 days				
Control	24.4±1.46 (12) n.s.	18.4±1.63 (10) n.s.	24.4±1.39 (9) n.s.	22.3±2.09 (11) n.s.
Hypoxia	18.5±1.57 (11)	15.8±1.24 (11)	20.5±1.65 (9)	18.3±1.81 (11)

the infant rats were exposed to a N_2–O_2 environment in a sealed 22-litre plastic cage for 30 min. All experiments started around 4 h after the onset of light. The oxygen content of the gas mixture was 6% and the mixture was let through the cage via inlet and outlet holes at a rate of about 4 liter/min. Control animals were kept in a similar open box exposed to room air. The cages were kept on a preheated (35–36°C) table and the room temperature was 27°C.

All animals were injected subcutaneously with NSD 1015, 100 mg/kg (3-hydroxybensylhydrazine HCl, synthetized in this laboratory by Dr P. Lindberg), 30 min before sacrifice. The infant rats were killed by decapitation immediately after 30 min exposure to the gas mixture. The whole brain (without olfactory lobes) was quickly removed and dissected on an ice-cold glass plate into the following parts: (1) "Striatum" including corpus striatum and limbic forebrain, (2) hemispheres including hippocampus, (3) diencephalon ("midbrain") and (4) lower brain stem. Cerebellum was identified and discarded. DA is the predominating catecholamine in part (1) and NA in parts (2), (3) and (4). Most of the 5-HT containing nerve cell bodies are found in part (4). For details of the dissection procedure, see Carlsson & Lindqvist (1973).

Immediately after the dissection procedure the brain parts were frozen on dry ice. In the 4 days old rats 10 parts were pooled, in the 14 days old 4 parts were pooled and in the 28 days old 2 parts were pooled and weighed. The brain samples were stored in a freezer at −70°C, in no case for more than 2 months.

After thawing, the pooled brain parts were homogenized in 10 ml of 0.4 N perchloric acid containing 5 mg $Na_2S_2O_5$ and 20 mg EDTA. The homogenates were centrifugated at about 10000×g for 10 min at 0°C, and the supernatant purified on a strong cation exchange column (Dowex 50-X-4) (Atack & Magnusson 1978). The separated amines and precursors were analyzed spectrofluorometrically according to previously described techniques (see Atack 1977).

Statistical analysis was performed using Student's *t*-test. *P*-values larger than 0.05 were considered not significant.

Table 2. *Tryptophan levels (μg/g wet weight) in various rat brain regions at different ages during normoxia and hypoxia*

Shown are mean ±S.E. Figures within parentheses indicate the number of experiments. Comparisons by *t*-test. n.s.=not significant

Age	Hemispheres	Striatum	Midbrain	Brain stem
4 days				
Control	6.0±0.43 (9) n.s.	6.5±0.46 (9) n.s.	6.7±0.49 (9) n.s.	6.2±0.40 (9) n.s.
Hypoxia	6.6±0.54 (9)	7.6±0.65 (9)	7.4±0.59 (9)	7.1±0.68 (9)
14 days				
Control	7.0±0.49 (10) n.s.	7.2±0.32 (10) n.s.	5.1±0.59 (10) n.s.	6.5±0.53 (10) n.s.
Hypoxia	7.0±0.31 (10)	6.9±0.21 (10)	5.1±0.33 (10)	6.4±0.26 (10)
28 days				
Control	5.5±0.20 (12) n.s.	5.1±0.25 (12) n.s.	5.5±0.28 (12) n.s.	5.5±0.32 (12) n.s.
Hyoxia	5.6±0.33 (12)	5.9±0.35 (12)	5.5±0.27 (12)	5.3±0.29 (12)

RESULTS

Tyrosine and tryptophan levels

Tyrosine and tryptophan levels tended to decrease in the various brain areas with advancing age. Exposure to hypoxia did not sigificantly alter the tyrosine levels in the different brain parts, except for a decrease noted in the brain stem at 14 days of age (Table 1). During 30 min of exposure to 6% O_2–94% N_2, no alteration in tryptophan levels was noted compared to controls (Table 2).

Dopa and 5-HTP accumulation after NSD 1015

The Dopa accumulation after NSD 1015 in the various brain parts increased significantly over the developmental period studied (Fig. 1).

In the 4 days old animals, there were significant decreases in Dopa accumulation during 30 min 6% hypoxia in all brain regions studied except for the hemispheres (Fig. 1). At 14 days of postnatal age, hypoxia caused a significant decrease in Dopa accumulation after NSD 1015 in the hemispheres and midbrain (Fig. 1), and in the 28 days old animals a marked decrease was noted in all brain regions (Fig. 1).

An increase in 5-HTP accumulation after NSD 1015 administration was noted in the various brain parts with increasing age (Fig. 2).

During the 30 min exposure to 6% hypoxia, a marked decrease in 5-HTP accumulation was noted in all brain regions (hemispheres, striatum, midbrain and brain stem) at all ages studied (Fig. 2). The relative decrease in the different brain areas appered to be similar for the various ages studied.

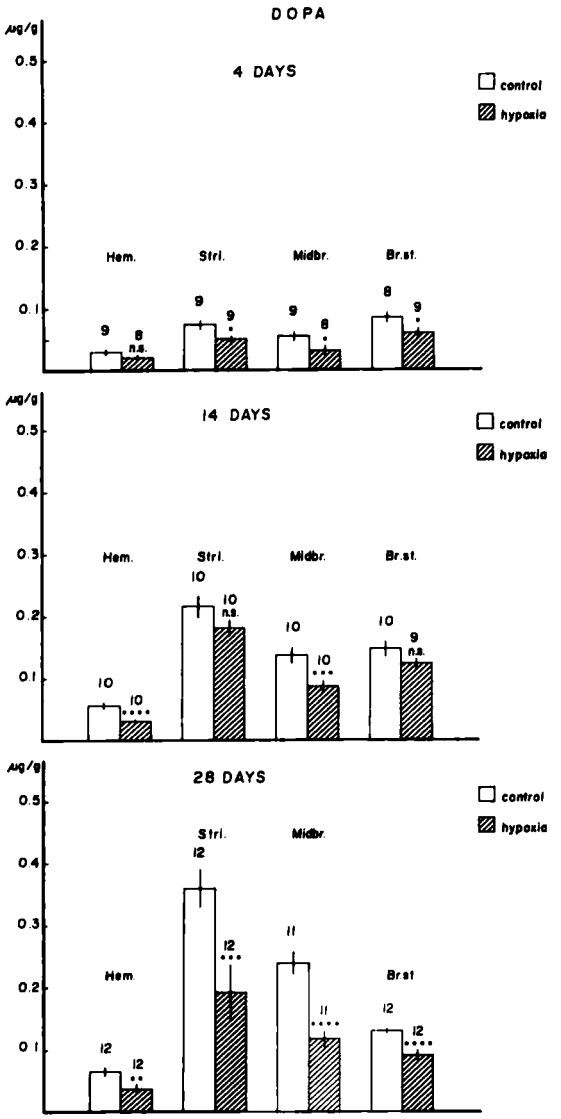

Fig. 1. Brain tyrosine hyroxylase activity in 4, 14 and 28 days old rats during hypoxia. Tyrosine hydroxylase activity was measured in various brain regions (*Hem.*, hemispheres; *Strl.*, striatum; *Midbr.*, "midbrain"; *Br.st.*, brainstem), by means of Dopa-accumulation after inhibition of aromatic L-amino acid decarboxylase with NSD 1015, injected subcutaneously 30 min before sacrifice. Shown are means ±S.E. Figures indicate the number of observations. Comparisons by *t*-test. * $P<0.05$, ** $P<0.01$, *** $P<0.005$, **** $P<0.001$, n.s.=not significant.

DISCUSSION

Experimental animal studies have shown that different forms of severe oxygen deprivation, such as hypoxia, anoxia or ischemia, have one common neuropathological denominator (Brierly et al. 1973), resulting in a highly predictable form of neuronal damage. These neuronal alterations are found mainly in areas in the brain which seem to exhibit "selective vulnerability" to oxygen deprivation (Brierly et al. 1973).

However, before cell death occur in the brain, oxygen deprivation initiates rapid and profound alterations in metabolic activity. The changes in oxidative reactions during hypoxia, anoxia or ischemia have been extensively studied in the adult (Siesjö & Nilsson 1971, Siesjö & Plum 1971) and the neonatal animal (Jilek 1970, Vannucci & Duffy 1976). Before changes in oxidative and carbohydrate metabolism are detected in the brain,

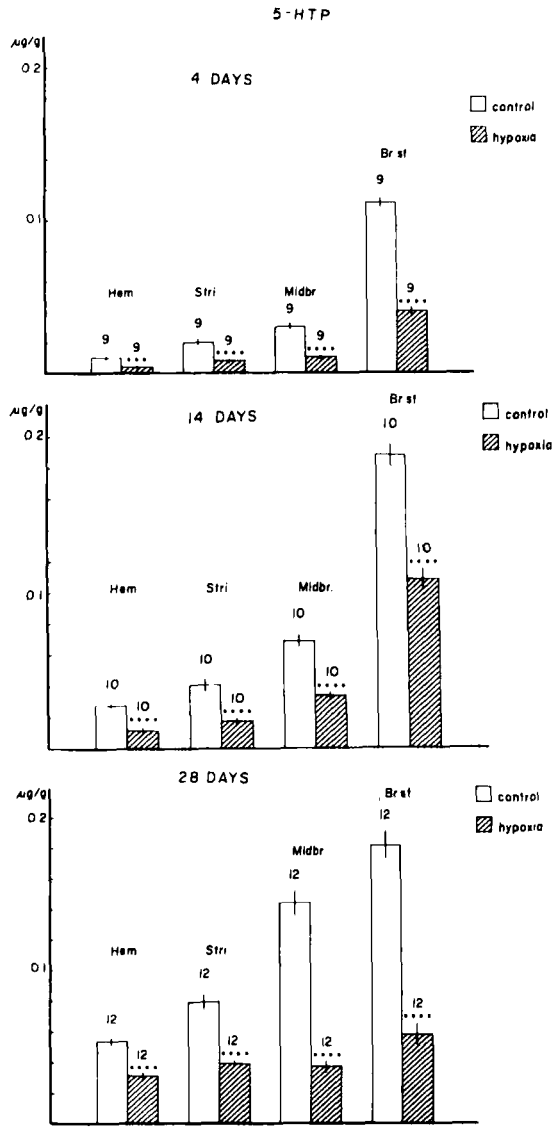

Fig. 2. Brain tryptophan hydroxylase activity in 4, 14 and 28 days old rats during hypoxia. Tryptophan hydroxylase activity was measured in various brain regions (*Hem.*, hemispheres; *Stri.*, striatum; *Midbr.*, "midbrain"; *Br.st.*, brainstem), by means of 5-HTP-accumulation after inhibition of aromatic L-amino acid decarboxylase with NSD 1015, injected subcutaneously 30 min before sacrifice. Shown are means ±S.E. Figures indicate the number of observations. Comparisons by *t*-test. *** $P<0.005$, **** $P<0{,}001$, n.s. =not significant.

alterations in the synthesis and degradation of the monoamine neurotransmitters DA, NA and 5-HT occur (Davis & Carlsson 1973, Hedner et al. 1977*a*, Hedner et al. 1977*b*).

In the present study we have used NSD 1015, which is a potent inhibitor of the second enzyme of both the catecholamine and the indoleamine synthetic pathways, to measure tyrosine hydroxylase and tryptophan hyroxylase activity. This can be achieved as the intermediate amino acids Dopa and 5-HTP, respectively, accumulate in a linear manner during the first 30 min after injection (Carlsson et al. 1972).

During 30 min hypoxia there were, generally, significant decreases in tyrosine hydroxylase activity in the different brain areas in the neonatal as well as in the 4 weeks old animals. Concerning 5-HT, no apparent differences in sensitivity to hypoxia between the different neuronal populations were evident. The inhibition of tryptophan hydroxylase was extensive in all brain areas and the relative decrease appeared to be similar for the various ages studied. The decrease in Dopa accumulation appeared to be more pronounced in the striatum at 28 days (47%) than at 14 (n.s.) and 4 days (32%) of postnatal age. One possible explanation for this discrepancy could be that monoamine neurons (mainly DA in striatum) are more metabolically active in older animals and therefore more sensitive to hypoxia.

However, the response of tryptophan hydroxylase to hypoxia did not seem to be correlated to the outgrowth of the serotonin neurons or other maturational events occurring in the rat brain during development, such as e.g. capillary proliferation.

The levels of hypoxia used in the present study are not likely to produce permanent neuronal damage in the rat brain. However, as a more severe oxygen deprivation causes permanent changes in brain monoamine metabolism (Hedner et al., to be publ.), these mechanisms may possibly be relevant factors in certain childhood behavioral disorders.

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