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ADAPTIVE CHANGES INDUCED BY HIGH ALTITUDE IN THE DEVELOPMENT OF BRAIN MONOAMINE ENZYMES[1]

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Exposure to high altitude (HA) affects neurotransmitter levels in the adult brain and induces a number of neurologic and behavioral disturbances. The present work was undertaken to investigate the effects of chronic exposure to a moderate hypoxic environment (natural altitude of 3800 m, 12.8% 0_2 in inspired air) on the development from birth until adulthood of brain monoamine enzymes in rats. The activity of synthesizing (tyrosine and tryptophan hydroxylase) and catabolizing (catechol-*O*-methyl transferase and monoamine oxidase) enzymes was studied in discrete brain areas (cerebral cortex, cerebellum, mesodiencephalon, hypothalamus, corpus striatum, and pons medulla) and was shown to be selectively affected by HA, depending on the age of the animal and the brain region. In general, enzyme activity was less susceptible to HA during the first week after birth than at later ages, some brain areas such as the hypothalamus showing significant alterations in some enzymes throughout development, and in all enzymes at adulthood. Furthermore, in all brain areas and at all ages, tyrosine and tryptophan hydroxylase were more affected by HA than the catabolizing enzymes, and their activity was increased in some areas (e.g., cerebral cortex and cerebellum) but decreased in other areas (e.g., hypothalamus, mesodiencephalon, corpus striatum). These enzymatic changes and the corresponding alterations in precursor amino acids, particularly tryptophan, seem to be due more to the direct effect of hypoxia on oxygen-dependent enzymes, than to the stress. It appears that an hypoxic environment may provoke both early and long-term alterations in catecholamine and serotonin metabolism, thus neurotransmitter imbalances may explain some of the alterations in neurologic and endocrine development characteristic of the hypoxic animal.

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INTRODUCTION

Exposure to high altitude (HA) is a complex form of stress where the contribution of individual components to the major pathophysiologic changes—sympathetic hyperactivity (1, 2) and adrenocortical hyperfunction (3, 4)—cannot be easily distinguished.

There is good evidence from our (5) and other laboratories (6) that oxygen deficiency (hypoxia) represents the primary stressor, since several effects of HA on brain maturation have been duplicated in decompression chambers. Hypoxia and exposure to HA during development induce a wide range of neurologic and neurochemical alterations in several animal species (5, 7, 8). In humans, some behavioral disorders of early childhood (e.g., infantile autism) considered secondary to prolonged hypoxia (9) have been correlated with alterations in monoamine metabolism (10, 11). With respect to the latter, it has been shown that in the adult rat brain short-term exposure to hypoxia or simulated HA decreases transitorily tryptophan hydroxylase (TPH) (7, 12–14), tyrosine hydroxylase (TH) (7, 12–15) and monoamine oxidase (MAO) (14) activity, whereas serotonin (5-HT) levels are unchanged (12–14), and norepinephrine (NE) and dopamine (DA) levels are transitorily decreased (16, 17).

The oxygen deficiency at natural and simulated HA is expected to affect the neurotransmitter pathways not only by inhibiting the oxygen-dependent enzymes such as TH, TPH, and MAO, but also indirectly, through the sympathoadrenal activation due to stress. As a matter of fact, many models of experimental stress are known to alter both central and peripheral neurotransmission through a stimulation of catecholamine (CA) and 5-HT neurons and an increase in the activity of the monoaminergic synthetic enzymes TH, TPH, and dopamine-β-hydroxylase (18–23), whereas MAO and catechol-*O*-methyl transferase (COMT) can be either induced or depressed (24–26) in selected brain regions. Thus the neurotransmitter homeostasis at HA is supposed to be modulated by the interaction of both inhibitory and stimulatory influences of hypoxia and stress, respectively.

The normalization of monoamine rate of synthesis and levels after the first few hours of exposure to hypoxia (12, 16, 27) and stress (19, 22, 23) testifies to the transient nature of these changes and underlines the efficiency of the adaptive mechanisms in the adult brain. It remains, however, unknown whether or not and to what degree the developing brain is capable of a similar adaptability to a stressfull environment. Based on this lack of evidence, the major purpose of the present investigation was to study whether prolonged exposure to HA induced selective alterations

in development of the enzymes which produce and inactivate brain monoamines. Rats were therefore exposed to a natural hypoxic environment throughout gestation and during postnatal development until adulthood.

EXPERIMENTAL PROCEDURE

Male and female 2-month-old Long-Evans rats were divided into two groups: one served as control and was maintained at sea level (altitude: 76 m, 20% O_2 in inspired air) on the Berkeley campus; the other animals were transported to the Barcroft Laboratory of the White Mountain Research Station, one of the experimental laboratories with biochemical facilities maintained by the University of California (altitude: 3800 m, equivalent to 12.8% O_2 in inspired air at sea level). After a month of acclimatization, the rats were bred and newborns (first generation, F_1 rats) were maintained at high altitude until 60 days of age. All animals were kept under strictly controlled and similar conditions of temperature, diet, and constant 12-hr periods of light and dark. At 21 days of age all animals were separated from their mothers, placed 3 per cage and fed Purina Rat Chow and water ad libitum. Sacrifice (by decapitation) was at 1, 7, 12, 20, and 60 days of age, and at constant times in the day and in the season, in order to avoid possible seasonal and circadian variations.

Preparation of Tissues. All dissections of the brain were performed over ice and soon after tissue samples were frozen over dry ice and stored at −80°C until assayed. Samples were pooled according to sex and litter, and pools from different litters were combined. Separation of the brain regions was performed as follows.

Cerebral cortex included hippocampus, amygdala, and septum; 1-day-old-cortex included also the basal ganglia. Olfactory bulbs were discarded. Cerebellum was isolated by cutting its peduncular connections with the brain stem. Corpus striatum was separated from the neocortex at the radiation of the corpus callosum and from the diencephalon at the sulcus terminalis. The mean weight at 60 days was 127.7 ± 2.5 mg (N = 96). Dissection of the entire hypothalamus was limited anteriorly by the margin of the optic chiasma, laterally by the lateral fissures, and posteriorly by the margin of the mammillary body. The block was about 2.5 mm deep from the basal surface of the hypothalamus. The average weight of the hypothalamic region in control rats at 60 days was 65.7 ± 1.0 mg (N = 51). The brain stem was divided into mesodiencephalon and pons medulla by an oblique cut running from the posterior border of the inferior colliculus on the dorsal surface to the anterior border of the pons on the ventral surface. Pons medulla was separated from spinal cord by cutting below the obex.

Whereas male and female samples were combined (50%–50%) at all ages for the hypothalamus and corpus striatum, at 1 day of age for the cortex, at 1, 7, and 12 days for the mesodiencephalon and cerebellum, and at 1 and 7 days for the pons medulla, tissues were assayed with respect to sex in all other regions and ages.

Enzyme Assays. The following methods were used for the different assays:

Tyrosine Hydroxylase (TH). From 25 to 100 μl of 10% or 20% homogenate (w/v) in 0.3 M sucrose were used according to the method of Waymire et al. (28). The substrate for the reaction included (final conc): 80 μM L-tyrosine plus 20 μM [1-^{14}C] L-tyrosine (54.6 mCi/mmol specific activity; New England Nuclear Corp., NEN); 2 mM Na-phosphate buffer (pH 6.1); 2.5 mM 6,7-dimethyl-5,6,7,8-tetrahydropterine (Calbiochem); 40 mM 2-mercaptoethanol, and 1 mM $FeSO_4$. Pyridoxal-5-phosphate-enriched (5 nmol) hog kidney decarboxylase was used for the reaction. The $^{14}CO_2$ developed was trapped by a folded filter paper embedded with 200 μl Hyamine (NEN) and contained in a plastic center well.

Tryptophan Hydroxylase (TPH). Determinations were carried on 500 μl of 20% or 30% homogenate in 0.3 M sucrose containing 0.1 M Tris-acetate buffer (pH 8.1), according to a modification (29) of Ichiyama's method (30). The incubation medium included 0.1 M Tris-acetate buffer (pH 8.1), 10 mM iproniazid phosphate, 2 μM [1-^{14}C]L-tryptophan (39.9 mCi/mmol specific activity, NEN). The test tubes were incubated for 1 hr at 37°C with shaking; after stopping the reaction, a second incubation of 1 hr was performed with shaking. The procedure for trapping the $^{14}CO_2$ developed in the reaction was the same as for the TH reaction. Inasmuch as labeled tryptophan concentration (2×10^{-6} M) was below the K_m of the decarboxylase for tryptophan (2×10^{-3} M) (30, 31), a direct decarboxylation of tryptophan was unlikely; however, the high-altitude-provoked changes in endogenous levels of tryptophan (Table I) perhaps did influence TPH activity. The specificity of the reaction was furthermore verified with the competitive inhibitor parachlorophenylalanine (PCPA): 1×10^{-4} M PCPA inhibited the reaction by approximately 50% (32).

Catechol-O-Methyl Transferase (COMT). A slight modification (33) of Axelrod's method (34) was followed; the incubation medium included 100 μl of 10% homogenate containing 5 μl of 10% Triton-X, 10 μl 10% $MgCl_2$, 0.5 μmol DL-epinephrine, 10 μM *S*-adenosyl methionine (SAMe), and 2 μM [^{14}C] methyl-SAMe (57.8 mCi/mmol specific activity, NEN). [^{14}C]Metanephrine formed was extracted with toluene–isoamyl alcohol (3:2 v/v).

Monoamine Oxidase (MAO). The microfluorimetric method of Kraml (35) as described by Nagatsu (33) was followed; 50 μl of 10% homogenate and 0.17 mM kynuramine hydrobromide (Sigma Chemical Co.) were used. The fluorescent product 4-hydroxyquinoline was measured at 315 nm excitation, 380 nm emission.

TABLE 1

EFFECTS OF HIGH ALTITUDE ON TRYPTOPHAN LEVELS[a] IN SEVERAL BRAIN REGIONS OF THE RAT

Region	Treatment	Age (days) 7	Age (days) 12
C. cortex	SL	19.3 ± 0.7 (12)	16.2 ± 0.6 (12)
	HA	19.7 ± 0.3 (12)	19.2 ± 1.1 (12)
	P	N.S.	<0.05
Cerebellum	SL	12.5 ± 0.8 (6)	10.6 ± 0.2 (6)
	HA	17.9 ± 2.4 (6)	14.8 ± 0.9 (6)
	P	<0.05	<0.005
Hypothalamus	SL	39.4 ± 0.3 (6)	41.4 ± 1.4 (6)
	HA	47.8 ± 2.4 (6)	49.6 ± 2.1 (6)
	P	<0.01	<0.01
Pons medulla	SL	21.9 ± 1.8 (6)	20.0 ± 0.6 (12)
	HA	25.1 ± 1.3 (6)	29.1 ± 1.1 (12)
	P	N.S.	<0.001
Mesodiencephalon	SL	12.4 ± 0.4 (6)	15.6 ± 0.4 (6)
	HA	18.3 ± 1.6 (6)	20.1 ± 1.3 (6)
	P	<0.01	<0.01
Corpus striatum		Not assayed	

[a] Values are μg/g wet weight ± SEM. Number of experiments is given in parentheses. SL = sea level controls; HA = high altitude. P = significance on Student's *t* test.

Tryptophan and Tyrosine Levels. Brain tryptophan (36) and tyrosine (37) levels were measured by fluorimetric methods.

Protein Concentration. This was determined by the method of Lowry et al. (38).

Statistical Evaluations. Results were analyzed with the Student's t test.

RESULTS

Enzyme activity was expressed in terms of proteins (specific activity) and tissue weight (total activity). It is well known that age-related changes in the ratio of water to solids may influence the calculations of the biochemical measurements in the developing brain (39); therefore, we have chosen to present our results and to discuss them as specific activity. In most cases, data of specific activity coincided with those of total activity. The data shown in the figures represent combined male (50%) and female (50%) samples. Whenever separate samples could be assayed, the sex differences, if any, are discussed in the text.

High-altitude animals displayed an early, long-lasting increase of plasma corticosterone levels compared with sea level rats (40), thus indicating that the hypoxic environment did provoke stress. In view of the many technical difficulties involved in measuring oxygen saturation in very young animals, blood oxygen saturation was not measured; nevertheless, evidence in several species shows that low ambient oxygen concentration markedly reduces organ and blood oxygen levels (41, 42), and the increased hematocrit value reported (43) in rats born and raised at high altitude represents and indirect measure of the reduced blood oxygenation.

Effects on Survival, Body Growth, and Gross Behavior

Survival was dramatically affected by HA: hypoxic animals showed a 45% mortality during the preweaning period as compared to 2% in the sea level controls. After weaning, survival increased and became similar to that of control animals. As previously reported, exposure to HA impaired body growth during the entire experimental period and induced marked cardiac hypertrophy (43). HA animals also displayed some grossly observable neurologic symptoms such as tremors, restlessness, hyperactivity, and aggressiveness, and reduced weight of most brain regions, particularly cerebellum.

Developmental Patterns

The developmental profiles of enzyme activity in controls resembled those already described in the literature (44–47) and therefore will be

summarized here only briefly. In both animal groups, developmental patterns were age-, brain region-, and enzyme-specific. For example, in the cortex (Figure 1) and cerebellum (Figure 2) still undergoing rapid maturation after birth, almost all activity levels were higher in adults than in the newborn, although peak activity was often reached prior to

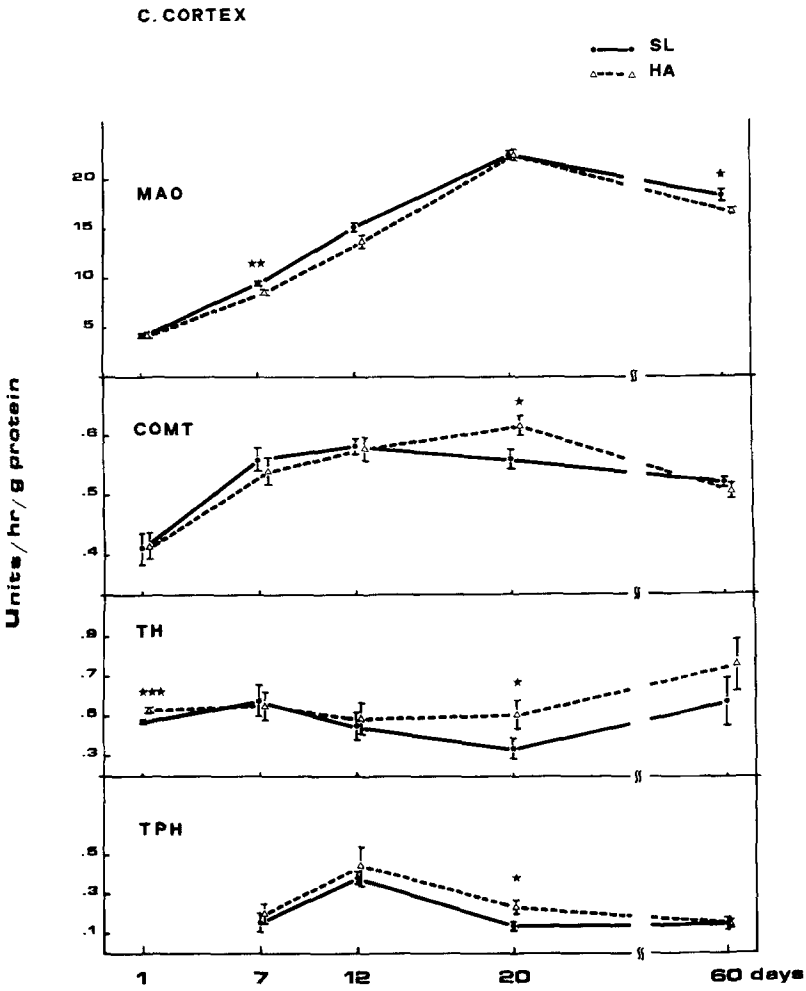

Fig. 1. Development of TPH, TH, COMT, and MAO activity in the rat cerebral cortex at sea level (SL; 76 m) and high altitude (HA; 3800 m). TPH activity is expressed as nmol/hr/g protein; other activities are μmol/hr/g protein. Vertical lines represent SEM from 6 experiments (1 day) or 12 experiments (remaining ages). Pooled samples from one or more litters (half males, half females) were used. $^{*}P < 0.05$; $^{**}P < 0.02$; $^{***}P < 0.005$.

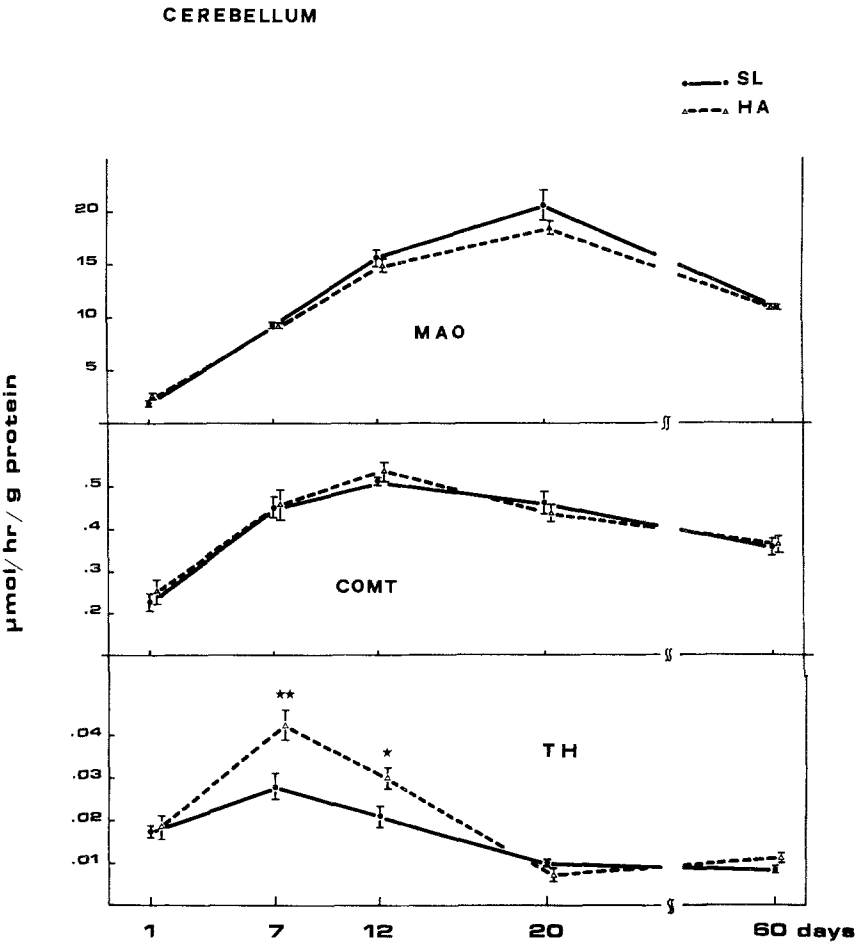

FIG. 2. Development of TH, COMT, and MAO activity in the rat cerebellum at sea level (SL; 76 m) and high altitude (HA; 3800 m). Activity is expressed as μmol/hr/g protein. Vertical lines represent SEM from 6 experiments (1, 7, and 12 days) and 12 experiments (remaining ages). Pooled samples from one or more litters (half males, half females) were used. $^{*}P < 0.05$; $^{**}P < 0.02$

adulthood at 12 or 20 days. Conversely, in the hypothalamus (Figure 4) and pons medulla (Figure 6), which develop primarily during fetal life, enzymes showed an already high activity at 1 day of age, sometimes exceeding that of the adult, except for MAO for which activity progressively increased with increasing age. Enzyme activities in the corpus striatum (Figure 5) were also higher in the adult than in newborns, only TH reaching a peak at weaning, then declining.

Effects of High Altitude on Monoamine Enzymes

High altitude did affect TH and TPH activity mainly at the same ages and in the same brain regions, as summarized below.

Tyrosine Hydroxylase. Despite a qualitative similarity in developmental profiles, sea-level and high-altitude animals showed marked quantitative differences in TH activity which increased in hypoxia in some brain regions (cortex, cerebellum, and pons medulla) and decreased in others (mesodiencephalon, hypothalamus, and corpus striatum). Thus, in the cortex (Figure 1), TH activity was already significantly higher in HA than sea level rats at 1 day (by 14%) and remained higher until 60 days of age, with a 59% increase at 20 days; similarly, in the cerebellum (Figure 2) TH activity was increased in HA rats at 7 (by 51%) and 12 (by 40%) days of age and in the pons medulla (Figure 6) by 24% at 7 days. Conversely, in the mesodiencephalon, TH activity was always lower in hypoxic rats, particularly at day 12 (Figure 3), and in the hypothalamus (Figure 4) and corpus striatum (Figure 5) it became significantly lower at adulthood.

Tryptophan Hydroxylase. The activity of TPH in the areas where it was measured showed the same dichotomy as did TH. It was indeed higher in HA rats than controls in the cortex (Figure 1), particularly at 20 days (by 70%); lower in the mesodiencephalon (Figure 3) at 60 days (by 34%) and in the hypothalamus (Figure 4) at all ages and particularly at 60 days (by 37%); no significant differences were observed in the corpus striatum.

Catechol-O-Methyl Transferase and Monoamine Oxidase. The effects of hypoxia on these enzymes were more sporadic and less marked than those on the hydroxylases, but when present, they were always characterized by decrease in activity. Thus, whereas little change was observed in the cerebellum (Figure 2) and mesodiencephalon (Figure 3), both COMT and MAO were generally lower in the hypothalamus (Figure 4) of HA adult rats as compared to controls; MAO was also significantly lower in the cerebral cortex (Figure 1) at 7 and 60 days, and in the corpus striatum (Fig. 5) and pons medulla (Figure 6) at 7 days.

Sex-Related Differences in Enzyme Sensitivity to High Altitude

Even though previous studies have shown distinct sex differences in enzyme activity at sea level (48), no consistent sex-related differences in the responsiveness of monoamine enzymes to the hypoxic environment were observed in these experiments; only sporadic differences occurred, as in the cerebral cortex where, in females, MAO activity was 14% lower than in males at high altitude.

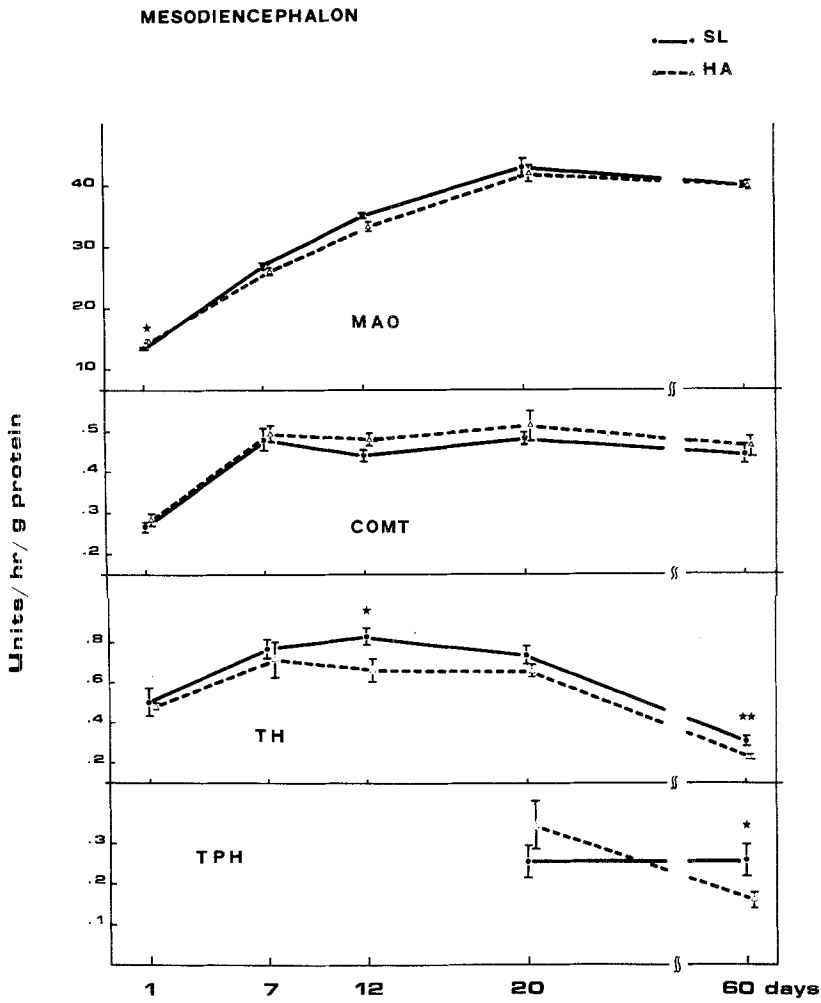

FIG. 3. Development of TPH, TH, COMT, and MAO activity in the rat mesodiencephalon at sea level (SL; 76 m) and high altitude (HA; 3800 m). TPH activity is expressed as nmol/hr/g protein; other activities are μmol/hr/g protein. Vertical lines represent SEM from 6 experiments (1, 7, and 12 days) or 12 experiments (other ages). Pooled samples from one or more litters (half males, half females) were used. $^{*}P < 0.05$; $^{**}P < 0.02$.

Tyrosine and Tryptophan Levels

Because TPH and TH activity seem to depend on precursor amino acid levels (49, 50), brain levels of tryptophan and tyrosine were measured. High altitude did not affect tyrosine levels in cortex, cerebellum, hypothalamus, and mesodiencephalon; in pons medulla tyrosine levels

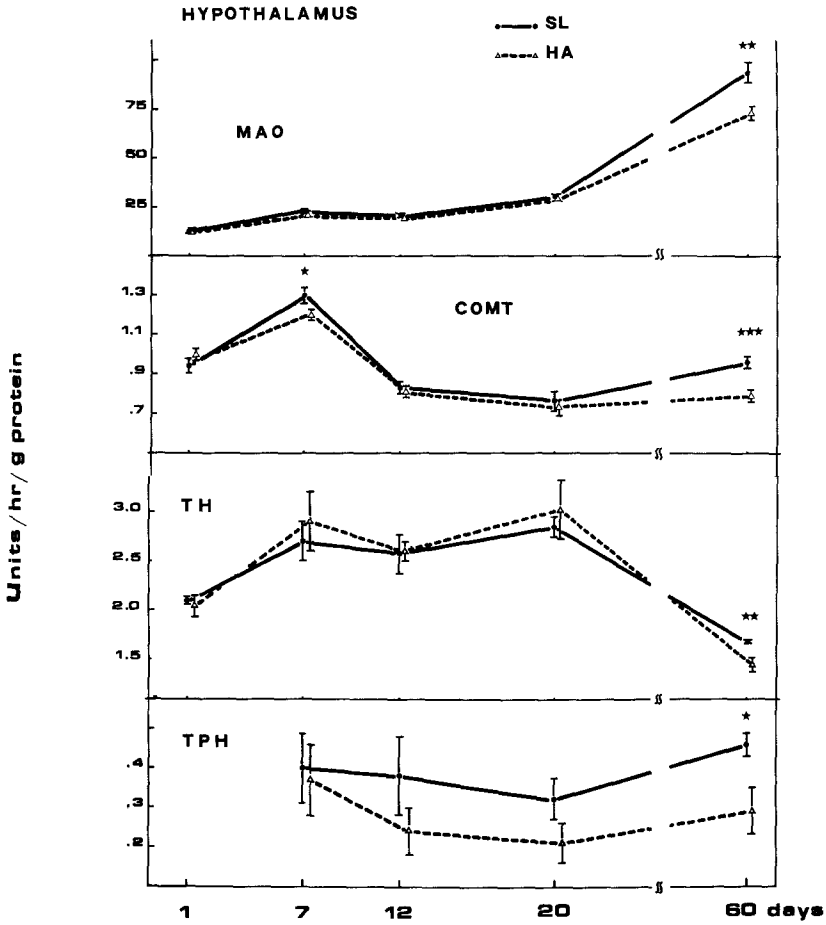

FIG. 4. Development of TPH, TH, COMT, and MAO activity in the rat hypothalamus at sea level (SL; 76 m) and high altitude (HA; 3800 m). TPH activity is expressed as nmol/hr/g protein; other activities are μmol/hr/g protein. Vertical lines represent SEM from 6 experiments. Pooled samples from one or more litters (half males, half females) were used. $^{*}P < 0.05$; $^{**}P < 0.02$ or < 0.01; $^{***}P < 0.001$.

were higher at 7 days (sea level: 51.8 ± 1.2; high altitude: 68.2 ± 6.3, μg/g wet wt, $P < 0.05$), and 12 days (sea level: 36.4 ± 0.9; high altitude: 47.9 ± 2.2, μg/g wet wt, $P < 0.001$), respectively.

Tryptophan levels (Table I), although generally unchanged early in development and in adulthood, were almost constantly increased at high altitude at 12 days of age in the cortex (+18%), pons medulla (+45%), hypothalamus (+20%), mesodiencephalon (+29%), and cerebellum (+40%); in the latter two areas a 47% and a 43% increase occurred also

at 7 days of age. The changes in amino acid concentrations, where present, might be related to the HA-provoked changes in TPH and TH activity. TPH has a low affinity to the substrate, and usual brain concentrations of tryptophan are quite far from the saturation (51).

DISCUSSION

It is well-known that the fetus and newborn are more capable than the adult of surviving acute hypoxia. However, in the present study, chronic

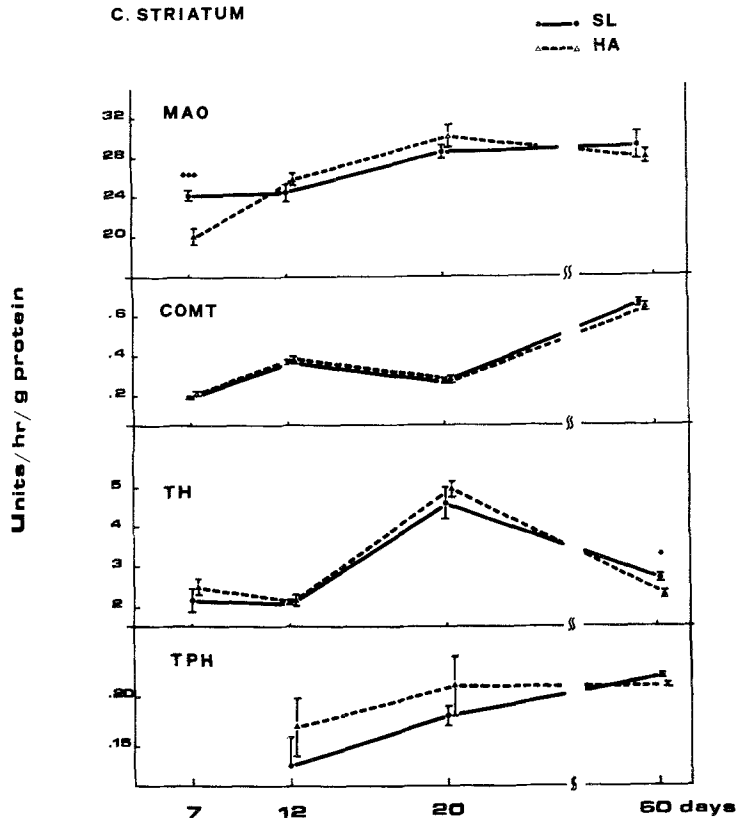

FIG. 5. Development of TPH, TH, COMT, and MAO activity in the rat corpus striatum at sea level (SL; 76 m) and high altitude (HA; 3800m). TPH activity is expressed as nmol/hr/g protein, other activities are μmol/hr/g protein. Vertical lines represent SEM from 6 experiments. Pooled samples from one or more litters (half males, half females) were used. $^{*}P < 0.05$; $^{***}P < 0.005$ or < 0.001.

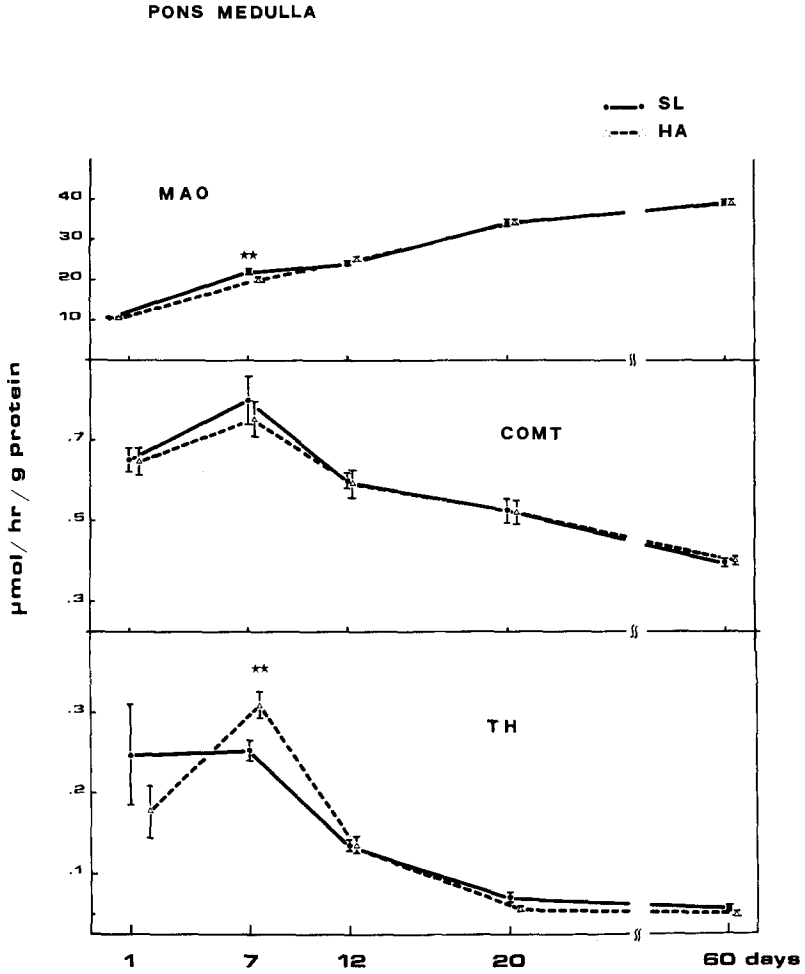

FIG. 6. Development of TH, COMT, and MAO activity in the rat pons medulla at sea level (SL; 76 m) and high altitude (HA; 3800 m). Activity is expressed as μmoles/hr/g protein. Vertical lines represent SEM from 6 experiments (days 1 and 7) or 12 experiments (remaining ages). Pooled samples from one or more litters (half males, half females) were used. $^{**}P < 0.02$ or < 0.01.

exposure to a natural hypoxic environment did induce long-lasting alterations in the development of enzymes involved in the synthesis and catabolism of monoamines. These changes became manifest at different ages and to varying degrees for each enzyme and brain area, the synthesis enzymes being more sensitive than the catabolic ones (14, 52).

Whereas during the first week after birth, several of the enzymes

showed a relative responsiveness to high altitude (HA), starting from day 12—a highly vulnerable period of accelerated brain maturation (53)—and continuing until adulthood, enzyme activity was significantly altered by HA in most regions considered. Of particular relevance to the overall balance of monoamines in the brain of HA rats is the finding that TH and TPH activity was increased as early as 1 and 7 days in the cerebral cortex and cerebellum—two areas relatively immature at birth in the rat (Figures 1 and 2)—and less consistently in the pons medulla (Figure 6). On the other hand, a consistent reduction in TH and TPH activity occurred in the mesodiencephalon, hypothalamus, and corpus striatum (Figures 3, 4, and 5) of 60-day-old rats.

Inasmuch as consistent changes (decrease) in the activity of catabolizing enzymes were present only in the hypothalamus (Figure 4), modulation of the monoaminergic homeostasis at HA would depend primarily on the stimulation or depression of the synthetic enzymes.

Any correlation of present results with the monoamine balance in vivo is hard to draw; however, it is tempting to relate the overall decrease in the activity of hypothalamic enzymes with the marked decrease in gonadotropin levels and consequent alterations in reproductive functions we have previously found in HA rats (5, 54). The relationships among hypothalamic monoamines, gonadotropin-releasing hormones, and secretion of pituitary gonadotropins are indeed well known (55).

Although hypoxia per se was expected to uniformly decrease the activity of oxygen-dependent hydroxylases and MAO, present results point out that the nonspecific effects of the stress may superimpose on the enzymic hypoactivity (if any), due to low oxygen concentration, in selected brain regions. The importance of the stress at HA was emphasized in a previous study on the adrenals, where both synthetic and catabolic monoaminergic enzymes were strongly induced early in development (40). Enzymic changes did parallel the HA-provoked increase of plasma corticosterone levels (56), thus suggesting a rather nonspecific production of new enzyme proteins due to stress.

The stress component at HA would be more effective on adrenal than brain enzymes, for anatomical and functional reasons; conversely, the direct effect of hypoxia on oxygen-dependent enzymes would be the primary altering factor for the brain.

Our results do not allow us to conclude whether the reduction of enzyme activity in most brain regions is due to neuronal damage rather than to an alteration in the number of neurons. Both possibilities seem to be open, since hypoxia can provoke neuronal degeneration in vitro (57), and the weight of most brain areas was reduced at HA.

Another possible mechanism by which HA might influence the devel-

opment of monoaminergic enzymes is by altering the levels of precursor amino acids. In the present experiment, tryptophan levels were significantly affected in several brain areas, but not at all ages. Changes in tryptophan levels may conceivably influence TPH activity, thus reflecting on the rate of 5-HT synthesis, as previously seen in rats developed at HA (54).

In conclusion, altered monoamine metabolism in specific brain areas, at certain ages, may occur as a result of an imbalance between changes in precursor availability due to stress, and altered enzyme development due both to hypoxia and stress. Therefore, at high altitude, multifactorial influences combine to assure survival and minimize long-term neurological and functional deficits during development.

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