Brain Tryptophan Hydroxylation: Dependence on Arterial Oxygen Tension

Abstract. *The accumulation of cerebral 5-hydroxytryptophan after decarboxylase inhibition was decreased in rats maintained at arterial O_2 tensions below 60 mm-Hg. In contrast, brain lactate was stable above 40 mm-Hg and brain adenosine triphosphate, adenosine diphosphate, and adenosine monophosphate were unchanged above 30 mm-Hg. There was a linear correlation of brain 5-hydroxytryptophan accumulation to cerebral venous O_2 tension. Cerebral tryptophan hydroxylase appears to have a poor affinity for oxygen and to be affected by slight hypoxia. The resultant decreases in monoamine neurotransmitter metabolism may explain the behavioral changes of mild oxygen deprivation.*

Cerebral dysfunction is one of the earliest signs of hypoxia in man. Errors in reading tests could be demonstrated with exposure to 16 percent oxygen (*1*). Acute mountain sickness occurs in 12 percent oxygen and consciousness is lost with exposure to 6 to 8 percent oxygen in man (*2*). However, cerebral oxygen consumption continues unchanged with exposure to 7 percent oxygen or more (*3*). Furthermore, the earliest biochemical changes of hypoxia, a drop in phosphocreatine and a modest rise in lactate, occur in rats exposed to 8 percent oxygen or less; these early changes may be a result of an intracellular acidosis rather than a failure in brain aerobic metabolism (*4, 5*). Thus, the functional changes of moderate hypoxia have not been associated with detectable alterations in cerebral energy production.

Oxygen is also utilized by the brain for the synthesis of the neurotransmitters dopamine, noradrenaline, and serotonin. The two enzymes that limit the synthesis rate of these neurotransmitters are tyrosine and tryptophan hydroxylase. Both enzymes require a pteridine cofactor and molecular oxygen to function. In vitro data indicate that neither of these enzymes is fully saturated in the range of brain oxygen tensions (*6*).

The in vivo activity of both tyrosine and tryptophan hydroxylase can be estimated in the brain by measuring the accumulation of dopa and 5-hydroxytryptophan (5-HTP) in the presence of an inhibitor of the aromatic amino acid decarboxylase [NSD 1015 (3-hydroxybenzyl hydrazine)] (*7*). Unanesthetized rats placed in low oxygen environments showed decreases in the in vivo activity of both enzymes as measured by the administration of NSD 1015 (*8*). The enzymes thus appeared very sensitive to hypoxia, but other factors such as hypocapnia, acid-base changes, or hypothermia may have affected the results. Furthermore, NSD 1015 may itself alter energy metabolism. In the present study we estimated brain tryptophan hydroxylase activity in vivo in lightly anesthetized animals. Blood oxygen and carbon dioxide tensions and *p*H values were carefully monitored and the animals were killed by freezing the brain in situ in order to measure lactate, pyruvate, and high-energy phosphates in addition to 5-HTP.

Male Wistar rats, weighing about 300 g, were anesthetized with divinyl ether; they were then tracheotomized, immobilized with tubocurarine chloride (1.8 mg/kg, intraperitoneally), and connected to a rodent respirator with an inflow of 70 percent N_2O and 30 percent O_2. Blood pressure was monitored through a femoral artery catheter and 100-μl samples of arterial blood were drawn for analysis. Rectal body temperature was maintained close to 37°C. A sagittal incision was made in the skin over the skull to accommodate a plastic funnel to be used in freezing the brain in situ later on. After a control period, NSD 1015 was administered intraperitoneally (100 mg/kg), and the oxygen concentration of the respiratory inflow was varied with nitrogen so as to give arterial P_{O_2} values of down to 28 mm-Hg while maintaining 70 percent N_2O.

Thirty minutes after the administration of NSD 1015 (*9*), cerebrospinal fluid (CSF) was sampled and the brains were frozen in situ. The frozen brains were split into two parts; one half was analyzed for 5-HTP (*10*), and the other half for organic phosphates, lactate, and pyruvate (*11*).

Cerebral venous P_{O_2} was derived from a curve relating the arterial and cerebral venous P_{O_2}'s [see MacMillan and Siesjö (*11*)]. This relation was unchanged in rats that had been treated previously with NSD 1015 (100 mg/kg).

Six animals treated with NSD 1015 were kept at an arterial P_{O_2} of 80 mm-Hg or higher (80 to 166 mm-Hg) and could thus be considered controls. Data

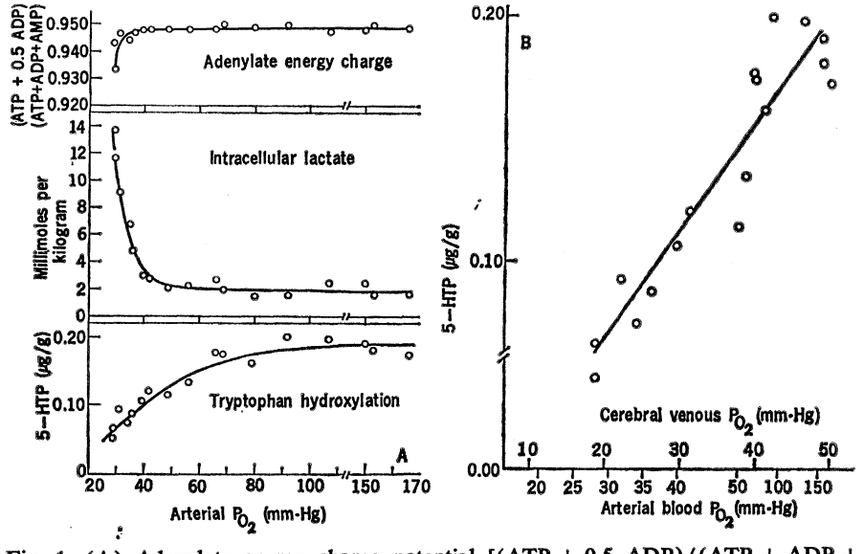

Fig. 1. (A) Adenylate energy charge potential [(ATP + 0.5 ADP)/(ATP + ADP + AMP)], intracellular lactate in millimoles per kilogram wet weight [brain lactate — 0.03 (blood lactate) — 0.15 (CSF lactate)]/0.61, and tryptophan hydroxylation [accumulation of 5-HTP 30 minutes after NSD 1015 (100 mg/kg, intraperitoneally)] plotted as a function of the arterial P_{O_2}. Each point represents the mean arterial P_{O_2} of three determinations on a single animal during the 30 minutes after administration of NSD 1015. (B) Tryptophan hydroxylation as a function of the cerebral venous P_{O_2} in rat brain. The accumulation of 5-HTP 30 minutes after the administration of NSD 1015 is plotted against the mean cerebral venous P_{O_2} during the 30-minute period. The cerebral venous P_{O_2} is an estimate of oxygen concentration in brain tissue. Each point represents an individual rat, whose venous P_{O_2} was calculated from the constant relation between arterial P_{O_2} and cerebral venous P_{O_2} (*14*). The diagonal line was calculated by regression analysis as the best fit. The correlation coefficient was .93 ($P < .001$).

Table 1. Rat brain high-energy intermediates after administration of NSD 1015 (3-hydroxybenzyl hydrazine). Anesthetized rats were given 100 mg of NSD 1015 per kilogram, and their arterial blood P_{O_2}, P_{CO_2}, and *p*H were monitored for 30 minutes. After blood and CSF samples were obtained from the rats, their brains were frozen in situ. Adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), phosphocreatine (PCr), lactate (La), and pyruvate (Py) were determined. The values are expressed in millimoles per kilogram wet weight ± standard error of the mean. The control values are taken from MacMillan and Siesjö (*12*). Statistical analysis was done by Student's *t*-test; N.S., not significant.

	ATP	ADP	AMP	PCr	La	Py
	(Millimoles per kilogram wet weight)					
Control ($N = 5$)	3.08 ± 0.01	0.258 ± 0.003	0.041 ± 0.001	5.16 ± 0.04	1.61 ± 0.03	0.122 ± 0.003
NSD 1015 ($N = 6$)	3.09 ± 0.02	0.272 ± 0.003	0.038 ± 0.001	4.49 ± 0.10	1.65 ± 0.12	0.097 ± 0.006
P	N.S.	< .01	N.S.	< .001	N.S.	< .01

in Table 1 compare the cerebral concentrations of adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), phosphocreatine, lactate, and pyruvate of these animals to the corresponding concentrations obtained in uninjected animals subjected to the same anesthetic and operative procedures (*12*). These data demonstrate that NSD 1015 had no significant effect on the tissue concentrations of ATP or lactate. There were slight drops in pyruvate and ADP, and a more pronounced decrease in phosphocreatine in the group treated with NSD 1015. The lactate and pyruvate concentrations in CSF were similar (results not shown).

Figure 1A relates the calculated adenylate energy charge (*11*), the intracellular lactate concentration, and the 5-HTP accumulation to the arterial P_{O_2}. The adenylate energy charge was stable even at low arterial P_{O_2}'s and in only two animals was there a small fall (by 0.5 to 1.5 percent). There was an increase in intracellular lactate below a P_{O_2} of 40 to 45 mm-Hg, corroborating previous findings (*4, 5*). However, the 5-HTP accumulation was clearly reduced at arterial P_{O_2} values below 60 mm-Hg, and at the lowest P_{O_2}'s the concentration was reduced to 30 percent of normal.

Since the venous P_{O_2} more closely reflects the mean tissue oxygen tension than does arterial P_{O_2}, the 5-HTP concentration was related to the cerebral venous P_{O_2}. Figure 1B shows a linear correlation between venous P_{O_2} and 5-HTP concentration. Regression analysis yielded a correlation coefficient of .93 ($P < .001$).

The striking stability of the organic phosphates measured in the present hypoxic animals is consistent with previous observations on animals that did not receive NSD 1015 (*5*). Such results reflect the efficiency of the mitochondrial respiratory enzymes in utilizing tissue oxygen (*13*). In contrast, tryptophan hydroxylase is apparently not maximally saturated with oxygen in vitro even at 100 percent oxygen and a pressure of 1 atm (*6*). The accumulation of 5-HTP after decarboxylase inhibition was closely correlated to the cerebral venous P_{O_2}, indicating a relation between the activity of tryptophan hydroxylase and tissue oxygen tensions in vivo. This linear relation suggests that the cerebral tryptophan hydroxylase enzyme is unsaturated in the range of the oxygen concentrations in brain tissue found in rats breathing room air (*6*). Thus, rats exposed to 100 percent oxygen show an increased rate of tryptophan hydroxylation compared to rats breathing room air, measured by administration of NSD 1015 (*8*) or by the increase in serotonin after a monoamine oxidase inhibitor (*14*).

The drop in brain phosphocreatine concentrations in NSD 1015–treated animals was unexpected. In hypoxic animals treated with NSD 1015, the adenylate energy charges were not different from untreated hypoxic animals, which suggests that NSD 1015 does not interfere with high-energy phosphate production. Furthermore, hypoxic decreases in the synthesis of serotonin and catecholamine can be measured by other pharmacologic methods (*15*) when NSD 1015 is not given. However, the possibility that NSD 1015 induces changes in cellular energy which make the tryptophan hydroxylase enzyme more sensitive to hypoxia cannot be excluded.

Brain tryptophan hydroxylase is not saturated with tryptophan in normal animals (*16*). There is no change in brain tryptophan levels in rats exposed to 5.6 percent oxygen for 1 hour (*15*) or after the administration of NSD 1015 (*7*).

In the present experiments there were clear changes in the brain 5-HTP concentrations at arterial P_{O_2} values of below 60 mm-Hg in rats treated with NSD 1015. These changes reflect an impairment in tryptophan hydroxylation even at mild levels of hypoxia. Tryptophan hydroxylase appears to compete poorly with mitochondrial enzymes for available oxygen. Decreases in the hydroxylases activities may mean that there are changes in the synthesis of serotonin with mild oxygen deprivation. Such a change may play a role in the functional effects of hypoxia.

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percent, respectively, of the tissue weight. The adenylate energy charge was calculated as (ATP + 0.5 ADP)/(ATP + ADP + AMP).
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Methyltetrahydrofolic Acid Mediates N- and O-Methylation of Biogenic Amines

Abstract. *A variety of mammalian and avian tissues* N- *and* O-*methylate indoleamines and phenylethylamines, with methyltetrahydrofolic acid as the methyl donor. Because it is considerably more efficient than* S-*adenosylmethionine, methyltetrahydrofolic acid may be the natural methyl donor in this reaction. With methyltetrahydrofolic acid, serotonin is* O-*methylated to 5-methoxytryptamine, a novel indoleamine in mammalian brain.*

The *N*-methylation of biogenic amines may play a major role in certain mental illnesses (*1*). Axelrod described an enzyme that *N*-methylated a variety of biogenic amines, including indoleamines, but the enzyme could not be demonstrated in tissues other than the rabbit lung (*2*). It has been reported that chick and human brains (*3*) and a variety of dialyzed mammalian tissues (*4*) contain enzymes that can *N*-methylate indoleamines. All of these enzymes use *S*-adenosylmethionine (AMe) as a methyl donor and, except for the rabbit lung enzyme, their activity is low (*2–4*). Laduron (*5*) observed that dopamine can be *N*-methylated to epinine with methyltetrahydrofolic acid (MTHF) as the methyl donor. We now report the *N*- and *O*-methylation of several biogenic amines by extracts of mammalian and avian tissues. The primary methyl donor appears to be MTHF.

Tissues from male rats (150 to 200 g), male rabbits (1.5 kg), and 5-day-old Leghorn chicks (mixed sex) were homogenized in ten volumes of 0.005*M* sodium phosphate buffer (*p*H 7.9), and the homogenates were centrifuged at 100,000*g* for 60 minutes. We dialyzed some supernatant preparations for 12 hours against 100 to 200 volumes of the same sodium phosphate buffer. Incubation mixtures for the enzyme assay contained, at final concentrations, sodium phosphate buffer, *p*H 7.9, 0.005*M*; amine substrate, 5 m*M*; [^{14}C]MTHF (50 mc/mmole; Amersham), 1 μM; or [^{14}C]AMe (50 mc/mmole; New England Nuclear), 1 μM; together with tissue enzyme (0.5 to 2.0 mg of protein per milliliter) in a final volume of 0.5 ml. The mixture was incubated for 30 to 60 minutes at 37°C; 1 ml of 0.5*M* borate buffer, *p*H 10, was added and the mixture was added to 6 ml of an organic solvent, selected on the basis of the amine substrate. After shaking the mixture for 10 minutes, it was centrifuged, a 5-ml portion of the organic phase was transferred to a counting vial, and the solvent was evaporated to dryness at 80°C in a chromatography oven. We dissolved the residue in 2 ml of ethanol, added 10 ml of toluene phospor, and counted the radioactivity. Controls, consisting of incubation mixtures that lacked substrate amine, showed radioactivity similar to that of the blanks obtained by heating the enzyme preparation. Enzyme activity was linear for at least 60 minutes. Methylated products were identified by thin-layer chromatography in three solvent systems (*6*). In all cases the radioactive peaks coincided with *N*- or *O*-methylated forms of the amines.

As reported, both undialyzed and dialyzed supernatant preparations of rabbit lung methylated serotonin and tyramine with AMe as the methyl donor (*2*) (Table 1). Supernatant preparations from undialyzed rat lung, rat brain, and rabbit brain failed to significantly methylate either substrate with AMe (*7*), while undialyzed chick brain preparations methylated amines with AMe (*3*). However, except for the enzymes from rabbit lung, which methylated amines as efficiently when dialyzed or not dialyzed, methylation by enzymes from other tissues was greatly enhanced by dialysis.

Dialyzed supernatant preparations of rabbit lung methylated serotonin and tyramine 4 to 6 times more efficiently with AMe than with MTHF. In all other tissues, tyramine methylation was considerably greater with MTHF than with AMe and we did not detect any serotonin methylation with AMe. In all tissues examined, serotonin was methylated with MTHF 1.5 to 3 times more efficiently than was tyramine.

The MTHF requiring methylating enzyme was partially purified from rat brain by ammonium sulfate fractionations and chromatography (*8*). The materials that precipitated when the solutions were 30 to 45 percent and 45 to 60 percent saturated with ammonium sulfate had similar activities

Table 1. Species and tissue distribution of methyltransferase activity. Tissues were homogenized in ten volumes of 5 μM sodium phosphate buffer (*p*H 7.9). After dialysis, the solutions were centrifuged at 100,000*g* and the supernatant was assayed for enzyme activity. Serotonin (5 m*M*) (S) or tyramine (5 m*M*) (T) were substrates and *S*-adenosylmethionine (1 μM) or 5-methyltetrahydrofolic acid (1 μM) were methyl donors. Data are presented as the mean of three experiments whose results varied less than 20 percent. Enzyme activity is expressed as picomoles of methyl group added to the substrate per milligram of protein in 1 hour.

Tissue	AMe			MTHF			Ratio of enzyme activity with AMe to activity with MTHF	
	S	T	S/T	S	T	S/T	S	T
Rabbit lung	32.0	24.0	1.33	8.0	4.0	2.0	4.0	6.0
Rabbit brain	0	0.05	0	2.6	1.4	1.9	0	0.04
Rabbit liver	0	0.2	0	2.1	1.2	1.7	0	0.08
Rat brain	0	0.4	0	3.0	1.3	2.3	0	0.31
Rat liver	0	1.2	0	4.3	1.4	3.1	0	0.90
Rat lung	0	0.35	0	4.0	1.3	3.1	0	0.27
Rat heart	0	0.4	0	8.0	3.0	2.7	0	0.14
Chick brain	0	1.0	0	6.0	4.0	1.5	0	0.25
Chick heart	0	2.0	0	26.0	11.0	2.3	0	0.18