BEHAVIORAL, METABOLIC, AND ENZYMATIC STUDIES OF A BRAIN INDOLEETHYLAMINE N-METHYLATING SYSTEM

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Although there have been many good reasons to look for a brain indoleamine N-methylating enzyme since the introduction of the Harley-Mason amine methylation hypothesis of schizophrenia (Osmond and Smythies 1952) and Axelrod's exciting and systematic elucidation of various amine-methylating enzymes in the central nervous system (Axelrod 1965), our own particular interests in this field started in 1964 when we were studying the effect of the intravenous infusion of various amine precursors on sleep patterns in man. We reported a 5-hydroxytryptophan (5-HTP)-induced increase in rapid eye movement sleep (Mandell, Mandell, and Jacobson 1965). At the same time we noted that occasionally there was a disruption of sleep during 5-HTP infusions, with episodes of bizarre mentation. Whereas 5-hydroxy-tryptophan alone in small doses (150 mg i.v. over 6 to 8 hours) would usually produce some degree of sedation in our subjects, higher doses (200 mg or more), or smaller doses given in combination with a monoamine oxidase inhibitor as a pretreatment before intravenous amino acid load produced behavioral and psychic activation. We then became aware of the wide variety of studies by a number of investigators (Kety 1961; Pollin, Cardon, and Kety 1961; Kline, Simpson, and Sacks 1967; Himwich *et al.* 1970) that demonstrated similar reversals of the indoleamino acid-produced sedation with monoamine oxidase inhibitor pretreatment.

The Harley-Mason hypothesis quite generally states that some mental illness might result from a normal biogenic amine being methylated in an unusual way or to an unusual extent to produce hallucinogenic amines and resulting behavioral aberrations. Derived from the studies of the relative peripheral and central pharmacological potency of various O-and N-methylated amines, it has been a

seductive hypothesis indeed. Although attention generated by this hypothesis generally focused on such phenylethylamines as mescaline or dimethoxyphenylethylamine, the theory can be examined just as well within the context of the indoleethylamines. Following Axelrod's elucidation of many similar amine-methylating systems in the brain, and a number of studies of induced behavioral aberrations with indoleamino acid loads accompanied by pretreatment with monoamine oxidase inhibitors, it was natural to speculate about the possibility that an indoleethylamine might be N-methylated if its normal pathway of oxidative deamination were blocked.

Having noted these observations while doing sleep research, we began to search for a dependable animal model manifesting this behavioral effect in order to begin to study some of its neurochemical correlates. We chose the newborn White Leghorn chick because of its reduced blood-brain barrier to peripherally administered amines during its first thirty days of life, as well as the stereotypy of its behavioral responses to drugs. Following behavioral work on the chick, we did correlative isotopic 5-hydroxytryptophan turnover studies with and without a monoamine oxidase inhibitor. Then we addressed the problem more directly by searching for the presence of an indoleethylamine N-methyltransferase enzyme in the chick brain. We then extended our studies to search for this enzyme in various mammalian experimental animals including man, and we have recently begun to study the behavioral affects of methylated indoleethylamines in rats. These studies were done in an attempt to get a coherent description of the availability, characteristics, and importance of an indoleethylamine N-methylating system in the brain. The following is a brief summary of our work to date.

METHODS

Behavioral studies on five-day-old White Leghorn chicks were carried out following loads of 5-hydroxytryptophan administered intravenously into the jugular vein with and without pargyline hydrochloride (5 mg/kg) pretreatment, using an activity platform connected through a cumulative counter to a multichannel kymograph (Mandell, Mandell, and Jacobson 1965). Behavioral evaluation of the relative central potencies of bufotenine and O-methylbufotenine administered intraventricularly in freely moving adult male albino rats (weight, 300 grams) was carried out using the technique of Segal and Mandell (1970).

Metabolic turnover studies were done in the whole brain and the blood content of labeled 5-HTP, 5-hydroxytryptamine (5-HT), bufotenine (BUFO), and 5-hydroxyindoleacetic acid (5-HIAA) in thirty White Leghorn cockerels that received 5-hydroxytryptamine-2-^{14}C (10.5 mCi/mmole). Animals receiving 5-HT were killed three minutes

after injection, and those receiving 5-HTP were decapitated thirty minutes after injection. The radioactivity of the metabolites was determined by solvent extraction, ultracentrifugation, thin layer chromatography (TLC), and scintillation counting. The brains were removed rapidly, weighed, and homogenized in acidified ethanol (0.01 percent HCl) maintained at 4° C. Blood was treated in a similar manner. In addition, the lung tissue of four animals was collected. Ten milligrams of ascorbic acid and 10 to 30 µg of appropriate carriers (5-HTP + 5-HT ± BUFO ± 5-HIAA) were added during the extraction procedure. An equal amount of water was added and the sample frozen briefly. The extracts were thawed and centrifuged at 75,000 x G (4° C) for ninety minutes. The supernatant was lyophilized and resolubilized in 0.5 ml of the cold acidified ethanol. A 10 percent aliquot of the concentrated extract was chromatographed bidimensionally on cellulose TLC using the solvent system of methanol-butanol-benzene-water-formic acid (40:30:20:10:1) in the first dimension, and all of these except formic acid in the second dimension. The compounds were visualized under ultraviolet light after ferricyanide oxidation and ethylenediamine condensation, or after spraying with a modified Ehrlich's reagent (10 percent *p*-dimethylaminobenzaldehyde). The areas of cellulose in which 5-HTP, 5-HT, BUFO, and 5-HIAA were located, collected, and placed in vials containing PPO (2,5-diphenyloxazole, 0.5 gm/100 ml toluene) as liquid scintillation medium and thixotropic gel powder (Cab-O-Sil, Packard), thoroughly mixed in a shaker, and counted in a Beckman LS II liquid scintillation spectrometer with external standard quench corrections. Counts per minute (count/min) recorded on data tape were converted to disintegrations per minute (dpm) and the data further computed using the Beckman Omega Data Reduction System. In addition to the known indole metabolites, two additional areas on the chromatograms of blood and liver were occasionally found to contain low amounts of ^{14}C-containing material. These spots are at present unidentified and not included in the chemical analyses.

Enzyme assays were carried out on tissue from brains of chick, rat, sheep, and man. The three human brain specimens were collected as follows: One piece of brain tissue was taken from the prefrontal cortical area of a 54-year-old woman during a neurosurgical decompression procedure following a cerebral hemorrhage. A second piece of tissue was taken from the parietal cortical area of a 5-day-old infant during a shunting procedure for congenital hydrocephalus. Regional human brain studies were done on tissue from a third brain from a 17-year-old boy who died suddenly of a testicular carcinoma. The brain was removed and the various pieces homogenized and frozen within two hours following death. The enzyme assay for indoleethylamine N-methyltransferase in some experiments was done using a method characterized by a high blank due to the poor specificity of the procedure involving isoamyl alcohol extraction of radioactive product (Morgan and Mandell 1969). A less polar and therefore more

specific solvent for extraction was substituted for isoamyl alcohol by using tryptamine as the substrate. This permitted the use of toluene or *n*-heptane as the extraction solvent of radioactive methylated product and resulted in a more sensitive assay with a much reduced background.

Our most recently used assay was done as follows: Brain tissue was homogenized at high speed in a blender for sixty seconds in 0.1 M K_2PO_4 buffer (made up of a mixture of sodium mono and dibasic phosphates, pH 7.9) 1/1.5, w/v. This was centrifuged at 50,000 x G for twenty minutes. The supernatant was used as "homogenate" in some of our assays. Further purification by ammonium sulfate precipitation was carried out with the 40-60 percent ammonium sulfate precipitate as the enzyme source. This was purified further by using Sephadex G-100 and G-200 columns equilibrated with the 0.1 M K_2PO_4 buffer. A typical assay incubation mixture contained 100 µl of 0.1 M K_2PO_4 buffer, 50 µl containing 2 µM of tryptamine (as the hydrochloride), 100 µl of enzyme preparation (containing 0.1 to 0.5 mg protein), and 50 µl of a solution of S-adenosylmethionine (SAM)-^{14}C-methyl that contained 130,000 cpm and 20 mµM of SAM. The final volume of the incubation mixture was 300 µl. Incubation at 37° produced toluene-extractable radioactivity in a linear fashion with time, protein concentration, and substrate level. The reaction was stopped by adding 0.5 ml of sodium borate buffer (0.5 M, pH 10). The product was extracted by the addition of 5 ml of toluene, shaken in Vortex shaker for forty-five seconds and centrifuged at 900 x G for three minutes. The tube was then pertubated slightly to break up the existing emulsion and centrifuged again at 900 x G for three minutes. The samples were then placed in a freezer (-20° C) for at least three hours. Then the toluene phase was poured into counting vials containing toluene-based fluor with 10 percent Biosolv BBS-3, and counted in a Beckman LS-250 scintillation counter with external standard quench corrections. Specific product radioactivity was determined by chromatography and scintillation counting following pooling of toluene extracts and lyophilization with known carriers (indole known standards were obtained from J. Biel of Aldrich Chemical). Chromatography of products was done by cellulose thin layer chromatography and various solvent systems.

RESULTS

Our laboratory, among others, has reported that in the young chick in which 5-hydroxytryptamine enters the central nervous system, systemically administered 5-HT produces behavioral and physiological signs of sedation that are similar to natural sleep, differing only in the degree of muscular relaxation (Mandell and Spooner 1969). In our hands, this is a monotonic, dose-dependent phenomenon. Figure 1 shows the rather typical sleeping posture induced in a baby chick by

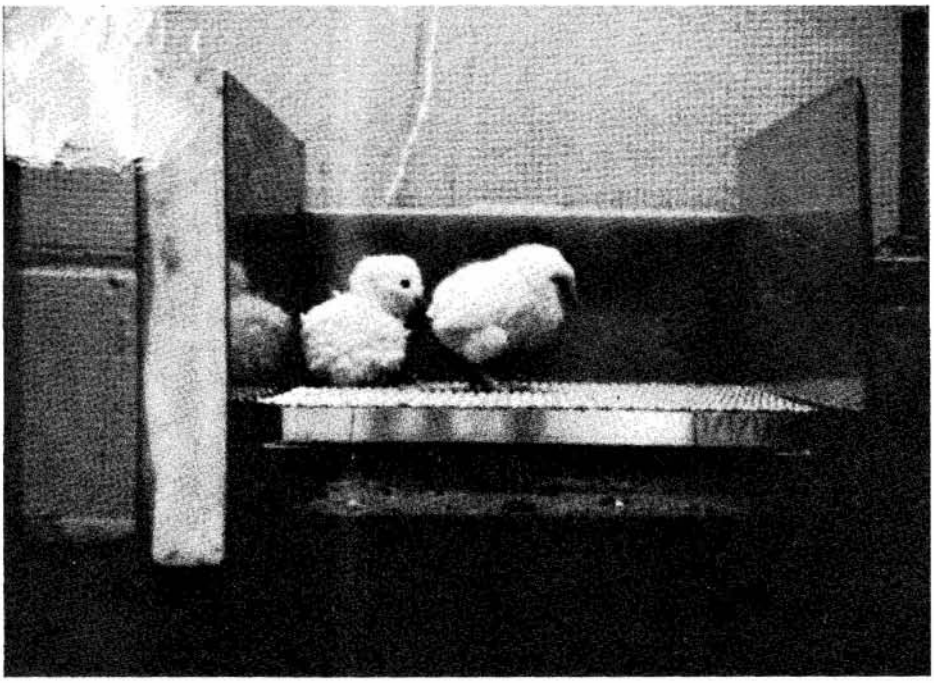

Figure 1. Typical response of chick to intravenous administration of 5-HT. Note relatively normal sleep posture and wing attitude. This is a monotonic, dose-dependent phenomenon.

intravenous 5-HT administration. The effect of this dose of 5-HT (0.1 mmole/kg) could become a peculiar kind of hyperactive state and bizarre posture (Figure 2) by pretreatment with a monoamine oxidase inhibitor, pargyline hydrochloride (5 mg/kg). In addition to hyperactivity, propulsive gait, and aggressive pecking, there was an interesting wing posture which has been seen as a rather unique response by chicks to the administration of either hallucinogens or high doses of amphetamines. Dimethyltryptamine and bufotenine, particularly, produced this sort of wing-spread posture. Since the 5-HT alone could not produce this phenomenon at any dose level, it is tempting to speculate that the bizarre posture and behavior might involve a mechanism other than 5-HT conservation alone induced by the monoamine oxidase inhibitor. In his well-known 1961 report in *Science*, Axelrod suggested the presence of an enzyme in rabbit lung (a nonspecific N-methyltransferase) that could function in the speculated shunt diagrammed in Figure 3. The question was, of course,

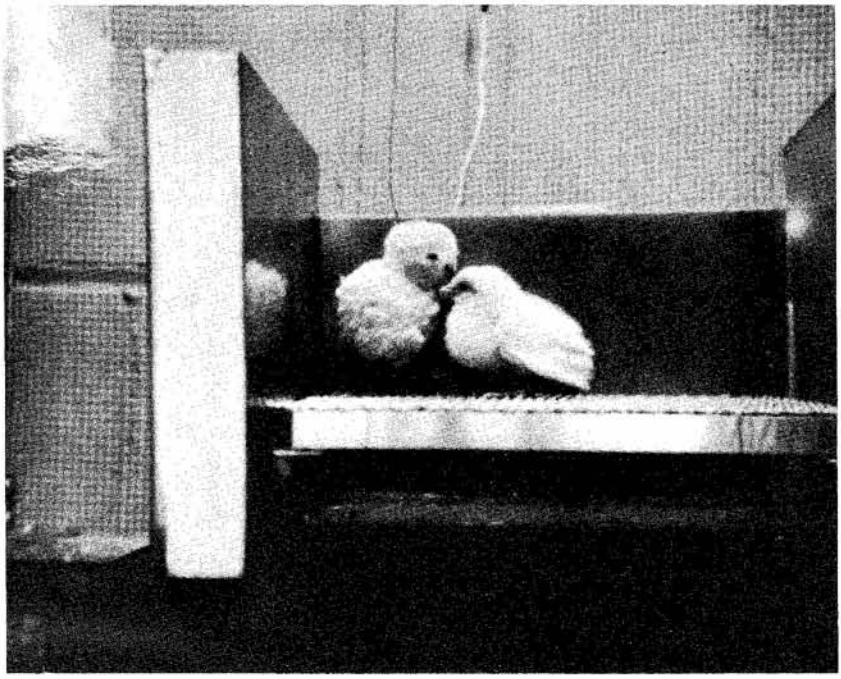

Figure 2. Typical response of chick when the intravenous administration of 5-HT was preceded by dose of a monoamine oxidase inhibitor (pargyline hydrochloride, 5 mg/kg). Note particularly the splayed attitude of wings. This response was quite characteristic of the actions of methylated indolealkylamines such as dimethyltryptamine and bufotenine.

whether a demonstrable increase or presence of an N-methylated indoleethylamine could be noted when behavioral activation was induced by the administration of an indoleamino acid following a monoamine oxidase inhibitor. As described before, ^{14}C-5-hydroxytryptophan was administered intravenously to chicks with and without pargyline hydrochloride (5 mg/kg). Figure 4 demonstrates metabolic reflections of monoamine oxidase inhibition (an increase in radioactive serotonin and a decrease of 5-hydroxyindoleacetic acid) associated with a doubling of radioactive bufotenine. The actual amount of radioactivity was relatively small, with a relatively high degree of variance. These results seemed far from striking. Figure 5 represents the results of the same kind of chemical determination done in the lung of the chick. Here the increase in bufotenine radioactivity seemed to

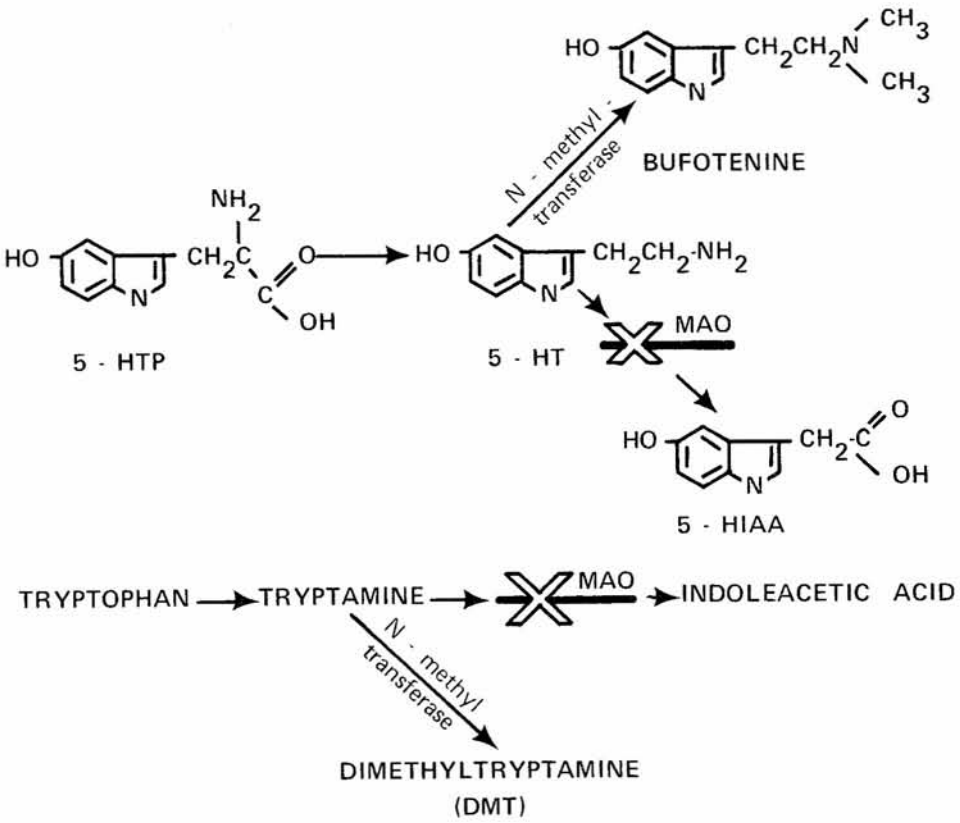

Figure 3. Diagrammatic representation of mechanisms speculated to underlie the reversal of 5-HT-induced sedation with monoamine oxidase inhibitor pretreatment. It is hypothesized that inhibition of the normal oxidative degradation of the indolealkylamines leads to more substrate being shunted through previously insignificant transmethylation pathways.

be considerably larger. It was not possible, at this stage of the chick's maturity, to determine whether the brain's radioactive bufotenine came from the lungs or the brain.

We took another approach to this problem. We attempted to find an enzyme capable of N-methylating indoleethylamines in the brain itself. Although Axelrod had not reported such a brain enzyme, he nonetheless had worked out enough parameters in similar transmethyl-

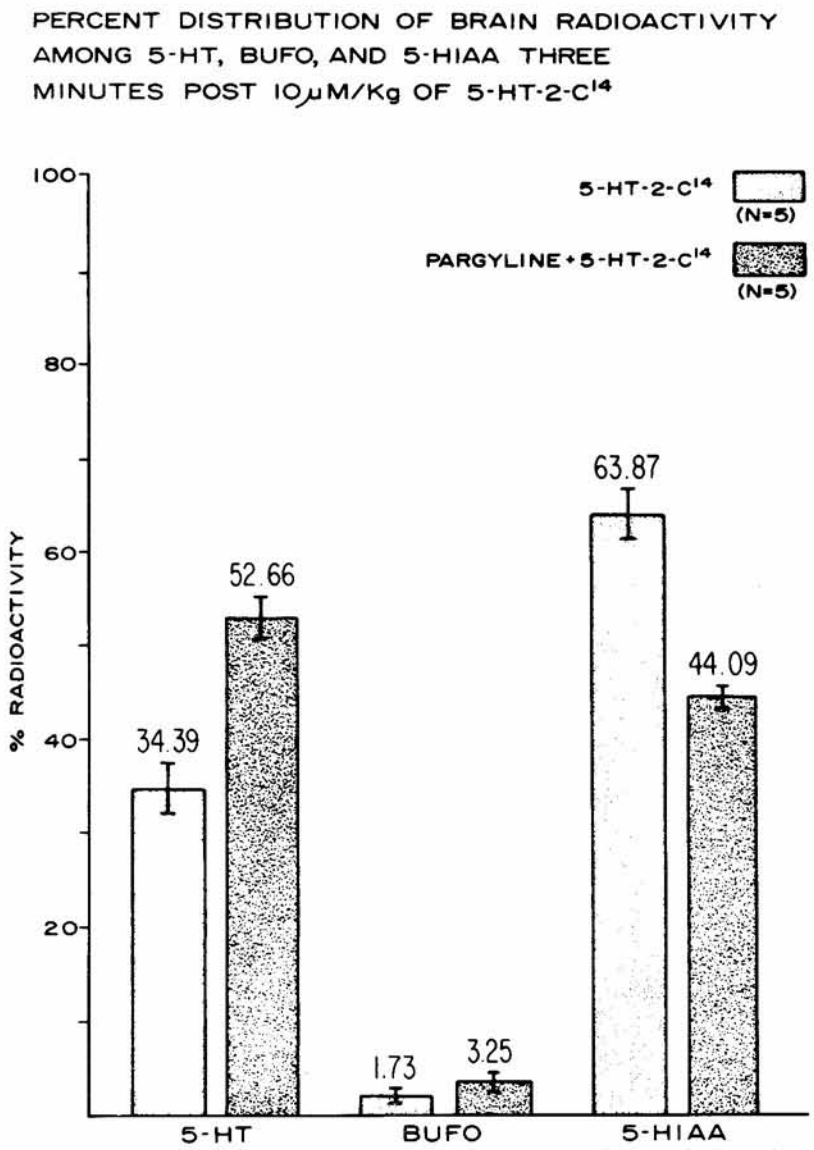

Figure 4. Metabolic distribution of radioactivity in brain following infusion of 10 μM/kg of 5-HT-2-^{14}C with and without monoamine oxidase inhibitor pretreatment. Note expected increase in radioactive 5-HT, decrease in radioactive 5-HIAA, and accompanying increase in labeled bufotenine. This latter change failed to reach significance.

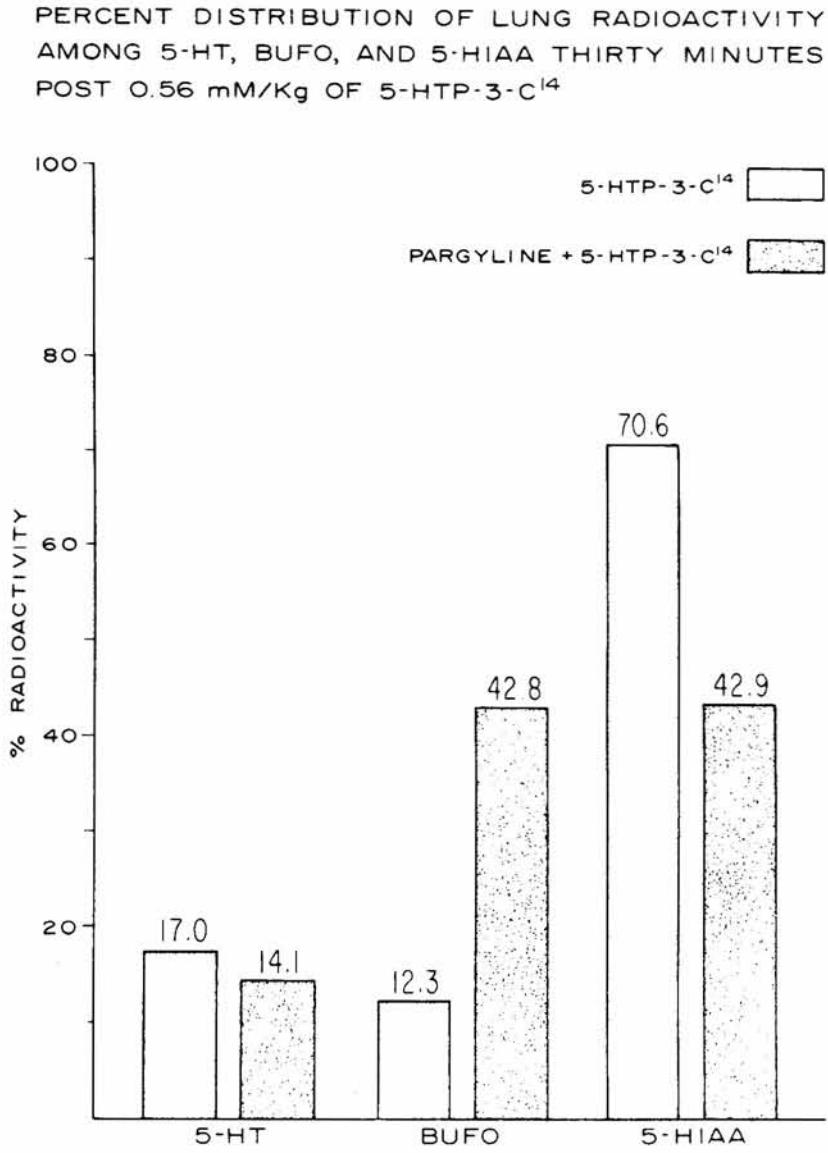

Figure 5. Experimental results, similar to Figure 4, in chick lung. Note larger increase in radioactive bufotenine.

ase assays (histamine, catecholamines, and nucleic acids) that we felt that we could begin to look for such an enzyme in chick brain. A wide variety of assay techniques were tried, and our newest version is described in some detail in the "Methods" section. Our initial studies showed that chick brain "homogenate" could catalyze the production of isoamyl alcohol-extractable radioactivity from S-adenosylmethionine-^{14}C-methyl, which was a function of the indoleamine substrate level, was linear with time and protein concentration, and that this activity was capable of being enriched about ten-fold using ammonium sulfate precipitation and a Sephadex G-200 column. Table 1 demonstrates this progressive enrichment using isoamyl alcohol-extractable radioactivity as the dependent variable.

Using a discontinuous sucrose gradient of 0.32 M, 0.8 M, and 1.2 M sucrose, centrifugation of wide-clearance brain homogenate at 100,000 x G for ninety minutes evidenced two peaks of indoleethylamine N-methyltransferase activity in the supernatant and the supramitochondrial particulate fraction (Figure 6). Table 2 is a summary of our studies of the substrate specificity for N-transmethylation of chick brain homogenate. Note that four indoleethylamines seem capable of being methyl acceptors in this system. Table 3 summarizes the regional specific activity in the rat brain, using isoamyl alcohol-extractable radioactivity. Recent studies of sheep brain in our laboratory have indicated that the caudate, which has relatively low activity in this study on rats,seems to show remarkably high enzyme activity in the sheep.

Table 4 represents perhaps one of the more interesting aspects of our studies of brain indoleethylamine N-methyltransferase activity. It seems that this kind of enzyme activity is demonstrable in human brain. It is interesting that the amygdala, medulla, and uncus have the highest specific activity of the parts measured. I apologize for the irregularity of this brain dissection; it occurred because of the limits of time and arrangements in the university morgue. Since only a small amount of human brain material was available, an attempt was made to get some idea whether this human brain enzyme activity was specific for indoleethylamines, or whether it resembled the nonspecific aromatic amine N-methylating system described by Axelrod in 1961. Table 5 shows that 100,000 x G supernatant fraction of the homogenate had relatively high activity for indoleethylamines and low activity for the phenylethylamines and histamine. Fractionation by ammonium sulfate precipitation (35 to 45 percent) led to a decrease in histamine N-methyltransferase activity, relatively no change in phenylethylamine N-methyltransferase activity, and relatively higher values for the indoleethylamine substrates. These findings suggest substrate specificity for indoleethylamines. Holding the ionic strength constant, the pH optimum for the N-methylation of serotonin was determined using the 100,000 x G supernatant fraction of human brain homogenate (Figure 7). This

TABLE 1

Activity of Indoleethylamine N-Methyltransferase from Whole Chick Brain

ENZYME SOURCE	SPECIFIC ACTIVITY*
Supernatant fraction of whole brain homogenate	0.26
45% - 55% saturated $(NH_4)_2SO_4$ precipitate	0.87
Sephadex gel filtration fraction	2.54

*Activity is expressed as mμM N-methylserotonin formed per milligram of protein per 90 minutes.

TABLE 2

Substrate Specificity of Chick Brain Indoleethylamine N-Methyltransferase

SUBSTRATE	RELATIVE ACTIVITY %
5-Hydroxytryptamine	100
5-Methoxytryptamine	88
Tryptamine	60
N-Methyltryptamine	47
Normetanephrine	6
5-Hydroxy-N,N-dimethyltryptamine	--

Enzyme source was Sephadex G-200 column eluate, the fraction containing 0.1 mg of protein.

TABLE 3

Regional Specific Activity of Rat Brain
Indoleethylamine N-Methyltransferase*

REGION	SPECIFIC ACTIVITY**
Medulla	364 ± 48.7
Cerebellum	354 ± 33.1
Pons	299 ± 49.6
Mesencephalon	258 ± 26.0
Olfactory Bulb	158 ± 43.3
Hypothalamus	154 ± 12.3
Amygdala	152 ± 12.8
Occipital Pole	143 ± 19.5
Caudate	121 ± 21.3
Frontal Pole	105 ± 22.0

*N = 6. 5-HT as substrate and isoamyl alcohol as product extraction solvent.

**Mean ± S.E.M. as mμM of 5-HT methylated/gm/hr.

TABLE 4

Regional Specific Activity of Human Brain Indoleethylamine N-Methyltransferase*

BRAIN	REGION	SPECIFIC ACTIVITY**	SOURCE
Infant male	parietal cortex	95	biopsy
Adult female	frontal cortex	103	biopsy
Adolescent male	frontal cortex	<30	two hours postmortem
	orbital surface of frontal lobe	69	
	ventral midbrain (long tracts included)	74	
	amygdala	136	
	medulla	189	
	uncus	218	

*5-HT was substrate and isoamyl alcohol the product extraction solvent. Each value is mean of three determinations.

**mμM of 5-HT methylated/gm/hr.

TABLE 5

Substrate Affinities of Methylating Enzymes in Human Brain Homogenate*

SUBSTRATE	FRACTION	
	100,000 x G SUPER.	NH_4 SO_4; 35-45%
5-HT	100	100
Tryptamine	95	111
N-methyl-5-HT	94	79
N-methyltryptamine	62	55
Norepinephrine	0	7
Normetanephrine	41	40
Histamine	1110	134

*Brain homogenate relative to 35 to 45 percent ammonium sulfate cut for various biogenic amine methyl acceptors (see text). 5-HT as reference substrate (i.e., equal to 100 percent).

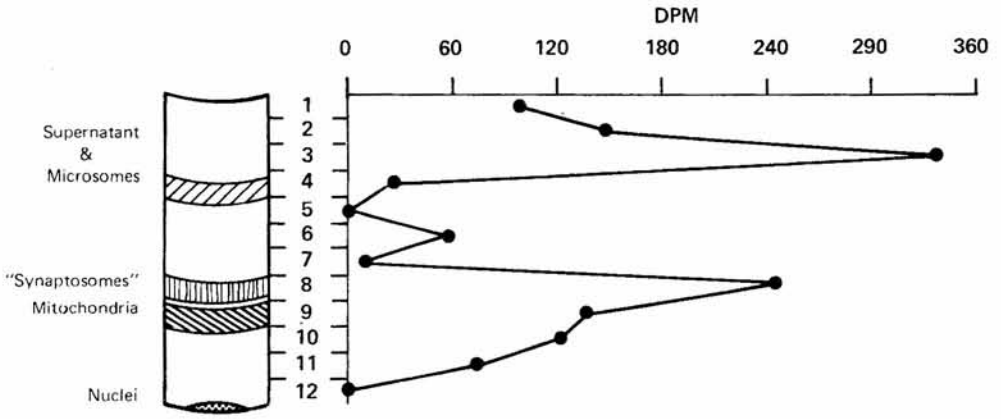

Figure 6. Subcellular localization in chick brain of indoleethylamine N-methyltransferase. Incubation mixture contained 50 µl of serial 3-ml fractions from the sucrose gradient. 5-HT was the substrate. The three layers of sucrose were 0.32 M, 0.8 M, and 1.2 M sequentially. The gradient was centrifuged at 100,000 x G for 90 minutes.

was the same as was demonstrated by Axelrod for lung enzyme.

Because of the shortage of human brain material, we have recently switched to using sheep brain. Table 6 summarizes studies of the relative substrate specificity of the brain enzyme from sheep brain during enrichment procedures. We are currently using tryptamine as the indoleamine substrate and toluene extraction of product. This increases the sensitivity and decreases the blanks. During these purification procedures, it seems that the indoleethylamine N-methyltransferase activity increases while the histamine decreases, and the phenylethylamine fails to make a significant appearance in these experiments. It seems that indoleethylamine N-methyltransferase is discriminable from other already known brain N-methyltransferases. This activity was linear over time, for protein and substrate concentration within the constraints of the reaction conditions described in the previous section. Boiled enzyme demonstrated no activity. The Km for SAM was 2×10^{-5}. The Km for 5-HT was 9×10^{-4}. The Ki for chlorpromazine was 10^{-3}, and for lithium 2×10^{-4}.

Chromatograms of the toluene-extractable radioactivity, lyophilized and spotted on cellulose thin layer chromatographic plates, were run in three solvent systems: isopropanol-H_2O-NH_4OH (50:50:3);

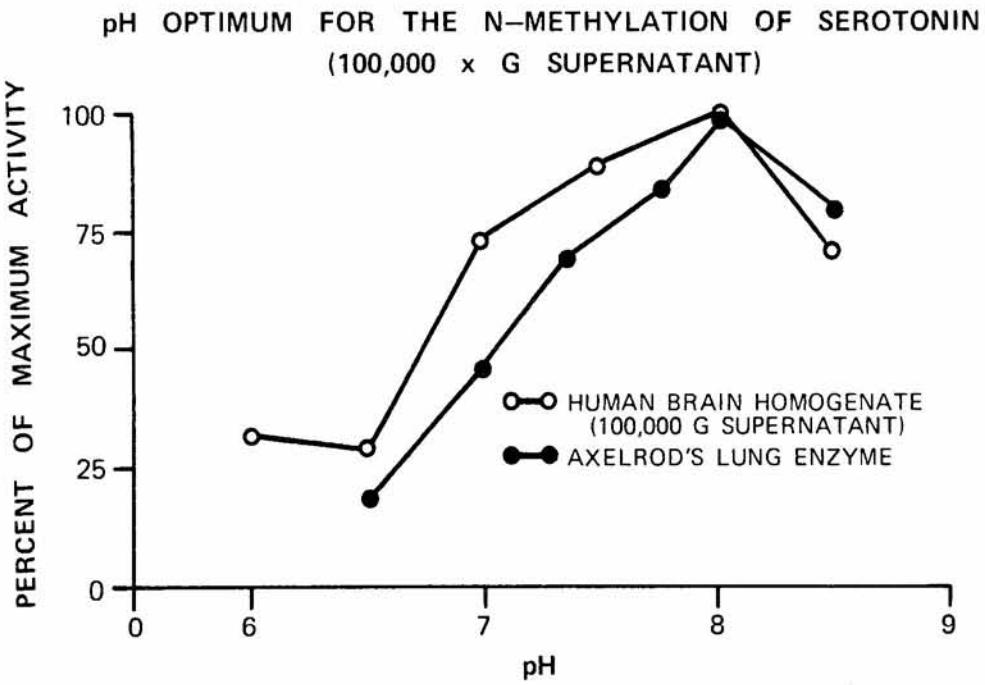

Figure 7. The pH optimum for human brain indoleethylamine-N-methyltransferase activity. Note similarity to Axelrod's lung enzyme (Axelrod 1961).

TABLE 6

Substrate Specificity and Purification of Mammalian Brain Indoleethylamine N-Methyltransferase (Sheep)*

SUBSTRATES	SPECIFIC ACTIVITIES OF FRACTIONS**		
	100,000 x G Super.	35-45% NH_4SO_4	G-200
Tryptamine	0.22	0.86	2.13
Histamine	7.13	1.23	0.17
Normetanephrine	0.04	-	-
Norepinephrine	-	-	-

*Enzyme was purified with ammonium sulfate precipitation and Sephadex G-200 column fractionation. Values below 0.02 mμM/mg/hour were regarded as zero.

**as mμM of substrate methylated/mg protein/hour.

isopropanol-H_2O-NH_4OH (100:10:2.5) and 8 percent NaCl in H_2O. Tryptamine, monomethyltryptamine, and dimethyltryptamine were seen as contiguous in one dimension, with a radioactive peak occurring in the area of monomethyltryptamine (Figures 8, 9, and 10). The first chromatogram is elegantly clean (Figure 8). The second and third systems (Figures 9 and 10) are not as clear. What seem to be multiple products have confused us somewhat, especially in light of the linearity of product-radioactivity with incubation time of the assay. I believe it is safe to say that, in terms of the chromatography of products, we have more work to do.

Since it seems that less than one-tenth of one percent of an isotopic dose of bufotenine enters the brain of an animal possessing a fully matured blood-brain barrier (Himwich 1969), it is not surprising that reports of the central actions of this agent have been negative (Turner and Merlis 1959). The structure of bufotenine is so similar to O-methylbufotenine (a known potent hallucinogen at 600 µg/dose; Gessner *et al.* 1960), that we guessed that the blood-brain barrier-crossing ability of the two compounds accounted for the difference in their potency. We believed that if we could eliminate the blood-brain barrier as a factor, we might find bufotenine to be very close in potency to its more lipid-soluble methylated congener. Using intraventricular infusions of both compounds in freely moving rats (Segal and Mandell 1970), and monitoring the drug-induced motor activity, we were able to demonstrate that bufotenine is somewhat more centrally active than O-methylbufotenine in producing hyperactivity (Figure 11). In addition, bufotenine produced bizarre boxing motions in the rat rather consistently at these low doses. Thus it seems that bufotenine, if produced in the brain, may be a potent biological agent.

CONCLUSION

We have presented some highly suggestive evidence that there may be an indoleethylamine N-methyltransferase enzyme in mammalian brain that can turn serotonin into N-methylated derivatives that may be biologically active. The enzyme is relatively low in specific activity and has a relatively high Km. One can argue that, with a Km in the 10^{-3} range, serotonin would never be in concentration high enough for the enzyme to function, or that serotonin in the brain might never reach a level at which the function of this enzyme might be physiologically significant. This may be true under normal circumstances. During a state such as chronic amphetamine intoxication, however, or during treatment with a monoamine oxidase inhibitor, it is possible that such an indoleethylamine-methylating shunt could become kinetically feasible. It is tempting to speculate that the amphetamine psychoses might result from such a mechanism following the amphetamine-induced inhibition of monoamine oxidase. Another

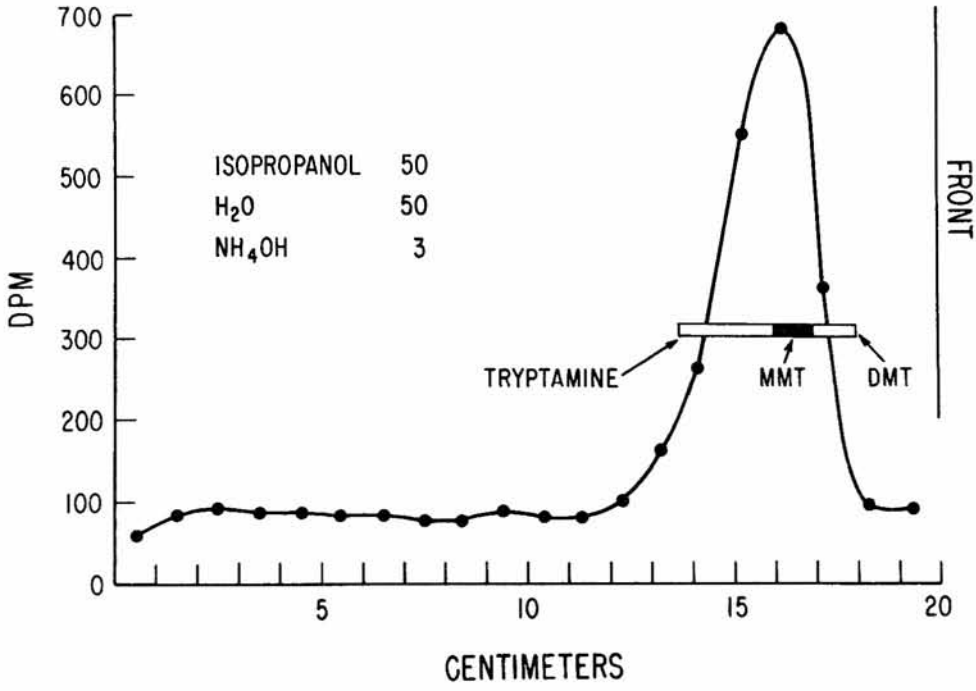

Figure 8. Cellulose thin layer chromatography in one dimension of toluene-extractable radioactivity following assay of sheep brain indoleethylamine N-methyltransferase, using tryptamine as substrate. Enzyme source was Sephadex G-200 column eluate which was 20X enriched from whole brain homogenate.

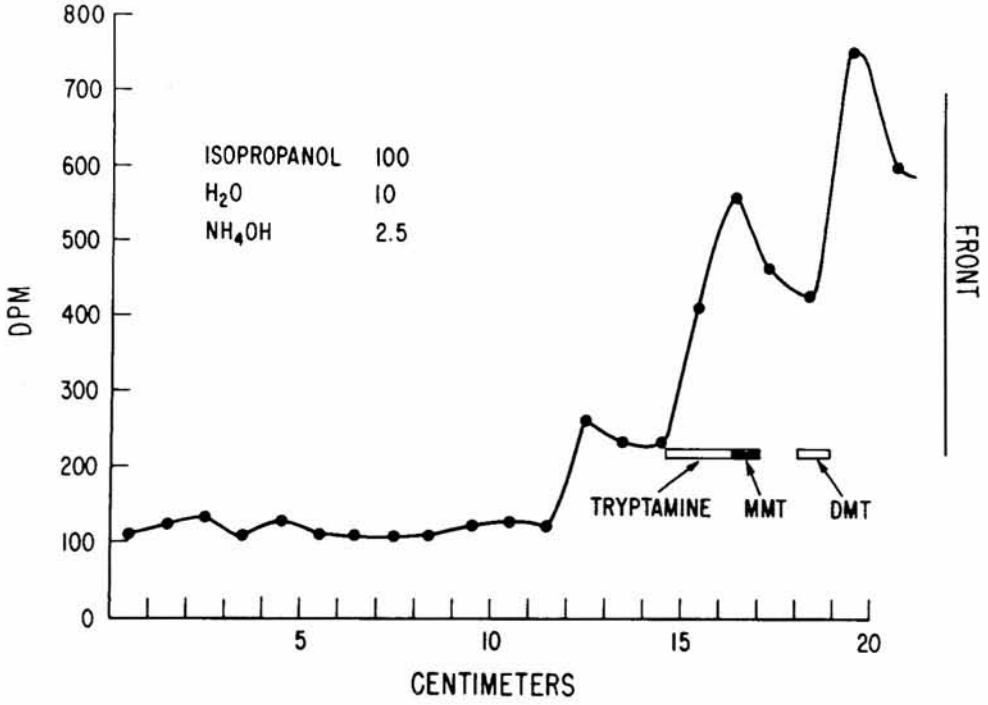

Figure 9. Cellulose thin layer chromatography of toluene-extractable radioactive product(s) as in Figure 8, with another set of solvent ratios.

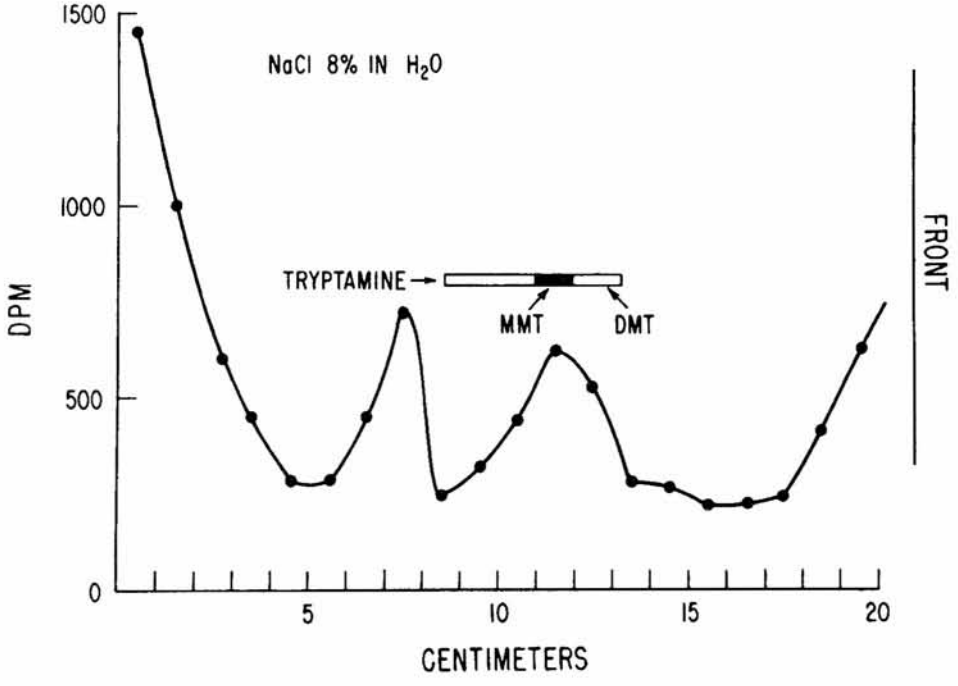

Figure 10. Cellulose thin layer chromatography of toluene-extractable radioactive product(s) as in Figure 8, with another solvent system.

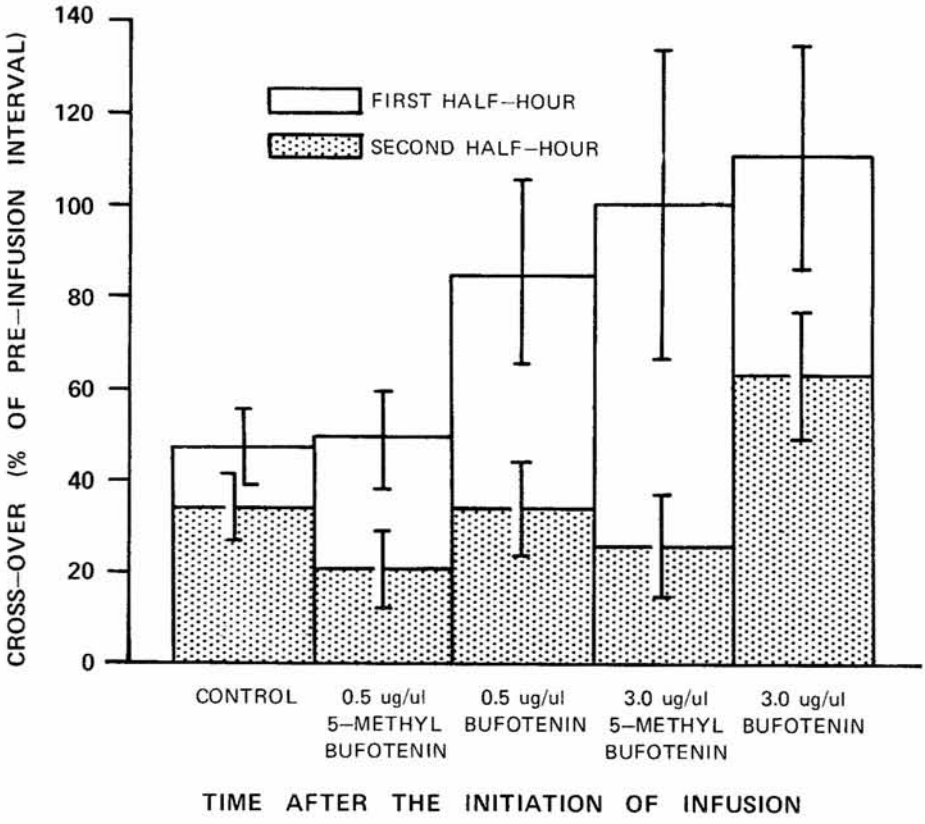

Figure 11. Effects on gross motor activity of intraventricular infusion of bufotenine and 5-methylbufotenine in freely moving rats. N = 6. Infusion rate was 1 µl/3 minutes. Note comparability of the potency of these two compounds when the blood-brain barrier is removed as a factor.

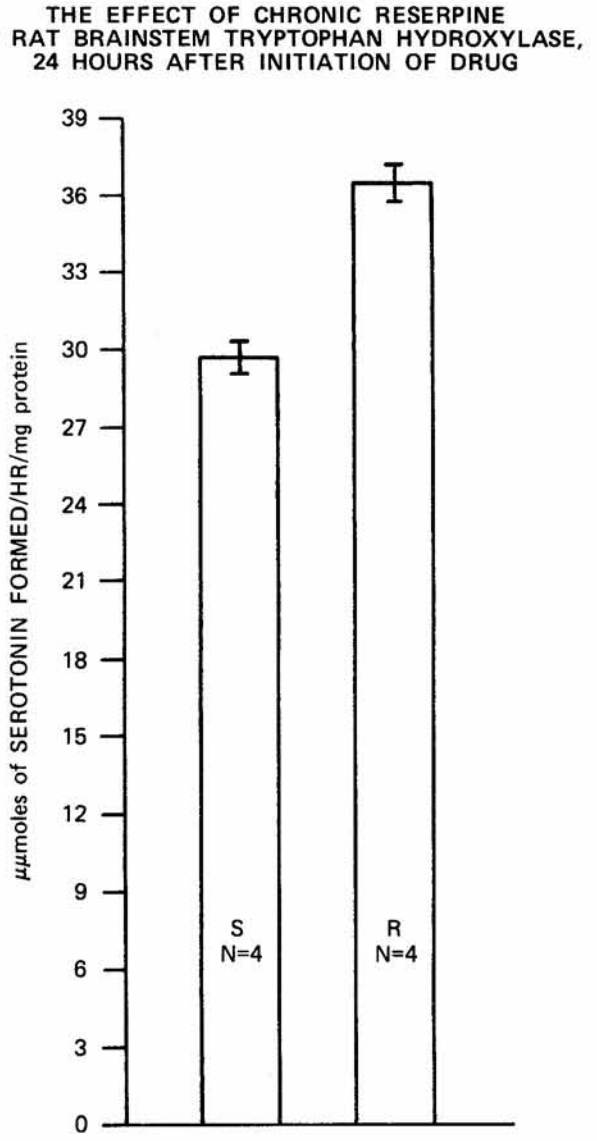

Figure 12. Effect of 1.5 mg/kg of reserpine given subcutaneously in two injections, twelve hours apart, on pons-midbrain tryptophan hydroxylase activity. See text.

possibility for the development of circumstances that could lead to unusual increases in serotonin substrate levels is currently being explored in our laboratories--that of a drug- or behavior-induced increase in tryptophan hydroxylase activity. Figure 12 shows preliminary data from this work. In this adaptation of the tryptophan hydroxylase assay of Ichiyama *et al.* (1970), note that reserpine administration can increase the specific activity of this rate-limiting enzyme in the biosynthesis of serotonin. Perhaps other drugs and conditions can do this as well. A third possibility for an indoleethylamine substrate reaching meaningful concentrations for transmethylation would be its shift in subcellular location to a pool that would be protected from monoamine oxidase and be available for transmethylation. This possibility is difficult to explore experimentally. Since the mono- and dimethylated tryptamines are poor substrates for brain monoamine oxidase (Blaschko and Levine 1966), it seems that once the indoleethylamines are N-methylated they can resist degradation long enough to be physiologically significant. We have recently demonstrated that methylated indoleamines competitively inhibit the oxidative deamination of serotonin by monoamine oxidase *in vitro*. Perhaps once this methylation process is allowed to begin, it could propagate itself.

ACKNOWLEDGMENT

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