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SHORT COMMUNICATIONS

Indoleamine-*N*-methyl transferase in human lung

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AN ENZYME which transfers the methyl group from S-adenosylmethionine to the amino group of a variety of aromatic amines was discovered by Axelrod[1] and found to be present in relatively high content in rabbit lung. Studies from our laboratory have demonstrated that *N*-methyltryptamine is the most active indoleamine substrate for this enzyme; *N,N*-dimethyltryptamine was identified as the reaction product.[2] Recently, a similar methyltransferase was isolated from chick, sheep and human brain.[3,4] It is apparently a more specific methylase in that it does not *N*-methylate phenylethylamine substrates, and it has a relatively high affinity for the substrate serotonin.

We report here the presence of indoleamine-*N*-methyl transferase activity in human lung. Its properties are qualitatively similar to those of the rabbit lung enzyme, although there are some quantitative differences between the enzymes from the two sources. *N*-methyltryptamine is the substrate with the lowest K_m for both enzymes; its reaction product has been identified as *N,N*-dimethyltryptamine. These results suggest that a physiological role for this enzyme in humans may be the formation of dimethyltryptamine.

MATERIALS AND METHODS

Tissue samples were obtained within 12 hr post mortem and stored at −20°. Indoleamine-*N*-methyl transferase was prepared from lung and brain by homogenization of the tissue in 3 vol. of 0·15 M KCl containing 10^{-4} M dithiothreitol and 10^{-4} M EDTA. The homogenate was centrifuged at 50,000 *g* for 30 min, and the supernatant dialyzed against 50–150 vol. of 10^{-4} M dithiothreitol-10^{-4} M EDTA for 20 hr at 5°. The dialyzed preparation was centrifuged at 25,000 *g* for 15 min at 0° to remove insoluble material formed upon dialysis. In the initial experiments (see Table 1) the supernatant was concentrated 10- to 20-fold in an Amicon ultrafiltration cell with a UM-10 Diaflo membrane (2 atm at 4°), and the concentrate used as the enzyme preparation. For further purification, the supernatant was fractionated directly with ammonium sulfate, and the 40–60 per cent fraction was dialyzed and passed through a Sephadex G-150 column as described previously.[2] The active fractions were pooled and concentrated 10-fold by ultrafiltration.

Indoleamine-*N*-methyl transferase activity was measured by minor modifications of the procedure of Axelrod.[1] Reaction mixtures contained 30 μmoles potassium phosphate buffer, pH 7·9, 4 mμmoles S-adenosylmethionine-methyl-^{14}C, 0·02–0·8 μmoles substrate and 0·01–0·05 ml of enzyme in a total volume of 0·1 ml. Mixtures were incubated 60–120 min at 37° and the radioactivity in the methylated products (extracted into water-saturated isoamyl alcohol) measured as outlined previously.[2] Protein was measured by the method of Oyama and Eagle.[5]

S-adenosylmethionine-methyl-^{14}C was purchased from the New England Nuclear Corp.; serotonin creatinine sulfate, *N*-methylserotonin oxalate, tryptamine, *N*-methyltryptamine, *N,N*-dimethyltryptamine and *N.N*-dimethylserotonin were purchased from the Aldrich Chemical Company. All chemicals were reagent grade commercial products.

RESULTS AND DISCUSSION

Data on the occurrence of indoleamine-*N*-methyl transferase in human lung are presented in Table 1. Enzyme activity was detected in five of seven lung samples; its specific activity ranged from 0·09 to 0·26 nmole of dimethyltryptamine formed per milligram of protein per hour. No relationship between the case data and the specific activity of the enzyme is apparent from these few cases. Enzyme activity was not observed in extracts of three brain specimens prepared and assayed by an identical procedure. This is in contrast to the data in a recent report on the properties of indole(ethyl)amine-*N*-methyltransferase from human brain,[4] and studies with additional specimens are required. No enzyme activity was detected in an extract of whole human blood in agreement with the results of Axelrod and Cohn.[6]

TABLE 1. OCCURRENCE OF INDOLEAMINE-*N*-METHYL TRANSFERASE IN HUMAN LUNG

Sample No.	Case data: Sex	Age	Post-mortem interval (hr)	Cause of death	Enzyme specific activity*
1	M	24	4	Acute intravenous narcotism	0·26
2	F	35	3	Glioblastoma of frontal lobe	Not detected†
3	M	15	12	Multiple injuries	0·21
4	M	45	4	Acute barbiturate intoxication	0·11
5	F	23	5	Acute barbiturate intoxication	0·11
6	M	62	8	Occlusive coronary arteriosclerosis	0·09
7	M	31	7	Multiple injuries	Not detected†

* Reaction mixtures (0·1 ml) contained 30 μmoles phosphate buffer, pH 7·9, 0·2 μmole *N*-methyltryptamine HCl, 4 nmoles S-adenosylmethionine-methyl-^{14}C and enzyme. Control samples in which the *N*-methyltryptamine was omitted were run simultaneously. Enzyme preparations were concentrates of the dialyzed supernatants of centrifuged tissue extracts. Incubation was for 2 hr at 37°. The specific activity is defined as millimicromoles of [^{14}C]dimethyltryptamine formed per milligram of protein per hour. Corrections were made for radioactivity extracted in the absence of substrate.

† Indicates no further incorporation of radioactivity into product above that obtained when substrate was omitted from reaction mixtures.

A partial purification of indoleamine-*N*-methyl transferase is summarized in Table 2. Detectable enzyme activity could not be found in the 50,000 *g* supernatant fraction of lung homogenates using either *N*-methyltryptamine or serotonin as substrate. Following dialysis of these supernatants and centrifugation at 27,000 *g*, then concentration of the supernatants by ultrafiltration, there was a 2- to 3-fold increase in the incorporation of radioactivity from S-adenosylmethionine-methyl-^{14}C into extractable product above that obtained when substrate was omitted from the reaction mixtures. Failure to detect enzyme activity in the 50,000 *g* supernatants which had not been dialyzed may be due to the presence in crude lung extracts of a naturally occurring inhibitor of the methylase, of high concentrations of a competing substrate, or of a contaminant which interferes with the read-out of enzyme activity. Further purification (ca. 25-fold) of the enzyme was achieved by ammonium sulfate fractionation and chromatography on Sephadex G-150. The methodology for these two steps was the same as that described for the purification of the rabbit lung enzyme.[2] The ammonium sulfate fractionation and molecular sieve chromatographic properties of the human lung enzyme are similar to those of the rabbit lung enzyme. At the same stage of purification, the specific activity of the enzyme from the rabbit tissue was approximately seven to ten times greater than that from the human tissue.

The reaction product of the methylation *in vitro* of *N*-methyltryptamine by the human lung enzyme was characterized as *N*,*N*-dimethyltryptamine by thin-layer chromatography of the isoamyl alcohol extracts on Silica gel G using a propanol-1N ammonium hydroxide (5 : 1) solvent system (R_f for

TABLE 2. PURIFICATION OF INDOLEAMINE-*N*-METHYL TRANSFERASE

Step	Specific activity*: Lung No. 5	Lung No. 6
Soluble supernatant fraction	Not detected	Not detected
Dialyzed-centrifuged supernatant	0·07	0·05
Ammonium sulfate fraction 40–60%	0·35	0·26
Sephadex G-150 concentrate	1·90	1·23

* The starting material was 100 g of lung No. 5 and 65 g of lung No. 6. For the details of the assay *in vitro* and the definition of enzyme specific activity see text and Table 1.

dimethyltryptamine = 0·62), then by reverse isotope dilution analysis. ^{14}C-labeled dimethyltryptamine recovered from the Silica gel plates by elution with methanol was co-crystallized from an ethereal solution to constant specific radioactivity with a known amount of authentic carrier compound. The dimethyltryptamine content in the isolates ranged from 80 to 90 per cent. *N,N*-dimethyltryptamine was further identified by combined gas–liquid chromatography–mass spectrometry of the trimethylsilylated (TMSi) derivative. Its retention time (1·5 min) and mass spectrum were identical to those of the TMSi derivative of the authentic reference compound analyzed under the same column and spectrometer conditions. Mass signals of 202 and 260 corresponding to the *m/e* values for the indole ring fragment and the molecular ion of the TMSi derivative were recorded. The details of the analytical techniques used to characterize *N,N*-dimethyltryptamine will be reported elsewhere.*

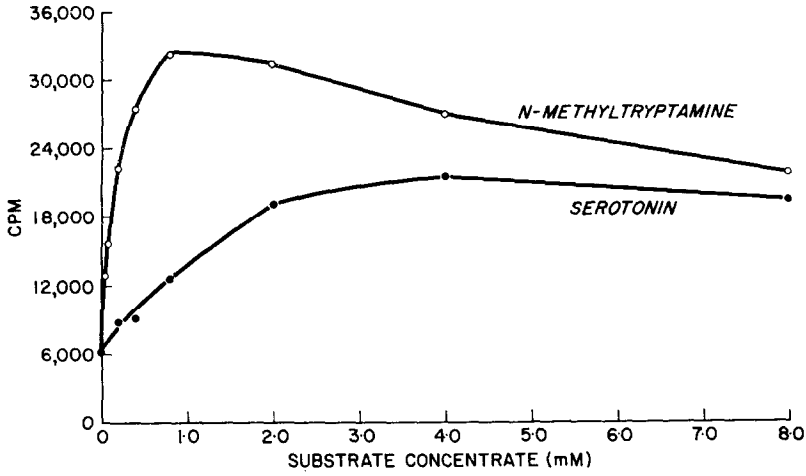

FIG. 1. Methylation of *N*-methyltryptamine and serotonin by indoleamine-*N*-methyl transferase.

The data in Fig. 1 demonstrate that *N*-methyltryptamine is superior to serotonin as a substrate for the partially purified enzyme. *N*-methyltryptamine exhibits an apparent substrate inhibition when present in relatively high concentration (8×10^{-3} M) in the assay *in vitro* and has the lowest K_m in the series of four related substrates tested (Table 3). The K_m values were determined as described by Dixon and Webb.[7] The order of substrate affinity qualitatively parallels that obtained in the earlier studies with partially purified rabbit lung indoleamine-*N*-methyltransferase.[2] Quantitatively, the K_m values are about two to four times higher with the human lung enzyme. *N,N*-dimethyltryptamine and *N,N*-dimethylserotonin (bufotenin) reduce the incorporation of radioactivity into the isoamyl alcohol phase by 68–90 and 50–63 per cent respectively, when tested at 2×10^{-3} M with the four indoleamine substrates at the same concentration (Table 3). Apparent product inhibition had also been demonstrated with the rabbit lung enzyme.[2]

TABLE 3. SUBSTRATE SPECIFICITY AND INHIBITION BY DIMETHYLTRYPTAMINE AND DIMETHYLSEROTONIN OF INDOLEAMINE-*N*-METHYL TRANSFERASE

Substrate (2×10^{-3} M)	K_m (M)	Per cent inhibition produced by: Dimethyltryptamine (2×10^{-3} M)	Per cent inhibition produced by: Dimethylserotonin (2×10^{-3} M)
N-methyltryptamine	$2{\cdot}8 \times 10^{-4}$	69	59
N-methylserotonin	$5{\cdot}7 \times 10^{-4}$	90	58
Tryptamine	$1{\cdot}2 \times 10^{-3}$	68	63
Serotonin	$2{\cdot}3 \times 10^{-3}$	70	50

* R. W. WALKER, H. S. AHN, L. R. MANDEL and W. J. A. VANDEN HEUVEL, *Analyt. Biochem.* (in press).

N,N-dimethyltryptamine has been found to be a potent psychotomimetic agent when administered parenterally or by inhalation to man.[8,9] This compound has recently been reported to occur in blood and urine of acute schizophrenics.[10,11] The present report provides evidence for the existence of an enzyme in human lung tissue which catalyzes the formation *in vitro* of this hallucinogen from *N*-methyltryptamine and S-adenosylmethionine. A similar enzyme activity had previously been detected in small amounts in one out of four human lungs.[1] The role of indoleamine-*N*-methyl transferase and of its reaction products *N,N*-dimethyltryptamine and bufotenin in the etiology of mental aberrations remains to be established. Studies on the relationship of *N,N*-dimethylated indoleamines to psychoses are awaited with interest.

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Inhibition of catechol-*O*-methyltransferase by S-adenosylhomocysteine and S-adenosylhomocysteine sulfoxide, a potential transition-state analog*

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WE HAVE been investigating several aspects of nonenzymic[1] and enzymic transmethylation reactions. One of our main goals in these studies has been the elucidation of a molecular mechanism for the transmethylation reaction in order to design more effective regulators of this important biological process. The recent report of Deguchi and Barchas[2] concerning the inhibition of phenethanolamine *N*-methyltransferase by S-adenosylhomocysteine (SAH) prompts us to report similar findings with catechol-*O*-methyltransferase (COMT), EC 2.1.1.6. Based on our conclusions regarding the nature of the transition state in nonenzymic methyl transfer reactions,[1] we have also investigated the inhibition of COMT by S-adenosylhomocysteine sulfoxide (SAHO), a possible transition-state analog for the enzyme-catalyzed process.

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