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Heterogeneity of N- and O-Methyltransferases

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SUMMARY

The methyltransferases phenylethanolamine *N*-methyltransferase, histamine *N*-methyltransferase (EC 2.1.1.8), catechol *O*-methyltransferase (EC 2.1.1.6), and hydroxyindole *O*-methyltransferase, from tissues of several species, were subjected to starch block electrophoresis. Adrenal phenylethanolamine *N*-methyltransferase and pineal hydroxyindole *O*-methyltransferase were heterogeneous among different species with respect to electrophoretic mobility on starch block, heat stability, and substrate specificity, but they were homogeneous within a given species. Multiple forms of histamine *N*-methyltransferase and catechol *O*-methyltransferase occurred in tissues within a given species as well as among different species. They had different electrophoretic mobilities, heat stabilities, and kinetic properties.

INTRODUCTION

Several methyltransferases are involved in the activation and inactivation of the biogenic amines norepinephrine, histamine, and serotonin. These enzymes catalyze the *N*-methylation of norepinephrine and other β-hydroxylphenylamines (phenylethanolamine *N*-methyltransferase) (1, 2), the *O*-methylation of catecholamines (catechol *O*-methyltransferase, EC 2.1.1.6) (3), the *N*-methylation of histamine (histamine *N*-methyltransferase, EC 2.1.1.8) (4), and the *O*-methylation of *N*-acetylserotonin (hydroxyindole *O*-methyltransferase) (5).

In view of the marked species differences in the total activity and tissue distribution of these enzymes, a study was undertaken to ascertain whether biochemically distinguishable forms of the enzyme possessing different catalytic properties could be identified. This report describes certain physicochemical properties of such distinct forms. Phenylethanolamine *N*-methyltransferase, catechol *O*-methyltransferase, histamine *N*-methyltransferase, and hydroxyindole *O*-methyltransferase exhibited multiple molecular forms in different species and, in certain cases, within the tissues of the same animal.

MATERIALS AND METHODS

Adult male animals were used unless otherwise stated. Tissues from rats (Sprague-Dawley), mice (NIH stock), mongrel dogs and cats, and guinea pigs (Hartley strain) were removed, placed on cracked ice, and homogenized within 1 hr after the animals had been killed. Pineal glands from quails (*Corturnix corturnix japonica*) were kindly supplied by Dr. Jean Lauber, University of Alberta, shipped in Dry Ice, and examined 1 week later. Bovine tissues were removed within 1 hr after the cattle had been killed in a local slaughterhouse and were stored

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for 1 week at −10°. Human tissues, obtained within 8 hr from two normal young men who had been killed in an accident, were stored at −10° for 1 day. Tissues were homogenized, and the supernatant fractions were subjected to starch block electrophoresis within 8 hr. All experiments were repeated two to five times with different tissue preparations from each species.

Tissues were homogenized in 3–10 volumes of ice-cold water, depending on the tissue, and centrifuged at 100,000 × *g* for 30 min in a refrigerated Spinco centrifuge. The fatty layer was removed by aspiration, and the clear supernatant fraction was subjected to starch block electrophoresis. A starch block was prepared in 0.05 M sodium barbital buffer, pH 8.6, as described previously (6). A 1–2-ml aliquot of the supernatant fraction, containing 2–10 units of enzyme activity (1 unit is equivalent to 1 mμmole of *N*- or *O*-methylated product formed per hour), was applied at the origin. Electrophoresis was carried out at 4° for 18 hr at 360 V and 80 mamp. The starch block was cut into 0.5-inch segments, and the enzyme was eluted from each segment with 2 ml of 0.05 M sodium phosphate buffer, pH 7.9.

To examine the efficacy and reproducibility of the electrophoretic technique, supernatant fractions from dog and rat adrenals, containing equal amounts of phenylethanolamine *N*-methyltransferase activity, were mixed and inserted into a starch block. After electrophoresis there were two distinct peaks of enzyme activity, which were the same as those obtained when the extracts from each animal were subjected separately to electrophoresis (Fig. 1A). After electrophoretic separation, the peak tube of phenylethanolamine *N*-methyltransferase activity from rat adrenals was eluted and again subjected to starch block electrophoresis. There was no change in mobility of the enzyme purified by electrophoresis. Duplicate determinations gave the same electrophoretic pattern.

An aliquot from each eluate was transferred to a 15-ml centrifuge tube and assayed for various methyltransferases by a modification of procedures described previously.

Phenylethanolamine *N*-methyltransferase (2), histamine *N*-methyltransferase (4), catechol *O*-methyltransferase (7), and hydroxyindole *O*-methyltransferase (5) were measured as follows.

Phenylethanolamine N-methyltransferase. DL-Phenylethanolamine (25 μg), ^{14}C-methyl-*S*-adenosylmethionine (New England Nuclear Corporation) (1.0 mμmole; 50 μCi/μmole), sodium phosphate buffer (0.05 M, pH 7.9), and enzyme preparation to make a final volume of 250 μl were incubated for 1 hr at 37°. The reaction was stopped by addition of 0.5 ml of sodium borate buffer, pH 10, and the ^{14}C-methylphenylethanolamine formed enzymatically was extracted with 6 ml of toluene containing 3% by volume of isoamyl alcohol. A 4-ml aliquot of the extract was transferred to a counting vial containing 1 ml of ethanol and 10 ml of phosphor, and the radioactivity was measured.

Histamine N-methyltransferase, hydroxyindole O-methyltransferase, and catechol O-methyltransferase. The procedure was similar to that used for phenylethanolamine *N*-methyltransferase, except that the following substrates were used: histamine dihydrochloride (25 μg) for histamine *N*-methyltransferase, *N*-acetylserotonin (50 μg) (Regis Chemical Company, Chicago) for hydroxyindole *O*-methyltransferase, and *l*-norepinephrine bitartrate (25 μg) for catechol *O*-methyltransferase. $MgCl_2$ (2 μmoles) was added to the incubation mixture for the catechol *O*-methyltransferase assay. The enzymatic products ^{14}C-methylhistamine, ^{14}C-melatonin, and ^{14}C-metanephrine formed in the respective reactions were extracted into a 6-ml mixture of toluene and isoamyl alcohol (3:2 by volume).

K_m values were determined by Wilkinson's method (8), using a digital computer and the FORTRAN program written by Cleland (9). Statistical differences were obtained by the procedure of Steele and Torrie (10).

RESULTS

Separation of different forms of phenylethanolamine N-methyltransferase from mammalian adrenal glands. The adrenal gland was used as a source of phenylethanolamine *N*-methyltransferase because the enzyme is highly localized in this organ (2). The electrophoretic separations of phenylethanolamine

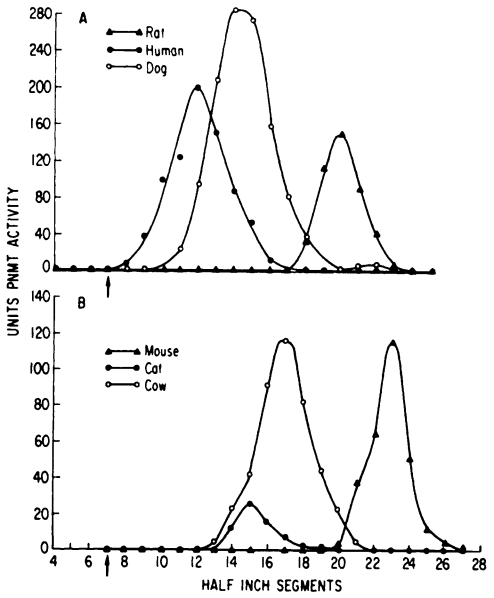

FIG. 1. *Electrophoretic mobility on starch block of adrenal phenylethanolamine N-methyltransferase (PNMT) from several mammalian species*

Electrophoresis was performed on supernatant fractions in 0.05 M sodium barbital buffer, pH 8.6, at 4° for 18 hr. Arrows indicate origin.

N-methyltransferase obtained from human, dog, mouse, rat, cat, and cow adrenal glands are shown in Fig. 1A and B. These illustrate electrophoretic patterns obtained for adrenal glands from six species, as revealed by separations on two starch blocks. Human, dog, and rat adrenals contain a distinct form of phenylethanolamine *N*-methyltransferase, as shown by their differing electrophoretic mobilities (Fig. 1A). The electrophoretic mobilities of phenylethanolamine *N*-methyltransferases obtained from cow and cat adrenals were similar (Fig. 1B), but the phenylethanolamine *N*-methyltransferases from these species differed in electrophoretic mobility from that of the mouse (Fig. 1B). The cat and cow phenylethanolamine *N*-methyltransferases exhibited electrophoretic mobilities resembling that of the dog. In many experiments, mouse phenylethanolamine *N*-methyltransferase was similar in electrophoretic mobility to that of the rat. To distinguish further among the phenylethanolamine *N*-methyltransferase activities from various species, peak tubes were diluted to contain similar enzyme activities, and stability to heat was examined (Table 1). Although the cat phenylethanolamine *N*-methyltransferase had an electrophoretic mobility similar to those of dog and cow, the cat enzyme was considerably less stable to heat. The rat phenylethanolamine *N*-methyltransferase was also more heat-stable than that of the mouse. These results suggest the existence of at least five distinguishable forms of phenylethanolamine *N*-methyltransferase among the six mammalian species examined.

TABLE 1

Heat stability of phenylethanolamine N-methyltransferase from various species

Peak tubes in Fig. 1A and B were diluted to contain approximately similar concentrations of phenylethanolamine *N*-methyltransferase. Aliquots were heated for 2 and 5 min at 48° in 0.05 M sodium phosphate buffer, pH 7.9. Results are expressed as percentage of phenylethanolamine *N*-methyltransferase activity remaining.

Species	Heat stability	
	2 min	5 min
	% enzyme remaining	
Rat	90	65
Mouse	50	20
Cat	55	20
Human	40	20
Dog	88	70
Cow	90	68

Phenylethanolamine *N*-methyltransferase methylates β-hydroxyphenylamine derivatives, but not phenylethylamines (2). The relative activities of phenylethanolamine *N*-methyltransferases separated electrophoretically from various species were examined with respect to their ability to *N*-methylate several phenylamine derivatives. The phenylethanolamine *N*-methyltransferases obtained from rat, human, and cow methylated phenylethanolamine derivatives, but not phenylethylamines (Table 2). However, dog phenylethanolamine *N*-methyltransferase showed small but definite activity toward some phenylethylamine derivatives. Dog and human phenylethanolamine *N*-methyltransferases methylated norephedrine to greater extents than did rat phenylethanolamine *N*-methyltransferase (Table 2).

TABLE 2

Substrate specificity of phenylethanolamine N-methyltransferase

Phenylethanolamine *N*-methyltransferases obtained from peak tubes (Fig. 1A and B) were incubated with 0.1 μmole of substrates and cofactors as described in MATERIALS AND METHODS. The ^{14}C-methylated products were extracted as described previously (2). Results are expressed as percentage of relative activity.

Substrate	Rat	Human	Dog	Cow
	%	%	%	%
DL-Phenylethanolamine	100	100	100	100
Phenylethylamine	<1	<1	4	0
DL-Normetanephrine	70	70	71	80
3-Methoxy-4-hydroxyphenylethylamine	<1	0	3	0
DL-Norephedrine	2	10	18	
D-Amphetamine	0	0	0	0

Histamine N-methyltransferase. The electrophoretic mobility of histamine *N*-methyltransferase was examined in several guinea pig tissues, and in human, cow, cat, dog, and guinea pig liver. The electrophoretic mobility of this enzyme was first studied in guinea pig stomach, liver, lung, and brain (Fig. 2A and B). All these tissues showed a single peak of activity with similar electrophoretic mobility. The livers of human, dog, and cat were then subjected to starch block electrophoresis and examined for histamine *N*-methyltransferase activity. The results, shown in Fig. 2B, reveal at least three distinct forms of histamine *N*-methyltransferase. Dog and guinea pig liver histamine *N*-methyltransferases had similar electrophoretic mobilities but differed from those of human and cat liver. The human and dog also had a smaller peak of histamine *N*-methyltransferase activity, which, to achieve adequate definition, required larger amounts of histamine *N*-methyltransferase activity to be applied to the starch block (Fig. 2C). The dog and human each showed two peaks of histamine *N*-methyltransferase activity: a major, slow-moving peak in the dog, and a larger, faster-moving peak in the human (Fig. 2C).

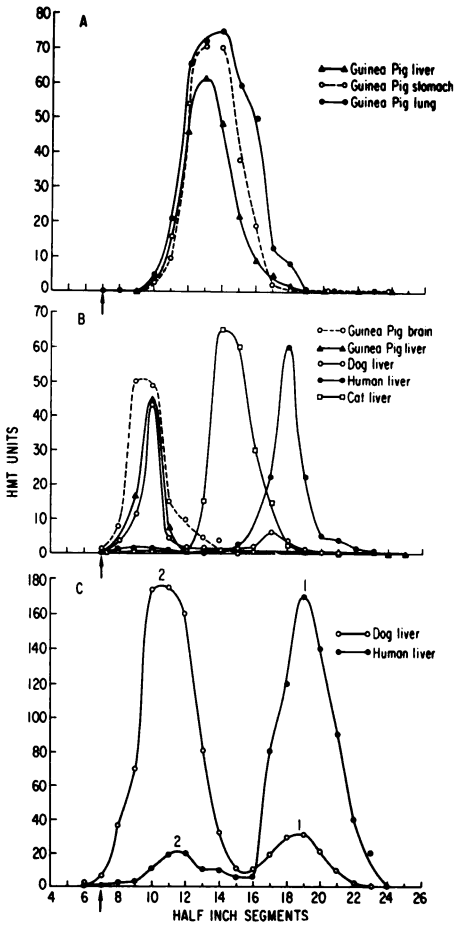

FIG. 2. *Electrophoretic mobility on starch block of histamine N-methyltransferase (HMT) from several mammalian species and tissues*

Conditions were the same as described in Fig. 1 and MATERIALS AND METHODS.

Heat stability and K_m values with respect to histamine were determined for the histamine *N*-methyltransferases electrophoretically separated from various species (Table 3). Cat histamine *N*-methyltransferase was most heat-stable, and the human peak 2 enzyme was least stable. There was also a wide range of K_m values for histamine. The two human enzymes showed a 40-fold difference in K_m values, whereas two dog enzymes showed a smaller difference, which was not statistically different.

Catechol O-methyltransferase. The electrophoretic mobilities of catechol *O*-methyl-

TABLE 3

Some properties of histamine N-methyltransferase from various species separated by starch block electrophoresis

Peak tubes (Fig. 2A–C) were diluted to contain similar concentrations of histamine *N*-methyltransferase. Aliquots were heated at 51° in 0.05 M sodium phosphate buffer, pH 7.9, for 3 min. Results are expressed as the percentage of histamine *N*-methyltransferase activity remaining. K_m values were obtained before heating, using varied amounts of histamine and a constant amount of *S*-adenosylmethionine (2.5×10^{-6} M). Peak 2 (human) K_m was significantly different from peak 1 ($p < 0.001$). K_m for cat was significantly different from that of all other species ($p < 0.05$). Dog peaks 1 and 2 were not statistically different.

Species	Heat stability	K_m
	% enzyme remaining	*μM ± SEM*
Cat	63	81 ± 22
Dog, peak 2	49	10 ± 2.3
Dog, peak 1	55	24 ± 7.3
Guinea pig	49	
Human, peak 1	46	950 ± 97
Human, peak 2	33	20 ± 0.4

transferases from rat heart, kidney, brain, and liver and from cat, human, and dog liver were examined. The rat tissues exhibited two isozymes of catechol *O*-methyltransferase after starch block electrophoresis (Fig. 3A and B). In the rat, isozymes from one tissue had the same electrophoretic mobility as the corresponding isozymes from the other tissue. The heat stability and K_m values of the two catechol *O*-methyltransferase isozymes from rat liver were found to differ (Table 4). Two electrophoretically distinguishable forms of catechol *O*-methyltransferase were also separated from human, dog, and cat livers (Fig. 3B and C). These isozymes appeared to have different electrophoretic mobilities, heat stabilities, and K_m values from those of the rat (Table 4). In the dog and cat the fast-moving peak was the major isozyme, whereas in the rat and human the slower-moving peak had most enzyme activity. Because of the extreme instability of human catechol *O*-methyltransferase after starch block electrophoresis, K_m values could not be determined.

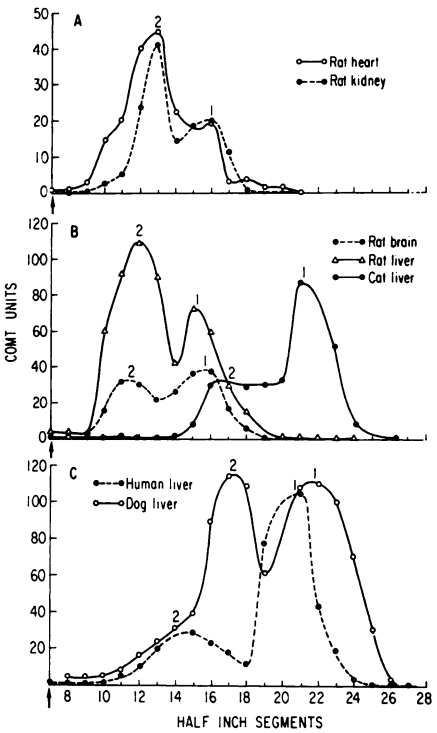

FIG. 3. *Electrophoretic mobility on starch block of catechol O-methyltransferase (COMT) from several mammalian species and tissues*

Conditions were the same as described in Fig. 1 and MATERIALS AND METHODS.

Hydroxyindole O-methyltransferase. The melatonin-forming enzyme hydroxyindole *O*-methyltransferase is uniquely localized in the pineal gland (5). The electrophoretic behavior of this enzyme obtained from a mammal (cow) and a bird (quail) was studied. Each had a single peak of hydroxyindole *O*-methyltransferase activity. The electrophoretic mobility of the quail hydroxyindole *O*-methyltransferase was faster than that of the cow (Fig. 4). The hydroxyindole *O*-methyltransferases from these species differed markedly in heat stability and K_m values (Table 5). Previous work has shown that cow and quail hydroxyindole *O*-methyltransferases also differ in substrate specificity (11). Quail hydroxyindole *O*-methyltransferase *O*-methylates both serotonin and *N*-acetylserotonin, whereas cow hydroxyindole *O*-methyltransferase is highly specific for *N*-acetylserotonin.

TABLE 4

Properties of catechol O-methyltransferases from various species

Peak tubes (Fig. 3A–C) were diluted to contain similar concentrations of catechol *O*-methyltransferase. An aliquot was heated at 51° in 0.05 M sodium phosphate buffer, pH 7.9, for 3 min. Results are expressed as percentage of catechol *O*-methyltransferase activity remaining. K_m values were obtained before heating, using varied amounts of norepinephrine and a constant amount of *S*-adenosylmethionine (2.5×10^{-6} M). Cat peak 1 K_m differs from all other species ($p < 0.05$). The K_m value for catechol *O*-methyltransferase rat peak 1 differs from that of peak 2 ($p < 0.05$).

Species	Heat stability	K_m
	% enzyme remaining	*μM ± SEM*
Human, peak 2	50	
Human, peak 1	48	
Dog, peak 2	54	39 ± 2.7
Dog, peak 1	15	39 ± 4.9
Cat, peak 1	7	57 ± 15
Rat, peak 2	33	43 ± 12
Rat, peak 1	45	194 ± 58

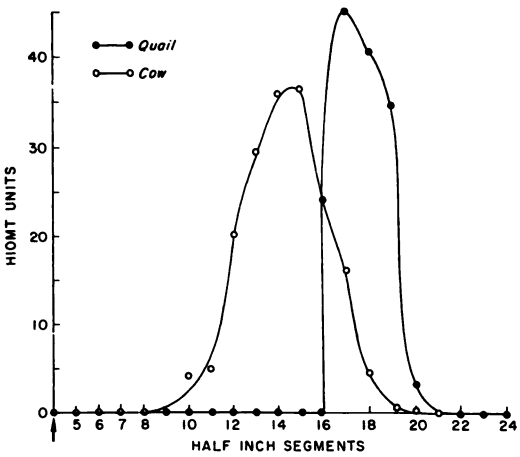

FIG. 4. *Electrophoretic mobility on starch block of cow and quail hydroxyindole O-methyltransferase (HIOMT)*

Conditions were the same as described in Fig. 1 and MATERIALS AND METHODS.

DISCUSSION

These results show that species differences exist in the physicochemical properties of the methyltransferase enzymes involved in biogenic amine synthesis and metabolism. Under the conditions of these experiments, phenylethanolamine *N*-methyltransferase and hydroxyindole *O*-methyltransferase appeared to be electrophoretically homogeneous within a given species, but species differences in adrenal phenylethanolamine *N*-methyltransferase and pineal hydroxyindole *O*-methyltransferase were identified by starch block electrophoresis and heat stability. Although these enzymes were homogeneous by starch block electrophoresis with sodium barbital buffer at pH 8.6, other conditions of electrophoresis or physicochemical techniques might uncover heterogeneity. Previous work has revealed different forms of phenylethanolamine *N*-methyltransferase in frog and rat (12). Rat phenylethanolamine *N*-methyltransferase was induced by corticoids, whereas the frog phenylethanolamine *N*-methyltransferase was not. Histamine *N*-methyltransferase and catechol *O*-methyltransferase were heterogeneous electrophoretically within a given species. These multiple forms of histamine *N*-methyltransferase and catechol *O*-methyltransferase were further distinguished by differences in heat stability and kinetic properties. Previously, rat liver catechol *O*-methyltransferase was shown to exhibit at least two forms of activity by acrylamide electrophoresis (13).

Isozymes have become a commonly en-

TABLE 5

Properties of hydroxyindole O-methyltransferase from cow and quail

Peak tubes (Fig. 4) were diluted to contain similar concentrations of hydroxyindole *O*-methyltransferase. An aliquot was heated at 51° in 0.1 M sodium phosphate buffer, pH 7.9, for 3 min. Results are expressed as percentage of hydroxyindole *O*-methyltransferase activity remaining. K_m values were obtained before heating, using varied amounts of *N*-acetylserotonin and a constant amount of *S*-adenosylmethionine (2.5×10^{-6} M). Hydroxyindole *O*-methyltransferases from quail and cow were statistically different ($p < 0.001$).

Species	Heat stability	K_m
	% enzyme remaining	*μM ± SEM*
Cow	35	51 ± 5.8
Quail	2	4.8 ± 0.65

countered biological phenomenon (14). Availability of multiple forms of isozymes has been shown to permit selective and flexible regulation of physiologically important reactions. A variety of mechanisms whereby isozymes act to allow finer metabolic control have been documented (14). For example, Stadtman has described differential feedback inhibition of several isozymes at branched metabolic pathways (14). Fritz has reported allosteric control of lactate dehydrogenase-5 by oxalacetate (15). With respect to the multiplicity of enzymes involved in the synthesis and degradation of biogenic amines, the distinct physiological roles performed by each isozyme remain to be elucidated. Possibly some forms are subject to induction by steroids or drugs, whereas others are not (16). Certain isozymes may represent synthetic or degradative by-products without additional physiological significance. Some of these isozymes may even be artifacts, arising during the processes of tissue homogenization and electrophoresis, although their markedly different physicochemical properties suggest that they do exist within the cell.

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