Biochemical Pharmacology, Vol. 33, No. 18, pp. 2911–2913, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

ACTIVATION OF ADRENAL PHENYLETHANOLAMINE *N*-METHYLTRANSFERASE BY PHOSPHATE

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(*Received* 11 *October* 1983; *accepted* 13 *February* 1984)

Abstract—Bovine adrenal phenylethanolamine *N*-methyltransferase is activated by inorganic phosphate. Addition of potassium phosphate to the enzyme assay mixture increased the enzyme activity 5- to 8-fold depending on the purity of the enzyme. Neuraminidase treatment of the enzyme decreased the activation by one half. The activity of the enzyme was also increased by other negatively charged ions, including chloride and sulfate. However, the activation was less than 3-fold, and pretreatment with neuraminidase did not alter the degree of activation. The high degree of activation was specific to bovine adrenal enzyme. Rat adrenal enzyme was activated less than 2-fold by phosphate and not affected at all by chloride and sulfate. These results suggest that bovine adrenal phenylethanolamine *N*-methyltransferase is a glycosylated protein, containing sialic acid moieties, and that this carbohydrate moiety plays a role in the activation of this enzyme. These results are also consistent with our previous finding [D. H. Park, E. E. Baetge, B. B. Kaplan, V. R. Albert, D. J. Reis and T. H. Joh, *J. Neurochem.* **38**, 410 (1982)] that the difference in characteristics between bovine and rat adrenal phenylethanolamine *N*-methyltransferase is due to posttranslational modification of the enzyme.

Phenylethanolamine *N*-methyltransferase (PNMT) (EC 2.1.1.28, *S*-adenosyl-L-methionine:phenylethanolamine *N*-methyltransferase) catalyzes the conversion of norepinephrine to epinephrine. PNMT is localized primarily in the adrenal medulla [1, 2] and in relatively smaller amounts in discrete regions of brain [3]. Charge differences, inferred from electrophoretic mobility, have been reported to exist among adrenal PNMT of various species, although their molecular weights, function, substrate specificity and other biochemical characteristics are similar [4–14]. Antibodies to bovine adrenal PNMT strongly inhibit both rat and bovine enzyme, whereas antibodies produced against rat PNMT inhibit less the activity of bovine and monkey enzyme than that of rat enzyme [9, 13]. Although the molecular basis behind these differences remains unknown, posttranslational modification of the enzyme is implicated as partly responsible for them [13, 14].

In the present study, we show that bovine adrenal PNMT is a partially glycosylated protein, which is markedly activated by phosphate. The presence of sialic acid residues probably plays a role in the ability of the enzyme to be activated by phosphate.

MATERIALS AND METHODS

Materials. D,L-Phenylethanolamine and neuraminidase (type X) were purchased from the Sigma Chemical Co. (St. Louis, MO); *S*-[*methyl*-^{14}C] adenosyl-L-methionine (56.2 mCi/mmole) and Aquassure were obtained from the New England Nuclear Corp. (Boston, MA); meta-aminophenyl boronic acid immobilized on agarose gel (Glycaffinity column) was from Isolab Inc. (Akron, OH).

PNMT assay. PNMT activity was measured by our modification [13] of the method of Axelrod [1] using 0.5 mM instead of 1 mM phenylethanolamine in the absence or presence of an appropriate amount of potassium phosphate (adjusted to pH 8.6) or other ions.

Preparation of PNMT. For crude extracts of bovine and rat adrenal glands, the 100,000 *g* supernatant fraction of the homogenates was used. The activities used for the activation experiment were as follows: bovine, 18.7 pmoles/15 min; rat, 21.5 pmoles/15 min. Purified enzymes were prepared as described elsewhere in detail [13]. The purified bovine and rat PNMT activities used for the studies were 34.1 pmoles/10 min and 58.8 pmoles/10 min, respectively, unless otherwise specified.

Neuraminidase treatment. Purified rat and bovine adrenal PNMT (78.9 pmoles/10 min and 67.2 pmoles/10 min respectively) were pretreated separately with 0.5 units (1 unit is expressed as 1 μmole of *N*-acetylneuraminic acid formed per min at pH 5.0 at 37°) of neuraminidase at 37° for 10 min in the presence of 30 mM sodium acetate buffer, pH 5.0, in a final volume of 100 μl. After stopping the neuraminidase reaction by immersing the reaction tube in an ice-water bath, a 50-μl aliquot was assayed for PNMT activity as described above.

Separation of glycosylated and nonglycosylated PNMT in the purified preparation. The glycosylated form of PNMT was qualitatively separated from the nonglycosylated form in the purified PNMT preparation by the method described in the Isolab manual. In

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brief, purified PNMT was mixed with the Isolab sample preparation reagent containing alkaline solution of glycine, $MgCl_2$, surfactant and sodium azide, and applied to a column (1 × 1 cm) of meta-aminophenyl boronic acid immobilized on agarose gel. The nonglycosylated form was eluted with Isolab first fraction elution reagent consisting of alkaline solution of glycine, $MgCl_2$ and sodium azide, and the glycosylated form was eluted with Isolab second fraction elution buffer composed of sorbitol as described in the manual.

RESULTS

Activation of PNMT by phosphate. PNMT activity in the crude bovine adrenal PNMT preparation was enhanced by phosphate in a concentration-dependent manner as shown in Fig. 1. The activity increased 680% over the control at 190 mM phosphate. In contrast, phosphate activation of the rat adrenal enzyme was minimal, increasing 77% over the control at 30 mM phosphate, and gradually decreasing at the higher concentrations (Fig. 1). The degree of activation was less when purified preparations of both bovine and rat PNMT were used (Fig. 2), although the activation patterns for crude and purified enzyme were similar. The activity of the purified enzyme increased by 390% for bovine enzyme and by 43% for rat enzyme.

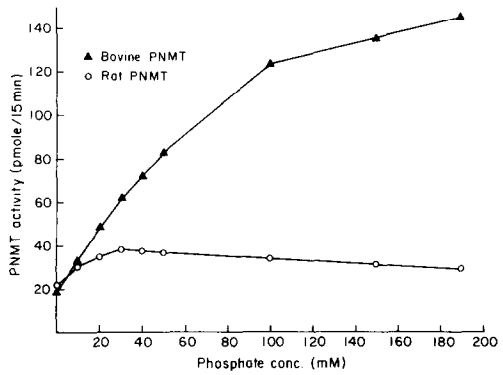

Fig. 1. Activation of phenylethanolamine *N*-methyltransferase in crude tissue extracts by phosphate. Crude enzyme preparations (100,000*g* supernatant fractions) were assayed as described in Materials and Methods in the presence of various amounts of potassium phosphate. The amounts of adrenal enzymes used were as follows: bovine enzyme, 8.7 μg; rat enzyme, 8.5 μg. Each point is the average of duplicate determinations.

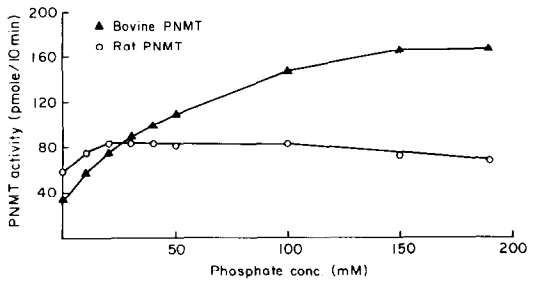

Fig. 2. Activation of purified phenylethanolamine *N*-methyltransferase by phosphate. The activity of purified enzyme was measured as presented in Materials and Methods with various amounts of phosphate. The amounts of adrenal enzymes used were as follows: bovine enzyme, 0.12 μg; rat enzyme, 0.17 μg. Each point is the average of duplicate determinations.

Activation of adrenal PNMT by phosphate after pretreatment of the enzyme with neuraminidase. After pretreatment of purified bovine PNMT with neuraminidase, the enzyme activity was gradually enhanced by phosphate in a concentration-dependent manner and reached 261% of the control at 200 mM (Table 1). Neuraminidase pretreatment of purified rat enzyme prevented activation of the enzyme by phosphate (Table 1).

Effects of various anions on PNMT activity. The activity of purified bovine enzyme was also increased

Table 1. Activation of adrenal phenylethanolamine *N*-methyltransferase (PNMT) by phosphate after pretreatment of the enzyme with neuraminidase*

	Rat PNMT† (% of control)	Bovine PNMT‡ (% of control)
Control	100	100
50 mM Phosphate	100	182
100 mM Phosphate	83.4	215
150 mM Phosphate	85.3	251
200 mM Phosphate	72.7	261

* Purified rat or bovine adrenal PNMT preparation was pretreated with 0.5 units of neuraminidase at 37° for 10 min in the presence of 30 mM sodium acetate buffer, pH 5.0. Each value is the average of three determinations.

† PNMT activity of rat control (no addition of phosphate) was 39.7 pmoles/10 min at 37°.

‡ PNMT activity of bovine control (no addition of phosphate) was 33.5 pmoles/10 min at 37°.

Table 2. Effects of various salts on phenylethanolamine *N*-methyltransferase (PNMT) activity*

		Rat adrenal PNMT† (% of control)	Bovine adrenal PNMT‡ (% of control)
NaCl	50 mM	104	157
	100 mM	102	196
	200 mM	85	227
KCl	50 mM	114	168
	100 mM	105	195
	200 mM	94	229
Na_2SO_4	50 mM	96	191
	100 mM	86	216
	200 mM	71	237
Tris-Cl	50 mM	110	105
	100 mM	109	109
	200 mM	105	111

* Each value is the average of three determinations.

† PNMT activity of rat control (no addition of salt) was 67.5 pmoles/10 min at 37°.

‡ PNMT activity of bovine control (no addition of salt) was 64.2 pmoles/10 min at 37°.

in a concentration-dependent fashion by other anions, including chloride and sulfate. The maximum enhancements of the activity were slightly greater than twice control levels in the presence of 200 mM anions (Table 2). In contrast, the addition of positive ion such as Tris to the assay mixture did not change the activity of purified PNMT as shown in Table 2. The activity of purified rat adrenal PNMT was not enhanced by the presence of either cation or anions.

Qualitative estimation of glycosylated form present in the purified adrenal PNMT preparation. To determine whether there is any relationship between the activation of PNMT and the extent of glycosylation, we qualitatively estimated the proportion of PNMT in glycosylated form by subjecting purified adrenal PNMT to phenylboronic acid affinity chromatography. The data obtained were as follows: purified bovine PNMT, 213 pmoles/30 min for nonglycosylated form (80%) and 52 pmoles/30 min for glycosylated form (20%); purified rat PNMT, 103 pmoles/30 min for nonglycosylated form (97%) and 3 pmoles/30 min for glycosylated form (3%).

DISCUSSION

To better understand the basis of the species differences in adrenal PNMT, our purpose in the present study was to demonstrate differences in the activation mechanisms of bovine and rat adrenal PNMT.

Our studies have shown that only bovine PNMT was glycosylated and that marked activation of the enzyme by phosphate ion occurred only when the glycosylated form of PNMT was present. These results suggest that the presence or absence of carbohydrate moieties on the enzyme may be largely responsible for the species differences observed. The degree of activation by phosphate was much greater with crude enzyme preparation than with purified enzyme. This may be due to partial removal of carbohydrate moieties from the enzyme during purification, since our qualitative estimate of glycosylated form in the purified bovine PNMT is about 20%. After neuraminidase pretreatment, bovine PNMT was still moderately activated by phosphate and a similar degree of activation was also observed with other anions, including chloride and sulfate. The neuraminidase-insensitive carbohydrate moieties may be responsible for this portion of activation. Since rat enzyme contains neither sialic acid nor carbohydrate moieties, no such activation of rat PNMT is expected.

Although our studies provide no unequivocal evidence, it is indicated from the above observation that activation of the bovine enzyme may require a more negatively charged environment at the active site since the bovine enzyme is less negatively charged than rat enzyme [4, 13]. It has been reported in a similar case [15–17] that the rate-limiting enzyme in the biosynthesis of catecholamines, tyrosine hydroxylase, was also activated by various anions and by phosphorylation. This activation of tyrosine hydroxylase has been postulated [16] to be mediated through a negative control on the active site by a cationic regulatory site. Phosphorylation would covalently modify the charge of this regulatory site. The active fragment obtained by limited proteolysis of the enzyme could not be activated by anions or phosphorylation.

Thus, it is tempting to speculate that modification of the ionic environment of the active site through mediation of carbohydrate or, particularly, a sialic acid moiety may be one of the important mechanisms in the regulation of bovine, but not rat, adrenal PNMT activity *in vivo*.

Acknowledgement—This work was supported by NIH Grants MH 24285 and HL 18974.

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