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단 신

쥐 뇌의 Phosphatidylethanolamine *N*-메틸전달효소에 대한 몇 화학물질의 영향

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Effects of Several Chemicals on Phosphatidylethanolamine *N*-methyltransferase in Rat Brain

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Phosphatidylethanolamine (PE) is converted to phosphatidylcholine (PC) via three sequential methylation of PE by phosphatidylethanolamine N-methyltransferase (PEMT).[1] This methylation pathway in brain has been shown to occur in both *in vivo*[2,3] and *in vitro* by various subcellular fractions.[4-7] The role of PEMT has been studied in various ways.[8-10] Many reports implicated action of this enzyme to a possible modulator for certain biological signal transductions such as nerve growth factor,[11] histamine,[12] and protein kinase C.[13] In the liver, one form of PEMT (2) has been isolated and cloned.[14]

In the course of examining the effect of exogenous phospholipids on PEMT in brain homogenate, we found that the highest specific activity of PEMT was localized in nuclear envelopes and significant amount of the activity was detected in cytosolic fraction.[6] Upon this observation we re-optimized the assay condition for PEMT after addition of sufficient amounts of phospholipid substrates and revised subcellular distribution of PEMT in rat brain.[15]

In this report, as a part of characterization of brain PEMT, effects of several chemicals known to affect PEMT activity was investigated in both nuclear and cytosolic subfractions.

EXPERIMENTAL

Materials. Phosphatidyl N-monomethylethanolamine, dipalmitoyl (PME), phophatidyl N,N-dimethylethanolamine, dipalmitoyl (PDE), egg phosphatidylcholine (PC), S-adenosylmethionine (SAM), S-adenosylhomocysteine, N-ethylmaleimide, aldosterone, hydrocortisone, 3-deazaadenosine and trypsin were obtained from Sigma Chemical Co. Spermine tetrahydrochloride was from Fluka. TLC plastic sheets of silica gel 60 F_{254} was obtained from Merck AG. Phosphatidylethanolamine was prepared by transphosphatidylation of egg PC in the presence of ethanolamine and cabbage phospholipase D.[7] Radioactive S-adenosyl-L-[methyl-^{3}H]-methionine was prepared by a reaction of rat liver methionine adenosyltransferase.[16] L-[methyl-^{3}H]-methionine (specific activity, 72.2 Ci/mmol) was purchased from New England Nuclear Co. Rats were supplied by Animal Breeding Laboratory of Seoul National University.

Subcellular fractionation. Wistar rats (3~4 week old) were killed by decapitation and the brains were homogenized by a glass-teflon tissue homogenizer driven by a motor at 850 rpm, four-five up-and-down strokes with 10 vol of 0.32 M

sucrose. The homogenate was centrifuged at 100,000×g for 1 hr and the supernatant was used as cytosolic fraction. For neuronal nuclei fraction (N 1), the homogenation was carried out in 5 volumes of 2.0 M sucrose and filtered through two layers of muslin. The filtrate was centrifuged in 1.8 M : 2.4 M sucrose discontinuous density gradient.[7]

Assay of PEMT. The methylation of phospholipids was measured by the incorporation of radioactive methyl group from SAM.[5-7] Briefly, the incubation medium was consisted of 100 μl of solution containing 10 mM carbonate buffer (pH 10.0), 10 mM $MgCl_2$, 200 μg of PE, 200 μg of PME, 200 μg of PDE, [^{3}H-methyl]-SAM (specific activity, 600 μCi/μmol) and appropriate amount of enzyme source. After 2 hr incubation at 37 °C, reaction was quenched by adding 3 mL of chloroform/methanol/HCl mixture (2:1:0.02, v/v/v). The organic extract was applied to a silica gel plate and the plate was developed employing a solvent system of chloroform/methanol/n-propanol/water (2:2:3:1, v/v/v/v). The product lipid was scraped and counted using an LKB 1219 Rackbeta scintillation counter. The enzyme activity was expressed in pmol/mg protein/hr and was determined from at least two separate measurements with duplicate samples.

RESULTS AND DISCUSSION

In order to characterize the PEMT in neuronal nuclei (N1) and cytosolic fractions, several chemicals known to affect PEMTs such as hormones, inhibitors and protease were examined. *Table* 1 summarized the effects of selected chemicals on PEMT in N1 subfraction. Aldosterone which stimulates Na^+ transport has been shown to facilitate the methylation of phospholipid.[17] Contrary to this report, this hormone showed rather slight inhibition at a concentration of 10 mM. Similarly spermine tetrahydrochloride and hydrocortisone had no activational effect on the PEMT at the same concentration. In the case of inhibitors, N-ethylmaleimide (NEM) and S-adenosylhomocystein (SAH), PEMT activities were inhibited to below 10% of original activities at 1 mM concentration. However PEMT specific inhibitor 3-deazaadenosine did not affect the N1 activities at all. The insensitivity of PEMT in N1 fraction seems to be unusual compared with PEMTs from other sources.[9,18] When trypsin was added into the incubation media in a concentration of 1 mg/ml, about 90% of PEMT activities were destroyed. When comparing the three activities after treatments of inhibitors and trypsin, slight differences could be observed. Generally PME synthesizing activity was less sensitive to inhibitors and trypsin digestion than PDE or PC synthesizing activities. These differences might provide another evidence for an existence of multiple types of PEMTs in N1 fraction.

For cytosolic PEMT, NEM and SAH showed similar inhibitory effects at 1 mM concentration. Since NEM is known to react with sulfhydryl groups in proteins, the inhibitory effect implies an involvement of -SH group in catalytic activity of

Table 1. Effects of several chemicals on N1 PEMT activities

Chemical	mM	PME		PDE		PC	
		Specific[a]	%[b]	Specific	%	Specific	%
None		6.04	100.0	20.60	100.0	14.11	100.0
Aldosterone	10	5.01	82.8	17.90	87.1	12.60	89.3
Spermine tetra-hydrochloride	10	5.20	86.1	18.04	87.6	14.90	105.6
Hydrocortisone	10	4.02	66.3	16.92	82.2	12.90	91.4
N-ethylmaleimide	1	0.55	9.1	0.63	3.1	0.59	4.2
S-adenosyl-homocysteine	1	0.34	5.3	0.62	3.0	0.25	1.8
3-deazaadenosine	1	6.20	102.1	19.30	93.9	15.94	113.0
Trypsin	1 mg/ml	0.60	9.9	1.61	7.8	0.86	6.1

[a]Specific activity (CH_3- incorporation, pmol/mg protein/hr). [b]Percent activity, the proportion to the original activity.

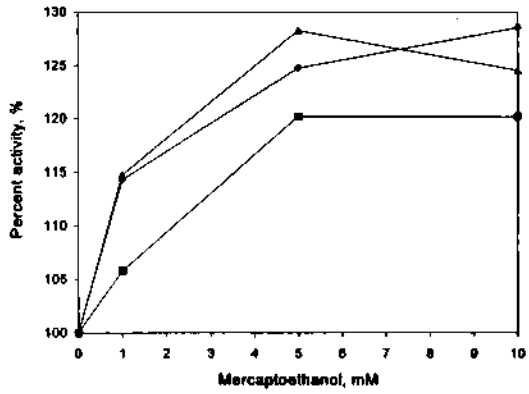

Fig. 1. Effects of mercaptoethanol on cytosolic PEMT activities. PME, -■-; PDE, -◆-; PC, -▲-.

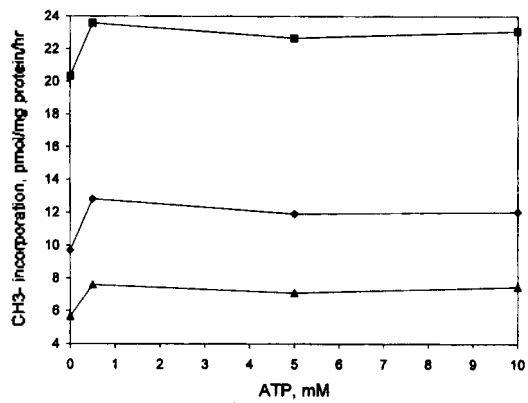

Fig. 2. Effects of ATP on cytosolic PEMT activities. PME, -■-; PDE, -◆-; PC, -▲-.

PEMT. The NEM result coincides with previous report[19] and agreed well with the stimulatory effect of mercaptoethanol observed on cytosolic PEMT (*Fig.* 1). The three steps of methylation processes in cytosolic PEMT was activated approximately 20-30% at 5 mM mercaptoethanol compared with the activities in the absence of mercaptoethanol. A preliminary data of ATP effect on PEMT revealed stimulatory effect of more than 20% in all three methylation processes (*Fig.* 2). Thus a possibility of phosphorylation of PEMT exists and could offer a clue for regulatory pathway of the enzyme in cellular function. In view of the importance of lipid polymorphism in cellular function, the conversion of PE to PC probably influence the membrane fluidity as well as nonbilayer-forming potential in the cellular membranes.[20] Therefore PEMT deserves further study in the aspect of enzymology as well as biological functions.

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