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DISTINCT EFFECTS OF PHOSPHATIDYLETHANOL ON THREE TYPES OF RAT BRAIN PROTEIN KINASE C

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INDEXING WORDS

protein kinase C; phosphatidylethanol; ethanol

SYNOPSIS

Protein kinase C plays a crucial role in signal transduction for activating cellular function. Phosphatidylserine and Ca^{2+} are essential for the activation of protein kinase C, and diacylglycerol which is produced in the receptor-mediated hydrolysis of inositol phospholipids, increases the affinity of this enzyme for phosphatidylserine and Ca^{2+}. In brain tissues, protein kinase C has been shown to be separated into three fractions, Type I, II, and III by hydroxyapatite column chromatography, and cDNA analysis has revealed that they correspond to γ-, β I-, β II-, and α-cDNA, respectively. Phospholipase D has been known to catalyze the transphosphatidyl reaction between various membrane phospholipids and alcohols. In fact, phosphatidylethanol has been found in many tissues including brain of ethanol-treated rats. This report

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Abbreviations used are: HPLC, high performance liquid chromatography; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid.

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describes the different responses of three distinct forms of protein kinase C to phosphatidylethanol. Phosphatidylethanol can replace phosphatidylserine at high Ca^{2+} concentrations for the activation of Type I, II, and III protein kinase C. However, phosphatidylethanol can activate only Type I enzyme at physiological Ca^{2+} concentrations, which is expressed exclusively in the central nervous tissue. Consideration of these results suggests the possibility that ethanol may exert some effect on the signal transduction in neuronal tissue, via changes in protein phosphorylation.

INTRODUCTION

Protein kinase C is a Ca^{2+}- and phospholipid-dependent protein kinase involved in the signal transduction mediating a wide variety of cellular responses to growth factors, hormones, neurotransmitters, and other modulators of cellular activation.[13] Recent sequence analysis of cDNA clones from brain libraries indicates the presence of several subspecies of protein kinase C, such as α, β I, βII, and γ that have structures similar to one another. These cDNA clones α, β, and γ are shown to be encoded by different genes,[4] and β I and β II are derived from alternative splicing of a single RNA transcript.[15] Chromatography on a hydroxyapatite column has shown that rat brain protein kinase C can be resolved into three fractions, Type I, II, and III.[7,11,15] The structure of each type has been identified by comparison of these enzyme fractions with the enzymes that are expressed in COS 7 cells transfected by the cDNA-containing plasmids.[11,15] Type I of protein kinase C has the structure encoded by α-sequence obtained from brain cDNA library. Type II is an unequal mixture of the two enzymes determined by β I- and β II-sequences, which differ from each other only in the carboxy terminal end regions of about 50 amino acid residues. Type III has the structure of γ-sequence. The kinetic and catalytic properties of these three types are slightly different from one another.[8,16]

Although phosphatidylserine is the sole phospholipid effective for the activation of protien kinase C in the presence of a small amount of unsaturated diacylglycerol and micromolar concentrations of Ca^{2+}, other species of phospholipids such as

phosphatidylethanolamine, phosphatidylinositol, and sphyngomyelin, modulate the activity of this enzyme considerably.[9] Phosphatidylethanol, which has a structure similar to phosphatidylethanolamine, is detected in brain, kidney, liver, and skeletal muscle of ethanol-treated rats.[1,2,3] Phosphatidylethanol is a transphosphatidylated metabolite of exogenous ethanol and phosphatidylcholine by phospholipase D reaction.[5,18] The brain synaptosomal membrane has the highest activity of phospholipase D.[12] The production of phosphatidylethanol by phospholipase D can produce the alteration of phospholipid composition in the biological membrane and might account for some of the physiological effects of ethanol. This communication describes the different responses of three distinct forms of protein kinase C to phospholipids including phosphatidylethanol and phosphatidylmethanol.

EXPERIMENTAL PROCEDURES

Materials and chemicals

Phosphatidylethanol and phosphatidylmethanol were prepared as described.[18] Phosphatidylserine and 1,2-diolein were obtained from Serdary Research Laboratories. Phosphatidylethanolamine was obtained from Avanti Polar-Lipids.

Purification and assay of protein kinase C

Protein kinase C was purified from rat brain soluble fraction by DE-52 (Whatman), threonine-Sepharose, and TSK phenyl-5PW (Toyo soda) column chromatographies, and was separated into three fractions, Type I, II, and III by chromatography on a hydroxyapatite column connected to a Pharmaica HPLC system as described.[11,15] Each type of protein kinase C was apparently homogenous upon sodium dodecyl sulfate polyacrylamide gel electrophoresis. Protein kinase C was assayed by measuring the incorporation of ^{32}P from [γ-^{32}P]ATP into H1 histone as described elsewhere.[10] The reaction mixture (0.25 ml) contained 20 mM Tris-HCl at pH 7.5, 200 μg/ml calf thymus H1 histone, 10 μM[γ-^{32}P]ATP (50-150 cpm/pmol), 5 mM magnesium acetate, 0.01 mM EGTA (from enzyme fraction), various amounts of $CaCl_2$, each phospholipid, and 1,2-diolein. Phospholipid and 1,2-diolein were mixed in a small amount of chloroform, dried under a nitrogen stream, and dispersed in 20 mM Tris-HCl at pH 7.5

by vigorous Vortex mixing followed by sonication for 5 min at 4C. The incubation was carried out for 3 min at 30C, and the reaction was terminated by the addition of 25% trichloroacetic acid. Acid precipitable materials were collected on a nitrocellulose membrane and the radioactivity was determined by liquid scintillation counter.

RESULTS

As described elsewhere,[7,11,15] the brain protein kinase C was resolved into three major fractions, Type I, II, and III upon a hydroxyapatite column chromatography. Table I shows the relative distribution of the enzyme activity among the subspecies present in several regions of central nervous tissue and some other tissues. The results show the variability of the expression pattern in each tissues examined. In particular, Type I enzyme encoded by γ-subspecies was detected only in central nervous tissue including whole brain, cerebral cortex, cerebellum, and spinal cord. Any of the other tissues so far tested contained no Type I enzyme. The enzyme subspecies isolated from rat brain

Table 1 Distribution of protein kinase C activity among the enzyme subspecies isolated from various tissue preparations.

Tissue	Protein kinase C activity (% total)		
	Type I	Type II	Type III
Whole brain	26	49	25
Cerebral cortex	20	63	17
Cerebellum	52	34	14
Spinal cord	3	50	47
Liver	-	31	69
Spleen	-	68	32
Kidney	-	18	82

Table 1 Tissue samples were homogenized in 20 mM Tris-HCl at pH 7.5 containing 0.25 M sucrose, 10 mM EGTA, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 20 μg/ml leupeptin, and centrifuged at 100,000 x g for 60 min. The supernatant was applied to a DE-52 column, followed by resolution of the enzyme subspecies on a hydroxyapatite column connected to an HPLC system (Pharmacia). Protein kinase activity was assayed with 0.3 mM $CaCl_2$, 8 μg/ml phosphatidylserine, and 0.8 μg/ml 1,2-diolein as described under "EXPERIMENTAL PROCEDURES."

required phospholipid for their catalytic activity, and phosphatidylserine was the most active at physiologically low concentrations of Ca^{2+}. Figure 1 shows the effect of phospholipids on protein kinase C activity in various concentrations of Ca^{2+}. At the high concentrations of Ca^{2+} (3.0×10^{-4}M), phosphatidylethanol and phosphatidylmethanol, could activate three types of protein kinase C as well as phosphatidylserine. At the physiological concentrations of Ca^{2+} (3.0×10^{-6}M), phosphatidylserine could activate all types of protein kinase C significantly, however phosphatidylethanol and phosphatidylmethanol could replace the effect of phosphatidylserine only for Type I enzyme, and Type II, and III enzyme could not be activated by phosphatidylethanol and phosphatidylmethanol at the lower concentrations of Ca^{2+}. At physiological Ca^{2+} concentrations, phosphatidylserine could be replaced by phosphatidylethanol and phosphatidylmethanol in activating only Type I enzyme of protein kinase C, and these phosphatidylalcohols were less effective for Type II, and III enzyme activation at this Ca^{2+} level. Neither ethanol nor methanol affected the enzyme activation at comparable concentrations (data

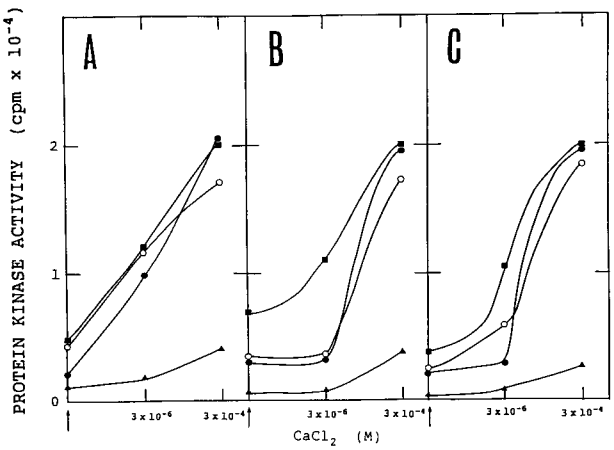

Figure 1 The activation of three types of protein kinase C by various concentrations of Ca^{2+}. Protein kinase C was assayed with 0.8 μg/ml 1,2-diolein, 4 μg/ml of each phospholipid and various concentrations of Ca^{2+}, as described under "EXPERIMENTAL PROCEDURES." A, Type I; B, Type II; C, Type III.
(●), phosphatidylethanol; (○), phosphatidylmethanol; (■), phosphatidylserine; (▲), phosphatidylethanolamine. Where indicated by arrows, EGTA (3 mM) was added instead of Ca^{2+}.

not shown). Figure 2 shows the further evidence of the type-specific activation of protein kinase C by phosphatidylalcohols. At fixed Ca^{2+} concentrations of its physiological level, phosphatidylserine activated three types of protein kinase C dose dependently. Activation of Type I enzyme by phosphatidylserine could be replaced by phosphatidylethanol and, to lesser extent, by phosphatidylmethanol. Phosphatidylalcohols were ineffective on Type II and III activation even at the high concentrations. In the absence of diacylglycerol, this Type I enzyme-specific activation by phosphatidylalcohols and phosphatidylserine was not observed (Figure 3).

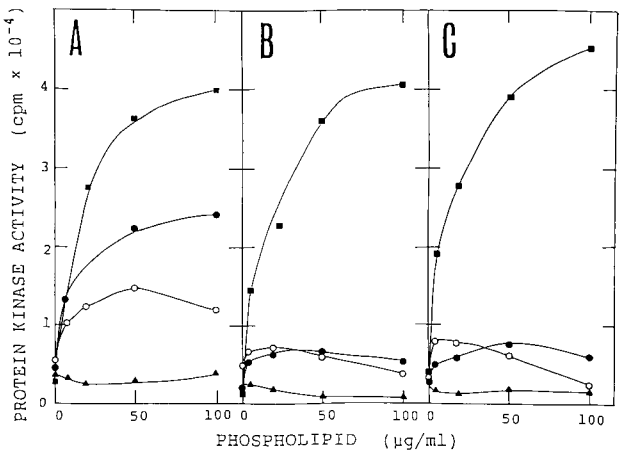

Figure 2 The activation of three types of protein kinase C by various concentrations of phospholipids. Protein kinase C was assayed with 3 μM $CaCl_2$, 0.8 μg/ml 1,2-diolein, and various concentrations of each phospholipid as described under "EXPERIMENTAL PROCEDURES." A, Type I; B, Type II; C, Type III.
(●), phosphatidylethanol; (○), phosphatidylmethanol; (■), phosphatidylserine; (▲), phosphatidylethanolamine.

DISCUSSION

Phospholipase D of plant origin was reported as early as 1967 by Dawson,[5] and Yang et al.[18] to catalyze the phosphatidyltransferase reaction of phosphatidylcholine. Mammalian phospholipase D has been subsequently shown to produce phosphatidylethanol,[6,12] and the brain synaptosomal membrane possesses the highest activity. Although, ethanol is one of the most common psychotropic agents, the mechanism of its behavioral and neurolog-

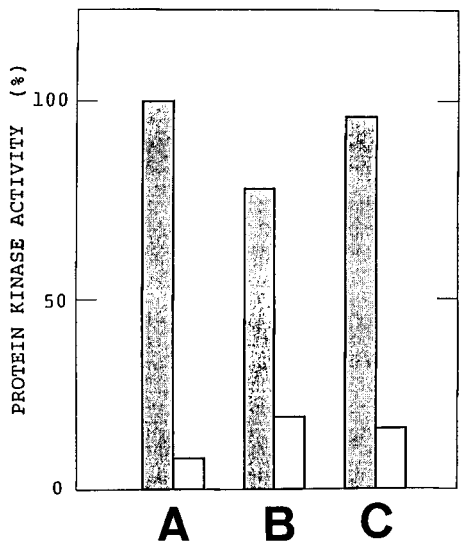

Figure 3
Effect of 1,2-diolein on Type I protein kinase C. Type I protein kinase C was assayed with 3 μM $CaCl_2$, 4 μg/ml of each phospholipid, and with (shaded bar) or without (open bar) 0.8 μg/ml 1,2-diolein, as described under "EXPERIMENTAL PROCEDURES." A, phospatidylserine; B, phosphatidylethanol; C, phosphatidylmethanol.

ical effects remains to be clarified. At present, it is generally accepted that ethanol exerts its biological effects on the lipids of the cell membrane.[17] It was only recent that the formation of phosphatidylethanol by the reaction catalyzed by phospholipase D was in fact demonstrated in various tissues from ethanol-treated rats.[1,2,3] This study suggests that this species of phospholipids can substitute for phosphatidylserine to activate protein kinase C at high Ca^{2+} concentrations, and that in physiological Ca^{2+} concentrations only central nervous tissue-specific Type I enzyme can be activated by phosphatidylalcohols dose dependently. The presence of diacylglycerol is essential for this activation. A possibility arising to further study, therefore, is that phosphatidylethanol, a product of transphosphatidyl reaction of exogenous ethanol in central nervous tissue may modulate the signal transduction through chnages in protein phosphorylation by activating Type I enzyme of protein kinase C. Additional members of the protein kinase C family has been isolated.[14] It is also necessary to investigate the effects of phosphatidylethanol on the new members of this enzyme family.

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