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PROTEIN KINASE C AND DOPAMINE RELEASE—I

MEASUREMENT BY THIOPHOSPHORYLATION

CECILIA T. GIAMBALVO*

Rhode Island Psychiatric Research and Training Center, Institute of Mental Health, Cranston, RI; Neurobiology Section, Division of Biology and Medicine, Brown University, Providence, RI; and Department of Pharmacology, College of Pharmacy, University of Rhode Island, Kingston, RI, U.S.A.

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Abstract—To examine the hypothesis that protein kinase C (PKC) plays a role in the release of dopamine (DA) in the nigrostriatal pathway, a new thiophosphorylation procedure was developed to monitor PKC activity. In this method, tissues were incubated with adenosine 5′-[γ-thio^{35}S]triphosphate, and the transfer of the γ-thiophosphoryl group to histones or endogenous substrate proteins was measured. The thiophosphorylation showed a marked dependency on both calcium and lipids, and the endogenous substrate proteins being thiophosphorylated were similar to those reported as being specific substrates of PKC using [^{32}P]ATP. Furthermore, the thiophosphorylation activity measured in the presence of calcium and lipids did not reflect cAMP-dependent or calmodulin-dependent protein kinase activities. Besides providing an accurate measure of PKC activity, thiophosphorylation has the advantage that it measures a phosphorylating activity that is independent of phosphatase activity because the thiophosphorylated substrates are resistant to the action of phosphatases.

The molecular mechanisms underlying the release of neurotransmitters is not known [1]. One current hypothesis relates to phosphorylation of proteins, including synapsin I [2] and tubulin [3], which are associated with synaptic vesicles. The calmodulin-dependent and the calcium, lipid-dependent protein kinase may be involved since the depolarization-induced increase in protein phosphorylation in synaptosomes can be blocked by neuroleptics [4], drugs that can inhibit both kinase activities *in vitro* [5].

Protein kinase C (PKC) is a calcium and phospholipid requiring enzyme [6] that has a high specific activity in brain tissues [7]. Its role in the transduction of extracellular signals into the cell was initially based on the observation that stimulation of platelets with thrombin led to a breakdown of membrane phosphatidylinositides and the production of diacylglycerol. The latter activated PKC activity, leading to the phosphorylation of a 40K protein and the subsequent release of serotonin [8]. A similar cascade of events for the release of other neurotransmitters has been postulated [9]. Indirect evidence for this hypothesis comes from the ability of phorbol esters to enhance the release of dopamine, norepinephrine and acetylcholine from neuronal cultures and striatal slices *in vitro* [10–12]. To examine this hypothesis *in vivo*, a new thiophosphorylation procedure was developed.

Thiophosphorylation is the transfer of the γ-thiophosphoryl group from adenosine 5′-[γ-thio^{35}S]triphosphate to substrate proteins by protein kinases. Both cAMP-dependent kinase and phosphorylase kinase have been shown to be capable of thiophosphorylating muscle proteins to the same extent as ATP albeit at a slower rate [13]. The advantage of thiophosphorylation over phosphorylation is that the thiophosphorylated proteins are resistant to the action of phosphatases, such that one can directly measure phosphorylation activity rather than a net balance of phosphorylation and dephosphorylation activities.

METHODS

Male, Sprague–Dawley rats (300 g) were killed, and the striata were dissected and homogenized in piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES) buffer (20 mM, pH 6) containing 20 mM $MgCl_2$ and 1 mM ouabain. The homogenate was centrifuged at 15,000 *g* for 20 min. The supernatant fraction was used as the source of soluble PKC activity. The pellet was rinsed twice with 3 mM ethyleneglycolbis (amino-ethylether)tetra-acetate (EGTA) (pH 7), resuspended in buffer, and then used as the source of particulate PKC activity.

PKC activity was determined by the thiophosphorylation of histones (type III S from calf thymus) with [^{35}S]ATP (DuPont, NEN). Tissue fractions (0.05 to 0.1 mg protein) were incubated at 30° for 3 min with the addition of various concentrations of calcium (5–150 μM), phosphatidylserine (PS, 3–75 μg/ml), diolein (0.3–7.5 μg/ml), histones (17 μM), and ATPγS (40 μM, 15 Ci/mol) in a final volume of 0.25 ml. The reaction was terminated by the addition of 2 ml of 25% trichloroacetic acid (TCA). The precipitated proteins were collected on Whatman GFB filters, rinsed three times with 1.25% TCA, and then

* Correspondence: Dr. C. T. Giambalvo, RI Psychiatric Research and Training Center, Institute of Mental Health, Louis Pasteur Building, 4th Floor, Cranston, RI 02920.

counted in a liquid scintillation counter. Blanks, prepared by adding TCA to the tissues prior to the addition of radioactive ATP, were subtracted from all the readings to calculate the net phosphorylation activity, which was expressed as pmol thiophosphate transferred per min per mg tissue. Basal activity was measured by incubating the tissues without added calcium, PS or diolein. PKC activity was determined by subtracting the basal activity from the total phosphorylation activity. This represented a 2- to 3-fold increase in cpm above the basal activity.

The cAMP-dependent protein kinase and the calmodulin-dependent protein kinase activities were determined in particulate membrane fractions under the same conditions as for measuring the calcium/lipid-dependent protein kinase activity except for the inclusion of cAMP (10 μM) in the presence or absence of EGTA (0.5 mM), or calmodulin (500 units) with calcium (15 μM) in the incubation medium. The cAMP-dependent activity was calculated as the net activity stimulated by cAMP in the presence of EGTA. The calmodulin-dependent activity was calculated as the stimulation by calmodulin above basal activity with no added calmodulin.

Phosphatase activity was determined by the addition of an 80–fold excess of unlabelled ATPγS to the incubation medium after a 4-min incubation period, and then following the decline in radioactivity with time. The large excess of unlabeled ATP would have greatly diminished the contribution of radioactivity from the phosphorylation process, thus allowing one to measure the decrease in radioactivity with time as an index of the de-phosphorylation activity.

Endogenous protein phosphorylation was carried out by incubating the soluble and particulate tissue fractions isolated from the striatum and containing 1 mg/ml protein for 3 min at 30° with calcium (5–150 μM), PS (3–75 μg/ml) and diolein (0.3–7.5 μg/ml) or calcium and calmodulin (150–500 units), and ATPγS (40 μM, 0.022 mCi/ml) in a final volume of 72.5 μl. The reaction was terminated by addition of 25 μl of a stop solution containing 9% sodium dodecyl sulfate (SDS), 15% glycerol, 0.5 mg/ml bromphenol blue, and 50 mM Tris, pH 6.8. The homogenate was boiled for 4 min. Mercaptoethanol (2.5 μl) was added to the cooled samples which then sat overnight. SDS-polyacrylamide gel electrophoresis of the phosphorylated samples was carried out on 12% SDS gels (60 μm/lane). After completion of electrophoresis, the gels were stained with Coomassie brilliant blue, destained, treated with Enhance (New England Nuclear), and then dried under vacuum. The dried gels were exposed to Kodak XRP-1 film for 1 week with the aid of Cronex Hi-Plus intensifying screens (Dupont).

RESULTS

Protein kinase activity was analyzed by the thiophosphorylation of exogenous histones with ATPγS in the presence of calcium and lipids. The reaction was linear for at least 5 min of incubation and was linear within the tissue concentration of 0.5 to 3.0 mg (Fig. 1). The apparent K_m for ATPγS was calculated

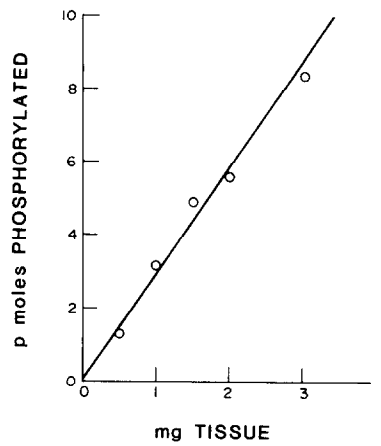

Fig. 1. Changes in PKC activity with tissue concentration. Various amounts of particulate tissues were incubated with histones, calcium, lipid and ATPγS and analyzed for PKC activity as described in the text. Results are expressed as picomoles of proteins being thiophosphorylated per minute.

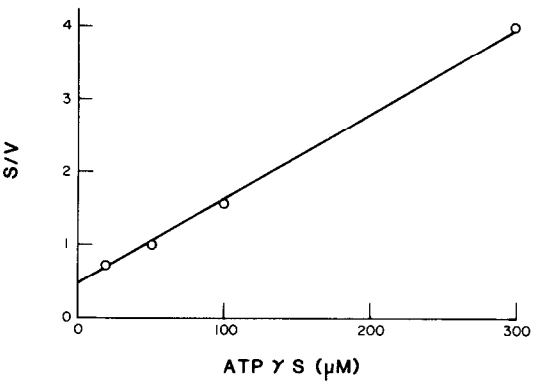

Fig. 2. Kinetic plot of PKC for ATPγS. Particulate tissues were incubated with histones, calcium, lipid and various concentrations of ATPγS for 3 min as described in the text. Results are expressed as substrate concentration (S; μM ATPγS) versus $S/V \times 10^{-1}$; V is the enzyme activity in picomoles per minute per milligram of tissue.

Table 1. Effect of pH on PKC activity

$[Ca^{2+}]$ (μM)	Soluble PKC (cpm)		Particulate PKC (cpm)	
	pH 6	pH 7.4	pH 6	pH 7.4
5	361	206	519	232
15	490	303	835	316
50	636	405	911	495
150	1060	456	920	540

Tissues were separated into soluble and particulate fractions and were analyzed for calcium- and lipid-dependent activity as described in the text. The phosphorylating activity represents the mean of three determinations which varied less than 5%.

Table 2. Effect of rinsing particulate membranes with EGTA on PKC activity

$[Ca^{2+}]$ (μM)	PKC activity (pmol/min/mg tissue)		
	No rinse	Rinse 1×	Rinse 2×
5	0.69	1.06	2.43
15	0.86	3.15	4.12
50	1.44	5.33	6.63

Tissue homogenates were centrifuged, and the pellet was either resuspended in buffer or rinsed once or twice with 3 mM EGTA (pH 7) and then resuspended in buffer. Protein kinase C activity was determined in the presence of lipids (10 μg/ml PS, 1 μg/ml diolein) and various concentrations of calcium as described in the Methods.

to be 39 ± 3 μM and the V_{max} was 10 ± 0.2 pmol/min/mg tissue (Fig. 2). The apparent K_m for histones was 0.65 ± 0.20 μg/ml.

The thiophosphorylating activity of the soluble and particulate protein kinase was dependent on the pH of the incubation medium (Table 1). Activity was higher at pH 6 than at pH 7.4 for any concentration of calcium examined. Moreover, the effects of the drug treatments were qualitatively the same when the tissues were incubated at either pH. For this reason, pH 6 was used for all subsequent experiments.

Particulate membrane fractions were found to have a higher activity if they had been pretreated with EGTA (Table 2). Rinsing the tissue once with EGTA resulted in a 3-fold increase in activity (at 15 μM calcium). Further rinsing with EGTA increased the activity by another 31%. Thus, all tissues were rinsed twice with EGTA prior to analysis.

The thiophosphorylation represents PKC activity since it showed a marked dependency on both calcium and lipids. Double-reciprocal plots (Figs. 3 and 4) showed that the soluble and particulate PKC activities were increased with an increase in the amount of calcium added to the incubation medium. Moreover, the presence of diolein decreased the K_m for calcium in a dose-dependent manner. In the soluble fraction, the K_m values were 83, 14, 7 and 2 μM in the presence of 0, 0.75, 2.5 and 7.5 μg/ml of diolein respectively; in the particulate fraction, the K_m values were 36, 8 and 2 μM in the presence of 0.25, 0.75 and 2.5 μg/ml of diolein respectively. The V_{max} was not changed.

The endogenous proteins that were thiophosphorylated were very similar to those reported as specific substrates for PKC [14]. SDS-polyacrylamide electrophoresis, followed by autoradiography, showed that the following proteins in the soluble fraction were thiophosphorylated: 95K, 73K, 58K, 47K, 45K, 41K, 21K and 17K (Fig. 5). Of these, the 47K, 73K and 87K proteins showed a marked increase in thiophosphorylation in the presence of calcium and lipids. In the particulate fraction, the following proteins were thiophosphorylated: 73K, 62K, 51K, 45K, 21K and 17K (Fig. 6). The 17K and 21K proteins showed an especially marked dependence on both calcium (Fig. 6, left panel) and lipids (Fig. 6, right panel). Calcium or lipid alone did not show a dose-dependent increase in thiophosphorylation of these protein bands.

The thiophosphorylation measured in the presence of calcium and lipid does not reflect calmodulin-dependent protein kinase activity. The preferred substrates being phosphorylated in the presence of calmodulin were the 51K and 62K proteins which were not affected by the addition of calcium and lipids. On the other hand, the presence of calcium and lipids greatly stimulated the phosphorylation of the 17K and 21K proteins (Fig. 7), even though the latter were also being phosphorylated by calmodulin.

The thiophosphorylation also does not reflect cAMP-dependent protein kinase activity. The presence of calcium inhibited the phosphorylation

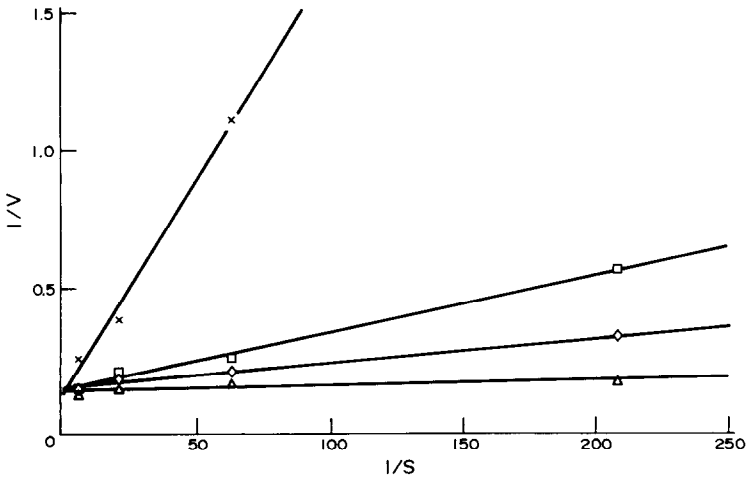

Fig. 3. Effect of lipids on the kinetic parameters of soluble PKC for calcium. Tissues were incubated with histones and ATPγS and various amounts of calcium in the absence of lipids (×—×), or in the presence of lipids: 1 μg/ml diolein and 10 μg/ml PS (□—□), 3 μg/ml diolein and 30 μg/ml PS (◇—◇), or 9 μg/ml diolein and 90 μg/ml PS (△—△). Results are depicted in a double-reciprocal plot of $1/S$ vs $1/V$, where S is the calcium concentration in micromolar and V the activity in picomoles per minute per milligram of tissue. Each point is the mean of three determinations which vary less than 5%.

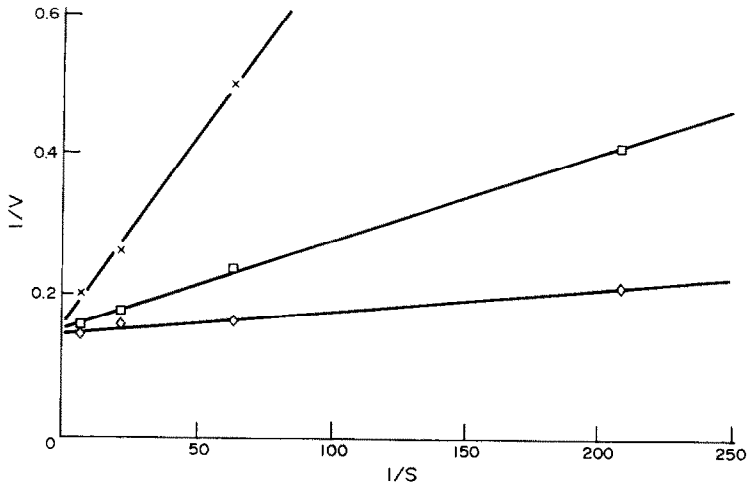

Fig. 4. Effect of lipids on the kinetic parameters of particulate PKC for calcium. Tissues were incubated with histones and ATPγS and various concentrations of calcium in the presence of various amounts of lipids: 0.3 μg/ml diolein and 3 μg/ml PS (×—×), 1 μg/ml diolein and 10 μg/ml PS (□—□), or 3 μg/ml diolein and 30 μg/ml PS (◇—◇). Results are depicted in a double-reciprocal plot of $1/S$ vs $1/V$ as described in the legend of Fig. 3.

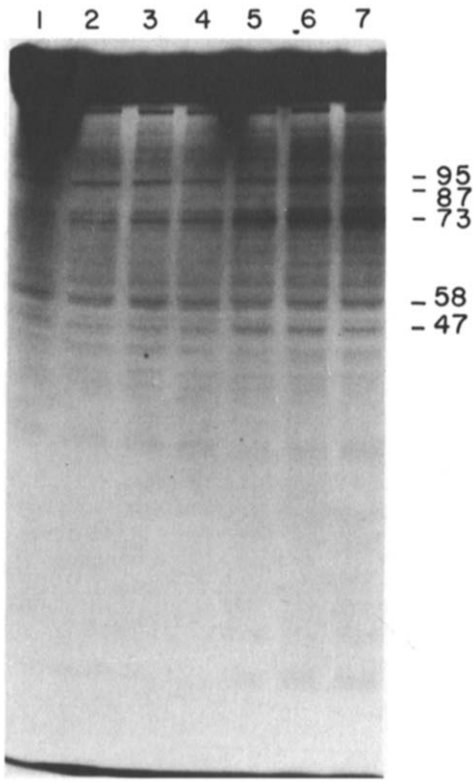

Fig. 5. Thiophosphorylation of endogenous substrate proteins by soluble PKC. Thiophosphorylation was carried out as described in the text. The conditions for the various lanes were as follows: 1, contained 0.5 mM EGTA and no added Ca^{2+} or lipid; 2, contained no Ca^{2+} or lipid; 3, with 5 μM Ca^{2+}; 4, with 15 μM Ca^{2+}; 5, with 30 μg/ml PS and 3 μg/ml diolein; 6, with 5 μM Ca^{2+}, 30 μg/ml PS and 3 μg/ml diolein; 7, with 15 μM Ca^{2+}, 30 μg/ml PS and 3 μg/ml diolein. The numbers on the right indicate the $M_r \times 10^{-3}$.

induced by cAMP, which can be stimulated by the addition of EGTA (Table 3). Inclusion of protein kinase inhibitor also did not affect the phosphorylating activity stimulated by calcium and lipid.

Thiophosphorylated proteins were resistant to phosphatase activity (Fig. 8). Addition of an 80-fold excess of unlabeled ATPγS, after the initial incubation to dilute the radioactivity, did not lead to a time-dependent decline in the amount of [^{35}S]thiophosphate incorporated for at least 24 min.

DISCUSSION

Previous studies have shown that various protein kinases, including the cAMP-dependent protein kinase, the phosphorylase kinase and the myosin light chain kinase, can utilize ATPγS effectively as a substrate [13, 15]. The rate of thiophosphorylation is slower than that using [^{35}P]ATP, but the extent of the reaction and final level of activation of the

Table 3. Effects of calcium on cAMP-dependent phosphorylation and of protein kinase inhibitor on calcium/lipid-dependent phosphorylation

Treatment	Phosphorylating activity
cAMP	1.20 ± 0.27
cAMP + Ca^{2+}	1.07 ± 0.10
cAMP + EGTA	4.20 ± 0.81
Ca^{2+} + lipid	5.47 ± 0.64
Ca^{2+} + lipid + PKI	5.40 ± 0.79

Protein kinase activity was determined in the presence of calcium (15 ±M), lipids (1 μg/ml diolein, 10 μg/ml PS), protein kinase inhibitor (PKI, 40 units/ml), cAMP (10 μM) or EGTA (0.5 mM) as described in the text. Results are expressed as pmol phosphorylated above basal activity (with no added Ca^{2+} or lipid) per min per mg tissue.

Values are means ± SEM; N = three experiments. When cAMP was added in the presence of EGTA, the cAMP-dependent activity was obtained by subtracting the total activity from that due to addition of EGTA alone.

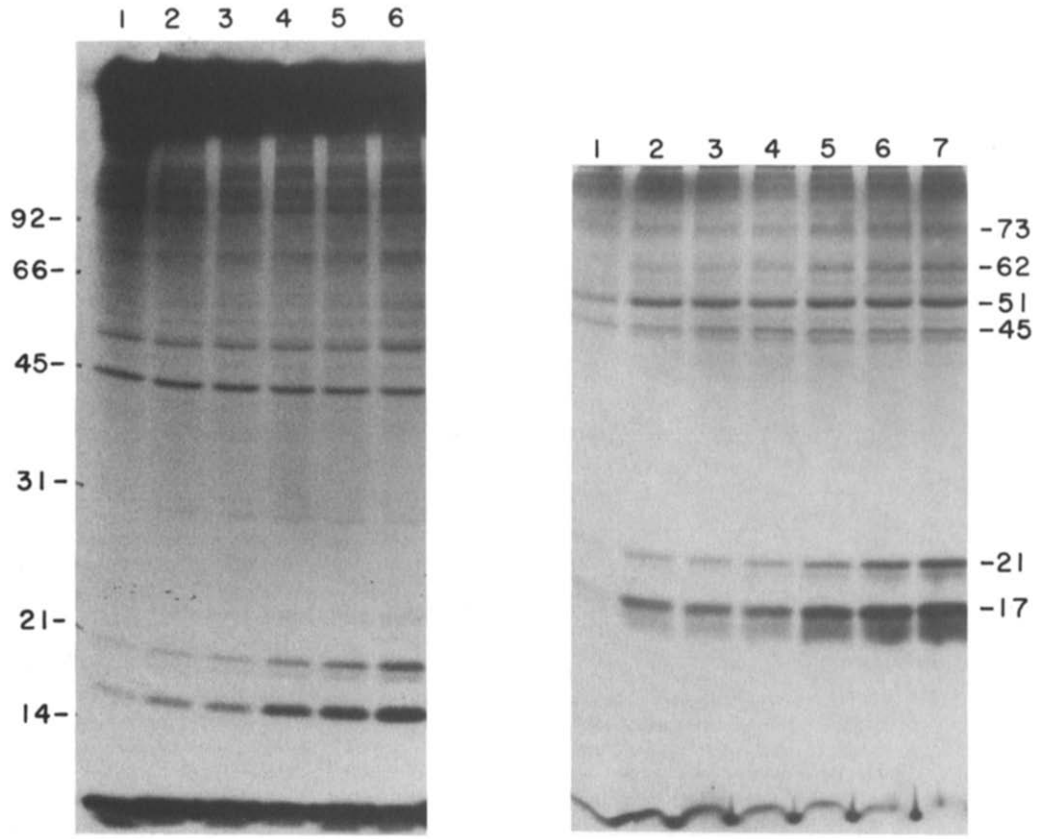

Fig. 6. Thiophosphorylation of endogenous substrate proteins by particulate PKC. Thiophosphorylation was carried out as described in the text. The amount of phosphorylation was increased with increases in the concentration of Ca^{2+} (left panel) or lipids (right panel). The conditions in the various lanes were as follows: (left panel) lane 1, with 0.5 mM EGTA; 2, with no Ca^{2+} or lipid; 3, with 5 μM Ca^{2+}; 4, with 3 μg/ml diolein and 30 μg/ml PS; 5, with 5 μM Ca^{2+}, 3 μg/ml diolein and 30 μg/ml PS; 6, with 15 μM Ca^{2+}, 3 μg/ml diolein and 30 μg/ml PS. The numbers on the left of the panel indicate the $M_r \times 10^{-3}$ of standard molecular weight markers. (Right panel) lane 1, with 0.5 mM EGTA, 2, with 0.3 μg/ml diolein and 3 μg/ml PS; 3, with 0.6 μg/ml diolein and 6 μg/ml PS; 4, with 2 μg/ml diolein and 20 μg/ml PS. Lanes 5–7 were the same as 2–4 except that they also included 50 μM Ca^{2+}. The numbers on the right of the panel indicate the $M_r \times 10^{-3}$.

enzyme are the same. Similarly, in this current study, it was shown that ATPγS can also be utilized by PKC. The K_m of PKC for ATPγS was higher than that for ATP (40 vs 4 μM), but the total activity was similar to that reported using ATP [16]. To compensate for the slower rate, the reaction was carried out for 3 min in this study, whereas 10- to 30-sec incubation periods were sufficient to label endogenous substrate proteins in other studies [17, 18]. In contrast to these findings, it has been suggested that ATPγS is not a very good substrate for measuring PKC activity [19]. The reason for this apparent discrepancy is not clear but may be related to differences in experimental conditions. For example, in this study, the reaction was done at pH 6 instead of pH 7.4. The activity was higher at pH 6 (Table 1), and since the changes in enzyme activity following pharmacological challenges with drugs were qualitatively similar at either pH, analyses at pH 6 would yield more accurate results. Another difference was in the pretreatment of tissues with EGTA, which increased the activity several fold (Table 2). This effect of EGTA may be due to an inactivation of calcium-dependent proteases which may degrade the enzyme, or to a depletion of endogenous calcium which is inhibitory at high concentrations. An increase in the calmodulin-dependent protein kinase activity by EGTA pretreatment has also been reported [20].

The nature of the calcium and lipid dependency of the thiophosphorylation reaction suggests that it was catalyzed by protein kinase C. Using histones as the substrate, an increase in the calcium concentration led to an increase in thiophosphorylation activity. Furthermore, addition of diolein and phosphatidylserine led to a dose-dependent decrease in the K_m for calcium (Figs. 3 and 4), which was very similar to that reported in the literature [19].

The increase in total thiophosphorylation was reflected in the individual substrate proteins. The 47K and 87K proteins in the cytoplasmic fraction, and the 17K and 21K proteins in the particulate fraction, known to be specific substrates for PKC [14, 17, 21], were thiophosphorylated in the presence of calcium and lipid in a dose-dependent manner not seen with calcium or lipid alone.

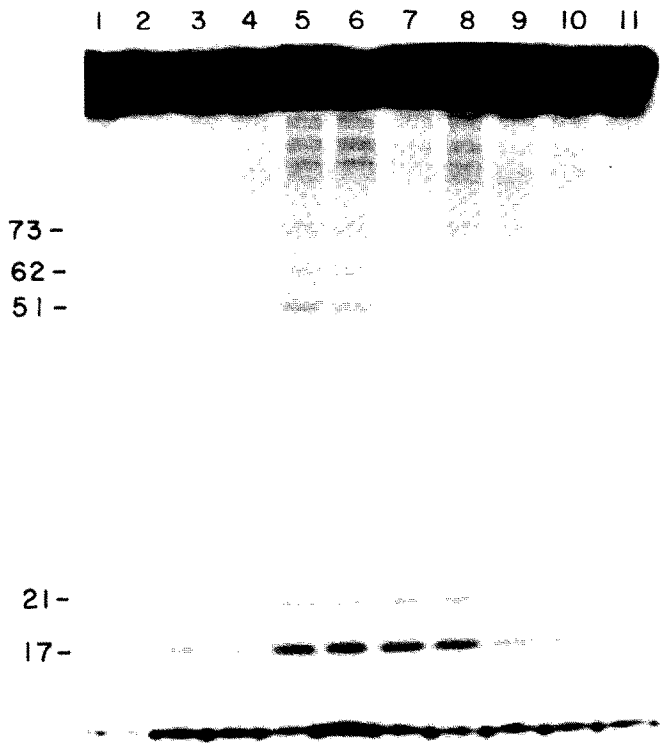

Fig. 7. Endogenous thiophosphorylation of particulate substrate proteins by Ca^{2+}/lipid and calmodulin-dependent protein kinases. The reactions were carried out as described in the text. The conditions for the various lanes are as follows: 1, with 0.5 mM EGTA; 2 and 11, with no added Ca^{2+}, lipid or calmodulin (CaM); 3 and 10, with 15 μM Ca^{2+}; 4 with 500 units of CaM; 5 and 6, with 15 μM Ca^{2+} and CaM; 7 and 8, with 15 μM Ca^{2+}, 3 μg/ml diolein and 30 μg/ml PS; 9, with 3 μg/ml diolein and 30 μg/ml PS. The numbers on the left indicate the $M_r \times 10^{-3}$.

The substrate specificity makes it unlikely that calmodulin-dependent kinase was mediating the thiophosphorylation seen in the presence of calcium and lipids. Addition of calmodulin selectively increased the thiophosphorylation of the 51K and 62K proteins (Fig. 7) [14], while addition of calcium and lipid increased the thiophosphorylation of the 17K and 21K proteins. It is unlikely that the thiophosphorylation was mediated by the cAMP-dependent protein kinase since calcium was inhibitory to the activity of this kinase (Table 3) [22]. However, the possibility that there are other calcium- and lipid-dependent protein kinases, besides PKC, that were responsible for the thiophosphorylating activity cannot be eliminated. As this manuscript was being completed, a report appeared showing that thiophosphorylation was just as effective as phosphorylation with [^{32}P]ATP in labeling the endogenous substrate proteins of PKC in adrenocortical membrane preparations [23].

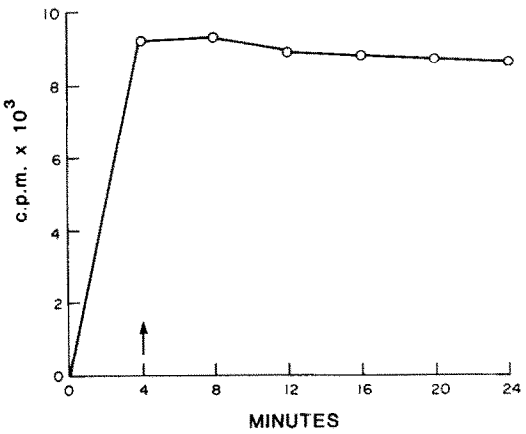

Fig. 8. Effect of phosphatase on the thiophosphorylated proteins. Tissues were incubated with histones and ATPγS (40 μM) for 4 min. An excess of unlabled ATPγS (3 mM) was then added (as indicated by the arrow) and incubation continued for another 20 min. Trichloroacetic acid was then added, and the radioactivity of the tissue precipitate counted. Results are expressed as counts per minute.

Thiophosphorylation provides a convenient and safe alternative to phosphorylation studies using [^{32}P]ATP. In addition, ^{35}S has a longer half-life (87 days). More importantly, it has the advantage that thiophosphorylated proteins are resistant to the action of phosphatases (Fig. 5). This allows one to measure kinase activity directly rather than the net balance of phosphorylation and dephosphorylation by protein kinase and phosphatase that is measured when ATP is used as the substrate.

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REFERENCES

1. Cooper JR and Meyer EM, Possible mechanisms involved in the release and modulation of release of neuroactive agents. *Neurochem Int* **6**: 419–433, 1984.
2. Nestler EJ and Greengard P, Protein phosphorylation in the brain. *Nature* **305**: 583–588, 1983.
3. Burke BE and DeLorenzo RJ, Ca^{2+} and calmodulin-dependent phosphorylation of endogenous synaptic vesicle tubulin by a vesicle-bound calmodulin kinase system. *J Neurochem* **38**: 1205–1218, 1982.
4. Robinson RJ, Jarvie PE and Dunkley PR, Depolarization dependent protein phosphorylation in rat cortical synaptosomes is inhibited by fluphenazine at a step after calcium entry. *J Neurochem* **43**: 659–667, 1984.
5. Mori T, Takai Y, Minakuchi R, Yu B and Nishizuka Y, Inhibitory action of chlorpromazine, dibucaine and other phospholipid-interacting drugs on calcium-activated, phospholipid-dependent protein kinase. *J Biol Chem* **255**: 8378–8380, 1980.
6. Takai Y, Kishimoto A, Iwasa Y, Kawahara Y, Mori T and Nishizuka Y, Calcium-dependent activation of a multifunctional protein kinase by membrane phospholipids. *J Biol Chem* **254**: 3692–3695, 1979.
7. Kuo JF, Anderson RGG, Wise BC, Mackerlova L, Salomonsoon I, Brackett NL, Katoh N, Shojiu M and Wrenn RW, Calcium-dependent protein kinase: Widespread occurrence in various tissues and phyla of the animal kingdom and comparison of effects of phospholipids, calmodulin and trifluoperazine. *Proc Natl Acad Sci USA* **77**: 7039–7043, 1980.
8. Sano K, Takai Y, Yamanishi J and Nishizuka Y, A role of calcium-activated phospholipid-dependent protein kinase in human platelet activation. Comparison of thrombin and collagen actions. *J Biol Chem* **258**: 2010–2013, 1983.
9. Nishizuka Y, Turnover of inositol phospholipids and signal transduction. *Science* **225**: 1365–1370, 1980.
10. Zurgil N and Zisapel N, Phorbol ester and calcium act synergistically to enhance neurotransmitter release by brain neurons in culture. *FEBS Lett* **185**: 257–261, 1985.
11. Matthies HJG, Palfrey HC, Hirning LD and Miller RJ, Down regulation of protein kinase C in neuronal cells: Effects on neurotransmitter release. *J Neurosci* **7**: 1198–1206, 1985.
12. Tanaka C, Fujiwara H and Fujii Y, Acetylcholine release from guinea pig caudate slices evoked by phorbol esters and calcium. *FEBS Lett* **195**: 129–134, 1986.
13. Gratecos D and Fischer EH, Adenosine 5′-*O*(3-thiotriphosphate) in the control of phosphorylase activity. *Biochem Biophys Res Commun* **58**: 960–967, 1974.
14. Wrenn RW, Katoh N, Wise BC and Kuo JF, Stimulation by phosphatidylserine and calmodulin of calcium-dependent phosphorylation of endogenous proteins from cerebral cortex. *J Biol Chem* **255**: 12042–12046, 1980.
15. Cassidy P, Hoar PE and Kerrick WGL, Irreversible thiophosphorylation and activation of tension in functionally skinned rabbit ileum strips by [^{35}S]ATPγS. *J Biol Chem* **254**: 1148–1153, 1974.
16. Turner RS, Raynor RL, Mazzei GJ, Girard PR and Kuo JF, Developmental studies of phospholipid-sensitive Ca^{2+}-dependent protein kinase and its substrates and of phosphoprotein phosphatases in rat brain. *Proc Natl Acad Sci USA* **81**: 3143–3147, 1984.
17. Wu WCS, Walaas SI, Nairn AC and Greengard P, Calcium phospholipid regulates phosphorylation of a M_r "87" substrate protein in brain synaptosomes. *Proc Natl Acad Sci USA* **79**: 5249–5253, 1982.
18. Lovinger DM, Colley PA, Akers RF, Nelson RB and Routtenberg A, Direct relation of long-term synaptic potentiation to phosphorylation of membrane protein F1, a substrate for membrane protein kinase C. *Brain Res* **399**: 205–211, 1986.
19. Wise BC, Glass DB, Chou JCH, Raynor RL, Katoh N, Schatzman RC, Turner RS, Kibler RJ and Kuo JF, Phospholipid-sensitive Ca^{2+}-dependent protein kinase from heart. II. Substrate specificity and inhibition by various agents. *J Biol Chem* **257**: 8489–8495, 1982.
20. Lau YS, Increase of calmodulin-stimulated striatal particulate phosphorylation response in chronic haloperidol-treated rats. *Brain Res* **307**: 181–189, 1984.
21. Akers R and Routtenberg A, Kinase C phosphorylates a protein involved in synaptic plasticity. *Brain Res* **334**: 147–151, 1985.
22. Kuo JF, Krueger BK, Sanes JR and Greengard P, Cyclic nucleotide-dependent protein kinases V. Preparation and properties of adenosine 3′,5′-monophosphate-dependent protein kinase from various bovine tissues. *Biochim Biophys Acta* **212**: 79–87, 1979.
23. Coyne MD, Luszczynska HM and Kunzi M, Evidence for protein kinase C in bovine adrenocortical membrane preparations using [^{35}S]thio-ATP as a phosphate donor. *J Cyclic Nucleotide Protein Phosphor Res* **11**: 433–444, 1987.