

Lead Activates Protein Kinase C in Immature Rat Brain Microvessels

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Lead Activates Protein Kinase C in Immature Rat Brain Microvessels. MARKOVAC, J., AND GOLDSTEIN, G. W. (1988). *Toxicol. Appl. Pharmacol.* **96**, 14-23. We investigated the effects of inorganic lead upon calcium-, phospholipid-dependent protein kinase (protein kinase C) in brain microvessels isolated from 6-day-old rat pups. We found that (a) in broken cell preparations, lead at micromolar concentrations activates this enzyme to an extent equivalent to that of micromolar calcium (10.3 ± 1.3 and 9.2 ± 1.6 pmol/mg/min, respectively) and (b) preincubation of intact microvessels with lead results in a translocation of protein kinase C from the soluble to the particulate fraction. The cytosolic kinase activity stimulated by lead has the same requirements for diacylglycerol and phospholipid as the calcium-stimulated enzyme, suggesting that lead activates the kinase by mimicking calcium. The hypothesis that lead affects protein kinase C activity through a mechanism similar to that of calcium is supported by the similar time courses of substrate phosphorylation and dephosphorylation mediated by lead and calcium. When intact microvessels are preincubated with micromolar concentrations of lead, the translocation of protein kinase C occurs in a dose- and time-dependent manner. The relocation is virtually complete at $0.1 \mu\text{M}$ lead and by 30 min of exposure. We propose that the sensitivity of protein kinase C to lead, described here in immature brain microvessels, makes this regulatory enzyme a potential mediator of lead toxicity. © 1988 Academic Press, Inc.

Lead is a toxic metal, widely distributed in the environment, that remains a significant health hazard, particularly to children (Committee on Environmental Hazards and Committee on Accident and Poison Prevention, 1987). The biochemical mechanisms mediating this toxicity are unclear, but in several biologic systems, lead alters calcium-mediated cellular processes (Pounds, 1984; Simons, 1986) and may mimic calcium in binding to regulatory proteins (Habermann and Richardt, 1986). Capillaries in the brain are a major target tissue for the toxic action of lead (Press, 1985). Pathologic studies in children poisoned with lead and in several animal models of lead toxicity reveal a breakdown in the normally tight blood-brain barrier (Goldstein, 1984; Clasen *et al.*, 1974). The formation of the blood-brain barrier is the result of the special structure of brain microvessels (Reese and Karnovsky, 1967) made up of

endothelial cells lacking fenestrae and transcellular channels. The cells are joined together by continuous complex tight junctions which limit transcapillary movement of proteins, organic molecules, and ions (Bradbury, 1985; Betz, 1986). *In vivo* studies demonstrate that lead accumulates in brain microvessels (Toews *et al.*, 1978; Thomas *et al.*, 1973) and markedly increases their permeability, especially in immature animals (Pentschew and Garro, 1966; Goldstein *et al.*, 1974; Roy *et al.*, 1974). We used microvessels isolated from immature rat brain to study the cellular mechanisms involved in this toxic reaction.

Brain microvessels are most vulnerable to lead early in development when they are proliferating (Press, 1977). The toxic injury results in loss of specific differentiated functions and a breakdown of the blood-brain barrier. In several other tissues, lead is known

to alter the metabolism of calcium (Pounds, 1984; Simons, 1986) and we believe that calcium-mediated events are likely cellular targets for the changes in brain capillary permeability induced by lead.

Protein kinase C, a calcium-, phospholipid-dependent enzyme closely associated with signal transduction, phosphorylates critical regulatory proteins and thus modulates cellular proliferation and differentiation (for review, see Nishizuka, 1986a). The enzyme is activated by diacylglycerol, a second messenger transiently produced by receptor-mediated hydrolysis of inositol phospholipids (Sano *et al.*, 1983). An additional second messenger, inositol trisphosphate, also resulting from this hydrolysis, ultimately affects the intracellular mobilization of calcium (Berridge and Irvine, 1984). We chose to investigate the effect of lead on protein kinase C because it is regulated by calcium (Takai *et al.*, 1979) and plays a pivotal role in the control of cellular growth and differentiation. We examined this effect both in broken cell preparations and in intact microvessels isolated from immature rat brain. Here we present evidence that micromolar concentrations of lead (a) stimulate diacylglycerol-activated, phospholipid-dependent protein kinase in cytosolic extracts of rat brain microvessels to an extent equivalent to that of micromolar calcium and (b) cause the translocation of this enzyme from the cytosol into the membrane, in intact microvascular cells, in a dose- and time-dependent manner.

METHODS

Materials. Six day-old Sprague-Dawley rat pups were obtained from Harlan (Haslett, MI). γ -[32 P]ATP (4000 Ci/mmol) was purchased from ICN Biomedicals, Inc. (Irvine, CA). 1,2-Diolein was obtained from P-L Biochemicals (Milwaukee, WI). Type III-S histone, phosphatidyl-L-serine, ATP, and all other reagents were obtained from Sigma (St. Louis, MO). Phosphocellulose paper (P81) was obtained from Whatman (Hillsboro, OR).

Isolation of rat brain microvessels. Microvessels were isolated from cortical hemispheres of 6-day-old Sprague-Dawley rat pups by modification of a technique previously described (Betz and Goldstein, 1981). The ani-

mals were killed by decapitation and the brains rapidly removed and placed in ice-cold Medium 199 buffered with 20 mM *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid (Hepes), pH 7.4 (buffer A). The brainstem, cerebellum, and meninges were discarded and the cortical shells, free of choroid plexus and ependyma, were minced in above buffer. Following homogenization (20 strokes, 390 rpm) in a Teflon/glass homogenizer (0.25 mm clearance), a 10% suspension (w/v) was centrifuged at 1000g for 10 min. To remove myelin and cellular debris, the pellet was suspended to 16% (w/v) in buffer A containing 15% dextran and centrifuged at 4000g for 10 min. The resulting pellet, consisting of free nuclei and capillary segments, was resuspended in buffer A and gently suctioned through a 118- μ m nylon mesh. To isolate the capillaries from nuclear debris, the suspension was subsequently passed over a 1.2 \times 1.5-cm column of 0.25-mm glass beads supported on a 53- μ m nylon mesh and washed extensively with the buffer. The capillary segments, retained on the column, were recovered by gentle agitation of the glass beads in same buffer, decanted, and collected by centrifugation at 500g for 5 min. The quality of each preparation was judged morphologically under phase microscopy (Fig. 1).

Incubation of microvessels. For preincubation with lead, the microvessels, prepared as described above, were divided into the desired number of aliquots, centrifuged at 500g for 5 min, and resuspended in buffer A containing specified concentrations of lead acetate (PbOAc). The capillary suspensions were incubated at 37°C for 45 min, unless otherwise noted. The suspensions were then pelleted by centrifugation at 500g for 5 min and prepared for protein kinase C assay as described below.

Extract preparation. To prepare a cytosolic extract for the assay of protein kinase activity, isolated microvessels were washed twice with ice-cold calcium-free phosphate-buffered saline, pH 7.4, sonicated for 15 sec in buffer containing 20 mM Hepes, pH 7.5, 2 mM ethylenediaminetetraacetic acid (EDTA), 2 mM ethyleneglycol-bis-(β -aminoethyl ether) *N,N,N',N'*-tetraacetic acid (EGTA), 0.25 M sucrose, and 10 mM β -mercaptoethanol (buffer B), and then centrifuged for 1 hr at 40,000g. The resulting supernatant fraction was used for all subsequent experiments except where otherwise noted. When appropriate, the pellets from the 40,000g centrifugation were solubilized in 0.03% Triton X-100 (in buffer B) and assayed for protein kinase C activity in the presence of 10 μ M CaCl₂, as described below.

Assay for protein kinase C. Protein kinase activity was assayed by a modification of the procedure described by Takai *et al.* (1979), measuring the incorporation of radiolabel from [32 P]ATP into endogenous cytosolic protein and exogenous lysine-rich histones. In a total volume of 250 μ l, the standard reaction mixture contained 20 mM Tris-HCl (pH 7.4), 5 mM MgCl₂, 5 μ g phosphatidylserine, 5 μ g 1,2-diolein (or equivalent volume of ddH₂O), 20 μ g lysine-rich histones, 0.2 nmol ATP, 2 μ Ci [32 P]ATP (4000 Ci/mmol), and 10 μ M CaCl₂ or 10 μ M PbOAc (un-

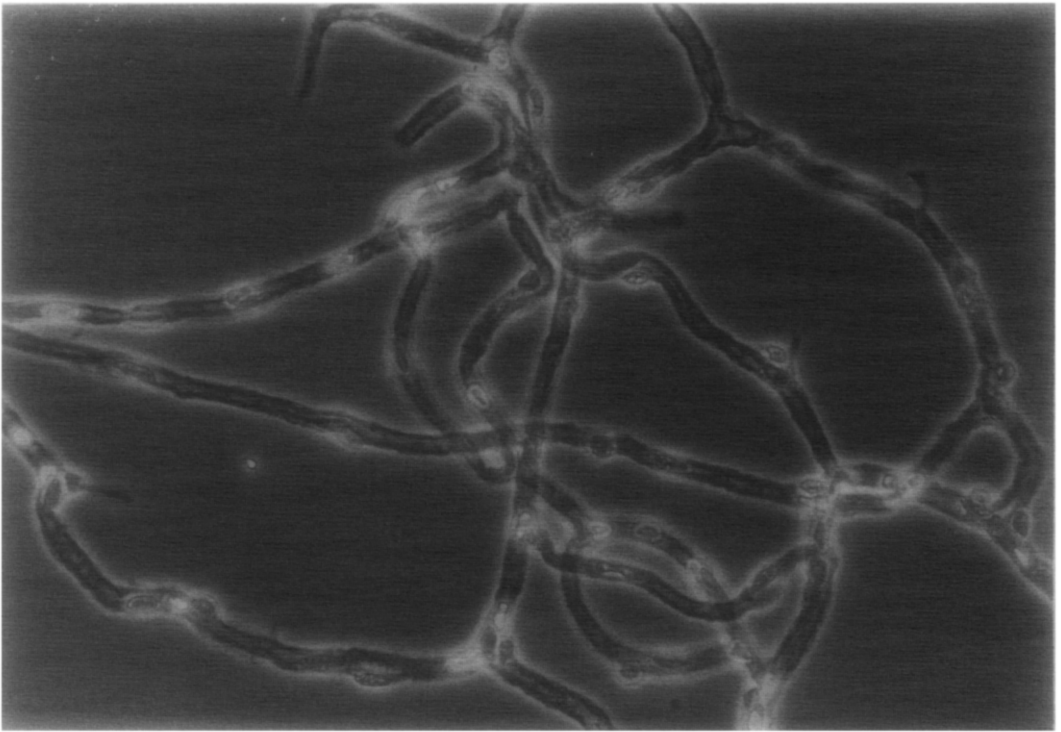


FIG. 1. Isolated immature microvessels. Microvessels were isolated from 6-day-old rats as described under Methods. Light phase contrast micrograph indicates segments of microvessels essentially free of debris ($\times 380$).

less otherwise noted). The reaction was initiated by addition of 100 μ l extract containing 5–15 μ g cytosolic or membranous protein, incubated for 5 min at 30°C, and terminated by addition of 75 μ l of 12 N glacial acetic acid. Phosphorylated proteins were collected by adsorption on P81 phosphocellulose papers. The filters were washed once in 30% acetic acid, twice in 15% acetic acid, and finally in acetone. The radioactivity was quantitated by Cerenkov counting and protein content determined by the method of Bradford (1976). Protein kinase activity was calculated as the difference in the activity in the presence and absence of 1,2-diolein.

RESULTS

The toxic effects of lead are thought, at least in part, to be due to its interference in calcium-mediated cellular function. Because brain capillaries are particularly vulnerable, purified microvessels provide a model system for the study of these effects. We investigated the ability of lead to affect protein kinase C in

(a) broken cell preparations of microvessels isolated from immature rat brain and (b) intact capillary segments.

Protein kinase C, in most tissues other than brain, is found in the cytosolic fraction (Kikawa *et al.*, 1982; Girard *et al.*, 1986). We determined that in immature brain microvessels, this enzyme is recovered mainly from the soluble fraction in contrast with that from 6-day-old whole brain where it is almost completely particulate (data not shown). Thus, all subsequent results using broken cell preparations were obtained with cytosolic fractions. Soluble protein kinase activity in disrupted cellular extracts was measured as diacylglycerol-specific incorporation of radiolabel from [32 P]ATP into exogenous lysine-rich histones and endogenous cytosolic proteins in the presence of lead or calcium.

Figure 2 illustrates the stimulation of soluble protein kinase as a function of the concen-

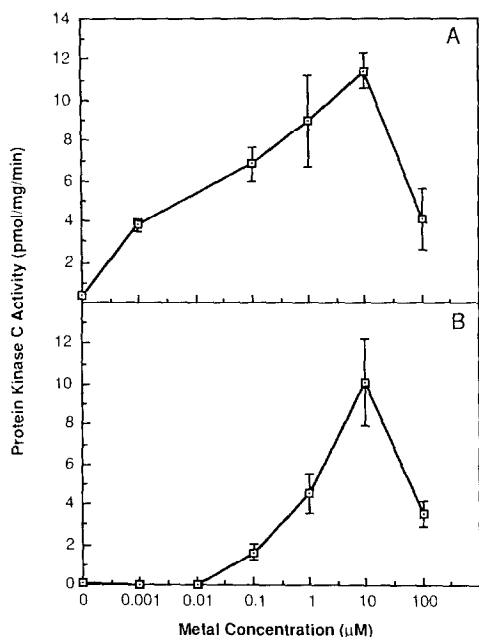


FIG. 2. Dose-dependent stimulation of protein kinase activity by calcium and lead. Cytosolic diacylglycerol-activated, phospholipid-dependent protein kinase was measured in the presence of varying concentrations (0–100 μM) of calcium (A) or lead (B). Values plotted are the means of three replicates \pm SE. Data points without error bars represent SE $<$ 5%. Equivalent results were obtained in three separate experiments.

tration of calcium (Fig. 2A) and lead (Fig. 2B). There was no detectible diacylglycerol-, phospholipid-dependent protein kinase activity in the absence of either cation. At concentrations of less than 1 μM , calcium appears to be more potent in the stimulation of protein kinase activity than lead. However, with increasing concentrations of both calcium and lead, kinase activity increased, peaked at 10 μM , and then declined. The decrease in activity observed in the calcium dose-response curves is thought to reflect a reduced affinity of protein kinase C for diacylglycerol at higher concentrations of calcium (for review, see Nishizuka, 1986a). The similar decline at higher concentrations of lead suggests an analogous mechanism for protein kinase activation.

Because of the apparent difference between calcium and lead in their ability to stimulate

protein kinase activity at lower concentrations (Fig. 2), we investigated the combined effects of these two cations upon protein kinase C. Figure 3 illustrates the effects of 0.1 μM calcium, of 0.1 μM lead, and of lead and calcium together in the stimulation of protein kinase activity. In this experiment, lead and calcium were equally potent in the activation of the enzyme. When lead and calcium were tested together, protein kinase C activity was somewhat greater than in the presence of either cation alone.

We then established that the kinase activity stimulated by 10 μM lead has the same requirements for diacylglycerol as the calcium-stimulated enzyme. With varying amounts of 1,2-diolein (0–30 $\mu\text{g}/\text{ml}$), we found no apparent difference in diacylglycerol-dependent activation of protein kinase between 10 μM calcium and 10 μM lead (Fig. 4). Similar experiments were performed using varying concentrations of phospholipid (phosphatidylserine, 0–25 $\mu\text{g}/\text{ml}$). Again, stimulation with either calcium or lead resulted in no significant difference in the phospholipid dose-dependent activation of protein kinase (data not shown). These results suggest that the cytosolic extracts of protein kinase activity stimulated by micromolar concentrations of lead are similar to if not the same as calcium-, phospholipid-dependent protein kinase (protein kinase C). Thus, lead may be activating this enzyme by mimicking calcium.

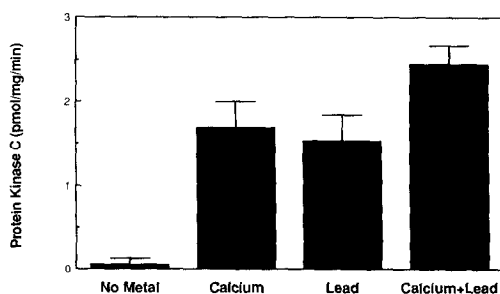


FIG. 3. Effects of 0.1 μM calcium and lead upon protein kinase activity. Cytosolic diacylglycerol-activated, phospholipid-dependent protein kinase was measured in the presence of 0.1 μM calcium, 0.1 μM lead, or both calcium and lead. Values plotted are the means of three replicates \pm SE.

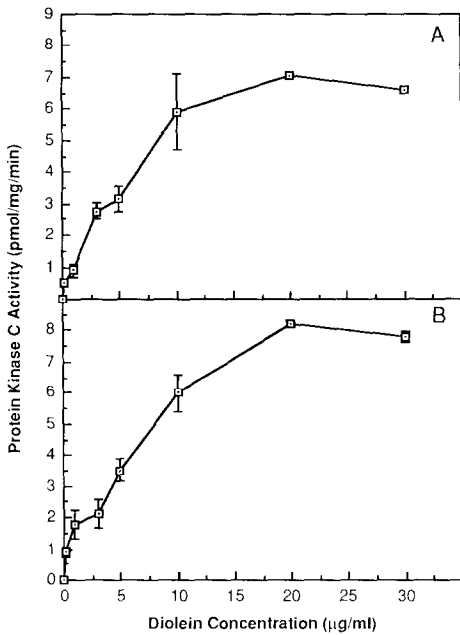


FIG. 4. Diacylglycerol-dependent activation of cytosolic protein kinase in the presence of calcium or lead. Diacylglycerol requirements of phospholipid-dependent protein kinase were tested in the presence of (A) $10 \mu\text{M}$ calcium or (B) $10 \mu\text{M}$ lead. CaCl_2 or PbOAc was added to the standard reaction mixture and the kinase activity assayed in the presence of various amounts of 1,2-diolein (0–30 $\mu\text{g/ml}$). Values plotted are the means of three replicates \pm SE. Data points without error bars represent SE $< 5\%$.

To further investigate this possibility, we determined the time dependence of substrate phosphorylation and dephosphorylation mediated by both calcium and lead. The time course of the reaction in the presence of $10 \mu\text{M}$ calcium or $10 \mu\text{M}$ lead is illustrated in Fig. 5. The data reflect quantities of phosphorylated protein resulting from the sum of phosphorylation and dephosphorylation reactions. We find that protein phosphorylation increased until 10 min of incubation time and then plateaued. After 30 min, the amount of phosphorylated protein decreased, reaching essentially zero by 1 hr. As well as reflecting reduced substrate phosphorylation, this decline also reflects phosphatase activity. The general shapes of the curves resulting from incubation by both cations are

very similar, suggesting a common mechanism of action for calcium and lead upon protein kinase C activity.

In order to demonstrate that the protein kinase C activation we observed is due to lead and not to the anionic component of lead acetate, we examined the effects of various lead salts for their ability to stimulate this enzyme. Lead acetate, citrate, and chloride all activate this enzyme at concentrations of $10 \mu\text{M}$ compared with basal levels (no lead or calcium). Sodium acetate has no significant effect on protein kinase activity (Table 1) indicating that the stimulation of protein kinase C is due to lead and not to an anionic interaction of acetate with the enzyme.

Having demonstrated that lead is able to stimulate soluble protein kinase C activity in broken cell preparations of immature brain microvessels, we then investigated its effects

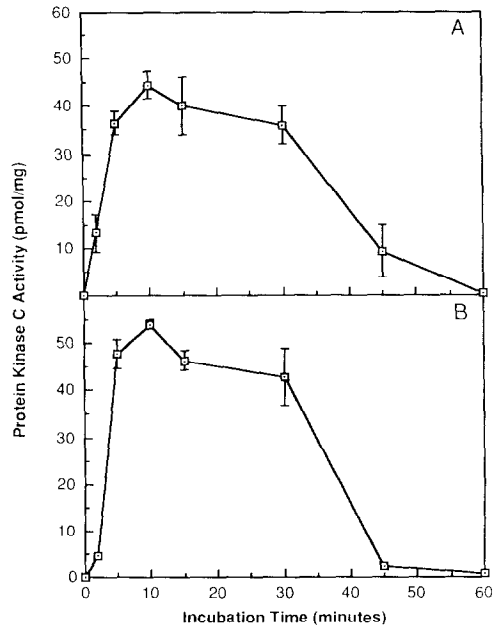


FIG. 5. Stimulation of cytosolic protein kinase activity by lead or calcium at variable incubation times. Diacylglycerol-stimulated, phospholipid-dependent protein kinase activity was assayed in the presence of (A) $10 \mu\text{M}$ calcium and (B) $10 \mu\text{M}$ lead at incubation times from 0 to 60 min. Values plotted are the means of three replicates \pm SE. Data points without error bars represent SE $< 5\%$.

TABLE I

EFFECT OF LEAD SALTS AND SODIUM ACETATE ON CYTOSOLIC PROTEIN KINASE ACTIVITY^a

Salt	N	Protein kinase C (pmol/mg/min)
None	3	0.16 ± 0.16
Pb(C ₂ H ₃ O ₂) ₂	3	10.13 ± 0.28
PbCl ₂	3	8.74 ± 1.43
Pb ₃ (C ₆ H ₅ O ₇) ₂ · 3H ₂ O	3	7.42 ± 0.67
NaC ₂ H ₃ O ₂	2	0.25 ± 0.25

^a Soluble protein kinase was activated by addition of 10 μ M specified compound to standard reaction mixture, omitting calcium (see Methods). Values represent means \pm SE.

on the intact capillary. When isolated microvessels were preincubated with micromolar concentrations of lead acetate, protein kinase C activity becomes redistributed from the cytosolic to the particulate fraction. Protein kinase C activity in lead-treated microvessels, subsequently fractionated into soluble and particulate components, was assayed in the presence of 10 μ M calcium. We find no change in total protein kinase C activity in response to lead, suggesting that this toxin redistributes but does not further activate the enzyme. Figure 6 illustrates the dose-dependent translocation of this enzyme in response to lead, with complete particulate recovery occurring between 0.01 and 0.1 μ M. At 1 μ M lead, the movement of protein kinase C from the cytosol into the membrane occurs in a time-dependent manner and is complete by 30 min (Fig. 7).

DISCUSSION

Protein kinase C phosphorylates various critical cell membrane and transport proteins (Hunter *et al.*, 1984; Witters *et al.*, 1985; Albert *et al.*, 1984) and thus represents a major site for regulation of cellular growth and differentiation (Nishizuka, 1984). Here, we present evidence that lead activates a brain microvessel protein kinase similar to protein

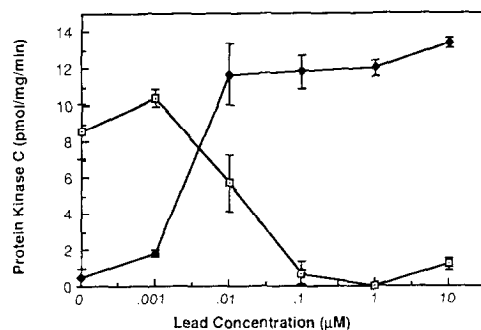


FIG. 6. Effect of varying concentrations of lead exposure on isolated microvascular protein kinase C. Microvessels were incubated with varying concentrations of lead (0–10 μ M) for 45 min at 37°C while under gentle agitation. Disrupted cell suspension was fractionated into soluble (\square) and particulate (\blacksquare) components and protein kinase C activity assayed as described under Methods. Values plotted are the means of three replicates \pm SE. Data points without error bars represent SE < 5%.

kinase C. The lead-induced activation occurs in a dose-dependent manner, peaking at 10 μ M, then declining, similar to that observed with calcium (Fig. 2). While the apparent V_{max} of this kinase for both cations is similar, at concentrations of less than 1 μ M, the affinity for calcium appears to be greater than that for lead. When cytosolic extracts were exposed to both 0.1 μ M calcium and 0.1 μ M

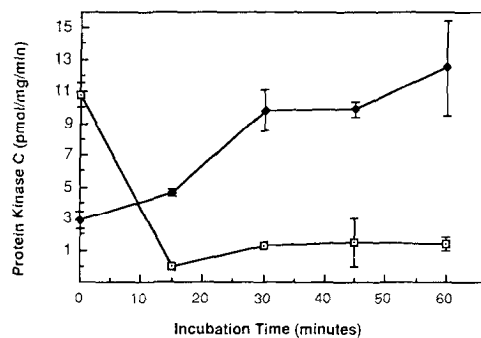


FIG. 7. Effect of lead exposure for varying intervals upon microvascular protein kinase C. Microvessels were exposed to 1 μ M lead at 37°C for varying incubation periods (0 to 60 min). Disrupted cell suspension was fractionated into soluble (\square) and particulate (\blacksquare) components and protein kinase C activity assayed as described under Methods. Values plotted are the means of three replicates \pm SE. Data points without error bars represent SE < 5%.

lead, the amount of protein phosphorylation increased compared to activation by each cation alone (Fig. 3). Thus, lead does not appear to antagonize the induction of this enzyme by calcium. Our observations are physiologically relevant in that intracellular calcium concentrations are known to be in the range of $0.1 \mu\text{M}$ (Pounds, 1984). To our knowledge, intracellular concentrations of lead have not been determined in exposed tissues. Further investigation of the kinetics of protein kinase C in response to calcium and lead, particularly at low concentrations, will require purification of protein kinase C. At $10 \mu\text{M}$ lead, the requirements of the protein kinase for diacylglycerol as well as for phospholipid were the same as those found at $10 \mu\text{M}$ calcium (Fig. 4), the standard condition for assay of protein kinase C. This suggests that lead mimics calcium in the activation of this enzyme *in vitro*.

An alternate explanation for the increase in substrate phosphorylation produced by lead is that instead of activating a kinase, lead inhibits a phosphatase responsible for phosphoprotein degradation. We believe that this explanation is unlikely in view of the similarity between the time courses of substrate phosphorylation and dephosphorylation produced by lead and calcium (Fig. 5). Furthermore, both cations have a nearly identical requirement for diacylglycerol and phospholipid in their stimulation of substrate phosphorylation. It should also be noted that the proteins targeted by protein kinase C can remain phosphorylated for long periods of time because they are generally more resistant to phosphatase action than the substrates of other protein kinases (Kikkawa and Nishizuka, 1986). Since lead-induced increase in substrate phosphorylation occurs within minutes, it is likely that this change represents stimulation of protein kinase rather than inhibition of phosphatase activity.

Having demonstrated that lead is able to stimulate a soluble protein kinase in disrupted immature brain microvessels, we then investigated the effects of this heavy metal upon protein kinase C in intact microvessels.

Exposure of the microvessels to micromolar concentrations of lead resulted in the disappearance of protein kinase C activity from the cytosol and its appearance in the membrane fraction. This apparent translocation was dose dependent and all the enzyme activity was recovered in the particulate fraction at $0.1 \mu\text{M}$ lead (Fig. 6). This effect was also time dependent and complete by 30 min of exposure to lead (Fig. 7). The "normal" blood lead concentration is between 5 and $25 \mu\text{g}/100 \text{ cc}$ of whole blood or 10^{-6} M (Mahaffey *et al.*, 1982). Because 95–98% is bound to red blood cells, approximately 10^{-8} M remains in the plasma (Clarkston and Kench, 1958). Thus, the concentration of lead which results in the apparent translocation of protein kinase C from the cytosol into the membrane is consistent with toxic exposures. This important regulatory enzyme, then, represents a possible mediator of lead toxicity.

The interaction between lead and calcium has been investigated in various biologic systems (for review, Simons, 1986). Exposure to lead enhances calcium uptake in several tissues (Pounds, 1984). We believe that the apparent redistribution of protein kinase C in response to lead exposure is a direct effect of the toxicant and not due to its influence upon calcium uptake because calcium alone does not translocate protein kinase C in the absence of specific agonists. To prove this hypothesis, additional investigation of the effect of lead upon phosphoinositol turnover and diacylglycerol formation is required.

Protein kinases, in response to specific stimuli, phosphorylate substrate proteins and thus alter the properties of those proteins to produce the physiological responses characteristic for the stimulating agent (Nessler and Greengard, 1984; Cohen, 1982). Protein phosphatases counter the actions of protein kinases by dephosphorylating their target proteins. The activation of protein kinases by hormones and polypeptide growth factors generally is thought to occur through receptor-mediated second messengers. When the appropriate agonist binds to a specific cell surface receptor, it can stimulate the metabo-

lism of phosphatidylinositol lipids in the cell membrane. This is accomplished through the activation of a specific phosphodiesterase (phospholipase C) presumably via a guanine nucleotide-binding protein (G protein) and results in the generation of two second messengers, inositol trisphosphate and diacylglycerol. Inositol trisphosphate mediates the mobilization of intracellular calcium from the endoplasmic reticulum, increasing calcium concentrations within the cell. Diacylglycerol activates protein kinase C, a calcium-dependent enzyme, by translocating it from the cytosol into the membrane. This redistribution can be accompanied by an increase in total activity (for review, see Kikkawa and Nishizuka, 1986). In isolated brain microvessels, as in several other tissues (Homma *et al.*, 1986; Chida *et al.*, 1986), further protein kinase C activation does not accompany translocation. Once protein kinase C becomes associated with the membrane, the enzyme phosphorylates critical regulatory proteins which may ultimately control growth and differentiation (Nishizuka, 1986b). Protein kinase C can, for example, regulate transmembrane signaling by phosphorylating and thus down-regulating membrane receptors such as that for epidermal growth factor (Hunter *et al.*, 1984).

We have shown that, in isolated immature brain microvessels, lead can substitute for calcium in the stimulation of soluble diacylglycerol-activated, phospholipid-dependent protein kinase in broken cell preparations. In addition, using intact microvascular cells, we found that exposure to lead produces a translocation of protein kinase C activity from the cytosol to the membrane. Lead is known to mimic calcium in various other biologic systems (Pounds *et al.*, 1982; Pounds and Mittelstaedt, 1983; Goldstein and Ar, 1983). Several components of the phosphoinositide turnover system are calcium dependent (Majerus *et al.*, 1985; Berridge and Irvine, 1984). These include the phosphodiesterases that convert inositol phospholipids to diacylglycerol and protein kinase C itself. By interacting with calcium in this second messenger

pathway, lead may ultimately affect growth and differentiation. Lead also alters another second messenger, cyclic AMP, by altering postsynaptic catecholamine action (Taylor *et al.*, 1978) and inhibiting adenylate cyclase (Nathanson and Bloom, 1975). 12-*O*-Tetradecanoyl phorbol-13-acetate (TPA), a phorbol ester tumor promoter that is a potent activator of protein kinase C (Niedel *et al.*, 1983; Castagna *et al.*, 1982; Kikkawa *et al.*, 1983), can phosphorylate the catalytic unit of adenylate cyclase. Purified protein kinase C is reported to phosphorylate this enzyme *in vitro* (Yoshimasa *et al.*, 1987). Lead, by activating protein kinase C, could therefore interact with other second messenger systems.

Inorganic lead is a mitogen for several cell types. Exposure of rat kidney cells to lead results in increased proliferation (Choie and Richter, 1972). In mouse kidney cells, lead stimulates DNA, RNA, and protein synthesis (Choie and Richter, 1974a,b). Both parenchymal and nonparenchymal rat liver cells, following a single exposure to lead, show an increase in the number of mitotic cells, as well as in total DNA and protein content (Columbano *et al.*, 1983). The mechanisms mediating the mitogenic effects of lead are unknown. Our results suggest that investigations of the activity and distribution of protein kinase C may be fruitful given the importance of protein kinase C in the regulation of growth.

Immature proliferating microvessels in brain are the ones most vulnerable to injury by lead (Press, 1985). A separation of tight junctions which normally seal the endothelial cells together in brain microvessels is found in lead encephalopathy (Lampert *et al.*, 1969). Support for a role of protein kinase C in this reaction is provided by the opening of tight junctions between kidney epithelial cells produced by the protein kinase C activator, TPA (Ojakian, 1981). Unregulated activation by lead of protein kinase C in immature microvessels could therefore produce separation of tight junctions and ultimately brain edema.

We propose that the ability of lead to activate protein kinase C, described here in immature brain microvessels, makes this regulatory enzyme a potential mediator of lead toxicity upon the blood-brain barrier. Abnormal activation of microvascular protein kinase C may also underlie lead toxicity in other tissues.

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