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Nicotinic and Muscarinic Agonists Stimulate Rapid Protein Kinase C Translocation in PC12 Cells

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Phosphoinositide hydrolysis, a major mechanism for signal transduction in neural cells, generates diacylglycerol, which can in turn activate protein kinase C (PKC). Although cholinergic agonists elicit phosphoinositide hydrolysis in neural tissues, little is known about activation of PKC by cholinergic agonists. PKC requires phosphatidylserine for activation, and in intact cells this lipid requirement is satisfied by binding of the enzyme to cell membranes. Therefore, in intact cells, activation of PKC is often associated with a decrease in cytosolic PKC activity accompanied by an increase in membrane-associated activity. We studied cholinergic-induced activation of PKC by examining changes in the subcellular distribution of the enzyme in PC12 cells treated with cholinergic drugs. Carbachol (1 mM) induced large and rapid increases in membrane-associated PKC activity; a maximal increase of 460% occurred after 5 sec of incubation. Carbachol-induced PKC translocation was concentration-dependent, with a biphasic dose–response curve yielding approximate EC_{50} values of 10^{-6} M and 10^{-4} M for the high- and low-affinity components, respectively. Experiments with selective cholinergic agents demonstrated that both muscarinic and nicotinic receptors are involved in carbachol-induced PKC translocation, but the response is predominantly mediated by nicotinic receptor stimulation. Muscarinic-induced association of PKC with cell membrane fractions was resistant to extraction by chelators, whereas nicotinic-mediated membrane binding was partially reduced by homogenization of cells in the presence of EGTA. Omission of calcium from the incubation medium or chelation of calcium with EGTA completely blocked muscarinic- and nicotinic-induced translocation. In addition, the calcium channel blocker nifedipine reduced the nicotinic response by 60%. These results indicate that carbachol-induced PKC translocation in PC12 cells is due mainly to activation of nicotinic receptors and is modulated by calcium influx through voltage-dependent calcium channels. These studies also suggest that stimulation of either nicotinic or muscarinic receptors generates diacylglycerol, which produces chelator-resistant binding of PKC to cell membranes.

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Protein kinase C (PKC) plays a key role in signal transduction by hormones and neurotransmitters that activate phosphoinositide hydrolysis. Breakdown of phosphatidylinositol bisphosphate (PIP_2) generates the intracellular second messengers inositol trisphosphate, which mobilizes calcium from intracellular stores (Berridge and Irvine, 1984), and diacylglycerol, which activates PKC by increasing the affinity of the enzyme for calcium and phospholipid (Nishizuka, 1984). PKC requires phosphatidylserine for activation, and in intact cells this lipid requirement is satisfied by association of the enzyme with cell membranes during activation (Kishimoto et al., 1980). Thus, tumor-promoting phorbol esters, which potently stimulate the enzyme by substituting for diacylglycerol (Nishizuka, 1984), cause a dramatic decrease in cytosolic PKC activity accompanied by a large increase in membrane-bound activity (Kraft and Anderson, 1983). Translocation of PKC activity from cytosolic to membrane fractions of intact cells has also been observed following receptor-mediated PIP_2 breakdown in some tissues (Liles et al., 1986; Niedel and Blackshear, 1986; TerBush and Holz, 1986), but receptor-mediated translocation has been more difficult to demonstrate than phorbol ester–induced translocation (Niedel and Blackshear, 1986).

Muscarinic cholinergic receptors are coupled to PIP_2 hydrolysis in brain and in several neural cell lines (Fisher and Agranoff, 1987). Certain neural responses to muscarinic stimulation appear to be mediated by PKC since they can be reproduced by treatment with phorbol esters. Such responses include inhibition of calcium-activated potassium currents in hippocampus (Malenka et al., 1986), blockade of the inhibitory effects of adenosine and $GABA_B$ receptor stimulation in hippocampus (Worley et al., 1987), and down-regulation of muscarinic receptors in the neuroblastoma cell line N1E-115 (Liles et al., 1986). In N1E-115, muscarinic stimulation is also associated with translocation of PKC activity to the cell membrane (Liles et al., 1986). Although nicotinic agonists stimulate phosphoinositide hydrolysis and PKC association with cell membranes in adrenal medullary cells (TerBush and Holz, 1986; Eberhard and Holz, 1987) and chick embryo myotubes (Adamo et al., 1985; Eusebi et al., 1987), nicotinic-mediated phosphoinositide turnover and PKC activation have not been clearly demonstrated in neural cells.

PC12 is a cell line that allows the study of both nicotinic and muscarinic modulation of PKC. PC12 cells express muscarinic receptors coupled to PIP_2 hydrolysis (Vicentini et al., 1986) and nicotinic receptors that resemble neuronal nicotinic receptors both in their resistance to α-bungarotoxin blockade (Patrick and Stallcup, 1977) and in their cross-reactivity with monoclonal antibodies to brain nicotinic receptors (Whiting et al., 1987).

PC12 cells treated with nerve growth factor (NGF) develop morphologic and biochemical characteristics of mature sympathetic neurons (Greene and Tischler, 1976; Dichter et al., 1977). NGF-differentiated PC12 cells express increased numbers of muscarinic receptors (Jumblatt and Tischler, 1982) and are more responsive to nicotinic stimuli (Amy and Bennett, 1983). Phorbol esters evoke a variety of responses in PC12 cells, including neurotransmitter release (Pozzan et al., 1984; Matthies et al., 1987), inhibition of voltage-dependent calcium uptake (Di Virgilio et al., 1986; Harris et al., 1986; Messing et al., 1986), and inhibition of muscarinic-induced phosphoinositide hydrolysis (Vicentini et al., 1985a). In addition, phorbol ester treatment causes PKC translocation to PC12 cell membranes (Matthies et al., 1987). In this report, we use NGF-differentiated PC12 cells as a model neural system to study cholinergic modulation of PKC. The results provide evidence for both nicotinic- and muscarinic-mediated activation of PKC in neural cells; they also indicate a role for voltage-sensitive calcium channels in mediating the nicotinic response.

Materials and Methods

Materials. DEAE cellulose (DE52) and P81 cellulose phosphate paper were purchased from Whatman. [γ-^{32}P]ATP was from Amersham. Phosphatidylserine was purchased from Avanti Polar Lipids. Histone III-S, sn-1,2 dioleoylglycerol (diC$_{18:1}$), carbachol, muscarine, leupeptin, phenylmethylsulfonyl fluoride (PMSF), 1,1-dimethyl-4-phenyl piperazinium iodide (DMPP), atropine, mecamylamine, and nifedipine were obtained from Sigma. NGF (2.5 S) was a gift from Dr. William Mobley.

Cell culture. PC12 cells were maintained at 37°C in a humidified atmosphere of 90% air and 10% CO_2 in Dulbecco's modified Eagle's medium (DMEM) containing 10% horse serum, 5% fetal calf serum, 50 U/ml penicillin, 50 μg/ml streptomycin, and 2 mM glutamine. Cells were subcultured onto collagen-coated 100 mm plastic dishes (Falcon) in medium supplemented with 50 ng/ml of 2.5 S NGF at a plating density of 3 × 10^6 cells per dish. Medium was changed 3 and 6 d after subculturing, and experiments were performed on day 7.

Subcellular fractionation. Cells were rinsed once in DMEM, preincubated at 37°C for 5 min in 4 ml of DMEM, and incubated at 37°C for various times in 4 ml of fresh DMEM containing indicated drugs. Incubations were terminated by aspirating the medium, rinsing once with 4 ml of ice-cold buffer containing 250 mM sucrose, 1 mM EDTA, 1 mM EGTA, and 20 mM Tris-HCl (pH 7.5), and scraping the cells from the dishes in 4 ml of 20 mM Tris-HCl (pH 7.5, 4°C), 50 μg/ml leupeptin, and 1 mM PMSF. This process took approximately 25 sec to complete. Thereafter, cells were kept on ice for up to 15 min during which time the subcellular distribution of PKC did not change. The samples were then homogenized on ice with 15 strokes of a tight-fitting teflon glass homogenizer, sucrose was added to a final concentration of 250 mM, and the cells were homogenized with another 5 strokes. After centrifugation at 100,000 × g for 60 min at 4°C, the supernatant (cytosol fraction) was applied to a 200 μl DE52 column (0.9 cm diameter) equilibrated with buffer A containing 20 mM Tris-HCl (pH 7.5 at 4°C), 1 mM EDTA, 1 mM EGTA, 12 mM 2-mercaptoethanol, and 10% glycerol. The column was washed with 2 ml of buffer A and eluted batchwise with 2.5 ml of buffer A containing 100 mM NaCl. All PKC activity was found in this fraction. The pellet (crude membrane fraction) was solubilized for 1 hr at 4°C in 4 ml of buffer A containing 1% Triton X-100 and centrifuged at 12,000 × g for 10 min to remove insoluble material prior to DEAE chromatography, as described for the supernatant.

PKC assay. The reaction mixture (final volume 100 μl) contained 50 μl of sample, 24 μg of phosphatidylserine, 0.8 μg of diC$_{18:1}$, 1 mM $CaCl_2$, 25 mM $MgCl_2$, 75 μg of histone III-S, and 2 nmol of [γ-^{32}P]ATP (2–4 × 10^5 dpm/nmol). Lipids were prepared by sonication in 20 mM Tris-HCl (pH 7.5 at 4°C). The reaction was started by adding $MgCl_2$, histone, and [γ-^{32}P]ATP at 30°C and was stopped after 3 min with 30 μl of a solution containing 0.2 M EDTA and 0.2 M ATP. Aliquots of 100 μl were spotted onto 2.5 cm^2 P-81 cellulose phosphate papers. Papers were washed in water (10 ml per paper) 4× and rinsed once in 95% ethanol prior to liquid scintillation counting. PKC activity was measured in triplicate samples as the difference between phosphorylation in the presence and absence of phosphatidylserine, diC$_{18:1}$, and Ca^{2+}. Enzyme activity was expressed as picomoles of ^{32}P incorporated per minute per milligram of cell homogenate. Results are reported in these units or as the percent of total PKC activity present in the membrane fraction. Protein was measured by the Bradford method (Bradford, 1976) with ovalbumin as the standard. Statistical comparisons between mean values were made by Student's t test, and differences were considered significant where p values < 0.05 were found.

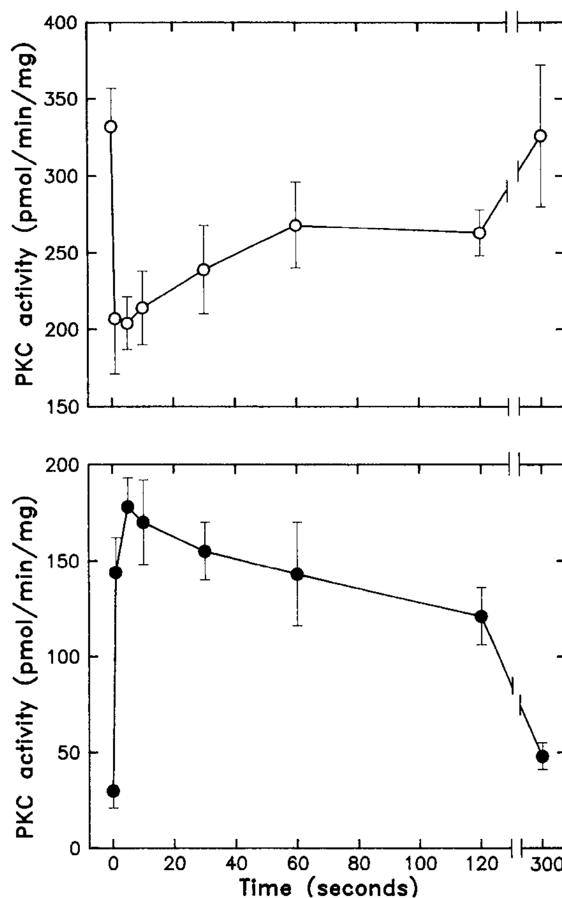

Figure 1. Time course of changes in the subcellular distribution of PKC in PC12 cells stimulated with 1 mM carbachol. Data shown are mean values ± SEM from 3 experiments, expressed as enzyme activity present in the cytosolic (○) or particulate (●) fraction of cells per mg of cell protein.

Results

PKC activity in unstimulated control PC12 cells was 336 ± 17 pmol/min/mg in the cytosol fraction and 77 ± 6 pmol/min/mg in the membrane fraction ($N = 21$). A similar predominance of cytosolic PKC activity has been noted in unstimulated cells from many other tissues (Nishizuka, 1984). As indicated in Figure 1, treatment of cells with 1 mM carbachol rapidly increased membrane-associated PKC activity. The effect was maximal by 5 sec, producing a 4.6-fold increase in membrane-associated activity, and was accompanied by a corresponding decrease in cytosolic activity. Total (membrane-associated plus cytosolic) activity in carbachol-stimulated cells was 103 ± 2% of total activity in control cells. Enzyme activity in the particulate fraction returned to levels equivalent to those found in unstimulated cells after 5 min of exposure.

The dependence of translocation on the concentration of carbachol is shown in Figure 2. Translocation occurred over a wide concentration range and the dose–response curve demonstrated

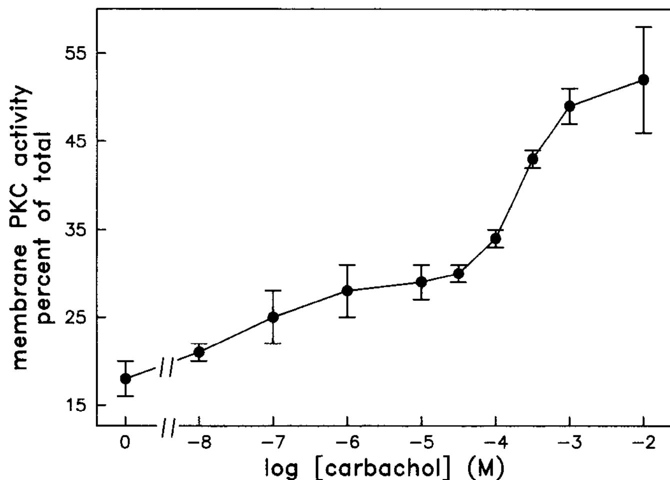

Figure 2. Stimulation of PKC translocation to PC12 membranes by incubation for 5 sec with carbachol. Data shown are mean values ± SEM from 3–6 experiments, expressed as the percent of total cellular PKC activity present in the particulate fraction.

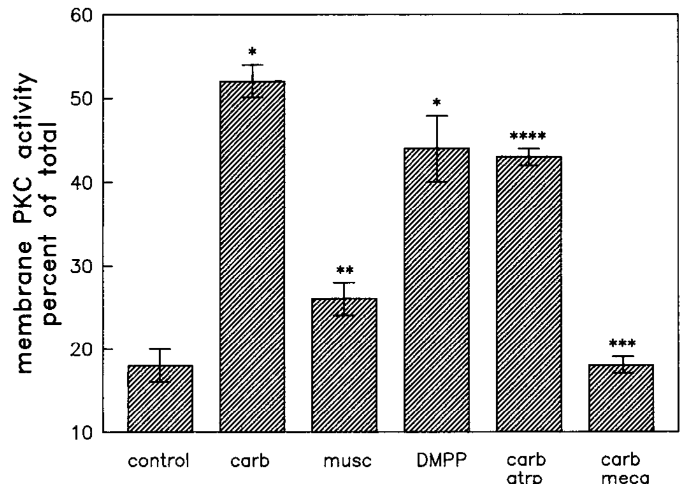

Figure 3. Effect of cholinergic agonists and antagonists on PKC translocation. Cells were preincubated with or without antagonists (atropine or mecamylamine) for 5 min and then incubated with indicated antagonists and agonists for 5 sec. Drugs were used at the following concentrations: 1 mM carbachol (*carb*), 1 mM muscarine (*musc*), 100 μM DMPP, 100 nM atropine (*atrp*), and 10 μM mecamylamine (*meca*). Data shown are mean values ± SEM from 3–6 experiments, expressed as the percent of total activity present in the particulate fraction. $p < 0.001$ (*) and $p < 0.03$ (**) relative to control; $p < 0.001$ (***) and $p = 0.05$ (****) relative to carbachol.

2 phases, with apparent EC_{50} values of approximately 10^{-6} M for the high-affinity and 10^{-4} M for the low-affinity component. The high-affinity phase corresponds to the range of carbachol concentrations that produce muscarinic stimulation of phosphoinositide hydrolysis in PC12 (Vicentini et al., 1986). The low-affinity phase approximates the concentrations of carbachol that stimulate nicotinic-mediated $^{22}Na^+$ (Patrick and Stallcup, 1977) and $^{86}Rb^+$ (Robinson and McGee, 1985) uptake in PC12 cells.

Since carbachol is both a nicotinic and a muscarinic agonist, and since the concentration–response curve shown in Figure 2 was biphasic, we considered whether activation of both cholinergic receptor subtypes might be involved in carbachol-induced translocation of PKC. As indicated in Figure 3, incubation of cells with 1 mM carbachol for 5 sec resulted in a shift of PKC activity such that 52 ± 2% of total activity was associated with the particulate fraction. Muscarine stimulated PKC translocation maximally at 300 μM, but the response was only 24% of that observed with 1 mM carbachol. In contrast, a maximally effective concentration (100 μM) of the selective nicotinic agonist DMPP nearly reproduced the response to carbachol. Atropine (100 nM), which completely inhibits muscarinic-stimulated phosphoinositide breakdown in PC12 (Vicentini et al., 1986), only slightly reduced the carbachol-stimulated increase in membrane PKC activity. In contrast, the nicotinic antagonist mecamylamine (1 μM) was far more effective. The predominance of a nicotinic effect persisted at later time points as well, since at 2 min of incubation with 1 mM carbachol, 1 μM mecamylamine inhibited PKC translocation by 81 ± 5%, whereas 100 nM atropine inhibited translocation by only 45 ± 8% ($N = 3$; $p < 0.02$ for inhibition by mecamylamine relative to inhibition by atropine). Neither atropine nor mecamylamine alone had any effect on PKC translocation (data not shown).

PKC activation and binding to cell membranes are modulated by calcium (May et al., 1985; Wolf et al., 1985; Gopalakrishna et al., 1986). Nicotinic receptor stimulation depolarizes cells, allowing for influx of extracellular calcium through voltage-dependent channels (Stallcup, 1979). Muscarinic stimulation activates a calcium influx in PC12 that is not dependent on depolarization and is resistant to organic calcium channel antagonist drugs (Pozzan et al., 1986). To determine whether influx of extracellular calcium is important in cholinergic-induced PKC translocation, we incubated cells with DMPP or muscarine in the presence and absence of calcium, EGTA, or the calcium channel antagonist, nifedipine. As shown in Figure 4 both muscarinic- and nicotinic-induced PKC translocation were completely inhibited in calcium-free buffer and in buffer containing 3 mM EGTA. Nifedipine (1 μM), which completely blocks calcium influx through dihydropyridine-sensitive, voltage-dependent calcium channels in PC12 cells (Toll, 1982), inhibited maximal DMPP-induced translocation by approximately 60%. In contrast, the response to muscarine was unaffected by nifedipine. Nifedipine alone had no effect on PKC translocation (data not shown).

Although calcium is required to initiate binding of PKC to cell membranes, binding induced by calcium alone is reversible and removed by extraction with calcium-chelating agents (Gopalakrishna et al., 1986). Phorbol esters and, to a lesser extent, diacylglycerol, stabilize membrane binding of the enzyme making binding resistant to removal by chelators (Gopalakrishna et al., 1986). To investigate whether cholinergic agonists caused membrane binding of PKC that was chelator-stable (i.e., due to calcium and diacylglycerol) or chelator-extractable (i.e., due only to elevated intracellular calcium), we compared cholinergic-induced PKC translocation in cells homogenized with and without 1 mM EGTA. As shown in Table 1, muscarine-induced translocation was unchanged by homogenization in EGTA, whereas DMPP-induced translocation was reduced 39% by this treatment. Since it was possible that 1 mM EGTA may not have chelated all of the calcium released during cell lysis, we also homogenized cells in 10 mM EGTA. Homogenization in 10 mM EGTA reduced membrane-associated PKC activity slightly further in control (7 ± 1%) and DMPP-treated cells (19 ± 2%), but the difference between these 2 values remained highly significant ($p < 0.007$; $N = 3$). Therefore, both muscarine and

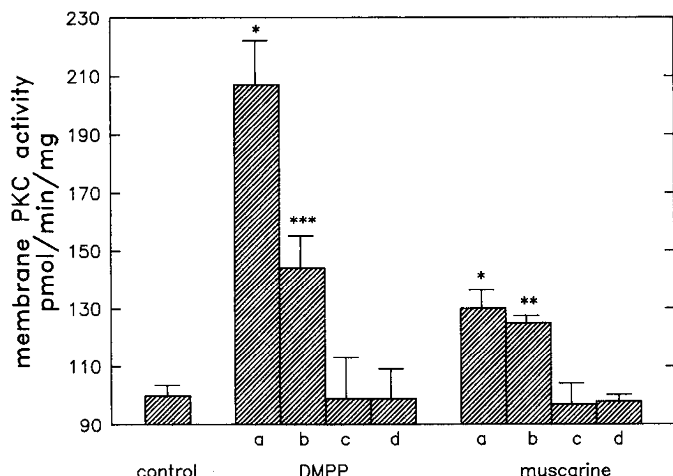

Figure 4. DMPP- and muscarine-stimulated PKC translocation in the presence and absence of calcium, nifedipine, and EGTA. PC12 cells were preincubated for 5 min in buffer containing 120 mM NaCl, 5 mM KCl, 1.4 mM $CaCl_2$, 0.8 mM $MgSO_4$, 1 mM NaH_2PO_4, 5 mM glucose, and 25 mM HEPES (pH 7.4). Cells in condition *b* were treated with 1 μM nifedipine during this period. Cells were then incubated for 5 sec in the same buffer with agonist alone (*a*), agonist plus 1 μM nifedipine (*b*), agonist plus 3 mM EGTA (*c*), or agonist without calcium (*d*). Data shown are mean values ± SEM from 3–6 experiments and are expressed as enzyme activity present in the particulate fraction per milligram of cell protein. Control values (in the absence of agonist) were equivalent in calcium-free and calcium containing buffers. $p < 0.001$ (*) and $p < 0.03$ (**) relative to control; $p < 0.01$ (***) relative to DMPP.

DMPP induced chelator-stable association of PKC with PC12 membranes.

Discussion

Our findings demonstrate that cholinergic stimuli induce rapid and marked increases in PKC activity in membranes of NGF-differentiated PC12 cells. The effect was evident by 1 sec, suggesting a role for PKC in early neural responses to cholinergic signals. We found that PKC translocation occurred via both muscarinic and nicotinic receptor stimulation. In PC12, nicotinic stimuli induce the release of ACh (Greene and Rein, 1977a), but nicotinic-mediated PKC translocation cannot be attributed to ACh release and subsequent muscarinic receptor stimulation, since a maximally effective concentration of muscarine produced a smaller effect than carbachol and the response to carbachol was reduced only slightly by atropine. Nicotinic stimuli also cause the release of other neurotransmitters, such as catecholamines (Greene and Rein, 1977b), from PC12 cells. However, phosphoinositide breakdown and PKC activation have not been reported in PC12 cells exposed to noncholinergic neurotransmitters produced by this cell line. Therefore, it is likely that PKC translocation was a direct result of nicotinic receptor activation and was not mediated by released neurotransmitters.

Although both nicotinic and muscarinic agonists caused PKC translocation, we found the response to nicotinic stimuli to be much greater. The association of PKC with cell membranes requires calcium, and increases in calcium enhance membrane binding of the enzyme (Wolf et al., 1985; Gopalakrishna et al., 1986, Akers and Routtenberg, 1987). Calcium influx through dihydropyridine-sensitive calcium channels accounted for much of the difference between nicotinic and muscarinic responses, since nifedipine inhibited DMPP-induced translocation to levels

Table 1. Membrane binding of PKC in cells homogenized with and without 1 mM EGTA

	Homogenization buffer	
Drug	No EGTA	1 mM EGTA
DMPP[a]	41 ± 4	25 ± 2
Muscarine	23 ± 1	23 ± 3
None	14 ± 1	12 ± 1

Values are expressed as the percent of total PKC activity present in the particulate fraction.

[a] $p < 0.02$ for cells homogenized in EGTA as compared to cells homogenized without EGTA.

observed with muscarine. The influx of calcium through dihydropyridine-insensitive calcium channels (Kongsamut and Miller, 1986) and through the nicotinic receptor (Stallcup, 1979) may explain why nifedipine failed to completely inhibit DMPP-induced translocation. In PC12 cells, muscarinic agonists elevate intracellular calcium by stimulating calcium influx through receptor-operated channels and inducing redistribution of calcium from cytoplasmic stores to the cytosol (Pozzan et al., 1986). However, the intracellular calcium concentration achieved with maximal muscarinic stimulation is only approximately one-half of that achieved by depolarization of PC12 cells (Pozzan et al., 1986). Therefore, nicotinic agonists, by depolarizing cells and stimulating calcium influx through voltage-dependent channels, may produce larger increases in intracellular calcium than do muscarinic agonists. Since the association of PKC with membranes varies with calcium concentration (Akers and Routtenberg, 1987), the higher concentrations of intracellular calcium achieved with nicotinic receptor stimulation may account for the greater extent of PKC translocation observed in cells treated with DMPP.

Although calcium is required for PKC binding to membranes, binding induced by calcium alone is reversible and is removed by calcium chelators (Gopalakrishna et al., 1986). In contrast, diacylglycerol, produced by phosphoinositide hydrolysis, stabilizes membrane binding of PKC so that binding becomes resistant to calcium-chelating agents (Gopalakrishna et al., 1986). We found that muscarine-induced translocation of PKC was entirely chelator resistant, suggesting that it is mediated by both diacylglycerol and calcium. In contrast, DMPP-induced translocation was partially reduced by homogenization in EGTA, suggesting that nicotinic-mediated binding of the enzyme to membranes involves two components, one mediated by calcium alone and the other by calcium and diacylglycerol.

The finding of chelator-stable binding suggests that nicotinic-receptor stimulation and the consequent rise in intracellular calcium may cause phosphoinositide hydrolysis and diacylglycerol formation in PC12 cells. Nicotinic-induced phosphoinositide hydrolysis has been demonstrated in adrenal chromaffin cells, where it is dependent on influx of extracellular calcium through voltage-dependent calcium channels (Eberhard and Holz, 1987). Therefore, in PC12 cells, phosphoinositide hydrolysis stimulated by depolarization-dependent calcium influx could be responsible for chelator-resistant association of PKC with membranes in cells treated with DMPP.

PKC tightly bound to PC12 membranes in the presence of both calcium and diacylglycerol is likely to be active. The functional state of PKC loosely bound to membranes by calcium

alone may vary in different tissues. Elevated intracellular calcium may either directly activate PKC (Akers and Routtenberg, 1987) or may serve to recruit PKC to the cell membrane, thereby priming it for subsequent activation by diacylglycerol (May et al., 1985; Wolf et al., 1985). Since the majority of PKC associated with membranes after nicotinic stimulation was chelator sensitive, it is likely that calcium-mediated binding of PKC plays an important role in normal signal transduction.

When we incubated cells in the absence of extracellular calcium or in the presence of 3 mM EGTA, muscarine and DMPP were unable to stimulate PKC transloction. It is unlikely that this was due to inhibition of drug binding, since ligand binding to cholinergic receptors can be measured in the absence of calcium (Jumblatt and Tischler, 1982; Whiting and Lindstrom, 1986). Prevention of extracellular calcium influx could explain the failure of DMPP to induce PKC translocation in cells incubated in calcium-free buffer. Incubation of PC12 cells in calcium-free buffer also inhibits muscarinic-stimulated calcium influx and can reduce muscarinic-induced PIP_2 hydrolysis and intracellular calcium mobilization (Vicentini et al., 1985b; Rabe et al., 1987). A similar sensitivity of receptor-mediated phosphoinositide hydrolysis to external calcium concentration has been noted in other neural tissues (Fisher and Agranoff, 1987). Muscarinic stimulation of PC12 cells incubated in calcium-free buffer for 1 to 4 min increases intracellular calcium to only 100–140 nM (Vicentini et al., 1985b, Rabe et al., 1987). Calcium at these concentrations does not enhance the association of PKC with rat brain synaptic membranes (Akers and Routtenberg, 1987) or erythrocyte vesicles (Wolf et al., 1985) *in vitro*. In our experiments, cells were exposed to calcium-free buffer for only 5 sec. Although this could block calcium influx, it is not known whether exposure for 5 sec is sufficient to reduce muscarine-stimulated phosphoinositide hydrolysis. Nevertheless, we speculate that incubation of PC12 cells in calcium-free buffer inhibited extracellular calcium influx and may have reduced phosphoinositide hydrolysis, thereby preventing free intracellular calcium from reaching concentrations sufficient to initiate PKC translocation in response to muscarine. Further work is needed to determine the influence of calcium-free buffer on muscarine-induced phosphoinositide hydrolysis after brief (e.g., 5 sec) periods of exposure.

The finding of cholinergic-induced PKC translocation suggests that PKC is involved in rapid responses of PC12 cells to cholinergic stimuli. Such responses include neurotransmitter release (Greene and Rein, 1977b; Rabe et al., 1987) and c-fos proto-oncogene transcription (Greenberg et al., 1986), both of which can be induced by treatment with phorbol esters (Greenberg and Ziff, 1984; Pozzan et al., 1984). We (Messing et al., 1986) and others (Di Virgilio et al., 1986; Harris et al., 1986) have found that phorbol esters reduce voltage-dependent calcium influx in PC12, suggesting that activation of PKC inhibits the function of voltage-dependent calcium channels in this cell line. Rane and Dunlap (1986) reported similar findings in dorsal root ganglion cells treated with the PKC activator 1,2-oleoylacetylglycerol. These studies, together with our present findings, suggest that ACh may modulate the function of voltage-dependent calcium channels via PKC. The stimulatory effects of nicotinic agonists on voltage-dependent calcium channels and PKC suggest a mechanism whereby PKC could be involved in a negative-feedback response that inhibits calcium-channel function. Such a mechanism could be involved in the termination of nicotinic-induced calcium signals in neural tissues.

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