Involvement of Protein Kinase C in Prostaglandin E2-induced Catecholamine Release from Cultured Bovine Adrenal Chromaffin Cells*

Manabu Negishi, Seiji Ito, and Osamu Hayaishi‡
From the Department of Cell Biology, Osaka Bioscience Institute, Suita, Osaka 565, Japan

We recently reported that prostaglandin (PG) E2 stimulated phosphoinositide metabolism in cultured bovine adrenal chromaffin cells and that PGE2 and ouabain induced a gradual secretion of catecholamines from the cells (Yokohama, H., Tanaka, T., Ito, S., Negishi, M., Hayashi, H., and Hayaishi, O. (1988) J. Biol. Chem. 263, 1119–1122). Here we examined the involvement of two signal pathways, Ca2+ mobilization and protein kinase C activation resulting from phosphoinositide metabolism, in the PGE2-induced catecholamine release. Either the Ca2+ ionophore ionomycin or 12-O-tetradecanoylphorbol 13-acetate (TPA) could enhance the release in the presence of ouabain, and ionomycin-induced release was additive to PGE2-induced release, but TPA-induced release was not additive. PGE2 dose-dependently stimulated the formation of diacylglycerol and caused the translocation of 4% of the total protein kinase C activity to become membrane-bound within 5 min. These effects were specific for PGE2 and PGE1 among PGs tested (PGE2 > PGE1 > PGF2α > PGD2). Furthermore, the phosphoinositide-specific phospholipase C inhibitor neomycin inhibited PGE2-induced accumulation of inositol phosphates, diacylglycerol formation, translocation of protein kinase C, and also stimulation of catecholamine release. Both PGE2- and TPA-induced release were inhibited by the depletion of protein kinase C caused by prolonged exposure to TPA, but ionomycin-induced release was not inhibited. We recently found that theamiloride-sensitive Na+, H+-antiport participates in PGE2-evoked catecholamine release (Tanaka, T., Yokohama, H., Negishi, M., Hayashi, H., Ito, S., and Hayaishi, O. (1990) J. Neurochem. 54, 86–95). In agreement with our recent report, PGE2 and TPA induced a sustained increase in intracellular pH that was abolished by the protein kinase C inhibitor staurosporine but not by thecalmodulin inhibitor W-7. Ionomycin also induced a marked increase in intracellular pH, but this increase was abolished by W-7 but not by staurosporine. These results demonstrate that PGE2-induced activation of the Na+, H+-antiport and catecholamine release in the presence of ouabain are mediated by activation of protein kinase C, rather than by Ca2+

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‡ To whom correspondence should be addressed: Osaka Bioscience Institute, 6-2-4 Furuedai, Suita, Osaka 565, Japan.

1 The abbreviations used are: PGs, prostaglandins; IPα, inositol phosphates; IPβ, inositol 1,4,5-trisphosphate; IPγ, inositol 1,4,5,6-tetrasphosphate; TPA, 12-O-tetradecanoylphorbol 13-acetate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; pH, intracellular pH; EGTA, [ethylenediamine(oxyethylenenitrilo)]tetraacetic acid.
of the secretagogue to induce release in a number of cell types, suggesting that protein kinase C also plays a role in exocytosis. Among putative in vivo substrates for protein kinase C, the Na⁺, H⁺-antipor exchanges extracellular Na⁺ for intracellular H⁺, producing cellular alkalinization and accumulation of intracellular Na⁺ (19). There has been increasing evidence indicating that the activation of Na⁺, H⁺-antipor by protein kinase C is involved in secretory processes (14). It has also been observed that Na⁺, H⁺-antipor in some cells is activated following an increase in the intracellular Ca²⁺ concentration, and Ca²⁺, calmodulin-dependent protein kinase has been suggested to mediate this activation (20). We recently demonstrated that the amiloride-sensitive Na⁺, H⁺-antipor system may be involved in the catecholamine release elicited by PGE₂ and ouabain (21). The purpose of the present paper is to characterize the signal-transduction mechanism(s) operative in PGE₂-induced catecholamine release from bovine chromaffin cells in the presence of ouabain. We report here that PGE₂ stimulates Na⁺, H⁺-antipor by activation of protein kinase C resulting from phosphoinositide metabolism and evokes a drastic catecholamine release from bovine chromaffin cells in concert with ouabain.

EXPERIMENTAL PROCEDURES

Materials—PGs were generous gifts from Ono Pharmaceuticals, Osaka, Japan; myo-[2-3H]inositol (20 Ci/mmol), [γ-32P]ATP (3000 Ci/mmol), and a diacylglycerol assay system were obtained from Amersham Corp. Agents obtained and commercial sources were as follows: staurosporine, forskolin, nigericin, N-1-methyl-5-phenylcarboxyfluorescein, Dojindo Laboratories (Kumamoto, Japan); and neomycin sulfate, Wako Pure Chemical Industries. All other chemicals were of reagent grade.

Cell Culture and Catecholamine Release—Chromaffin cells were prepared from bovine adrenal medulla by collagenase digestion and purification on Percoll gradients as described previously (22). Cells were cultured for 3–4 days prior to use in Dulbecco’s modified Eagle’s medium (or when measuring IPs, in Ham’s F-10 medium) supplemented with 10% heat-inactivated fetal calf serum, tyrosine arabinoside (2.8 μg/ml), streptomycin (100 μg/ml), penicillin (100 units/ml), and nystatin (250 units/ml). In experiments on catecholamine release, cells cultured in 24-well plates (2 x 10⁵ cells/well) were washed with HEPES-buffered saline solution containing 125 mM NaCl, 4.7 mM KCl, 2.2 mM CaCl₂, 1.2 mM MgCl₂, 1.2 mM KH₂PO₄, 15 mM NaHCO₃, 11 mM glucose, and 15 mM HEPES (pH 7.4), and the resultant formation of IPs and an increase in the intracellular Ca²⁺ concentration, and Ca²⁺, calmodulin-dependent protein kinase has been suggested to mediate this activation (20). We recently demonstrated that the amiloride-sensitive Na⁺, H⁺-antipor system may be involved in the catecholamine release elicited by PGE₂ and ouabain (21). The purpose of the present paper is to characterize the signal-transduction mechanism(s) operative in PGE₂-induced catecholamine release from bovine chromaffin cells in the presence of ouabain.

RESULTs

Effect of PGE₂, TPA, and Ionomycin on Catecholamine Release from Bovine Adrenal Chromaffin Cells—Recently we reported that PGE₂ caused phosphoinositide breakdown with the resultant formation of IPs and an increase in the intracellular Ca²⁺ concentration, as well as induced catecholamine release in the presence of ouabain from cultured bovine adrenal chromaffin cells (9). As a further study of the participation of the two signal pathways, protein kinase C and Ca²⁺, we examined the effect of TPA as a stimulator of protein kinase C or the Ca²⁺ ionophore ionomycin as an elevator of intracellular Ca²⁺. We also compared the effect among PGE₂, TPA, and ionomycin on catecholamine release with a half-maximal concentration of 10⁻⁶ M for PGE₂, 0.1 μM for TPA, or 1 μM for ionomycin, gave only a small release (1–2%) over the basal level of 1% by 30 min. On the other hand, the secretory response elicited by exposure of the cells to ouabain alone gradually increased over a 30-min incubation period, and ouabain markedly enhanced the stimulant-induced catecholamine release with a quite similar time course. To elucidate whether these two signal pathways are involved in the action of PGE₂, we examined the synergistic effect among PGE₂, TPA, and ionomycin on catecholamine release in the presence of ouabain. Fig. 1 shows the time courses of catecholamine release induced by ouabain alone and by the glycodeox plus PGE₂, TPA, or ionomycin. The stimulant alone, 1 μM PGE₂, 0.1 μM TPA, or 1 μM ionomycin, gave only a small release (1–2%) over the basal level of 1% by 30 min. On the other hand, the secretory response elicited by exposure of the cells to ouabain alone gradually increased over a 30-min incubation period, and ouabain markedly enhanced the stimulant-induced catecholamine release with a quite similar time course. To elucidate whether these two signal pathways are involved in the action of PGE₂, we examined the synergistic effect among PGE₂, TPA, and ionomycin on catecholamine release in the presence of ouabain. Fig. 2 shows the dose-response effect of TPA (Fig. 2A) or ionomycin (Fig. 2B) on PGE₂-induced catecholamine release. In the presence of ouabain, TPA dose-dependently potentiated catecholamine release with a half-maximal concentration of 10⁻⁶ M and reached the almost maximal level at 10⁻⁴ M. 4α-Phorbol 12,13-di-decanoate, an inactive phorbol ester analogue, did not stimulate catecholamine release up to 1 μM in the presence of ouabain (data not shown). Ionomycin alone did not stimulate the release up to 1 μM. In the presence of ouabain the level of...
Effects of PGE₂ on Diacylglycerol Formation and Translocation of Protein Kinase C Activity  Next, we sought to determine whether PGE₂ would stimulate diacylglycerol formation and translocation of protein kinase C activity from the cytosol to the membrane fraction. As shown in Fig. 3A, PGE₂ transiently elevated the diacylglycerol level within 5 min and then the level declined to almost the basal level by 30 min. PGE₂ dose-dependently caused accumulation of diacylglycerol with a half-maximal concentration of 20 nM and maximally elevated at the level 1.5-fold over the basal level at 10 μM (Fig. 3B). As shown in Fig. 4A, PGE₂ transiently translocated protein kinase C activity to the membrane fraction. Under the unstimulated condition, 9% of total protein kinase C activity existed in the membrane fraction. The level of protein kinase C activity in the membrane fraction reached maximum (14%) at 5 min and then declined to the basal level.

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by 10 min. The time course of the translocation of protein kinase C activity by PGE₂ was similar to that of diacylglycerol formation. The increase of protein kinase C translocation induced by PGE₂ was concentration-dependent, the half-maximal increase occurring at about 20 nM PGE₂ (Fig. 4B). A good correlation was observed between dose dependencies of PGE₂ for stimulation of diacylglycerol formation and for that of protein kinase C translocation. In contrast, 1 μM TPA gradually enhanced the translocation of protein kinase C from the cytosol to the membrane fraction over a 30-min incubation period, and 50% of the total activity was translocated by 30 min (Fig. 5A). Furthermore, this translocation was dose-dependent (Fig. 5B). The level in the membrane fraction began to increase at 10 nM TPA and was elevated to more than 60% of the total by 1 μM TPA. This increase well corresponded with the decrease of protein kinase C activity in the cytosol fraction. Comparison of the dose dependencies of TPA for the stimulation of catecholamine release in the presence of ouabain and that of protein kinase C translocation revealed that TPA at 10 nM caused almost maximal stimulation of catecholamine release when TPA translocated only 4% of the protein kinase C activity from the cytosol to the membrane fraction.

Furthermore, we compared the effects of various PGs on both diacylglycerol formation and translocation of protein kinase C activity. As shown in Table I, PGE₂ was the most efficient in producing an increase in both diacylglycerol formation and translocation of protein kinase C activity. PGE₂ had almost the same potencies, but PGF₂α and PGD₂ were less potent in this order. These parameters examined here were well correlated with the ability of each PG to displace [³H]PGE₂ from the membrane (27), to stimulate [³H]IP formation (28), and also to enhance catecholamine release in the presence of ouabain (21).

Effects of Neomycin on PGE₂-induced Catecholamine Release, [³H]IP Formation, Diacylglycerol Formation, and Translocation of Protein Kinase C Activity—The good correlation between the specificities of PGs for the parameters mentioned above suggests that the stimulation of phosphoinositide metabolism by PGE₂ induces diacylglycerol formation followed by translocation of protein kinase C activity to evoke catecholamine release.

To explore further this possibility, we examined the effects of a phosphatidylinositol-specific phospholipase C inhibitor, neomycin, on PGE₂-induced catecholamine release, [³H]IP formation, diacylglycerol accumulation, and translocation of protein kinase C activity. Fig. 6A shows the effect of neomycin on catecholamine release induced by various stimulants. Neomycin alone had no effect on catecholamine release, but inhibited PGE₂-induced catecholamine release in the presence of ouabain with a half-maximal concentration of 1 mM and completely inhibited it at 3 mM. In contrast, TPA- or ionomycin-induced catecholamine release in the presence of ouabain was not significantly inhibited by neomycin up to 1 mM. Fig. 6B shows the effect of neomycin on PGE₂-induced IP formation. Production of inositol bisphosphate and IP₃ was completely inhibited by neomycin alone had no effect on IP₃ accumulation or phosphoinositide metabolism by PGE₂.

**TABLE I**

Comparison of the effects of various PGs on protein kinase C activity in membrane fraction and diacylglycerol formation in chromaffin cells

Chromaffin cells were incubated for 5 min at 37 °C with various PGs (1 μM). Protein kinase C activity in the membrane fraction and diacylglycerol content were determined as described under "Experimental Procedures." Values shown are the mean ± S. E. of triplicate experiments.

<table>
<thead>
<tr>
<th>PG</th>
<th>PGE₂</th>
<th>PGE₁</th>
<th>PGF₂α</th>
<th>PGD₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of total</td>
<td>9.5 ± 0.5</td>
<td>13.0 ± 0.5</td>
<td>12.8 ± 0.5</td>
<td>11.3 ± 0.5</td>
</tr>
<tr>
<td>pmol/10⁶ cells</td>
<td>233 ± 8.9</td>
<td>365 ± 17</td>
<td>336 ± 6.4</td>
<td>270 ± 13</td>
</tr>
</tbody>
</table>

**Fig. 5.** Time course and dose dependence for TPA-induced translocation of protein kinase C. A. cells were incubated for the indicated times at 37 °C with 1 μM TPA. B. cells were incubated for 30 min at 37 °C with the indicated concentrations of TPA. Protein kinase C activity in the cytosol (○) or the membrane fraction (■) was determined as described under "Experimental Procedures." Values shown are means ± S. E. (n = 6). Student’s t test was examined for the protein kinase C activity in the membrane fraction in B. Significant differences from the control are represented (*, p < 0.05; **, p < 0.01).
on inositol monophosphate formation. Furthermore, 3 mM neomycin inhibited PGE2-induced diacylglycerol formation and the translocation of protein kinase C activity, as shown in Table II. These results demonstrate that PGE2-induced catecholamine release in the presence of ouabain is triggered by phosphoinositide metabolism.

Involvement of Protein Kinase C in PGE2-induced Catecholamine Release—Involvement of protein kinase C in PGE2-induced catecholamine release in the presence of ouabain is inferred from the evidence that TPA stimulates the release in concert with ouabain, that TPA replaces most of the action of PGE2 (Fig. 2A), and that PGE2 enhances diacylglycerol formation and subsequent translocation of protein kinase C activity resulting from the stimulation of phosphoinositide metabolism (Figs. 3A and 4A). To confirm the participation of protein kinase C in the action of PGE2, we studied the effect of the depletion of protein kinase C by prolonged exposure to TPA (29) on the catecholamine release. As shown in Fig. 7A, the treatment of the cells with TPA for 10 h had no significant effects on the basal release, ouabain-induced release and total content of catecholamine in the cells, but dose-dependently inhibited PGE2- or TPA-induced release in the presence of ouabain and completely suppressed it at 0.1 μM. In contrast, the same treatment with TPA had no effect on ionomycin-induced release in the presence of ouabain up to 0.1 μM TPA, but inhibited it by 24% at 1 μM. Fig. 7B shows dose dependency of TPA for the depletion of cellular protein kinase C activity in both cytosol and membrane fractions by the 10-h exposure to TPA. Protein kinase C activity in the membrane fraction began to decrease at 1 nM TPA and was completely depleted by 0.1 μM TPA. Protein kinase C activity in the cytosol fraction was also reduced dose-dependently by TPA with a half-maximal inhibitory concentration of 10 nM, and was completely depleted at 1 μM TPA. We also examined the effect of inhibitors of protein kinase C on catecholamine release and staurosporine (30), sphingosine (31), and poly-myxin B (32) markedly inhibited PGE2- or TPA-induced catecholamine release in the presence of ouabain, but the inhibitory effect on ionomycin-induced release was very weak (data not shown).

Effect of PGE2, TPA, or Ionomycin on pHi—Recently we reported that the amiloride-sensitive Na+·H+-antiport system participates in PGE2-evoked catecholamine release (21). Therefore, we examined the effect of PGE2, TPA, or ionomycin on the activity of Na+·H+-antiport by measuring pHi. As shown in Fig. 8, PGE2 (1 μM) or TPA (0.1 μM) gradually increased pHi (∆pHi = 0.1) over a 10-min period. Both of these increases were abolished by removal of the Na+ from the buffer or the addition of 1 mM amiloride, an inhibitor of the Na+·H+-antiport. Furthermore, these pH elevations were also inhibited by the protein kinase C inhibitor staurosporine (1 μM), but not by a calmodulin inhibitor, W-7 (100 μM), thus suggesting that the PGE2- or TPA-induced increase in pH is mediated by protein kinase C. Ionomycin (1 μM) markedly elevated pHi (∆pHi = 0.2) and this elevation was also abolished by the removal of Na+ or the addition of amiloride, but not by staurosporine (1 μM). On the other hand, W-7 (100 μM) strongly inhibited this elevation, but a weak calmodulin inhibitor, W-5 (100 μM), was much less effective. Removal of Ca2+ from the buffer completely abolished the pH elevation. These results suggest that ionomycin produces a Ca2+ influx and stimulates the Na+·H+-antiport activity via calmodulin.

**TABLE II**

**Effects of neomycin on translocation of protein kinase C and diacylglycerol formation**

After chromaffin cells were preincubated for 5 min at 37 °C with or without 3 mM neomycin, the cells were further incubated at 37 °C for 10 min with 1 μM TPA or for 3 min with 1 μM PGE2 or vehicle. Protein kinase C activity in the membrane fraction and diacylglycerol content were determined as described under "Experimental Procedures." Values shown are means ± S. E. of triplicate experiments.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Neomycin</th>
<th>Protein kinase C activity</th>
<th>Diacylglycerol formation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% of total</td>
<td>pmol/10^6 cells</td>
</tr>
<tr>
<td>None</td>
<td>−</td>
<td>9.7 ± 0.5</td>
<td>296 ± 10.6</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>10.0 ± 1.4</td>
<td>257 ± 6.1</td>
</tr>
<tr>
<td>TPA</td>
<td>−</td>
<td>29.3 ± 0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>29.3 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>PGE2</td>
<td>−</td>
<td>13.4 ± 0.5</td>
<td>422 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>9.8 ± 0.3</td>
<td>310 ± 7.5</td>
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**Fig. 7.** Effects of prolonged exposure to TPA on catecholamine release from chromaffin cells and protein kinase C activity in the cytosol and the membrane fraction. Cells were incubated for 1 h at 37 °C with the indicated concentrations of TPA. A, after TPA was removed, the cells were incubated for 30 min at 37 °C without (C) or with 100 μM ouabain (D), 1 μM PGE2 and 100 μM ouabain (E), 0.1 μM TPA and 100 μM ouabain (F), or 1 μM ionomycin and 100 μM ouabain (G). Catecholamine release was determined as described under "Experimental Procedures." B, after TPA was removed, protein kinase C activity in the cytosol (C) or the membrane fraction (O) was determined as described under "Experimental Procedures." Values shown are means ± S. E. of triplicate experiments.

**Fig. 8.** Effect of PGE2, TPA, or ionomycin on pHi of chromaffin cells. Bovine chromaffin cells (5 x 10^6 cells) were stimulated by 1 μM PGE2, 0.1 μM TPA, or 1 μM ionomycin. pH was recorded and calibrated as described under "Experimental Procedures." The scale on the left indicates the change in pH. Traces: a, buffer; b, in the presence of 1 mM amiloride; c, Na+ replaced by N-methyl-D-glucamine; d, in the presence of 1 μM staurosporine; e, in the presence of 100 μM W-5; f, in the presence of 100 μM W-7; g, Ca2+ replaced by 1 mM EGTA.
It was earlier demonstrated from our laboratory that PGE₂ can cause phosphoinositide breakdown with the resultant formation of IP₃ and increased intracellular Ca²⁺ in bovine adrenal chromaffin cells, as well as enhance catecholamine release in the presence of ouabain (9). Furthermore we reported that PGE₂ did not stimulate adenylate cyclase activity in bovine adrenal medulla (27) and that forskolin, a potent activator of adenylate cyclase, and the cAMP analogue dibutyryl cAMP did not cause catecholamine release from ouabain-treated cells (9), demonstrating that the release induced by PGE₂ was not mediated by cAMP.

Phosphoinositide breakdown generates two signal pathways, Ca²⁺ mobilization and protein kinase C activation (33). First we examined the respective participation of these two signal pathways in the stimulation of catecholamine release from chromaffin cells, using TPA and ionomycin, and the possibility of involvement of these pathways in PGE₂-induced catecholamine release. The effect of TPA and ionomycin was additive to each other (Fig. 2). About 60% of the level of PGE₂-induced catecholamine release in the presence of ouabain was replaced by TPA at 1 μM but not by ionomycin, indicating the involvement of the protein kinase C pathway in PGE₂-induced catecholamine release. Actually PGE₂ caused the formation of diacylglycerol (Fig. 3) and the translocation of a small portion (4%) of the total protein kinase C activity to the membrane fraction (Fig. 4). TPA also induced the translocation protein kinase C activity from the cytosol to the membrane fraction (Fig. 5), but the ability of TPA to increase membrane-bound protein kinase C was much stronger than that of PGE₂. However, from the dose dependences of TPA for the stimulation of catecholamine release in the presence of ouabain (Fig. 2A) and that of protein kinase C translocation (Fig. 5B), TPA at 10 nM caused almost maximal stimulation of the release by the translocation of only 4% protein kinase C activity to the membrane fraction. This percentage of protein kinase C translocation inducing almost maximal stimulation of the release by TPA was similar to that of protein kinase C translocation induced by PGE₂, indicating that the translocation of a small portion of protein kinase C activity by PGE₂ is enough to cause catecholamine release. Consistent with our results, TerBush et al. reported that TPA increased the percentage of membrane-bound protein kinase C activity in bovine adrenal chromaffin cells from less than 10% of the basal level to 20–60% within 30 min (34) but that translocation of as little as 2–3% of the cellular protein kinase C to the membrane enhanced Ca²⁺-dependent catecholamine secretion by 25–30% from the cells permeabilized by digitonin (35). Furthermore, the involvement of the protein kinase C pathway in PGE₂-induced release was supported by the results of inhibition of PGE₂-induced release by depletion of protein kinase C caused by prolonged exposure to TPA (Fig. 7).

Neomycin, an aminoglycoside antibiotic, binds strongly to polyphosphoinositides and then inhibits phosphoinositide-specific phospholipase C (36, 37). It has been shown that neomycin inhibits platelet aggregation, secretion, and phosphoinositide metabolism after stimulation by thrombin (38). Here we demonstrate that neomycin strongly inhibited PGE₂-evoked catecholamine release in the presence of ouabain (Fig. 6A). This inhibitory effect is thought to be due to the inhibition of PGE₂-induced accumulation of inositol bisphosphate and IP₃ (Fig. 6B) and resultant inhibition of diacylglycerol formation and subsequent translocation of protein kinase C (Table II). From the results of the effects of neomycin, we infer that PGE₂-evoked catecholamine release is mediated by stimulation of phosphoinositide metabolism and protein kinase C translocation. On the other hand, ionomycin at 1 μM induced a gradual formation of IP₃, diacylglycerol formation, and also protein kinase C activity in the membrane fraction (data not shown). However, the participation of ionomycin-induced phosphoinositide metabolism and subsequent activation of protein kinase C in ionomycin-induced catecholamine release in the presence of ouabain may be low because the inhibition of the release by neomycin and prolonged exposure to TPA was weak (Figs. 6A and 7A). It would be explained by the Ca²⁺, calmodulin pathway (Fig. 8).

The synergistic enhancement of ouabain for PGE₂-induced catecholamine release suggests that accumulation of Na⁺ is necessary for the release. Indeed, the inhibitory effect on the release by isoosmotic replacement of Na⁺ by N-methyl-D-glucamine indicates that the presence of extracellular Na⁺ is essential for PGE₂-induced release from bovine chromaffin cells (21). Furthermore, we reported that PGE₂ potentiated ouabain-induced Na⁺ accumulation and the following increase of Ca²⁺ uptake and that these accumulations and enhanced catecholamine release were inhibited by ethyloisopropyramilide, a potent and specific inhibitor of the Na⁺, H⁺-antiprot (21). These results suggest that the amiloride-sensitive Na⁺, H⁺-antiprot system participates in PGE₂-evoked catecholamine release. Therefore we investigated the mechanism of activation of Na⁺, H⁺-antiprot by PGE₂. Two mechanisms have been proposed to stimulate the Na⁺, H⁺-antiprot (39). The first mechanism for the stimulation of Na⁺, H⁺-antiprot is mediated by protein kinase C. Phorbol esters and synthetic diacylglycerol analogues, direct activators of protein kinase C, stimulate the activity of the Na⁺, H⁺-antiprot in various lines of cells (14). Thus it has been postulated that activation of protein kinase C by diacylglycerol is responsible for the stimulation of the Na⁺, H⁺-antiprot. Second, an increase in the free cytoplasmic Ca²⁺ concentration has also been reported to be responsible for the activation of the antiprot (39), and the Ca²⁺ ionophore A23187 and ionomycin mimic this effect on the antiprot (40, 41). As shown in Fig. 8, both TPA and ionomycin have the ability to activate the Na⁺, H⁺-antiprot in chromaffin cells, and TPA- or ionomycin-induced elevation of pH exclude inhibited by the protein kinase C inhibitor staurosporine or the calmodulin inhibitor W-7, respectively. These findings suggest that there are two different mechanisms for the activation of the Na⁺, H⁺-antiprot in chromaffin cells: one is the activation by protein kinase C and the other is that by Ca²⁺, calmodulin. PGE₂-induced activation of the Na⁺, H⁺-antiprot results from the activation of protein kinase C, not that of the Ca²⁺, calmodulin pathway because the elevation of pH, by PGE₂, was inhibited by staurosporine, but not by W-7.

The importance of protein kinase C in the secretory process has been demonstrated by the use of phorbol esters (14). It was previously reported by Brocklehurst (42) that TPA and A23187 stimulated catecholamine secretion from intact chromaffin cells but that this level was very low (≤5%). It has also been shown that TPA enhanced both protein phosphorylation and Ca²⁺-dependent catecholamine secretion in digitonin-treated chromaffin cells, thereby suggesting that protein phosphorylation caused by protein kinase C is responsible for the enhancement of Ca²⁺-dependent catecholamine secretion induced by phorbol esters (43). Added to the knowledge that protein kinase C is important in the secretory process (14), our present study demonstrates that protein kinase C, activated as a result of stimulation of phosphoinositide metabolism by PGE₂, modulates the function of the Na⁺, H⁺-antiprot,
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