Pardaxin, a New Pharmacological Tool to Stimulate the Arachidonic Acid Cascade in PC12 Cells

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ABSTRACT

The effect of Pardaxin, a neurotoxin that induces neurotransmitter release from neurons, on the arachidonic acid (AA) cascade was studied in PC12 cells. Both native and the synthetic Pardaxin selectively stimulated phospholipase A\(_2\) (PLA\(_2\)) activity (measured by \(^{3}H\)AA release) in the presence as well as in the absence of extracellular calcium. Pardaxin-stimulated PLA\(_2\) activity was also evident in the increased formation of lysophosphatidylcholine. Pardaxin analogs, lacking the \(\alpha\)-helical structure that is essential for insertion into the plasma membrane, were ineffective in stimulating the AA cascade in PC12 cells. Pardaxin stimulation of PLA\(_{2}\) was markedly inhibited by the nonselective PLA\(_{2}\) inhibitors bromophenacyl bromide and mepacrine, by methyl arachidonoyl fluorophosphonate, a dual inhibitor of calcium-dependent cytosolic PLA\(_{2}\) and the calcium-independent PLA\(_{2}\), and by bromoenoel lactone[(E)-6-(bromothielenetetrahydro-3-(1-naphthalenyl-2H-pyran-2-one), a highly specific inhibitor of calcium-independent PLA\(_{2}\). After Pardaxin treatment, there was increased release of AA metabolites produced by the cyclooxygenase pathway as expressed in an 8-fold increase of PGE\(_{2}\) release. The release of other eicosanoids, such as 6-keto-PGF\(_{1\alpha}\) and thromboxane B\(_{2}\), was also augmented. Pardaxin-induced PGE\(_{2}\) release was observed in calcium-free medium and in the absence of any increase in cytosolic calcium. Dexamethasone partially inhibited Pardaxin-induced PGE\(_{2}\) release. This effect was reversed by the type II corticosteroid receptor antagonist RU-38486. Our results indicate that Pardaxin stimulates release of AA and eicosanoids, independently of calcium, and suggest that calcium-independent PLA\(_{2}\) plays an important role in Pardaxin stimulation of the AA cascade.

Ionophore toxins belong to a large group of proteins of bacterial, plant and animal origin that alter plasma membrane permeability by forming small lesions (Harvey, 1990). The importance of this group of toxins is evident from the finding that of the several hundred toxins studied to date, a large group are ionophores. Although the pore/channel activity of these toxins was intensively investigated (Bernheimer and Rudy, 1986), their mode of interference with the cellular signal transduction pathways is largely unknown (Bloch-Shilderman et al., 1996). According to pore size and mechanism of cell membrane injury, ionophore toxins have been classified into three major groups (Thelestam and Mollby, 1979; Bloch-Shilderman et al., 1997): 1) thiol-activated toxins that form large pores, 2) toxins with surfactant activity on the plasma membrane and 3) toxins that form voltage-dependent ionic channels. Recent studies suggest that thiol-activated toxins such as streptolysin S (Abu-Ray et al., 1993a), surfactant toxins including melittin and mastoparan (Choi et al., 1992) and other toxins such as maitotoxin (Choi et al., 1990) and canatoxin (Barja-Fidalgo et al., 1991) activate the AA cascade in rat pheochromocytoma PC12 cells, rat brain synaptosomes and rabbit platelets.

Over the past ten years we have isolated and characterized a new ionophore toxin, Pardaxin, derived from the fish Parachirus marmoratus (Lazarovici et al., 1986, 1988; Shai et al., 1988). Pardaxin is an acidic, amphipathic and hydrophobic polypeptide composed of 33 amino acids (Lazarovici et al.,...
1986) that forms voltage-dependent pores in liposomes (Loew et al., 1985) and planar lipid bilayers (Lazarovici et al., 1992; Shi et al., 1995). The toxin exhibits a neurotoxic, excitatory activity toward neurons (Lazarovici, 1994), induces contraction of guinea pig ileum (Primor, 1986), hemolysis and reduction of blood pressure and is lethal to rats (Primor and Lazarovici, 1981). Pardaxin was proposed as a pharmacological tool for studying neurotransmitter release (Lazarovici and Lelkes, 1992) because it stimulates excotysis in a variety of neuronal preparations by both calcium-dependent and calcium-independent mechanisms (Lazarovici and Lelkes, 1992; Arribas et al., 1993). The major aim of the present study was to examine the ability of Pardaxin to stimulate the AA cascade in PC12 cells, in the presence or absence of [Ca]i.

Materials and Methods

Materials. The following drugs and chemicals were used in this study: [3H]AA (210 Ci/m mole), [3H]PEG2, [3H]TXB2, [3H]6-keto-PGF1α (120–200 Ci/m mole) and [3H]myoinositol (10–25 Ci/m mole) (New England Nuclear, Boston, MA), [3H]Dex (35–60 Ci/m mole) and ortho[32P]phosphate (10 μCi/ml) (Amersham, Buckinghamshire, UK), antibodies directed against PGE2, TXB2 and 6-keto-PGF1α (BioYeda, Rehovot, Israel), fura 2-AM (Molecular Probes Inc., Junction City, OR), indomethacin, EGTA, dextran, Dex, carbachol, charcoal, A23187, NDGA, PGE2, TXB2, 6-keto-PGF1α, esculetin, pBPB-4 and mepacrine (Sigma Chemical Co., St. Louis, MO), RU-28318 and RU-38486 (UCLAF-Roussel, Romanville, France) and RHC-80267, BEL and MAFP (Biomol, Plymouth Meeting, PA).

Toxins. Pardaxin, a natural toxin secreted from the glands of the fish Pardachirus marmoratus, was isolated by the liquid chromatography method as described (Lazarovici et al., 1986). The native toxin used in the present study was purified by HPLC and found to be homogeneous by SDS-gel electrophoresis and by amino acid analysis (Lazarovici et al., 1986). The primary structure of Pardaxin: NH2-GFFALIPKSSPLFTLSAVGSALSSSSGGQQ-COOH was determined by sequencing. Pardaxins were synthesized by the solid-phase method on (phenylacetamido) methyl-amino acid resin, as previously described (Shai and Oren, 1996). Coupling was carried out using dicyclohexylcarbodiimide and an appropriate dicyclohexylurea. Pardaxins were synthesized by the solid-phase method on (phenylacetamido) methyl-amino acid resin, as previously described (Shai and Oren, 1996). Coupling was carried out using dicyclohexylcarbodiimide and an appropriate dicyclohexylurea. Pardaxins were synthesized by the solid-phase method on (phenylacetamido) methyl-amino acid resin, as previously described (Shai and Oren, 1996). Coupling was carried out using dicyclohexylcarbodiimide and an appropriate dicyclohexylurea.

Cell viability. In the present study, subcytotoxic concentrations of Pardaxin were used. Pardaxin concentration was considered subcytotoxic when less than 10% cell death was obtained, as determined by trypan blue exclusion (Abu-Raya et al., 1993b).

PC12 cultures. PC12 cells were grown in DMEM supplemented with 7% fetal calf serum, 7% horse serum, 100 μg/ml streptomycin and 100 units/ml penicillin. The cell cultures were maintained in an incubator at 37°C in an atmosphere of 6% CO2. Medium was changed twice weekly, and the cultures were split at a 1:6 ratio once a week. Experiments were performed with PC12 cells grown on 6-well or small Petri dishes coated with equal parts of collagen (0.01 mg/ml collagen in 0.1 M acetic acid) and poly l-lysine (0.01 mg/ml) (Abu-Raya et al., 1993a).

Measurement of PLA2 activity. 1. AA release: PC12 cells were grown in 6-well dishes in serum-containing DMEM for 24 hr at 37°C. The growth medium was then removed and replaced with serum-free DMEM to which [3H]AA (0.5 μCi/ml) was added, and the plates were incubated for 4 hr. The medium (containing the nonincorporated isotope) was removed, and the cells were washed three times with PBS (138 mM NaCl, 8 mM Na2HPO4, 0.5 mM MgCl2 and 0.9 mM CaCl2, pH 7.4). The rinsed cells were then incubated with 1 ml of PBS supplemented with 20 mM glucose and 1 mg/ml fatty acid free-BSA for 10 min at 37°C (Fink and Guroff, 1990). Release assays were initiated by adding the tested compound in the presence or absence of different inhibitors, and the cultures were further incubated at 37°C for various intervals. At the indicated times, 200 μl of incubation medium was collected from each well and centrifuged for 10 min (1000 × g); then the supernatant was collected and the volume measured. Release of [3H]AA was measured in 100-μl aliquots in a liquid scintillation counter. The amount of protein in each well was determined according to Lowry (Lowry et al., 1951). The amount of AA released was expressed as counts per minute per milligram of protein, or as percentage of the control. 2. Changes in phospholipids content: PC12 cells were grown to confluence on small Petri dishes (2 × 106 cells/dish) in serum-containing DMEM for 24 hr at 37°C. The growth medium was removed and replaced with 1% delipidated serum containing [32P]orthophosphate (1 μCi/ml), and the cultures were incubated for 18 hr at 37°C. Thereafter, the medium containing nonincorporated isotope was removed, and the cultures were washed three times with PBS. The rinsed cells were incubated in PBS with 5 μM Pardaxin for 15 min at 37°C. After extensive washing, the lipid were extracted with cold n-propanol for 4 hr, evaporated and separated by two-directional thin-layer chromatography on precoated silica-gel plates (Merck, Darmstadt). 32P-labeled phospholipids were isolated by autoradiography (Kodak, XRP-54 film), followed by counting of the radioactive spots as previously described (Yavin and Zutra, 1977). Because the level of PE was not changed after treatment with Pardaxin, the levels of the major phospholipids are presented relative to the PE level.

Eicosanoid RIA. The cultures were exposed at 37°C for various intervals to different concentrations of Pardaxin. At the end of the experiment, the medium was removed and centrifuged at 4°C for 10 min at 1000 × g, and aliquots were removed for RIAs of the different eicosanoids. (Abu-Raya et al., 1993a,b). Standard curves were generated with the respective eicosanoids. After incubation of samples (or standard) for 18 to 24 hr with the appropriate antiserum and radioligands, free and bound compounds were separated by dextran coated with activated charcoal. Radioactivity was counted in a β-scintillation counter (LKB, Wallac Oy, Finland).

Measurement of [Ca]i levels. The concentration of [Ca]i, was measured using the fluorescent calcium chelator Fura 2, as previously described (Lazarovici and Lelkes, 1992). PC12 cells were collected and incubated at 20°C for 60 min in the dark in culture medium containing 5 μM Fura 2-AM. After washing, the cells were resuspended in fresh medium at a concentration of 2 × 106 cells/ml, in the presence or absence of Pardaxin, for 15 min in a SPEX Fluorolog 2 spectrofluorometer. [Ca]i was measured by the fluorescence obtained by excitation at 340 nm and emission at 510 nm. A 435-nm cut-off filter was used to reduce light scattering. After an initial equilibration of the fluorescent signal for 3 to 5 min, the base line remained stable over the duration of the experiment (15 min) (Lazarovici and Lelkes, 1992).

Measurement of PLC activity. PC12 cells were pretreated with [3H]myoinositol, and assays were performed as previously described (Fink et al., 1989). The [3H]inositol phosphates in the cultures were extracted with perchloric acid and analyzed by anion-exchange chromatography.

[3H]Dex binding. PC12 cultures grown to confluence on small Petri dishes (2 × 106 cells/dish) were washed with serum-free DMEM and incubated with different concentrations of [3H]Dex for 2 hr at 37°C in 1 ml of serum-free medium. To measure nonspecific binding, the same experiments were performed in the presence of 1 μM unlabeled Dex. In the competition experiments, cells were incubated with [3H]Dex (20 nM) and the respective antagonist (1 μM). At the end of the incubation, the cultures were cooled for 30 min at 4°C and...
Results

Stimulation of eicosanoid release in PC12 cells by Pardaxin. The effect of Pardaxin on PGE$_2$ release in the presence or absence of [Ca]$^{++}$ is presented in figure 1. Treatment of the cultures with 5 μM and 15 μM Pardaxin for 30 min in calcium-containing medium stimulated PGE$_2$ release by 5- and 13-fold, respectively, over that of the control (P < .01). In the absence of [Ca]$^{++}$, PGE$_2$ release in the presence of Pardaxin (5–15 μM) was between 50% and 70% of that obtained in the presence of [Ca]$^{++}$ (fig. 1). Pardaxin-induced PGE$_2$ release was detected as early as 5 min after addition of the toxin and reached a maximum at 30 min (data not shown). Much like native Pardaxin, synthetic Pardaxin (5 μM) stimulated PGE$_2$ release about 3-fold over that of the control (table 1). To verify the selective effect of Pardaxin on PGE$_2$ release, three Pardaxin structural analogs without α-helical structure (Shai and Oren, 1996) were tested. They proved to be ineffective in stimulating PGE$_2$ release (table 1).

The culture medium of PC12 cells treated with Pardaxin (5 μM) for 15 min was examined for other cyclooxygenase products. As shown in table 2, the release of TXB$_2$ (the stable metabolite of thromboxane) and 6-keto-PGF$_1$α (the stable metabolite of prostacyclin) was increased 2- and 3-fold, respectively, over that of the control. Preincubation of the cells for 30 min with 50 μM indomethacin (a cyclooxygenase inhibitor) completely blocked the basal release (data not shown), as well as the Pardaxin-stimulated release, of PGE$_2$, TXB$_2$, and 6-keto-PGF$_1$α (table 2).

Stimulation of AA release from PC12 cells by Pardaxin. Because the triggering of eicosanoid release by Pardaxin could be due to phospholipases stimulation, we explored this possibility by treating PC12 cultures labeled with [3H]AA with several concentrations of Pardaxin, in the presence or absence of [Ca]$^{++}$. In the presence of [Ca]$^{++}$, 1 μM and 10 μM Pardaxin stimulated AA release 2.3-fold (P < .05) and 10-fold, respectively (P < .01), vs. the control (fig. 2). In the absence of [Ca]$^{++}$, 1 μM and 10 μM Pardaxin stimulated AA release 1.6-fold (P < .05) and 7.1-fold, respectively (P < .05), vs. the control (fig. 2). Stimulation of AA release by Pardaxin (5 μM) was detected after 5 min of incubation, and maximal stimulation was measured after 30 min of incubation. The same time course of AA release was observed in the presence or absence of [Ca]$^{++}$ (fig. 3). A careful comparison of the kinetics of AA release observed in the presence and absence of [Ca]$^{++}$ indicates that upon short-term (5-min) incubation, the bulk (about 80%) of the Pardaxin-induced AA release was independent of [Ca]$^{++}$ whereas after 30 min, about 50% of the Pardaxin-induced AA release was independent of [Ca]$^{++}$ (fig. 3).

AA could be released because of PLA$_2$ activation or as a result of the combined action of PLC and diacylglycerol lipase. To examine the possibility that PLC stimulation is involved in AA release by Pardaxin, PC12 cells labeled with [3H]myoinositol were treated for 30 min with Pardaxin (5 μM) or bradykinin (1 μM). Whereas bradykinin induced accumulation of [3H]IP$_1$ to about 280 ± 25% over that of the control, Pardaxin did not induce [3H]IP$_1$ accumulation (100 ± 2%). These data suggest that Pardaxin does not stimulate PLC in PC12 cells. To confirm this finding, we treated PC12 cells with 50 μM RHC-80267, a DAG-lipase inhibitor (Allen et al., 1992). Table 3 shows that RHC-80267 completely suppressed bradykinin-induced AA release, but not Pardaxin-induced AA release. A similar lack of inhibition of Pardaxin-induced AA release was observed upon treatment of the cells with neomycin (data not shown), a phospholipase C inhibitor (Tsukada et al., 1994).

To verify further that AA release induced by Pardaxin is mediated mainly by PLA$_2$ activation, we examined the effect of Pardaxin on $^{32}$P-phospholipids content. As shown in table 4, the levels of the major phospholipid species PE, PI and PS were not changed after Pardaxin treatment. In addition, no formation of PA was observed. Because Pardaxin did not lead to PA generation, it appears that PLD is not activated and/or involved in Pardaxin-induced AA release. This finding is further supported by the fact that ethanol, which causes a loss of PA production in favor of phosphatidylethanol, did not affect Pardaxin-induced AA release in PC12 cells (data not shown). However, a reduction by about 30% in PC level was observed after Pardaxin treatment, which was accompanied by about a 40% increase LPC formation (table 4), a result that indicates PLA$_2$ activation. Because Pardaxin affects the level of PC but not that of PS or PE, these data suggest that PC is one of the major phospholipid sources utilized by Pardaxin for AA mobilization in PC12 cells.

To examine the effect of PLA$_2$ inhibitors on Pardaxin-induced AA release, we treated PC12 cells with different PLA$_2$ inhibitors (fig. 4). Treatment of the cells with Dex (1 μM) for 24 hr reduced by 60% AA release by Pardaxin. In contrast to this partial inhibition, treatment of PC12 cells for 15 min with the PLA$_2$ inhibitors pBPB (50 μM) and mepacrine (100 μM) (Mukherjee et al., 1994) blocked Pardaxin-induced AA release by 90% and completely, respectively. These inhibitors did not affect the basal release of AA from PC12 cells (data not shown).

To evaluate the role of calcium in Pardaxin-induced AA release, experiments with Fura 2-AM-loaded cells were per-
TABLE 1
The effect of native Pardaxin versus synthetic Pardaxin peptides on PGE₂ release from PC12 cells

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence b</th>
<th>Peptide-induced PGE₂ release (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>GFFALIKISSPLKFTLLSAGVSALSSGGQEQ</td>
<td>750 ± 55</td>
</tr>
<tr>
<td>Synthetic</td>
<td>GFFALIKISSPLKFTLLSAGVSALSSGGQEQ</td>
<td>660 ± 85</td>
</tr>
<tr>
<td>Analog [D]P⁷</td>
<td>GFFALIKISSPLKFTLLSAGVSALSSGGQEQ</td>
<td>ND</td>
</tr>
<tr>
<td>Analog [D]P⁷L¹⁹</td>
<td>GFFALIKISSPLKFTLLSAGVSALSSGGQEQ</td>
<td>ND</td>
</tr>
<tr>
<td>Analog [D]P²⁰L¹⁹</td>
<td>GFFALIKISSPLKFTLLSAGVSALSSGGQEQ</td>
<td>ND</td>
</tr>
</tbody>
</table>

*PC12 cells were incubated with Pardaxin peptides (5 μM) for 15 min at 37°C, and PGE₂ release was determined in the culture medium. The results are the mean ± S.E.M. of three experiments (n = 3). The basal release of PGE₂ (217 ± 82 pg/ml) was subtracted from all the experimental values.

Amino acids marked by bold letters were substituted with their α-ε-amino analogues; E=NH₂₃ stands for glutamic acid in which the two COOH groups were modified to two CO-NH₂CH₂NH₂ groups by transamination with ethylenediamine.

**ND**, nondetectable.

TABLE 2
The effect of indomethacin on Pardaxin-induced release of cyclooxygenase products in PC12 cells

<table>
<thead>
<tr>
<th>Eicosanoid (pg/ml)</th>
<th>Pardaxin</th>
<th>Pardaxin + Indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE₂</td>
<td>1034 ± 275*</td>
<td>ND b</td>
</tr>
<tr>
<td>TXB₂</td>
<td>81 ± 25*</td>
<td>ND b</td>
</tr>
<tr>
<td>6-keto-PGF₁α</td>
<td>23 ± 9*</td>
<td>ND b</td>
</tr>
</tbody>
</table>

*PC12 cells preincubated with or without indomethacin (50 μM) for 30 min were treated with Pardaxin (5 μM) for an additional 15 min in DMEM containing calcium. Eicosanoids were determined in the culture medium. The basal release of PGE₂ (287 ± 66 pg/ml), TXB₂ (85 ± 7 pg/ml) and 6-keto-PGF₁α (8 ± 1 pg/ml) was subtracted from all the experimental values. The results are the mean ± S.E.M. of three experiments (n = 6).

**ND**, nondetectable.

**P** < .01 vs. basal release.

**Fig. 2.** Dose-dependent stimulation of [³H]AA release from PC12 cells by Pardaxin in the presence (+Ca) or absence (−Ca) of [Ca]₀(+1 mM EGTA). Cells labeled with [³H]AA were incubated with the toxin for 30 min. The results are the mean ± S.E.M. of three experiments (n = 3).

PC12 cells were incubated with Pardaxin peptides (5 μM) for 15 min at 37°C, and PGE₂ release was determined in the culture medium. The results are the mean ± S.E.M. of three experiments (n = 3).
TABLE 4

<table>
<thead>
<tr>
<th>Phospholipid Class</th>
<th>Untreated</th>
<th>Pardaxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>3.6 ± 0.4</td>
<td>2.5 ± 0.3*</td>
</tr>
<tr>
<td>LPC</td>
<td>0.025 ± 0.002</td>
<td>0.05 ± 0.005*</td>
</tr>
<tr>
<td>PS</td>
<td>0.23 ± 0.003</td>
<td>0.23 ± 0.05</td>
</tr>
<tr>
<td>PI</td>
<td>0.39 ± 0.05</td>
<td>0.35 ± 0.06</td>
</tr>
<tr>
<td>PA</td>
<td>0.02 ± 0.005</td>
<td>0.02 ± 0.002</td>
</tr>
</tbody>
</table>

*P < .05 vs. untreated.

Fig. 4. The effect of PLA₂ inhibitors on AA release by Pardaxin. [³H]AA-labeled cells were preincubated for 24 hr with Dex (1 μM) or for 15 min with mepacrine (Mep, 100 μM) or pBPB (50 μM), before stimulation with Pardaxin (5 μM), for 15 min. The values are the mean ± S.E.M. of three experiments (n = 3). The basal release value of [³H]AA (1204 ± 123 cpm/mg protein) was subtracted from all experimental values. Release of AA in all experimental groups was lower (P < .01) than in the control (Pardaxin alone).

Fig. 5. The effect of [Ca²⁺], on [Ca²⁺] in response to Pardaxin. Fura 2-AM-loaded PC12 cells were treated with KCl (50 mM), ionomycin (20 μM), or Pardaxin (5 μM) in medium containing 2 mM CaCl₂ (+Ca²⁺) (panels A and B) or calcium-free medium containing 1 mM EGTA (-Ca²⁺) (panel B). Arrows indicate time of addition of the compound

TABLE 5

<table>
<thead>
<tr>
<th>Compound (concentration)</th>
<th>Pardaxin-Induced PGE₂ Release (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[³H]AA Release (cpm/mg protein)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Pardaxin (5 μM)</td>
<td>392 ± 11</td>
</tr>
<tr>
<td>Pardaxin + BEL (100 nM)</td>
<td>316 ± 7*</td>
</tr>
<tr>
<td>Pardaxin + RU-38486 (1 μM)</td>
<td>254 ± 13*</td>
</tr>
<tr>
<td></td>
<td>*P &lt; .05 vs. Pardaxin alone.</td>
</tr>
</tbody>
</table>

The inhibitory effect of BEL and MAFP on Pardaxin-induced AA release from PC12 cells
PC12 cells were labeled with [³H]AA, washed and either incubated for 15 min with BEL (5 μM) or MAFP (25 μM) or left untreated. The cultures were then incubated with Pardaxin (5 μM) for 15 min. The basal release of AA (2013 ± 178 cpm/mg protein) and the release in the presence of BEL or MAFP alone (1879 ± 126 and 2000 ± 155 cpm/mg protein, respectively) were subtracted from the experimental values. The values are the mean ± S.E.M. of three experiments (n = 3)

Fig. 6. Characterization of [³H]Dex-specific binding by PC12 cells. A) [³H]Dex specific binding at different concentrations. B) Competition between [³H]Dex (DEX, 20 nM) and corticosteroid receptor antagonists (1 μM). PC12 cultures (2 × 10⁶ cells/dish) were incubated with [³H]Dex for 2 hr at 37°C in the presence (nonspecific binding) or absence (total binding) of unlabeled Dex (1 μM). The specific binding data represent the mean ± S.E.M. of three experiments (n = 3).

Discussion

In the present study, we demonstrate that both native and synthetic Pardaxin trigger the AA cascade in PC12 cells. This
stimulation is mediated by activation of PLA$_2$, as reflected in the massive release of AA and various eicosanoids. Stimulation of the AA cascade in PC12 cells by Pardaxin was blocked by a variety of AA cascade inhibitors.

The activation of cellular phospholipases is thought to mediate signal transduction of certain receptors, and it has been related to a variety of cellular events (Serhan et al., 1996). A moderate increase in AA release is observed after receptor-mediated PLA$_2$ activation by different ligands such as nerve growth factor (Pink and Guroff, 1980), ACh (Felder et al., 1990a), serotonin (Felder et al., 1990b), histamine (Murayama et al., 1990), bradykinin (Allen et al., 1992) and epidermal growth factor (Schalkwijk et al., 1995). In the present study, we found that Pardaxin-induced AA release in PC12 cells was up to 10-fold greater than that in the control. Such strong stimulation of PLA$_2$ activity is typical of other cytolytic toxins (Abu-Raya et al., 1993a).

The effect of cytolytic toxins on PLA$_2$ stimulation may be explained by assuming a drastic change in plasma membrane phospholipid organization due to insertion of the $\alpha$-helical structures in the phospholipid bilayer. Indeed, Pardaxin, an $\alpha$-helical toxin, induced aggregation of PS vesicles (Lelek and Lazarovici, 1988); melittin, another $\alpha$-helical toxin, changed the phase-transition properties of the phospholipid bilayer (Mollay, 1976). It is well known that PLA$_2$ activity is very sensitive to the molecular interactions between the plasma membrane constituents, lateral surface pressure and the surface potential of the phospholipid interface (Jain and De Kloet, 1991), we characterized, for the first time, the corticosteroid receptors in PC12 cells as type II receptors (fig. 6B). This finding is also supported by the ability of the corticosteroid type II receptor antagonist RU-38486 to abolish Dex-induced inhibition of PGE$_2$ release in response to Pardaxin (table 6). Because PC12 cells are derived from a tumor of the adrenal medulla (Tischler and Greene, 1978), in which the chromaffin cells are exposed to high levels of glucocorticoids, it is not surprising that the low-affinity corticosteroid type II receptors that we found in the PC12 cells are similar to those observed in chromaffin cells (Betito et al., 1992).

The AA release after Pardaxin stimulation of PLA$_2$ results in the synthesis of the cyclooxygenase products in PC12 cells: mainly PGE$_2$ and to a lesser degree TXB$_2$ and 6-keto-PGF$_1\alpha$. Similar results were obtained with PC12 cultures under ischemic conditions (Abu-Raya et al., 1993b). In the present experiments, pretreatment of PC12 cells with indomethacin completely abolished the release of these eicosanoids (table 2). Our results point to a direct correlation between Pardaxin-stimulated AA release and PGE$_2$ production: 1) in the presence of $[\text{Ca}]_o$, Pardaxin caused greater amounts of AA (figs. 2 and 3) and PGE$_2$ (fig. 1) to be released than in calcium-free medium, and 2) inhibition of AA release in response to PLA$_2$ inhibitors (fig. 4) corresponded to a parallel reduction in Pardaxin-induced PGE$_2$ release (table 6 and data not shown).

Stimulation of the AA cascade and the subsequent formation of eicosanoids such as prostacyclin (PGI$_2$) in endothelial cells (Suttrop et al., 1985), leukotriene B$_4$ in polymorphonuclear leukocytes (Suttrop et al., 1987) or platelet-activating factor (PAF) in pulmonary artery endothelial cells (Suttrop et al., 1992) was reported for Staphylococcus aureus $\alpha$-toxin.
Other cytolsins, such as streptolysin S, Staphylococcus aureus toxin, and streptolysin O, might explain the wide spectrum of physiological and pathological effects caused by these toxins, such as neuro-transmitter release, pulmonary hypotension, blood coagulation, endothelial shock, edema and inflammation (Seegeier et al., 1984; Bhakdi et al., 1988, Snyder, 1990) observed upon exposure to such toxins.

As we have noted, Pardaxin, in contrast to maitotoxin (Choi et al., 1990), melittin and mastoparan (Choi et al., 1992) did not stimulate PLC. In addition, Pardaxin treatment did not affect PLD activity as measured by PA formation. The present study indicates that the AA release by this toxin was due mainly to PLA2 activity as measured by PA formation. The present study indicates that the AA release by this toxin was due mainly to PLAs enzymes and the development of PLAs inhibitors.

References


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