Differential Effects of the Protein Kinase C Activator Phorbol 12-Myristate 13-Acetate on Calcium Responses and Secretion in Adherent and Suspended RBL-2H3 Mucosal Mast Cells*

(Received for publication, June 7, 1995, and in revised form, December 11, 1995)

Patricia C. Wolfe§, En-Yuh Chang¶, Juaan Rivera§, and Clare Fewtrell‡

From the Department of Pharmacology, Cornell University, Ithaca, New York 14853 and §Section on Chemical Immunology, NIAMS, National Institutes of Health, Bethesda, Maryland 20892

Adhesion of RBL-2H3 mucosal mast cells to fibronectin-coated surfaces has been linked to changes in secretion and tyrosine kinase activity. We now show that adhesion affects the sensitivity of RBL cells to the protein kinase C activator phorbol 12-myristate 13-acetate (PMA). In suspended cells, PMA inhibited antigen-induced calcium influx (as measured by manganese influx) and changes in intracellular free calcium and had complex effects on antigen-stimulated secretion. However, in adherent cells PMA had little effect on these responses. Suspended cells only secreted in response to thapsigargin if they were co-treated with PMA, while adherent cells secreted in response to thapsigargin alone. The thapsigargin-induced secretion in adherent cells was inhibited by protein kinase C down-regulation and by the protein kinase C inhibitor GF 109203X, but not by calphostin C. We suggest that protein kinase C is constitutively activated in adherent cells, possibly due to modification of the regulatory domain of the enzyme.

The RBL-2H3 mucosal mast cell line has been used extensively as a model of stimulus secretion coupling (1). Activation of these cells by antigen leads to a complex series of events including tyrosine phosphorylation of various proteins (2, 3), emptying of intracellular calcium stores by inositol 1,4,5-trisphosphate (IP₃) (7,8), and influx of calcium across the plasma membrane (9–11). These events culminate in the secretion of various mediators of the inflammatory response (1, 8). It is clear that both the increase in intracellular calcium and protein kinase C activation are important steps in the signaling pathway and that these two signals act synergistically to promote secretion (12, 13).

Activation of protein kinase C with the phorbol ester phorbol 12-myristate 13-acetate (PMA), alone, does not induce secretion in rat basophilic leukemia (RBL) cells (12–14). Some laboratories have reported that PMA potentiates antigen-induced secretion at concentrations below 15 nM (12, 15), but other reports do not support this finding (13, 14). Nevertheless, there is general agreement that PMA markedly potentiates secretion in response to calcium ionophore (12–14). A similar synergism has been seen when protein kinase C is activated by PMA while intracellular calcium is increased by treatment with the endoplasmic reticulum Ca²⁺-ATPase inhibitors thapsigargin or cyclopiazonic acid (16). Additionally, the protein kinase C inhibitors staurosporine, Ro31–7549, and calphostin C have been shown to inhibit antigen-stimulated secretion (17). In general, it appears that the combination of protein kinase C activation and increases in intracellular calcium are sufficient to induce secretion.

In addition to promoting secretion, activation protein kinase C by PMA has a second, inhibitory effect on RBL cells in suspension (12, 13, 15). Increases in intracellular Ca²⁺ are inhibited at concentrations above 10 nM (12, 13, 15), possibly by the inhibition of phospholipase C-γ (13, 18), thus preventing phosphoinositide breakdown. Some groups have also shown that antigen-stimulated secretion is inhibited by high concentrations of PMA (12, 15), presumably due to the inhibition of the Ca²⁺ response.

In the past, experiments on RBL cells have been performed interchangeably with cells in suspension or with adherent cells. However, recent experiments have shown that adhesion itself affects RBL cell responses. Adhesion of RBL cells results in the tyrosine phosphorylation of several proteins including pp125FAK (19). In addition, antigen-stimulated secretion is enhanced in adherent RBL cells (20). In studying the effects of protein kinase C activation on secretion and calcium handling, we have discovered another effect of adhesion on RBL cell responses, namely a loss of sensitivity to the effects of the protein kinase C activator, PMA.

EXPERIMENTAL PROCEDURES

Sensitized RBL Cells—All experiments were performed with the secreting subline 2H3 of rat basophilic leukemia cells (21) maintained in monolayer culture in Eagle's minimum essential medium containing 10% fetal bovine serum, 8% newborn bovine serum, and antibiotics as described (22). For secretion experiments in adherent cells, 0.4 × 10⁶ cells in 0.3 ml of culture medium containing 0.18 μg/ml mIgE·DNP were added to 35-mm plastic culture dishes, each of which contained a 22 × 22-mm glass coverslip that had been scored down the middle. The cells were incubated overnight at 37 °C in a humid atmosphere containing 5% CO₂. For fura-2 experiments with adherent cells, aliquots of 5 × 10⁵ cells in 2.5 ml of culture medium containing 1 μg/ml mIgE·DNP were added to 35-mm plastic culture dishes, each of which contained a 22 × 22-mm glass coverslip that had been scored down the middle. The cells were incubated overnight at 37 °C in a humid atmosphere containing 5% CO₂. For fura-2 experiments with adherent cells, aliquots of 5 × 10⁵ cells in 2.5 ml of culture medium containing 1 μg/ml mIgE·DNP were added to 35-mm plastic culture dishes, each of which contained a 22 × 22-mm glass coverslip that had been scored down the middle. The cells were incubated overnight at 37 °C in a humid atmosphere containing 5% CO₂.

The abbreviations used are: IgE, immunoglobulin E; fura-2/AM, fura-2 acetoxymethyl ester; IP₃, inositol-1,4,5-trisphosphate; PMA, phorbol 12-myristate 13-acetate; RBL, rat basophilic leukemia; mIgE·DNP, mouse monodonal IgE anti-dinitrophenyl.

§Recipient of a National Science Foundation predoctoral fellowship.

¶Recipient of a fellowship for advanced predoctoral training in pharmacology/toxicology from the Pharmaceutical Research and Manufacturers of America Foundation. To whom correspondence should be addressed: Dept. of Pharmacology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853-6401.

1 The abbreviations used are: IgE, immunoglobulin E; fura-2/AM, fura-2 acetoxymethyl ester; IP₃, inositol-1,4,5-trisphosphate; PMA, phorbol 12-myristate 13-acetate; RBL, rat basophilic leukemia; mIgE·DNP, mouse monodonal IgE anti-dinitrophenyl.

2 F. C. Mohr, personal communication.
5% CO₂. For secretion and fura-2 experiments with cell suspensions, cells grown to confluence in a 75-cm² tissue culture flask were incubated overnight at 37 °C in 10 ml of culture medium containing 6 μg of mlGEdN.P.

Solutions—The standard saline solution used was a modified Tyrode's solution composed of 135 mM NaCl, 5 mM KCl, 1.8 mM CaCl₂, 5.6 mM glucose, 0.05% gelatin, and 10 mM HEPES adjusted to pH 7.4 with NaOH. Ice-cold quenching solution for secretion experiments contained 135 mM NaCl, 5 mM KCl, and 10 mM Na-HEPES (pH 7.4). For loading cells with fura-2, the saline solution contained 250 μM sulfipyrazone (Sigma) and 0.1% bovine serum albumin instead of gelatin to maximize uptake and retention of the dye (23, 24).

Reagents—Fura-2 acetoxyethyl ester (fura-2/AM) was purchased from Molecular Probes (Junction City, OR). Calphostin C and GF 109203X were purchased from Calbiochem-Novabiochem International (San Diego, CA). PMA, thapsigargin, and 4-methylumbelliferon-N-acetyl β-D-glucosaminide were purchased from Sigma. Stock solutions of fura-2/AM, PMA, thapsigargin, 4-methylumbelliferon-N-acetyl β-D-glucosaminide, calphostin C, and GF 109203X were prepared in dry dimethyl sulfoxide. Cells were never exposed to >0.2% dimethyl sulfoxide, and at this or lower concentrations, the solvent did not affect the responses of RBL cells. Purified mlGEdN.P. (25) was a gift from Barbara Baird and David Holowka, Department of Chemistry, Cornell University. The antigen used was bovine γ-globulin to which an average of 10 dinitrophenyl groups/molecule had been coupled (26), except for the measurements of translocation and tyrosine phosphorylation of protein kinase C, where the dinitropheenyl groups were coupled to bovine serum albumin.

Secretion—This was determined from the release of the granule-associated enzyme, β-hexosaminidase. Secretion was carried out in 24-well plates in which cells had been plated overnight and washed with saline solution, or in polystyrene tubes containing 0.5 × 10⁶ cells in saline solution. Secretion was initiated by antigen (1 μg/ml) added directly to the wells or tubes, and was terminated by adding ice-cold quenching solution to each well after 30 min for adherent cells or 60 min for suspended cells. These incubation times resulted in maximal secretion. An aliquot from each supernatant was assayed fluorimetrically for β-hexosaminidase (excitation 360, emission 450) using 4-methylumbelliferonyl-N-acetyl β-D-glucosaminide as the substrate. Secretion is expressed as a percent of the β-hexosaminidase content of the cells prior to stimulation.

Fura-2 Measurements in Cell Suspensions—For measurements of free ionized calcium, sensitized cells (10⁷/ml) were incubated with 0.5 μM fura-2/AM for 1 h at 37 °C. In manganese quench experiments, the free manganese concentration was 5 μM. Cells were then washed and resuspended in saline solution containing 250 μM sulfipyrazone and 0.05% gelatin. Three-ml aliquots of cell suspension (10⁶ cells/ml) were added to 3-ml acrylic cuvettes maintained at 37 °C and constantly stirred. Fura-2 fluorescence at 510 nm was monitored with a Perkin-Elmer LS-5 fluorescence spectrophotometer. Fura-2 was excited at 334 nm for measurements of free ionized calcium, or at 360 nm for manganese quench experiments.

Fura-2 Measurements in Adherent Cells—Sensitized cells on coverslips were washed twice and incubated for 45 min with 1 μM fura-2/AM for measurements of free ionized calcium, or 5 μM fura-2/AM for manganese quench experiments. After loading, the cells were washed twice and each coverslip half was placed in a holder made from a 1.5-ml centrifuge tube, which was then inserted into a 3-ml acrylic cuvette containing 2.5 ml of saline solution containing 250 μM sulfipyrazone and 0.05% gelatin. The temperature was maintained at 37 °C, and fluorescence was monitored as described above for suspended cells.

Measurement of Translocation and Tyrosine Phosphorylation of Protein Kinase C Isozymes Derived from Suspended and Adherent Cells—Suspended or adherent cells were sensitized and activated with antigen essentially as described above, with activation times of 1 min. In some experiments cells were treated with either 50 or 100 nM PMA for 2 min, followed by the addition, or not, of antigen for 1 min. In experiments using thapsigargin, cells were incubated with 500 nM thapsigargin for 5 min, to block ATPase activity and to achieve a maximal increase in intracellular calcium. All incubations were in 0.9 ml of saline solution. Following activation, 0.1 ml of a 10 × sonication buffer (27) was added and samples were immediately sonicated at 4 °C. Suspended cells were treated as described (27), while adherent cells were activated and sonicated directly in the 25-cm² flasks in which the cells were cultured. A 0.1-ml sample of the nucle-free sonicate was mixed with an equal volume of 2 × Tris-glycine SDS sample buffer for determination of the amount of protein kinase C isoforms present in the cells. The soluble and pelleted fractions were then recovered from the remaining nuclei-free sonicate as described (27). Pelleted fractions were resuspended to 0.9 ml, and 0.1-ml aliquots were removed from both soluble and pelleted fractions and mixed with the 2 × Tris-glycine SDS sample buffer as above. Proteins derived from the soluble and particulate fractions were resolved by SDS-PAGE (8%) and transferred to nitrocellulose for analysis of the relative amounts of protein kinase C isoforms present in each fraction. Analysis was by Western blots using the polyclonal or monoclonal antibodies described previously (28), except for the antibody to protein kinase C-γ, which was obtained from Transduction Laboratories, Lexington, KY.

Immunoprecipitation of protein kinase C isoforms for analysis of tyrosine phosphorylation was done as described previously (27). Triton X-100 (final concentration 0.5%) was added to the remaining volume of the particulate fraction (see above), and the detergent lysates were used for immunoprecipitation of the individual protein kinase C isoforms. Antibodies for immunoprecipitation have been described (28). Proteins were resolved and transferred to nitrocellulose as above. The tyrosine

![Image](http://www.jbc.org/)

**FIG. 1.** PMA shows both enhancing and inhibitory effects on antigen-stimulated secretion in RBL cell suspensions, but has much less effect on secretion in adherent cells. Antigen-stimulated β-hexosaminidase secretion was measured in suspended (A) and adherent (B) RBL-2H3 cells in the presence of the indicated concentrations of PMA. Spontaneous secretion was subtracted from stimulated secretion at each PMA concentration. Data are expressed as a fraction of control (antigen-stimulated secretion without PMA) and represent the mean and standard deviation of four experiments. Control secretion was 31.9 ± 10.5% in suspended cells and 45.2 ± 12.1% in adherent cells. Inset, a single experiment with suspended cells showing the mean range of two replicates. The antigen concentration was 1 μg/ml. Spontaneous secretion was 5.5 ± 1.7% in suspended cells and 8.3 ± 2.0% in adherent cells; it was unaffected by PMA.
phosphorylation of protein kinase C-α, -β, and -γ derived from suspended or adherent cells was analyzed by immunoblotting with a mouse monoclonal antibody to phosphotyrosine (4G10, Upstate Biotechnology, Inc., Lake Placid, NY). Tyrosine phosphorylation of the β isozyme was not assessed due to the unavailability of an immunoprecipitating antibody. Detection was by enhanced chemiluminescence, and relative quantitation of immunoblots was performed by densitometry as described (29).

RESULTS

Antigen-stimulated Secretion—We have examined the effect of the protein kinase C activator PMA on adherent and suspended RBL cells to determine whether cell adherence can explain the conflicting reports in the literature on the effects of PMA on antigen-stimulated secretion (12-15). In cell suspensions, concentrations of PMA higher than about 15 nM inhibited antigen-stimulated secretion, while lower concentrations of PMA potentiated secretion somewhat (Fig. 1A). The potentiation of secretion by low concentrations of PMA varied between experiments; Fig. 1A (inset) shows an experiment in which this potentiation was especially striking. In adherent RBL cells, however, PMA had only a small effect on secretion (Fig. 1B).

Fig. 2 shows that the protein kinase C inhibitor GF 109203X (30) inhibits antigen-stimulated secretion in both suspended and adherent RBL cells, thus confirming the central role of protein kinase C in secretion from RBL cells. Although high concentrations of PMA can abolish antigen-stimulated secretion from cells in suspension (Fig. 1A), while PMA has little effect on adherent cells (Fig. 1B), the results in Fig. 2 clearly demonstrate that protein kinase C activity is necessary for secretion in both adherent and suspended cells. This result supports previous studies showing that secretion can be recon-
Antigen-stimulated Calcium Responses—We also examined the effects of PMA on antigen-induced changes in intracellular calcium in both adherent cells and cell suspensions, using the fluorescent indicator fura-2. PMA completely abolished the antigen-induced increase of intracellular Ca\(^{2+}\) in suspended cells (Fig. 3A), as has been shown previously (13, 15). The IC\(_{50}\) for this inhibition was approximately 15 nM (Fig. 4A), in agreement with results from other groups (12, 15). However, in adherent cells, PMA had no significant effect on the antigen-stimulated Ca\(^{2+}\) response at any of the concentrations tested (Figs. 3B and 4B). In addition, GF 109203X was able to reverse the inhibition of the Ca\(^{2+}\) response by PMA in suspended cells (Fig. 3A), supporting the idea that this effect of PMA is due to activation of protein kinase C.

Responses to Thapsigargin—Thapsigargin and other inhibitors of the endoplasmic reticulum Ca\(^{2+}\)-ATPase (34) deplete intracellular stores of calcium and activate Ca\(^{2+}\) influx in RBL cells (16, 35–37), thus bypassing the IP\(_3\)-dependent pathway activated by antigen. If protein kinase C activation by PMA directly inhibits the Ca\(^{2+}\) influx pathway in suspended cells, then PMA should also prevent the thapsigargin-induced Ca\(^{2+}\) influx. However, since the Ca\(^{2+}\) response to antigen is completely abolished by PMA, a more likely possibility is that PMA is inhibiting the Ca\(^{2+}\) responses at, or prior to, the release of...
Ca\textsuperscript{2+} from stores. If this is the case then PMA should have no effect on the thapsigargin-induced activation of the calcium influx pathway as monitored by manganese influx, and this is shown in Fig. 6. This is in agreement with recent results obtained by Ali et al. (37), showing that the thapsigargin-induced increase in intracellular calcium is not inhibited by PMA. Since activation of protein kinase C by PMA is known to inhibit phospholipase C-\(\gamma\), it seems likely that PMA is inhibiting the Ca\textsuperscript{2+} response to antigen by preventing IP\textsubscript{3} production (13, 18).

Treatment of RBL cell suspensions with thapsigargin did not induce secretion unless the cells were also treated with PMA (Fig. 7A). This is consistent with work using another endoplasmic reticulum Ca\textsuperscript{2+}-ATPase inhibitor, cyclopiazonic acid (16). In contrast, thapsigargin alone was able to stimulate secretion in adherent cells, although co-treatment with 50 nM PMA enhanced thapsigargin-induced secretion (Fig. 7B). These data suggest that adherent cells have a constitutive protein kinase C activity that synergizes with thapsigargin to promote secretion.

Effects of Protein Kinase C Inhibitors—If the thapsigargin-induced secretion in adherent cells is indeed dependent on constitutive activity of protein kinase C, then the protein kinase C inhibitor GF 109203X should inhibit secretion in response to thapsigargin. This is shown in Fig. 8. PMA-induced down-regulation of protein kinase C in adherent cells (31) also inhibited thapsigargin-induced secretion, with complete inhibition of secretion after 6 h of incubation in 100 nM PMA (Fig. 8). These data support the idea that thapsigargin-induced secretion in adherent RBL cells is dependent upon a constitutive activity of protein kinase C.

Protein kinase C contains two functional domains: a regulatory domain that interacts with the physiological activator diacylglycerol and with PMA, and a catalytic domain that binds ATP and contains the kinase activity. We have shown that adhesion of RBL cells results in a marked loss of sensitivity to PMA as well as an increased activity of protein kinase C, which suggests that the regulatory domain may have been altered in some way. Since calphostin C acts on the the regulatory domain, we predicted that it would be unable to inhibit protein kinase C in adherent cells. Indeed, calphostin C failed to inhibit either antigen- or thapsigargin-induced secretion in adherent cells at concentrations that completely inhibited antigen-induced secretion in cell suspensions (Fig. 9). Since GF 109203X acts on the catalytic domain of protein kinase C (30), this inhibitor should affect suspended and adherent cells similarly, as was shown in Fig. 2.

Translocation and Tyrosine Phosphorylation of Protein Kinase C Isozymes from Suspended and Adherent Cells—The difference in the protein kinase C response to PMA of suspended versus adherent cells might be mediated by differential membrane translocation of protein kinase C isozymes. We therefore examined the distribution of isoforms in membrane and cytosolic fractions from suspended and adherent cells. Table I shows the relative amounts of each protein kinase C isozyme present in the membrane fraction from resting and antigen-stimulated cells, in the presence or absence of PMA. Prior to stimulation, the membrane-associated protein kinase C-\(\alpha\) was 3-fold higher in adherent cells, with 64.4% of this isozyme present in the membrane fraction of adherent cells as compared to 2.1% for suspended cells. A difference was also observed for protein kinase C-\(\epsilon\), where adherent cells had less
enzyme in the membrane fraction than suspended cells. The membrane-association of the β and δ isozymes (5–6% and 25–30%, respectively) was the same in adherent and suspended cells, prior to stimulation.

Activation of suspended and adherent cells by antigen also revealed a difference in the ability of the calcium-dependent protein kinase C-α and -β isozymes to translocate (Table I). There was a 5-fold increase in membrane-associated protein kinase C-α in both adherent and suspended cells in response to antigen, but again the extent of translocation was 3-fold higher in adherent cells. Antigen stimulation caused a 2–3-fold increase in membrane-associated protein kinase C-β in suspended cells, whereas a 6-fold increase was seen with adherent cells. In contrast, no difference between suspended and adherent cells was observed for translocation of protein kinase C-δ and -ε in response to antigen.

Treatment of cells for 3 min with 50 nM PMA (a concentration that effectively inhibited 75% of the secretory response of suspended cells) resulted in translocation to the membrane of all isozymes except γ. Although the extent of this translocation varied between isoforms (see Table I), no statistically significant differences were seen between adherent and suspended cells. However, the differential distribution of protein kinase C-α and -β in adherent and suspended cells that was seen in response to antigen appeared to be maintained in PMA-treated cells (Table I). No large differences were observed for protein kinase C-δ and -ε in response to antigen, since both of these isozymes were already localized to the membrane by PMA treatment (Table I). An additional experiment using 100 nM PMA showed a similar trend, although the PMA alone induced a more substantial translocation of isozymes and so additional translocation in response to antigen was not as great (data not shown). Thapsigargin-induced elevation of intracellular calcium in adherent and suspended cells did not affect membrane association of any of the isozymes except for protein kinase C-ε, which increased from 7.6 ± 4.6% to 21.3 ± 6.1% in adherent cells (n = 3).

Tyrosine phosphorylation of the individual protein kinase C isozymes was assessed by immunoprecipitation of the individual isozymes and immunoblotting of the resolved proteins with antibody to phosphotyrosine. Only protein kinase C-δ, which was previously shown to be tyrosine-phosphorylated (27), was tyrosine-phosphorylated in response to antigen or PMA. In resting cells a trace amount of tyrosine phosphorylation of protein kinase C-δ was also noted. However, in all cases the state of tyrosine phosphorylation of protein kinase C-δ from adherent and suspended cells was similar (data not shown).

**DISCUSSION**

In the past, there have been discrepancies in the literature describing the effects of PMA on antigen-stimulated secretion in RBL cells. Pecht and colleagues (12, 15) found that PMA potentiates secretion at low concentrations (<15 nM) and inhibits secretion at higher concentrations. However, Beaven's laboratory (13, 14) has shown that PMA has no effect on anti-
Adhesion Affects the Responsiveness of RBL Cells to PMA

One difference between these two sets of experiments is that Pecht’s group worked with cell suspensions while Beaven’s group worked with adherent cells. Our results clearly demonstrate that adherent RBL cells are markedly resistant to PMA. Only in suspended cells did PMA inhibit antigen-induced increases in intracellular \([\text{Ca}^{2+}]\), while Beaven’s group worked with adherent cells. Our results show that Beaven’s group worked with cells in suspension. These results suggest that several of the protein kinase C isozymes can become phosphorylated (27, 31) and that tyrosine phosphorylation of protein kinase C-δ occurs on the regulatory domain (43). However, this would not explain the potentiation of thapsigargin-induced secretion by PMA that is still seen in adherent cells (Fig. 7B). Another possibility is that the function of the regulatory domain is altered by phosphorylation. This is supported by data suggesting that several of the protein kinase C isozymes can become phosphorylated (27, 31) and that tyrosine phosphorylation of protein kinase C-δ occurs on the regulatory domain (43). However, we failed to detect any differences between adherent and suspended cells in the tyrosine phosphorylation of any of the protein kinase C isozymes. One mechanism suggested by the ability of thapsigargin to stimulate secretion in adherent cells without PMA treatment is that protein kinase C is activated in adherent cells when intracellular \([\text{Ca}^{2+}]\) is increased, even without diacylglycerol stimulation. However, in response to stimulation with thapsigargin, we did not detect membrane translocation of any of the protein kinase C isozymes except for protein kinase C-ε in adherent cells. The ability of thapsigargin to induce membrane translocation of protein kinase C-ε has been described previously in GH4C1 rat pituitary cells (44); since protein kinase C-ε is not calcium-dependent, this effect may be an indirect consequence of the thapsigargin-induced increase in intracellular \([\text{Ca}^{2+}]\) (44).

Another possibility is that cell adhesion may activate the kinase by inducing translocation of protein kinase C to the plasma membrane in a manner similar to activation by PMA or antigen. Our experiments suggest that protein kinase C-α and -ε are indeed differentially distributed in adherent versus suspended cells, with greater membrane translocation of protein kinase C-α in adherent cells, and of protein kinase C-ε in suspended cells (Table I). We also observed that with antigen stimulation the calcium-dependent protein kinase C-α and C-β isozymes were translocated to the membrane to a greater extent in adherent than in suspended cells (Table I). Protein kinase C-β is able to reconstitute antigen-induced secretion in permeabilized cells (31), while protein kinase C-α and -ε have been shown to inhibit phospholipase C-γ, thus preventing \(\text{IP}_{3}\) production and the release of calcium from stores (18).

### Table I

| Isozyme | Cells⁵ | −PMA² | +PMA³ | −Ag² | +Ag³ | −Ag² | +Ag³ |
|---------|--------|-------|-------|------|------|------|------|
| α       | S       | 2.1 ± 0.7 | 10.7 ± 7.1 | 9.7 ± 6.1 | 12.6 ± 2.5 |
|         | A       | 6.4 ± 0.6⁴ | 32.6 ± 5.8⁴ | 20.9 ± 19.4 | 50.5 ± 27.0 |
| β       | S       | 5.0 ± 3.2 | 13.0 ± 6.1 | 13.1 ± 9.2 | 24.6 ± 14.4 |
|         | A       | 5.8 ± 3.7 | 38.2 ± 8.2⁴ | 17.6 ± 15.3 | 43.4 ± 18.3⁴ |
| δ       | S       | 29.3 ± 4.9 | 70.9 ± 16.3 | 81.8 ± 7.0 | 88.2 ± 8.0 |
|         | A       | 25.1 ± 8.7 | 57.7 ± 8.8 | 74.9 ± 10.4 | 83.0 ± 9.7 |
| ε       | S       | 41.9 ± 21.3 | 88.0 ± 5.1 | 91.2 ± 12.1 | 95.6 ± 4.4 |
|         | A       | 7.6 ± 46⁶ | 74.2 ± 5.5 | 72.9 ± 18.8 | 93.3 ± 11.6 |

---

⁵ Values were derived from suspended (S) or adherent (A) cells.
² The membrane-associated isozyme was expressed as a percentage of the sum of the isoform in the membrane and cytosolic fractions, as determined by densitometry. Protein kinase C-δ did not translocate under any conditions studied and was therefore not included in the table.
³ The data for non-PMA-treated cells are from three separate experiments and show means ± SD.
⁴ Values are from three separate experiments using cells treated with 50 nM PMA for 2 min prior to stimulation with antigen.
⁵ Indicates a significant difference (p < 0.05, paired t test) when compared with the corresponding suspended cell experiment.
⁶ Indicates a significant difference (p < 0.05, paired t test) when compared with the corresponding suspended cell experiment.
Thus, both potentiating and inhibitory protein kinase C isozymes show differential distribution in adherent and suspended cells.

In conclusion, we have shown that following adhesion, RBL cells lose sensitivity to PMA and display a constitutive activity of protein kinase C, perhaps because the regulatory domain of protein kinase C has been altered in some way. Since mature mucosal mast cells reside in tissues, adherent cells should be more representative of mast cells in vivo. It is possible that the increase in protein kinase C activity represents a regulatory mechanism which allows mature, adherent mast cells to achieve greater sensitivity to intracellular Ca\(^{2+}\), thus leading to full physiological activation. Activation of protein kinase C when mast cells adhere may therefore be an important link between physiological stimulus and cell response in mast cells and perhaps in other cell types as well.

Acknowledgments—We thank J. Kuchtey and T. D. Kim for helpful comments on the manuscript.

REFERENCES

1. Fewtrell, C., and Metzger, H. (1981) in Biochemistry of the Acute Allergic Reactions (Becker, E. L., Simon, A. S., and Austen, K. F., eds) pp. 295–314, Alan R. Liss, Inc., New York.

2. Benhamou, M., Stephan, V., Robbins, K. C., and Siraganian, R. P. (1992) J. Biol. Chem. 267, 7310–7314.

3. Hamawy, M. M., Swaim, W. D., Minoguchi, K., de Feijter, A. W., Mergenhagen, S. E., and Siraganian, R. P. (1994) J. Immunol. 153, 4655–4662.

4. Padini, R., Jouvin, M.-H., and Kinet, J.-P. (1991) Nature 353, 855–858.

5. Beaven, M. A., Moore, J. P., Smith, G. A., Hesketh, T. R., and Metzger, H. (1988) J. Biol. Chem. 259, 7137–7142.

6. White, K. N., and Metzger, H. (1988) J. Immunol. 141, 942–947.

7. Mohr, F. C., and Fewtrell, C. (1987) J. Cell Biol. 104, 783–792.

8. Beaven, M. A., and Cunha-Melo, J. R. (1988) Prog. Allergy 42, 123–184.

9. Kanner, B. I., and Metzger, H. (1984) J. Biol. Chem. 259, 10188–10193.

10. Crews, F. T., Bohn, J. W., Ferry, E. L., Yamamoto, H., Molinaro, C. A., Sherman, L. A., Kliman, N. R., and Katz, D. H. (1980) J. Immunol. 124, 2728–2737.

11. Eisen, H. N., Kern, M., Newton, W. T., and Helmreich, E. (1959) J. Exp. Med. 110, 187–206.

12. Haleem-Smith, H., Chang, E.-Y., Szallasi, Z., Blumberg, P. M., and Rivera, J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 9112–9116.

13. Beaven, M. A., Guthrie, D. F., Moore, J. P., Smith, G. A., Hesketh, T. R., Metcalfe, J. C. (1984) J. Biol. Chem. 259, 7137–7142.

14. Falcone, D., and Fewtrell, C. (1995) J. Biol. Chem. 269, 1749–1756.

15. Sagi-Eisenberg, R., Lieman, H., and Pecht, I. (1985) J. Immunol. 134, 10188–10193.

16. Falcone, D., and Fewtrell, C. (1995) J. Biol. Chem. 269, 2118–2124.

17. Germano, G., Kazanietz, M. G., Blumberg, P. M., and Rivera, J. (1994) J. Biol. Chem. 269, 23102–23107.

18. Toullec, D., Pianetti, P., Coste, H., Belleveruge, P., Grand-Perret, T., Ajakane, M., Baudet, V., Bossin, P., Boursier, E., Loricile, F., Duhamel, L., Charon, D., and Kirilovsky, J. (1991) J. Biol. Chem. 266, 15771–15781.

19. Ozawa, K., Szaisslasi, Z., Kazanietz, M. G., Blumberg, P. M., Mischak, H., Mushinski, J. F., and Beaven, M. A. (1993) J. Biol. Chem. 268, 1749–1756.

20. Meritt, J. E., Jacob, R., and Hallam, T. J. (1989) J. Biol. Chem. 264, 1522–1527.

21. Fasada, H., Ghe, M., Matthews, G., and Penner, R. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 3068–3072.

22. Thastrup, O., Dawson, A. P., Scharff, O., Foder, B., Cullen, P. J., Drøbak, B. K., Bjerrum, P. J., Christensen, S. B., and Hanley, M. R. (1989) Agents Actions 27, 17–23.

23. Mohr, F. C., and Dunston, S. K. (1991) J. Biol. Chem. 266, 259, 1749–1756.

24. Ali, H., Maeyama, K., Sagi-Eisenberg, R., and Beaven, M. A. (1994) Biochem. J. 304, 431–440.

25. Hogg, N., and Landis, R. C. (1993) Cur. Opin. Immunol. 5, 383–390.

26. Hamawy, M. M., Mergenhagen, S. E., and Siraganian, R. P. (1994) Immunol. Invest. 5, 62–66.

27. Kobayashi, E., Nakano, H., Morimoto, M., and Tamaoki, T. (1988) Biochem. Biophys. Res. Commun. 159, 548–553.

28. Melloni, E., Pontremoli, S., Muehle, R., Sacco, O., Cakiroglu, A. G., Jackson, L. A., Klinman, N. R., and Katz, D. H. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 5832–5836.

29. Tamaoki, T., Nakano, H., Morimoto, M., and Kuroda, T., Nishizuka, Y. (1989) J. Biol. Chem. 264, 187–206.

30. Fewtrell, C., and Metzger, H. (1981) in Biochemistry of the Acute Allergic Reactions (Becker, E. L., Simon, A. S., and Austen, K. F., eds) pp. 295–314, Alan R. Liss, Inc., New York.

31. Fewtrell, C., and Metzger, H. (1981) J. Immunol. 124, 2728–2737.

32. Hogg, N., and Landis, R. C. (1993) Cur. Opin. Immunol. 5, 383–390.

33. Beaven, M. A., Guthrie, D. F., Moore, J. P., Smith, G. A., Hesketh, T. R., and Metzger, H. (1988) J. Biol. Chem. 264, 1522–1527.

34. Fasada, H., Ghe, M., Matthews, G., and Penner, R. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 3068–3072.

35. Thastrup, O., Dawson, A. P., Scharff, O., Foder, B., Cullen, P. J., Drøbak, B. K., Bjerrum, P. J., Christensen, S. B., and Hanley, M. R. (1989) Agents Actions 27, 17–23.

36. Ali, H., Maeyama, K., Sagi-Eisenberg, R., and Beaven, M. A. (1994) Biochem. J. 304, 431–440.

37. Hogg, N., and Landis, R. C. (1993) Cur. Opin. Immunol. 5, 383–390.

38. Hamawy, M. M., Mergenhagen, S. E., and Siraganian, R. P. (1994) Immunol. Invest. 13, 62–66.

39. Kobayashi, E., Nakano, H., Morimoto, M., and Tamaoki, T. (1988) Biochem. Biophys. Res. Commun. 159, 548–553.

40. Melloni, E., Pontremoli, S., Michetti, M., Sacco, O., Cakiroglu, A. G., Jackson, L. A., Klinman, N. R., and Katz, D. H. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 5832–5836.
Differential Effects of the Protein Kinase C Activator Phorbol 12-Myristate 13-Acetate on Calcium Responses and Secretion in Adherent and Suspended RBL-2H3 Mucosal Mast Cells

Patricia C. Wolfe, En-Yuh Chang, Juan Rivera and Clare Fewtrell

J. Biol. Chem. 1996, 271:6658-6665.
doi: 10.1074/jbc.271.12.6658

Access the most updated version of this article at http://www.jbc.org/content/271/12/6658

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 43 references, 26 of which can be accessed free at http://www.jbc.org/content/271/12/6658.full.html#ref-list-1