Intracellular free Ca\(^{2+}\) concentrations were monitored in vascular smooth muscle cells (VSMC) using the Ca\(^{2+}\)-sensitive dye fura II. Superfusion of VSMC with platelet-activating factor (S-PAF; 1–100 nM) increased cytosolic Ca\(^{2+}\) in a dose-dependent manner. The response was transient and returned to base line even though the agonist was still present. A second, higher dose of PAF did not elicit a response. The inactive optical isomer, R-PAF, was ineffective suggesting that the S-PAF response is specific and receptor-mediated. Pretreatment of VSMC with PAF attenuated angiotensin II-stimulated Ca\(^{2+}\) mobilization but not vasopressin-stimulated Ca\(^{2+}\) mobilization. Treatment of VSMC with PAF (10 nM) stimulated inositol trisphosphate and inositol tetrakisphosphate formation above control by 260 ± 15% and 195 ± 11%, respectively. Diacylglycerol levels also rose during PAF stimulation and remained increased over 15 min. Pretreatment of VSMCs with phosphol-12,13-myristate acetate (10 nM) for 30 min abolished both the PAF- and angiotensin II-induced increases in cytosolic Ca\(^{2+}\), but not the vasopressin-induced increase. Pretreatment of VSMC with dioctanoylglycerol (10 μM) abolished the S-PAF-, angiotensin II-, and vasopressin-induced elevation in cytosolic Ca\(^{2+}\). We propose that this desensitization is possibly mediated by diacylglycerol formed in response to PAF.

Platelet-activating factor (PAF)\(^*\) is a potent proaggregatory and proinflammatory compound, which is released by macrophages, platelets, and endothelial cells (1). PAF also possesses strong vasoactive properties causing either vasoconstriction or vasodilation depending on the vascular bed studied (2, 3). Recently we and others have reported that PAF antagonizes angiotensin II-induced vasoconstriction in the renal vascular bed (2) and pulmonary vascular bed (4, 5). In the kidney this antagonism was enhanced by indomethacin, thus ruling out a role of prostaglandins in mediating PAF-mediated vasodilation (2). The mechanism of action for PAF is not known; however, PAF may mediate its effects by increasing intracellular Ca\(^{2+}\) through a receptor-mediated release of intracellular Ca\(^{2+}\) (6–8) and/or Ca\(^{2+}\) influx (9–12). As with other agonists, PAF-induced Ca\(^{2+}\) mobilization is thought to require activation of phospholipase C. Once activated, phospholipase C generates inositol phosphates (13) and diacylglycerol (14). The inositol phosphates have been proposed to release intracellular Ca\(^{2+}\) and to function as mediators of Ca\(^{2+}\) influx from extracellular sources (13). Although the role of diacylglycerol produced by phospholipase C activity is not completely clear, oleoylacylglycerol and phorbol myristate acetate (PMA) have been shown to activate protein kinase C and inhibit cellular responses to a number of agonists (16–19) through mechanisms thought to involve phosphorylation of the receptor. Both homologous and heterologous desensitization have been proposed (14, 16, 19). In addition, protein kinase C activation may regulate inositol phosphate metabolism by phosphorylation and activation of phosphatases (14) or may regulate Ca\(^{2+}\) mobilization by modification of the Ca\(^{2+}\)-ATPase (20) or modification of channel proteins (21). Thus mediators produced in response to PAF may not only regulate the response of cells to this agonist but may also modify the subsequent response of cells to other vasoactive agents.

The purpose of the present study was to define the mechanisms by which PAF alters cell function and responsiveness. We have investigated the time course for PAF-induced changes in cytosolic Ca\(^{2+}\) levels, inositol phosphate production, and diacylglycerol formation in vascular smooth muscle cells. In addition, we have studied the effects of angiotensin II and AVP on Ca\(^{2+}\) mobilization. Finally, we have investigated both the desensitization of the PAF response and the effect of PAF on both the angiotensin II and the AVP responses. Since incubation of VSMC with either PMA or diCs could mimic both homologous and heterologous desensitization, we propose that activation of phospholipase C by PAF and subsequent diacylglycerol formation is a potential mechanism by which receptor function is altered in cells exposed to PAF.

**EXPERIMENTAL PROCEDURES**

**Materials—**S-PAF, lyso-PAF, PMA, dihydroxyatraminepentaoctetoc acid (DETPAC), EGTA, imidazole, ATP, and elastase were purchased from Sigma. Collagenase was from Worthington. Cardiolipin and dioleoylglycerol were from Avanti Lipids (Birmingham, AL); dithiotreitol was from Bachem (Torrance, CA); fura 2-AM ester and octyl-D-glycoside were from Behring Diagnostics; \([^{32}P]ATP\) (3000 Ci/mmole) and 2,3-\([^{3}H]\)myoinositol (20 Ci/mmole) from American Radiolabeled Chemicals (St. Louis, MO); 2,3-\([^{3}H]\)inositol 1-phosphate, \(-4,5\)-phosphate, \(-1,4,5\)-trisphosphate, and 1,3,4,5-tetraakisphosphate (5 Ci/mmole) as well as \([^{3}H]\)PAF (179 Ci/mmole) from New England Nuclear. Diacylglycerol kinase was prepared as a membrane prepa-
ration from Escherichia coli N 4830 by Drs. Honeycutt and Dougherty, Duke University. Bioactivity of PAF was checked by rabbit platelet aggregation. R-PAF was kindly provided by Dr. R. Wykle, Bowman Gray, Winston Salem. Dioctanoylglycerol was kindly provided by Dr. R. Bell, Duke University, Durham, NC.

Cell Culture—VSMC were isolated from the aortas of male Sprague-Dawley rats (250-300 g) according to Gunther et al. (22). Vessels were dissected free of adventitious tissue and cut open. Endothelial cells were removed by scraping the inside of the vessel with cotton gauze. The tissue was then minced and incubated for 45 min at 37 °C in a mixture of elastase/collagenase (elastase, 0.125 mg/ml; collagenase, 1 mg/ml; and soybean trypsin inhibitor 0.25 mg/ml in Hanks' balanced salt solution (HBSS) containing 0.2 mM Ca2+, pH 7.2. After incubation, the tissue was triturated through a 19-gauge needle, centrifuged at 700 × G, and resuspended in Dulbecco's modified Eagle's medium containing 10% fetal calf serum and seeded in T-25 cm² tissue culture flasks. Cells became confluent in 3-5 days. They were then passaged at a split ratio of 1:4 after releasing the cells from the flask by incubation for 5 min at 37 °C with trypsin/EDTA (0.25 g of trypsin/liter and 0.1 g of EDTA/liter). Cells were identified as smooth muscle cells by electron microscopy by the presence of myofibrils and dense bodies and appeared uniform through six passages.

Cytosolic Ca2⁺ Measurements in Resting and Stimulated VSMC—VSMC were seeded on glass coverslips (3 × 10⁶ cells/coverslip) and grown to confluence. They were then labeled for 1 h at 37 °C with 5 mM fluo 3-AM in HBSS containing 125 mM albumin and 10 mM HEPES at pH 7.4. The coverslips were placed into a cuvette and continuously superfused (bottom to top) with HBSS, pH 7.4, at a flow rate of 3 ml/min. Temperature of superfusate and cuvette was maintained at 37 °C. The cuvette was placed into the heated cuvette holder of a spectrofluorimeter (Kontron SFM 29) and the excitation spectrum from 310 to 410 nm was determined with the emission monitored at 510 nm (see Fig. 1). During an experiment, the relative fluorescence at 510 nm of cells alternately excited at 350 and 380 nm (Sa) was displayed on the monitor. Cytosolic Ca2⁺ concentration was calculated as described by Grynkiewicz et al., (23): (see Eq. (1)).

\[ [Ca^{2+}]_{i} = K_{d} \times \frac{(R - R_{min})}{(R_{max} - R)} \]

where \([Ca^{2+}]_{i}\) is the ratio of fluorescence of Ca2⁺-fura 2 complex is 224 nm (23). \(R\) is the ratio of fluorescence between the excitation wavelengths for each individual experimental procedure. \(R_{max}\) is the maximal fluorescence ratio of fura 2 measured in the presence of 2 mM Ca2⁺. \(R_{min}\) is the minimal fluorescence ratio measured in Ca2⁺-free medium supplemented with 10 mM EGTA. The dissociation constant of the Ca2⁺-fura 2 complex is 224 nM (22). PAF dissolved in the same buffer was added at the desired concentrations. After the times indicated, the incubation media were discarded and the reaction was stopped with 1 ml of ice-cold methanol.

Measurement of sn-1,2-Diacylglycerol—Confluent cultures of VSMC in T-25 cm² flasks were rinsed with HBSS containing 0.25% BSA and HEPES, pH 7.4, and used to measure diacylglycerol (29). PAF dissolved in the same buffer was added at the desired concentrations. After the times indicated, the incubation media were discarded and the reaction was stopped with 1 ml of ice-cold methanol. The samples were then passaged at a split ratio of 1:4 after releasing the cells from the flask by incubation for 5 min at 37 °C with trypsin/EDTA (0.25 g of trypsin/liter and 0.1 g of EDTA/liter). Cells were identified as smooth muscle cells by electron microscopy by the presence of myofibrils and dense bodies and appeared uniform through six passages.

Inositol Phosphate Production and Analysis—VSMC were seeded at a split ratio into T-25 cm² flasks and incubated for 72 h at 37 °C with 4 mM [3H]inositol dissolved in inositol-free medium 199 containing 10% fetal calf serum until they reached confluence. Before stimulation with PAF, cells were rinsed twice with HBSS supplemented with 0.25% albumin and 10 mM HEPES, pH 7.4. VSMC were preincubated with 20 mM LiCl 10 min prior to agonist exposure, since LiCl has been shown to inhibit the dephosphorylation of inositol monophosphates, bisphosphates, and triphosphates (25-27). PAF, dissolved in HBSS supplemented with 0.25% BSA and 10 mM HEPES, pH 7.4, was added to the cultured cells and incubated at 37 °C for appropriate times. The reaction was stopped by adding 0.5 ml of 50% trichloroacetic acid to 5 ml of media. The cells were scraped and transferred to polypropylene tubes. The flasks were rinsed and combined with the first wash. After centrifugation at 1200 × g, clear supernatants were transferred to glass test tubes and the tissue was disrupted with distilled water and neutralized to pH 7. Free inositol and the inositol phosphates were separated by anion exchange chromatography on 1.5-ml Bio-Rad AG 1X8 anion exchange columns (formate) (29). The eluents used were: (a) H₂O for free inositol 4-8 × 5 ml; (b) 5 mM tetraborate, 0.06 mM ammonium formate for glycerophosophoinositol 3 × 5 ml; (c) 0.2 M ammonium formate, 0.1 M formic acid for inositol monophosphates 4 × 5 ml; (d) 0.4 M ammonium formate, 0.1 M formic acid for inositol bisphosphates 5 × 5 ml; (e) 2 M ammonium formate, 0.1 M formic acid for inositol polyphosphates (IP) 5 × 5 ml; (f) 2 M ammonium formate, 0.1 M formic acid for inositol polyphosphates 4 × 5 ml. The first water wash was monitored for radioactivity to ascertain that all free [3H]inositol was eluted from the sample. All subsequent fractions were collected and mixed with 10 ml of Aquasol and the radioactivity was measured using a scintillation counter. Counts/minute for each eluate were combined and compared to untreated controls. Commercially available labeled standards were loaded onto separate columns and eluted as above to validate the procedure.

RESULTS

In these studies we used the Ca2⁺-sensitive intracellular dye, fura 2 to follow changes in [Ca2⁺]i. In Fig. 1, a representative experiment is given showing the relative fluorescence
AM as described under "Experimental Procedures," placed in a cuvette, and positioned in the heated tracing. Agonists were added directly to the superfusion buffer immediately before entering the cuvette. The excitation spectra of dye-loaded cells excited at 350 and 380 nm (upper and middle curve). As is evident, the ratio or base-line [Ca\textsuperscript{2+}] value for Ca\textsuperscript{2+} in response to increasing doses of PAF was significantly elevated above base line induced by were significantly elevated above base line (t* = P < 0.05). Both PMA and diC8 blocked PAF-induced Ca\textsuperscript{2+} mobilization at all doses of PAF (P < 0.05).

EGTA, the PAF responses were abolished. The rise in Ca\textsuperscript{2+} in response to increasing doses of PAF was significantly inhibited by pretreatment of cells with either PMA (10 nM) or diC8 (100 μM) (Fig. 2). Neither PMA nor diC8 had a significant effect on basal [Ca\textsuperscript{2+}]. Furthermore, when cells were treated with the inactive optical isomer of PAF, R-PAF, or diC8 (100 μM) (Fig. 2). Neither PMA nor diC8 had a significant effect on basal [Ca\textsuperscript{2+}]. Furthermore, when cells were treated with the inactive optical isomer of PAF, R-PAF, increased [Ca\textsuperscript{2+}] was not increased cytosolic Ca\textsuperscript{2+} (Fig. 1A). When cells were superfused with Ca\textsuperscript{2+}-free buffer containing 1 mM

for dye-loaded cells excited at 350 and 380 nm (upper and lower curves, respectively). The ratio is displayed as the middle curve. As is evident, the ratio or base-line [Ca\textsuperscript{2+}] value in these cells is constant throughout the experiment (up to 60 min), although the fluorescence values slowly decline due to photobleaching and dye leakage from the cells.

Superfusion of VSMC monolayers with PAF (1–100 nM) increased cytosolic Ca\textsuperscript{2+} concentration in a dose-dependent manner (Fig. 2). The effect on [Ca\textsuperscript{2+}], was transient even in the presence of continuous superfusion of PAF with cytosolic Ca\textsuperscript{2+} levels rising over 90–150 s and returning to baseline by 210 s (Fig. 4). A second dose of PAF given within 20 min of the first dose did not increase cytosolic Ca\textsuperscript{2+} (Fig. 1A). When cells were superfused with Ca\textsuperscript{2+}-free buffer containing 1 mM

EGTA, the PAF responses were abolished. The rise in Ca\textsuperscript{2+} in response to increasing doses of PAF was significantly elevated above base line (t* = P < 0.05). Both PMA and diC8 blocked PAF-induced Ca\textsuperscript{2+} mobilization at all doses of PAF (P < 0.05).

The mechanism of PAF-induced cell stimulation may involve receptor-coupled phospholipase C activation or receptor-operated Ca\textsuperscript{2+} channels. Inositol polyphosphates produced by phospholipase-mediated hydrolysis of phosphatidylinositol polyphosphates may activate both release of Ca\textsuperscript{2+} from intracellular stores and influx of extracellular Ca\textsuperscript{2+} (15). To study the time course and extent of PAF-induced inositol polyphosphate formation, cells which had been prelabeled with [3H]inositol were incubated with PAF (1–100 nM). Aliquots were taken after 3 min and inositol phosphates isolated and quantitated. The time course for PAF (10 nM)-induced inositol phosphate formation is given in Figs. 3 and 4. When cells are treated with PAF, IP\textsubscript{3} rises slowly to a peak value at about 3 min (Fig. 4), whereas inositol bisphosphate reaches a peak at 5 min (Fig. 3). IP was maximal at 3 min. Interestingly, the time course for formation of these mediators is slower than that reported for other systems and does not fit the notion of a precursor-product relationship for the changes in Ca\textsuperscript{2+} given in Fig. 4. Also, the dose-response curves for PAF-stimulation of Ca\textsuperscript{2+} mobilization and IP\textsubscript{3} formation are different (Fig. 5). Effects on Ca\textsuperscript{2+} mobilization reach a plateau between 10 and 100 nM PAF, whereas stimulation of IP\textsubscript{3} formation reaches peak at 10 nM with 100 nM PAF being less effective. The time course of PAF-induced diacylglycerol formation was also determined and is shown in Fig. 6. Significant increases in diacylglycerol levels were seen with 10 nM PAF after a 5-min incubation. As expected, this time course coincides with the initial rise in phosphoinositol 4,5-bisphosphate hydrolysis but unlike IP\textsubscript{3} formation is sustained.

![Figure 1](https://example.com/fig1.png)

**FIG. 1.** Effect of agonists on the fluorescence of fura 2-loaded VSMC. VSMC on glass coverslips were loaded with fura 2-AM as described under “Experimental Procedures,” placed in a cuvette, and positioned in the heated tracing. The ratio between the two values is plotted as the middle tracing (top portion of figure). The cells were superfused with HBSS containing 0.25% BSA at 3 ml/min. The relative fluorescence at 510 nm was followed with time as the cells were alternately excited at 350 nm (upper tracing) and 380 nm (lower tracing). The ratio between the two values is plotted as the middle tracing. Agonists were added directly to the superfusion buffer immediately before entering the cuvette. The excitation spectra of loaded cells was followed at the emission wavelength 510 nm and is shown at the top of the figure. Data are given for a representative experiment.

![Figure 2](https://example.com/fig2.png)

**FIG. 2.** Effect of PAF on cytosolic Ca\textsuperscript{2+} in VSMC. VSMC were loaded with fura 2 and placed in the spectrofluorimeter as described in the legend to Fig. 1. Increasing doses of PAF were added to the superfusate, and changes in Ca\textsuperscript{2+} were followed. Peak [Ca\textsuperscript{2+}], values were reached after 90 s and are plotted as the hatched bars for each dose. In some experiments the cells were pretreated with either PMA (10 nM, open bars) or diC8 (100 μM, dark bars) by incubation with either compound in HBSS plus 0.25% BSA for 30 min at 37°C after loading with fura 2. The cells were rinsed and placed in the cuvette, the superfusion was started, and the cells were stimulated with the indicated doses of PAF. Data were calculated as percent of control for each treatment and are given as mean ± S.E., n = 9. Peak [Ca\textsuperscript{2+}], induced by were significantly elevated above base line (t* = P < 0.05). Both PMA and diC8 blocked PAF-induced Ca\textsuperscript{2+} mobilization at all doses of PAF (P < 0.05).
between PAF and angiotensin I1 or AVP. Superfusion of VSMC on coverslips with angiotensin I1 increased cytosolic production was monitored using prelabeled cells as in Fig. 1. Pretreatment of VSMC with PAF (100 mM) by superfusion desensitized the cells toward angiotensin I1, resulting in a shift of the angiotensin I1 dose-response curve by 1 order of magnitude to the right. PMA (10 nM) and diCa (100 μM) pretreatment also significantly inhibited angiotensin II-induced increases in cytosolic Ca2+.

Since PAF has been reported to interfere with the action of other vasoactive agents, we wished to study the interactions between PAF and angiotensin II or AVP. Superfusion of VSMC on coverslips with angiotensin II increased cytosolic Ca2+ at a threshold concentration of the peptide of 1 nM (Fig. 6).

7). Maximal responses were obtained with 1 μM angiotensin II. Pretreatment of VSMC with PAF (100 nM) by superfusion for 5 min desensitized the cells toward angiotensin II, resulting in a shift of the angiotensin II dose-response curve by 1 order of magnitude to the right. PMA (10 nM) and diCa (100 μM) pretreatment also significantly inhibited angiotensin II-induced increases in cytosolic Ca2+.

AVP also caused a dose-dependent increase in cytosolic Ca2+ and was approximately as potent as angiotensin II in this preparation (Fig. 8); however, the threshold of the response may be higher for AVP (no significant changes were seen at 1–10 nM AVP). In contrast to the effects of PAF and PMA on the angiotensin II response, neither of these compounds at the same dose had an effect on AVP-induced increases in cytosolic Ca2+. Interestingly, however, diCa was an effective inhibitor of the AVP-induced elevation of cytosolic Ca2+ (Fig. 8).

Since conversion of PAF to lyso-PAF may explain in part the transient nature of the response of VSMC to this lipid, we investigated the metabolism of [3H]PAF in cultured cells. In these experiments, only a small fraction of the added PAF was metabolized to lyso-PAF and other unidentified products.
Data are given as the mean plotted as cuvette and stimulated with angiotensin I1 at the doses indicated. Pretreatment significantly reduced the angiotensin I1 response at after loading with fura 2.

In some studies changes in intracellular calcium were followed with [3H]PAF plus unlabeled PAF (10 nM) did not produce any labeled metabolites which interfered with the diacylglycerol assay (co-migrating with phosphatidic acid).

**DISCUSSION**

PAF-stimulated elevation of cytosolic Ca²⁺ has been reported in platelets (12), macrophages (9), endothelial cells (7, 8, 32), vascular smooth muscle cells (8) and in a cultured cell line derived from vascular smooth muscle cells (6). In most of these studies changes in intracellular calcium were followed using either ⁴⁰Ca²⁺ (7, 8) or the Ca²⁺ sensitive dye, Quin 2 (9, 12). In the present study we used fura 2, a Ca²⁺ binding dye, which has less Ca²⁺-buffering capacity than Quin 2 (24).

Differences exist depending on the method used. For example, the PAF-induced Ca²⁺ response is attenuated in the absence of extracellular Ca²⁺ when measured with Quin 2 or fura 2 but not with ⁴⁰Ca²⁺ (12, 33). Furthermore, PAF-stimulated Ca²⁺ increase in platelets and macrophages occurs within 30 seconds when measured with Quin 2 (9, 12) but within 1 min when measured as ⁴⁰Ca²⁺ release in endothelial cells (7, 8). In our preparation, PAF caused a rise in Ca²⁺ concentration which reached a peak within 1.5 min.

Interestingly, the time to peak of the Ca²⁺ response does not correlate with the time to peak of IP₃ and inositol triphosphate formation. In fact, the peak Ca²⁺ concentration is reached before there is a significant change in the amount of IP₃. Thus, although IP₃ has been proposed to be the intracellular signal for release of stored Ca²⁺ (15), it appears that other mechanisms regulate PAF-induced Ca²⁺ mobilization. For example, since our data indicate that the rise in intracellular Ca²⁺ is dependent on extracellular Ca²⁺, it is possible that PAF effects are mediated through a receptor-operated channel and thus independent of phosphatidylinositol polyphosphate hydrolysis. In fact, IP₃ and inositol tetrakishosphate formation and Ca²⁺ uptake may be mediated by different receptors, as pointed out by Nacchiache et al. (19) and Verghese et al. (34), who demonstrated that PAF increased cytosolic Ca²⁺ when phospholipase C activation was inhibited by pertussis toxin. It is also possible that the IP₃ levels we measured are much lower than those actually present due to rapid degradation or due to compartmentation and dilution as the total cell extract was prepared. In addition, since disparate dose-response curves for agonist-induced Ca²⁺ mobilization and IP₃ formation have been noted in other cells (35), it is possible that small increases in IP₃ lead to major changes in Ca²⁺ mobilization. However, diacylglycerol formation was also relatively slow in these cells, reaching significant levels between 3 and 5 min of incubation with PAF. The rate of diacylglycerol is difficult to determine since, as is the case with IP₃, this compound is rapidly metabolized to phosphatidic acid. Thus, whereas IP₃ and diacylglycerol appear to be produced with similar time courses, PAF-induced Ca²⁺ mobilization and phospholipase C activation are separate events in VSMC. A slow, prolonged time course for PAF-induced IP₃ formation has also been reported in endothelial cells (35).

The PAF response is transient and a second dose of PAF does not elict a response; i.e. PAF desensitizes its receptor. In a number of systems it has been shown that activation of protein kinase C leads to receptor phosphorylation and desensitization (32). PAF-induced Ca²⁺ mobilization in endothelial cells (32) has been shown to be blocked by pretreatment of the cells with activators of protein kinase C, PMA, and diacylglycerol. In addition, we found that pretreatment of VSMC with PMA or diC₈ attenuated the PAF response. Thus, together with our data which shows that PAF stimulates diacylglycerol formation, it is likely that desensitization results from activation of protein kinase C.

The exact mechanism for desensitization is not known but may involve direct receptor phosphorylation (42), inactivation of the receptor-phosphoprotein link (18), or direct interference with Ca²⁺ channels (21). Furthermore, other mechanisms must be operating to regulate the extent of the Ca²⁺ rise since Ca²⁺ reaches a peak at 90 s and returns to base line before diacylglycerol levels increase. Interestingly, it has been suggested that diacylglycerol formation in response to PAF stimulation functions in enhancing the response to PAF (36). This does not
seem to be the case in our system since diacylglycerol formation is slow and appears to be correlated with desensitization rather than initiation of the response. PAF treatment desensitizes VSMC to angiotensin II-induced increases in intracellular Ca²⁺ and shifted this dose response curve for angiotensin II by 1 order of magnitude to the right. In addition, the angiotensin II effect was also attenuated by PMA and diacylglycerol pretreatment. In other studies, angiotensin II receptor internalization was shown to correlate with the time course of diacylglycerol formation in VSMC (37), suggesting that PAF cross-desensitization is mediated by diacylglycerol formation. In contrast to this interaction, PAF did not alter the effect of AVP on Ca²⁺ mobilization in these cells and PMA pretreatment did not affect AVP-induced Ca²⁺ uptake. Likewise, AVP-induced Ca²⁺ uptake and phosphatidylinositol bisphosphate hydrolysis in liver cells have been found to be insensitive to PMA pretreatment (19) and to cross-desensitization by insulin (38). However, in these studies (19, 38), diC₆ inhibited AVP-induced Ca²⁺ mobilization. The disparity between the diC₆ and the PMA effects on AVP in our experiments is difficult to explain; however, different protein kinase C isoenzymes have been described which have different sensitivities to PMA and diacylglycerol (39).

PAF may have other effects and may act directly to alter receptor-ligand interactions. For example, fatty acids of different chain lengths and saturation have been shown to reduce angiotensin receptor affinity (40). Perhaps the C-16 alkyk group in the sn-1 position of PAF interferes with the angiotensin II receptor. Although it should be pointed out that the concentration of fatty acid necessary for this effect was 10 times higher than the concentrations of PAF used in our experiments. PAF may also indirectly alter cell function by metabolism to active compounds. A small fraction of PAF was found to be metabolized to lysophosphatidic acid and 1-alkyl-2-acyl-glycerol. 1-alkyl-2-acyl-glycerol was shown to have a weak activity on protein kinase C and to inhibit diacylglycerol-stimulated protein kinase C activity (41). These compounds may potentially mediate the PAF-induced desensitization and cross-desensitization, although it seems unlikely.

In summary, our data demonstrate a specific action of PAF on VSMC leading to the mobilization of Ca²⁺ through a mechanism which is not dependent on the formation of IP₃. The PAF response is transient and initial exposure to the agonist desensitized the cell toward a second challenge. In addition, PAF pretreatment results in a unique inhibitory action on angiotensin II-induced but not AVP-induced responses. Both the homologous and heterologous desensitization appear to be mediated by PAF-stimulated phosphatidylinositol bisphosphate hydrolysis and diacylglycerol formation. However, PAF itself may be metabolized to a biologically active compound which mediates these effects.

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