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EFFECTS OF SCALARADIAL, A NOVEL INHIBITOR OF 14 kDa PHOSPHOLIPASE A_2, ON HUMAN NEUTROPHIL FUNCTION

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Abstract—Scalaradial, a marine natural product with anti-inflammatory activity, has been shown to be a selective inhibitor of 14 kDa type II phospholipase A_2(PLA_2). We have examined the inhibition by scalaradial (0.1 nM to 10 μM) of neutrophil function (degranulation) in response to receptor-mediated activation [*N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP), 30 nM; leuokotriene B_4 (LTB_4), 100 nM; platelet-activating factor (PAF), 100 nM] and non-receptor-mediated stimuli [A23187 (1 μM) and thapsigargin (100 nM)]. Furthermore, we evaluated the ability of scalaradial to inhibit the increase in intracellular Ca^{2+} in response to fMLP, LTB_4, A23187, and thapsigargin as well as its ability to prevent either fMLP- or LTB_4-mediated elevation in inositol phosphate production (InsP). Scalaradial was a potent inhibitor of both receptor- (IC_{50} = 50–200 nM) and non-receptor- (IC_{50} = 40–900 nM) mediated degranulation. Although scalaradial inhibited the mobilization of Ca^{2+} induced by fMLP, LTB_4, and PAF, it did not affect the maximal Ca^{2+} levels attained with A23187 or thapsigargin. Neutrophil-binding studies with [^{3}H]fMLP and [^{3}H]LTB_4 would suggest that the effect of scalaradial on agonist-induced degranulation and increase in intracellular Ca^{2+} was not at the receptor level because 50-fold higher concentrations were required to have a significant effect on the binding of these agonists. To determine if scalaradial affected phosphatidylinositol selective phospholipase C (PI-PLC) activity, assays were conducted to monitor fMLP- and LTB_4-induced formation of InsPs using *myo*-[^{3}H]inositol-labeled U-937 cells. In these cells, 2.5 to 9-fold higher concentrations of scalaradial were required to inhibit PI-PLC activity than to inhibit agonist-induced degranulation of neutrophils, suggesting that the effects of scalaradial on Ca^{2+} and degranulation are not the sole result of blocking receptor activation of PI-PLC. Results obtained with receptor-mediated stimuli suggest that scalaradial may have direct effects on Ca^{2+} channels and InsP turnover, but inhibition of intracellular Ca^{2+} levels was not required for scalaradial to block degranulation since scalaradial was capable of inhibiting degranulation produced by either A23187 or thapsigargin, without changing the maximal Ca^{2+} levels obtained with these two stimuli. These results demonstrate that scalaradial can inhibit degranulation in the presence of micromolar intracellular Ca^{2+} concentration, thus supporting the hypothesis that a 14 kDa PLA_2 may be important in the regulation of neutrophil degranulation.

Key words: phospholipase A_2; degranulation; neutrophils; calcium mobilization

It is widely recognized that PLA_2† (EC 3.1.1.4) enzymes are critical for the generation of lipid inflammatory mediators from various cells [1]. What is not widely appreciated is the possible role for these enzymes in regulating exocytosis. Over the last decade, several reports have indicated that inhibition of PLA_2 activity not only prevents the release of arachidonic acid and its metabolites but also inhibits secretory processes. Addition of exogenous PLA_2 to isolated synaptic vesicles promoted the fusion of these vesicles [2]. Furthermore, pretreatment of rat brain synaptosomes and adrenal chromaffin cells with BPB, an irreversible inhibitor of PLA_2, prevented the depolarization-induced release of radiolabeled catecholamines [3, 4]. PLA_2 inhibitors have also been reported to prevent glucose-induced insulin and arachidonic acid release from pancreatic acinar cells [5]. More extensive studies examining the possible role for PLA_2 enzymes in exocytosis have been carried out in inflammatory cells. In rat peritoneal mast cells, inhibition of low molecular mass PLA_2 (14 kDa type II) by BPB or ONO RS-082 (another inhibitor of 14 kDa PLA_2), or by addition of a neutralizing antibody of PLA_2, produces a reduction in histamine release caused by either antigen or substance P [6, 7]. This inhibition of degranulation occurred without altering the antigen-induced increase in intracellular Ca^{2+} [8]. Both BPB and mepacrine have been reported to inhibit degranulation of human, rat, and guinea pig neutrophils as well as human eosinophils [9–12]. The

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† Abbreviations: BPB, bromophenacyl bromide; DB-PBS, Dulbecco's phosphate-buffered saline; fMLP, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine; HRO, horseradish peroxidase; InsP, inositol phosphate; LTB_4, leukotriene B_4; MPO, myeloperoxidase; PMNs, neutrophils; PLA_2, phospholipase A_2; PLC, phospholipase C; PI-PLC, phosphatidylinositol selective phospholipase C; and PAF, platelet-activating factor.

response to stimuli in human eosinophils or guinea pig neutrophils could be restored by the addition of exogenous arachidonic acid or lysophosphatidylcholine, respectively [9, 12]. Thus, these findings support the hypothesis that certain forms of PLA_2 play an integral role in exocytosis, possibly by providing free arachidonic acid or lysophospholipids to act as fusogens between the granular and plasma membrane.

Since it has been demonstrated that at least two forms of PLA_2 exist in inflammatory cells [1], it is important to determine which form may be involved in regulating exocytosis. Exploring the function of the individual forms of PLA_2 has been hampered by the lack of selective inhibitors. One compound that has been characterized extensively is manoalide. This marine sesterterpenoid was shown to be a potent inhibitor of the low molecular mass PLA_2 found in bee venom [4, 13] or human synovial fluid [14]. Unfortunately, manoalide proved to be non-selective as it was reported to inhibit PLC [15] and 5-lipoxygenase [16] and to block a number of Ca^{2+} channels, including the inositol 1,4,5-triphosphate (IP_3) sensitive channels, and both receptor-operated and voltage-gated Ca^{2+} channels [17]. This lack of selectivity limits the usefulness of manoalide in investigating the role of 14 kDa PLA_2 in cell function. Recently, another marine natural product, scalaradial, was characterized as a potent inhibitor of the 14 kDa PLA_2 [18], with 300-fold less activity against 85 kDa high molecular weight PLA_2 activity [19, ‡]. In human neutrophils, scalaradial or its 12 epi analogue inhibits cell free *sn*-2 acyl hydrolysis as well as A23187-induced arachidonic acid mass release [19, ‡], and PAF and LTB_4 production [19, 20]. The purpose of the present studies was to characterize the functional actions of this novel inhibitor of PLA_2 in human neutrophils and to determine its utility in examining the role of 14 kDa PLA_2 in stimulus–secretion coupling. In this report, we describe the ability of scalaradial to inhibit both receptor- and non-receptor-mediated degranulation of human neutrophils and the ability of scalaradial to inhibit fMLP- and LTB_4-induced formation of InsPs at a concentration greater than those required to alter degranulation. Furthermore, while scalaradial blocked receptor-mediated increases in intracellular Ca^{2+}, the maximal levels of Ca^{2+} produced by A23187 and thapsigargin were not affected by pretreatment with scalaradial and, therefore, its action on degranulation cannot totally be accounted for by its ability to alter intracellular Ca^{2+}.

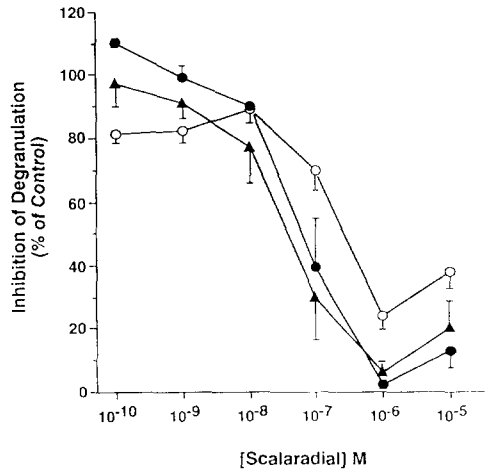

Fig. 1. Inhibition of agonist-induced degranulation of human neutrophils by scalaradial (-●-, 30 nM fMLP; -▲-, 100 nM LTB_4; -○-, 100 nM PAF). Data are expressed as a percent of the control response and are the means ±SEM of 3–6 experiments. Control release of myeloperoxidase (MPO) was 28.0 ± 5.8 horseradish peroxidase (HRO) U/L (fMLP), 18.3 ± 5.4 HRO U/L (LTB_4), and 4.6 ± 1.1 HRO U/L (PAF).

MATERIALS AND METHODS

Isolation of human neutrophils. Neutrophils (PMNs) were isolated from freshly drawn heparinized blood by gradient centrifugation using Ficoll (Histopaque 1077, Sigma Chemical Co., St. Louis, MO) followed by dextran sedimentation to remove erythrocytes. Any remaining erythrocytes were lysed with water for 30 sec, and isotonicity was restored using 10X DB-PBS without Ca^{2+} or Mg^{2+}. PMNs were isolated by centrifugation and were washed one additional time with isotonic DB-PBS prior to determining cell number and viability (using trypan blue dye exclusion). Cell number was adjusted to 0.75–1.5 × 10^7 cells/mL depending on the individual donor, and viability was always >95%.

Degranulation (release of myeloperoxidase). An aliquot (0.1 mL) of the above cell suspension was incubated in 1 mL of Earle's Balanced Salt Solution containing 20 mM HEPES buffer (pH 7.4) and 0.1% gelatin in the presence of 5 μg/mL of cytochalasin B for 5 min at 37°. Cells were pretreated for an additional 5 min with various concentrations of scalaradial (gift of Dr. R. Jacobs of the Marine Science Institute of the University of California, Santa Barbara, CA) or epi-scalaradial (purchased from Biomol, Springfield, PA) prior to addition of stimuli. Agonists were added, and the incubation was continued for an additional 30 min. The reaction was terminated by placement of the samples on ice followed by centrifugation. The supernatant fraction was removed and stored frozen (−30°) until assayed for myeloperoxidase activity. Preliminary experiments demonstrated that scalaradial and its isomer epi-scalaradial were identical in their abilities to inhibit degranulation of PMNs and to inhibit purified PLA_2 activity and, therefore, were used interchangeably.

Determination of myeloperoxidase activity. Myelo-

‡ Marshall L, Winkler JD, Griswold D, McCarte-Roshak A, Bolognese B, Huang L, Sung C-M, Webb EF, Chabot-Fletcher M and Jacobs R, Effects of scalaradial (SLD), a selective type II, phospholipase A_2 (PLA_2) inhibitor, on arachidonic acid (AA) metabolism of human monocyte and neutrophil (PMN). *Sixth International Conference, Inflammation Research Association*, White Haven, PA, September 20–24, 1992.

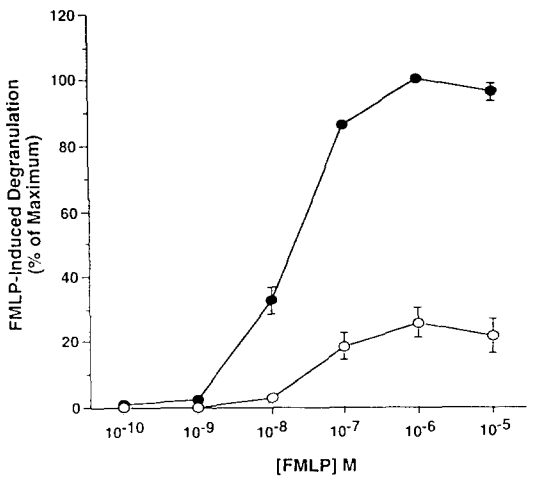

Fig. 2. Non-surmountable antagonism produced by scalaradial (50 nM) of fMLP-induced degranulation (-●-, control; -○-, + scalaradial). Data are expressed as a percent of the maximal fMLP response and are the means ±SEM of 4 experiments. Maximum release of MPO in the absence of scalaradial was 38.9 ± 1.8 HRO U/L.

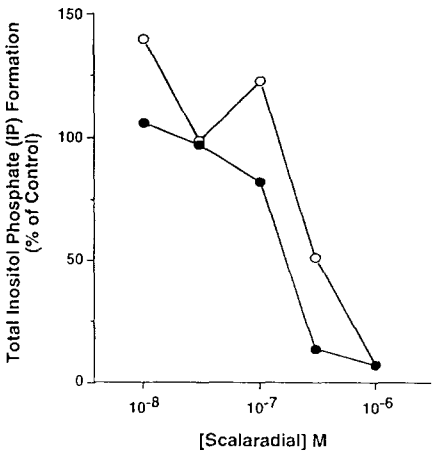

Fig. 4. Inhibition of fMLP- or LTB_4-induced formation of inositol phosphates in U-937 cells (-●-, 100 nM fMLP; -○-, 100 nM LTB_4). The data are the average of 2 experiments and are expressed as a percent of control response. In the absence of scalaradial, fMLP and LTB_4 increased the formation of InsP from an average of 46,200 dpm (basal) to 83,000 dpm (fMLP) and 78,400 dpm (LTB_4).

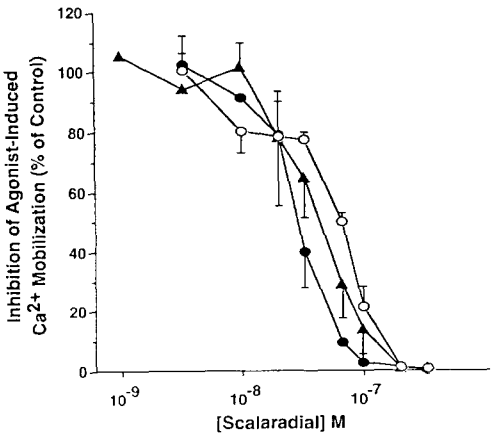

Fig. 3. Inhibition by scalaradial of agonist-induced Ca^{2+} mobilization (-●-, 30 nM fMLP; -▲-, 100 nM LTB_4; -○-, 100 nM PAF). The data are the means ± SEM of 3–4 experiments and are expressed as a percent of the control response. In the absence of scalaradial, the agonists increased intracellular Ca^{2+} concentrations to a mean response of 3.3 μM (fMLP), 2.0 μM (LTB_4), and 2.8 μM (PAF).

peroxidase activity was determined by a modification of the method of Bradley *et al.* [21] using *o*-dianisidine (Sigma) as substrate and horseradish peroxidase (Boehringer Mannheim Biochemicals, Indianapolis, IN) as a standard. Briefly, aliquots (50 μL) of supernatant were incubated with 100 μL of substrate (*o*-dianisidine, 0.53 mM; H_2O_2, 0.147 mM; final concentration) in 50 mM sodium phosphate buffer (pH 6.0). The reaction was terminated by the addition of 50 μL of 4 M sulfuric acid. Product formation was determined by measuring absorbance at 410 nm, and activity was determined by comparison to the standard curve using horseradish peroxidase. Data are expressed as percent of control (amount of myeloperoxidase released in the presence of agonist alone), and IC_{50} values were calculated by linear interpolation of the mean response.

Competitive receptor binding assays. [^{3}H]LTB_4 (sp. act. 140–210 Ci/mmol) and [^{3}H]fMLP (sp. act. 40–87 Ci/mmol) were obtained from New England Nuclear (Boston, MA). Scalaradial was evaluated for its ability to compete with [^{3}H]LTB_4 and [^{3}H]fMLP for their receptors on intact human PMNs using methods described previously [22]. Equilibrium binding studies for [^{3}H]LTB_4 with washed PMNs were performed at a cell concentration of 1–2 × 10^6 cells/0.5 mL in Hanks' Balanced Salt Solution (HBSS) with 0.1% ovalbumin and 0.2 nM [^{3}H]LTB_4. Total and non-specific binding of [^{3}H]LTB_4 were determined in the absence and presence of 1 μM unlabeled LTB_4 (synthesized by the Medicinal Chemistry Department of SmithKline Beecham Pharmaceuticals, King of Prussia, PA), respectively. For inhibition studies, scalaradial was preincubated with the cells for 10 min at 37° over the concentration range of 0.1 to 10.0 μM, and then cooled to 0°. [^{3}H]LTB_4 was added and the incubation continued for an additional 20 min at 0°. Unbound radioligand and competing compounds were separated from cell bound ligands by vacuum filtration through Whatman GF/C filters. Cell bound radioactivity was determined by liquid scintillation spectrometry. [^{3}H]fMLP binding assays were conducted under the same conditions as the LTB_4 assays, using a concentration of cells of 5 × 10^6 cells/0.3 mL and 5–7 nM [^{3}H]fMLP.

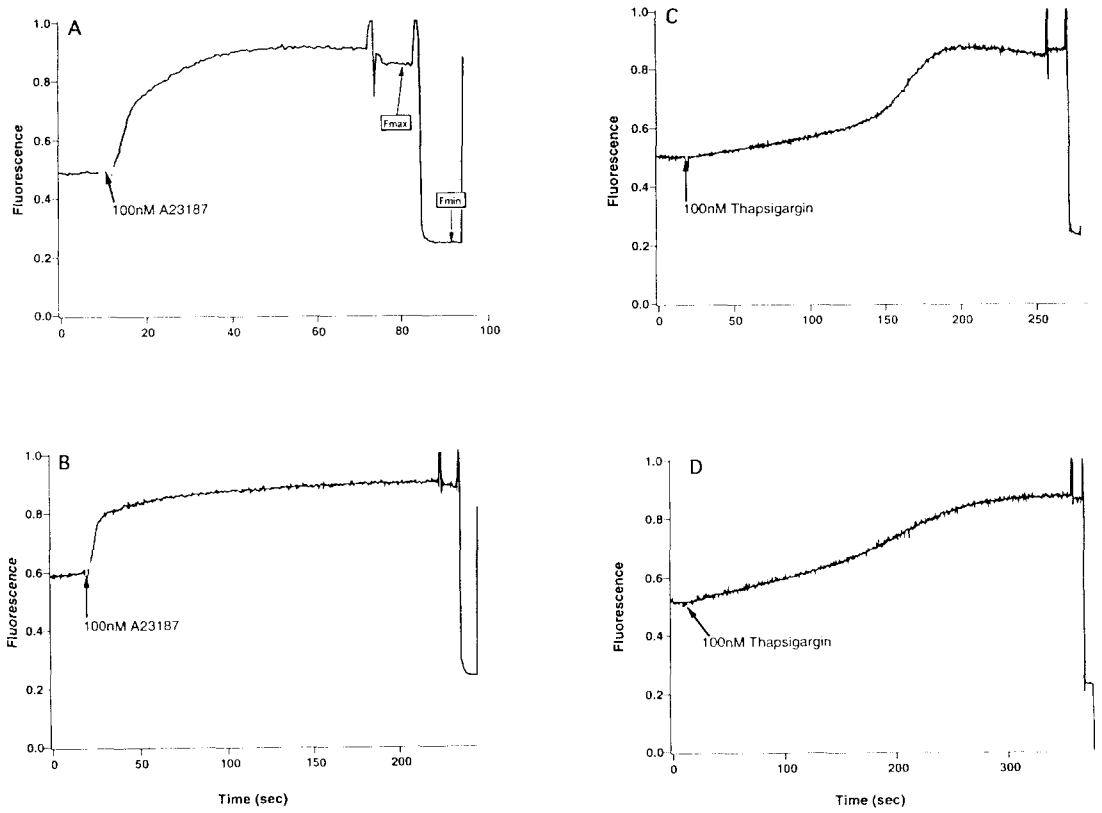

Fig. 5. Representative graph of the effect of scalaradial (1 μM) on intracellular Ca^{2+} concentration produced by A23187 (100 nM) or thapsigargin (100 nM). Panels A and C show the control response to A23187 and thapsigargin, and panels B and D show the response obtained in the presence of scalaradial. Similar results were obtained in 4 additional experiments.

The incubation time for determination of fMLP binding was 40 min at 0°, and non-specific binding was determined with 5 μM unlabeled fMLP (Sigma).

Measurement of intracellular Ca^{2+} *concentration.* Agonist-induced Ca^{2+} mobilization was determined using the calcium fluorescent probe fura 2 [23]. Isolated PMNs were suspended in Krebs Ringer Henseleit (KRH) buffer at 2×10^6 cells/mL containing 0.1% bovine serum albumin, $MgCl_2$ (1.1 mM), and HEPES (5 mM; pH 7.4). The diacetoxymethoxy ester of fura 2 (fura 2/AM; Calbiochem, San Diego, CA) was added at a concentration of 2 μM, and cells were incubated for 45 min at 37°. PMNs were centrifuged at 225 *g* for 5 min, resuspended in the same buffer, and incubated for an additional 20 min to allow complete hydrolysis of the entrapped ester. Cells were centrifuged and resuspended in KRH with 1.0 mM $CaCl_2$, 1.1 mM $MgCl_2$, 5 mM HEPES (pH 7.4) and 0.1% gelatin at 1×10^6 cells/mL. Cells (2×10^6 in 2 mL) were preincubated in a 1 cm^2 cuvette with cytochalasin B (5 μg/mL) for 2.5 min at room temperature, and then scalaradial was added and the incubation continued for an additional 5 min at 37°. The fluorescence of fura 2 containing PMNs was measured with a fluorimeter designed by the Johnson Foundation Biomedical Instrumentation Group and

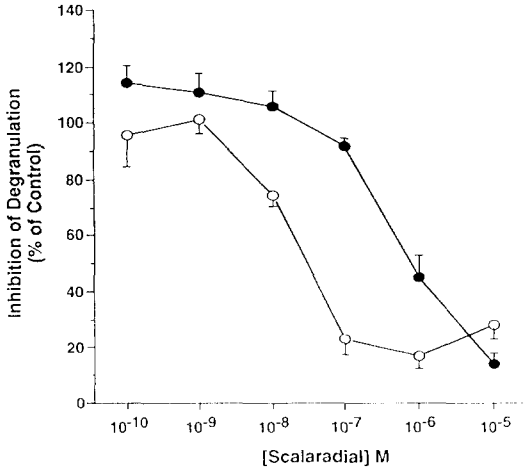

Fig. 6. Inhibition by scalaradial of A23187- (–●–, 1.0 μM) or thapsigargin-(–○–, 100 nM) induced degranulation. The data are expressed as a percent of the control response and are the means ±SEM of 4–7 experiments. Control release of MPO was 30.4 ± 3.9 (A23187) and 6.2 ± 1.0 (thapsigargin) HRO U/L.

equipped with a temperature control (37°) and magnetic stirrer. The excitation wavelength was 340 nm and the emission was 510 nm. After pretreatment with scalaradial, cuvettes were transferred to the fluorometer and fluorescence was monitored for 1 min to ensure a stable baseline before addition of stimuli. Fluorescence was monitored for an additional 2–7 min to obtain the maximal intracellular calcium levels. PMNs were used within 3 hr of fura 2 loading. The $[Ca^{2+}]_i$ was calculated as previously described [23].

Determination of phosphoinositide metabolism in U-937 cells. U-937 cells obtained from the American Type Culture Collection were grown in RPMI-1640 medium (GIBCO Laboratories, Grand Island, NY) supplemented with heat-inactivated 10% fetal bovine serum (HyClone Laboratories, Logan, UT). To differentiate U-937 cells into "macrophage-like" cells and to label the pool of phosphatidylinositol lipids, U-937 cells were seeded at a concentration of 1.25×10^5 cells/mL in 175 cm^2 T-flasks and were grown for 3 days in inositol-free RPMI-1640 medium with 10% heat-inactivated dialyzed fetal bovine serum, plus 2 μCi/mL of *myo*-[^{3}H]inositol (80 Ci/mmol; Amersham, Arlington Heights, IL) and 1.3% DMSO. After 3 days of incubation and upon reaching a density of 6–8 $\times 10^5$ cells/mL, cells were harvested by centrifugation at 200 *g* for 5 min. U-937 cells were washed three times in ice-cold KRH buffer with 1 mM calcium, 1.1 mM magnesium and 0.1% BSA, and were resuspended at a concentration of 1.7×10^6 cells/mL in the same KRH buffer with 50 mM lithium chloride but without BSA. Aliquots (0.6 mL) of this cell suspension were preincubated with various concentrations of scalaradial for 10 min at 37° in a shaking water bath. Agonists, either LTB_4 (10 nM) or fMLP (100 Nm), were added and the reaction was allowed to proceed for an additional 5 min. The reaction was stopped by the addition of 0.11 mL of 100% trichloroacetic acid (TCA; w/v), and the samples were placed on ice for 20 min prior to centrifugation (1750 *g* for 10 min). The pellets were washed once with 0.9 mL of distilled water, and the supernatant fractions were combined. TCA was extracted from the supernatant fraction with 3×5 mL washes of water-saturated diethyl ether. Excess ether was removed by evaporation under argon, and samples were neutralized by the addition of 0.15 mL of 0.1 M Tris base. Samples were stored at −40° until analysis of inositol metabolites by anion exchange chromatography.

^{3}H-Labeled inositol phosphates were separated by a modification of the method of Hawkins *et al.* [24]. The samples (1.0 mL) were filtered through Millipore HV filters (0.45 μm), and then were injected and fractionated on an anion exchange column (Beckman Ultrasil 10-SAX column, 25×0.46 cm) with a discontinuous gradient from 0 to 3.5 M ammonium formate (pH 3.7 with phosphoric acid). The flow rate was 1.25 mL/min, and 0.3-mL fractions were collected; 4 mL of Tru-Count (IN/US) was added to each fraction, and the radioactivity was determined by liquid scintillation spectrometry. The concentration required to inhibit 50% of the agonist-induced stimulation of total InsP formation was determined by linear interpolation of the data.

RESULTS

Pretreatment of neutrophils with scalaradial produced a concentration-dependent inhibition of fMLP-, LTB_4,- and PAF-induced degranulation with IC_{50} values of 70, 50, and 200 nM, respectively (Fig. 1). With regard to fMLP, the inhibition produced by scalaradial was non-surmountable, suggesting that scalaradial was not acting as an fMLP receptor antagonist (Fig. 2). To further explore this possibility, we determined the ability of scalaradial to inhibit [^{3}H]fMLP binding to neutrophils. At concentrations as high as 1 μM, scalaradial did not reduce significantly [^{3}H]fMLP binding while abolishing neutrophil degranulation. Higher concentrations of scalaradial did inhibit fMLP binding to neutrophils (data not shown). The IC_{50} value obtained for scalaradial at the fMLP receptor was 7.5 μM, which was about 100-fold greater than the IC_{50} value needed for inhibition of degranulation. Binding studies using [^{3}H]LTB_4 as the ligand showed a similar IC_{50} for scalaradial of 7.0 μM, which was 140 times higher than its IC_{50} value for inhibiting degranulation (data not shown). These data demonstrate that inhibition of degranulation produced by scalaradial does not appear to result from receptor antagonism.

Many receptor-mediated stimuli increase intracellular $[Ca^{2+}]$ in PMNs either by releasing it from intracellular stores or by increasing its influx [25], as part of the signal transduction process. To determine if scalaradial inhibits degranulation by preventing the rise in intracellular Ca^{2+}, we determined the ability of scalaradial to prevent the change in Ca^{2+} induced by fMLP, LTB_4, and PAF. Pretreatment with scalaradial produced a concentration-dependent inhibition of agonist-induced Ca^{2+} increases with a potency similar to that observed for degranulation. The IC_{50} values obtained for fMLP-, LTB_4-, and PAF-induced Ca^{2+} mobilization were 26.3, 48, and 62 nM, respectively (N = 3–4 experiments; Fig. 3). The inhibition of calcium increase appeared not to be competitive since similar IC_{50} values were obtained over a 100-fold concentration range of agonists (data not shown). These results suggest that the inhibition of agonist-induced degranulation by scalaradial may be mediated either by a blockade of Ca^{2+} channels or by an inhibition of PI-PLC.

To explore the possibility that scalaradial was acting by inhibiting PI-PLC, we determined its ability to inhibit the formation of [^{3}H]inositol phosphate products from U-937 cells equilibrium-labeled over 3 days with *myo*-[^{3}H]inositol. Pretreatment of U-937 cells with scalaradial produced a concentration-dependent reduction in inositol phosphate formation induced by fMLP or LTB_4 (Fig. 4). The IC_{50} values obtained were 180 and 450 nM, respectively, values 2.5 to 9-fold higher than that required for degranulation.

Since it was apparent from our studies that scalaradial had additional effects on signal transduction pathways, we evaluated its ability to inhibit degranulation by two stimuli that bypass these pathways, A23817 and thapsigargin. These agonists increase intracellular Ca^{2+} but through distinct mechanisms: A23187, a calcium ionophore, enhances the influx of extracellular Ca^{2+} through the plasma

membrane [25] and thapsigargin inhibits the Ca^{2+} pump in the endoplasmic reticulum [26]. Scalaradial did not prevent the increase in calcium concentration and subsequent saturation of intracellular fura 2 produced by either A23187 or thapsigargin (Fig. 5). Thus, since in the absence or presence of scalaradial both A23187 and thapsigargin saturated the fura 2, this indicates that the intracellular Ca^{2+} concentration produced by these two agonists was at least 2 μM. Using these two stimuli, pretreatment of PMNs with scalaradial produced a concentration-dependent inhibition of degranulation with IC_{50} values of 40 nM for thapsigargin and 900 nM for A23187 (Fig. 6). In these studies, scalaradial was as potent in blocking the degranulation response to thapsigargin as it was in inhibiting the response to the various receptor-mediated stimuli tested. Thus, scalaradial inhibited degranulation even in the presence of micromolar concentrations of intracellular Ca^{2+}.

DISCUSSION

In this report, we have examined the ability of scalaradial to inhibit both receptor- and non-receptor-mediated degranulation of human neutrophils. Pretreatment with submicromolar concentrations of scalaradial, which implicate its actions on the type II 14 kDa PLA_2 [19, *], produced a potent, concentration-dependent inhibition of fMLP-, LTB_4-, and PAF-induced degranulation. Similar findings were obtained using BPB with rat neutrophils and human eosinophils [9, 10] or mepacrine with RBL-2H3 [27]. In the present study, the inhibition of fMLP-induced degranulation was non-competitive, suggesting that scalaradial was not acting as a competitive antagonist at the fMLP receptor. We confirmed this by demonstrating that scalaradial did not reduce [^{3}H]fMLP binding to PMNs except at much higher concentrations. Thus, scalaradial appeared to inhibit the action of fMLP at a site beyond the receptor and initially supported the hypothesis that PLA_2, especially the 14 kDa form, was important for exocytosis.

However, closer characterization of the actions of scalaradial showed that it prevented the Ca^{2+} mobilization produced by all three agonists with a similar potency (IC_{50} = 25–60 nM). Also, scalaradial inhibited the agonist-induced elevation of InsP formation in U-937 cells, although 2.5- to 9-fold higher concentrations were required than were needed to inhibit degranulation. In a similar manner, manoalide, another PLA_2 inhibitor, which inhibits neutrophil degranulation [28], also inhibits phospholipase C and several Ca^{2+} channels [17, 29]. Our findings with scalaradial on agonist-induced calcium mobilization and InsP formation raise the possibility that scalaradial, like the other PLA_2 inhibitors, has additional pharmacological actions that may limit the interpretation of its actions in degranulation. One approach to eliminate these additional considerations was to bypass the signal transduction process in the plasma membrane using A23817 or thapsigargin as stimuli. Scalaradial did not prevent A23187- or thapsigargin-induced increase in intracellular levels of Ca^{2+} but inhibited both A23817- and thapsigargin-induced degranulation. These agents induce Ca^{2+} mobilization that is independent of PLC activation and the release of inositol phosphates in addition to receptor-mediated membrane transducers such as G proteins or other protein kinases. Although scalaradial has additional pharmacological actions, our results with A23187 and thapsigargin support the hypothesis that PLA_2 activity is important for neutrophil exocytosis.

In summary, scalaradial was a potent inhibitor of both receptor-mediated and non-receptor-mediated degranulation, and with selected stimuli this action was independent of any effect on Ca^{2+} concentration. Finally, our data support the hypothesis that PLA_2, especially the 14 kDa form, is important in exocytosis. However, our data also suggest that scalaradial may have additional effects on the Ca^{2+} signal transduction mechanism; therefore, caution should be used in interpretation of data using this compound.

* Marshall L, Winkler JD, Griswold D, McCarte-Roshak A, Bolognese B, Huang L, Sung C-M, Webb EF, Chabot-Fletcher M and Jacobs R, Effects of scalaradial (SLD), a selective type II, phospholipase A_2 (PLA_2) inhibitor, on arachidonic acid (AA) metabolism of human monocyte and neutrophil (PMN). *Sixth International Conference, Inflammation Research Association*, White Haven, PA, September 20–24, 1992.

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