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ACCELERATED COMMUNICATION

A Distinct G_i Protein-Coupled Receptor for Sphingosylphosphorylcholine in Human Leukemia HL-60 Cells and Human Neutrophils

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SUMMARY

The sphingolipids, sphingosylphosphorylcholine (SPPC) and sphingosine-1-phosphate (SPP), induce a rapid and transient rise in intracellular free calcium concentration ($[Ca^{2+}]_i$) in a variety of cell lines via activation of pertussis toxin-sensitive G protein-coupled receptors. We investigated whether these sphingolipids act on different receptors by testing the effect of varying concentrations of SPPC on $[Ca^{2+}]_i$ in human leukemia HL-60 cells, which have been found to be nonresponsive to SPP. SPPC potently (EC_{50} = 1.5 μM) and rapidly increased $[Ca^{2+}]_i$ in HL-60 cells in a pertussis toxin-sensitive manner. Differentiation of HL-60 cells through treatment with dibutyryl cAMP into granulocyte-like cells did not change the magnitude or the pertussis toxin sensitivity of the SPPC-induced $[Ca^{2+}]_i$ rise, indicating that the receptor for SPPC is constitutively expressed in HL-60 cells. SPPC did not activate phospholipase C or D in HL-60 cells. However, SPPC, but not SPP, stimulated the generation of superoxide anions in dibutyryl cAMP-differentiated HL-60 cells as well as in human neutrophils, suggesting that the SPPC receptor may play a role in the inflammatory defense against invading microorganisms. On the basis of these results, we conclude that there apparently is a heterogeneity of G protein-coupled receptors for sphingolipids in mammalian cells.

Within the past few years, there has been a surge in attention to the role of sphingolipids in the regulation of cellular events (1). In particular, certain metabolites of complex sphingolipids, such as SPH, ceramide, and SPP, have been implicated as putative intracellular messengers produced in cells after stimulation with tumor necrosis factor-α, interleukin-1, and platelet-derived growth factor (1-3). Another sphingolipid, SPPC, the *N*-deacylated derivative of sphingomyelin, was reported to be a mitogen for a variety of cells, including Swiss 3T3 cells (4, 5). The mitogenic effect of SPPC seemed to be very specific because SPPC stimulated cellular proliferation of quiescent Swiss 3T3 cells to a much greater extent than did SPP and other growth factors (4).

We recently demonstrated that SPP activates a high affinity G_i protein-coupled receptor in the plasma membrane of various cell types, leading to, for example, mobilization of calcium in a variety of different cells, inhibition of adenylyl cyclase, and activation of inwardly rectifying potassium channels in atrial myocytes (6, 7). PTX-sensitive calcium responses in human embryonic kidney cells were also observed with SPPC. However, although the maximal responses to SPP and SPPC were of similar magnitude, the slopes of the concentration-response curves of the two sphingolipids were significantly different. These findings prompted us to investigate whether SPPC and SPP act by stimulating different G protein-coupled receptors. We show that in human leukemia HL-60 cells, which are virtually nonresponsive to SPP, a G_i protein-coupled receptor for SPPC is expressed that mediates calcium mobilization and superoxide generation in dibutyryl cAMP-differentiated

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ABBREVIATIONS: SPH, sphingosine; SPPC, sphingosylphosphorylcholine; SPP, sphingosine-1-phosphate; BSA, bovine serum albumin; FMLP, *N*-formyl-methionyl-leucyl-phenylalanine; PMA, phorbol-12-myristate 13-acetate; PtdEtOH, phosphatidylethanol; PTX, pertussis toxin; $[Ca^{2+}]_i$, intracellular free calcium concentration; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ester)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

(granulocyte-like) HL-60 cells as well as in human neutrophils. On the basis of these results, we conclude that there is a heterogeneity of sphingolipid receptors in the plasma membrane of mammalian cells.

Experimental Procedures

Materials. SPPC and SPP were purchased from Biomol or Matreya. SPH was obtained from Calbiochem. Fatty acid-free BSA, lucigenin, lysophosphatidic acid, lysophosphatidylcholine, FMLP, and PMA were obtained from Sigma Chemical Co. Methanolic solutions of the lipids were dried under N_2, and the compounds were dissolved in 1 mg/ml BSA in phosphate-buffered saline. [9,10-^{3}H]-Oleic acid (10 Ci/mmol) and *myo*-[2-^{3}H]inositol (24.4 Ci/mmol) were obtained from DuPont-New England Nuclear. Fura-2/AM was obtained from Molecular Probes, RPMI 1640 medium from GIBCO, and PTX from List Biological Laboratories.

HL-60 cell culture and isolation of human neutrophils. HL-60 cells were cultured in RPMI medium supplemented with 10% horse serum, 100 units/ml penicillin, and 100 μg/ml streptomycin in 7.5% CO_2. Where indicated, HL-60 cells were differentiated into granulocyte-like cells through treatment with 0.5 mM dibutyryl cAMP for 72-96 hr. For efficient PTX treatment, HL-60 cells were preincubated for 24 hr with 100 ng/ml PTX as described previously (8, 9). To measure the effect of SPPC on cell division, HL-60 cells were washed two times with serum-free RPMI 1640 medium and grown in defined, serum-free RPMI 1640 growth medium supplemented with 1 mg/ml fatty acid-free BSA, 5 μg/ml human holotransferrin, 10 μg/ml human insulin, 3 μg/ml ascorbic acid, 2 ng/ml hydrocortisone, 20 μM ethanolamine, and various trace element compounds, as described in detail previously (10). Human neutrophils from peripheral blood of healthy volunteers were isolated through sedimentation of erythrocytes with dextran, followed by centrifugation through a Ficoll-Hypaque gradient and hypotonic lysis of residual erythrocytes according to Bøyum (11). Isolated cells were then resuspended in phosphate-buffered saline/glucose containing 137 mM NaCl, 2.7 mM KCl, 0.5 mM $MgCl_2$, 0.9 mM $CaCl_2$, 1.5 mM KH_2PO_4, 6.5 mM Na_2HPO_4, and 5.5 mM D-glucose.

Measurement of $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ was determined with use of the fluorescent calcium indicator dye Fura-2 in a Perkin Elmer LS-5B spectrofluorimeter equipped with a fast-filter device as described previously (12). Briefly, cells resuspended at ~0.5-1.0 × 10^6 cells/ml were incubated with 1 μM Fura-2/AM for 1 hr at 37° in a buffer containing 137 mM NaCl, 2.7 mM KCl, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$, 6.5 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 1 mg/ml BSA, and 5.5 mM D-glucose, pH 7.4. Thereafter, cells were washed twice, resuspended in fresh buffer, and used for fluorescence measurements within the next hour. To remove dye that leaked into the medium, aliquots were pelleted in a microcentrifuge, resuspended in fresh prewarmed medium without BSA, and immediately transferred to a thermostated cuvette (37°) in the spectrofluorimeter. Sphingolipids were added in vehicle solution (1 mg/ml BSA in phosphate-buffered saline). Excitation was alternating at 340 and 380 nm, with emission read at 495 nm. Fluorescence data were converted into Ca^{2+} concentration with the use of software supplied by the manufacturer. In some experiments, extracelluar Ca^{2+} was chelated by the addition of 5 mM EGTA 30 sec before SPPC exposure, resulting in 17 nM free extracellular Ca^{2+}.

Assays of phospholipases C and D. Measurements of phospholipase C and D activities were performed as described in detail previously (13). Cellular phospholipids were labeled by incubating HL-60 cells for 48 hr with [^{3}H]inositol (1.25 μCi/ml) or [^{3}H]oleic acid (2-2.5 μCi/ml) in growth medium. After the labeling period, the cells were equilibrated two times for 10 min at 37° in Hanks' balanced salt solution containing 118 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM D-glucose and buffered at pH 7.4 with 15 mM HEPES. Then, phospholipase C- and D-catalyzed formation of [^{3}H]-inositol phosphates and [^{3}H]PtdEtOH, respectively, was measured in response to the indicated stimulatory agents dissolved in vehicle solution (1 mg/ml BSA in phosphate-buffered saline) for 30 min at 37°. LiCl (10 mM) was added 10 min before the stimulatory agents when [^{3}H]inositol phosphate formation was monitored in a total volume of 100 μl containing 2 × 10^6 cells. For measurement of [^{3}H]PtdEtOH accumulation in a total volume of 400 μl containing 8 × 10^6 cells, ethanol (400 mM) was present. The reactions were stopped by the addition of 1 ml of ice-cold methanol to the tubes. The phospholipids and inositol phosphates were extracted and analyzed as described previously (13).

Measurement of superoxide anion generation. Superoxide anion generation in dibutyryl cAMP-differentiated HL-60 cells and isolated human neutrophils was measured with the lucigenin assay in a lumiaggregometer as described previously (14). The assays were performed in luminometer cuvettes at 37°, with the samples being continuously stirred at 300 rpm. HL-60 cells or isolated human neutrophils (2 × 10^6 cells/380 μl) were incubated for 2 min in phosphate-buffered saline containing 5 mM D-glucose and 0.1 μg/ml lucigenin to record base-line luminescence. Then, stimulatory agent or vehicle (1 mg/ml BSA in phosphate-buffered saline) in a volume of 20 μl was added, and the responses were recorded continuously. Chemiluminescence response to 1 μM PMA was used as positive control in PTX-pretreated and control HL-60 cells because the PMA response was unaffected by PTX treatment. The PMA response was ~2-3-fold higher than the response to 1 μM FMLP.

Results

Effect of SPPC on $[Ca^{2+}]_i$ in HL-60 cells. To test the hypothesis that SPPC and SPP act on different G protein-coupled receptors, we investigated the effect of SPPC on $[Ca^{2+}]_i$ in various cell lines that had been found to be virtually nonresponsive to SPP (6). Of these cell lines, HL-60 cells showed a significant increase in $[Ca^{2+}]_i$ in response to SPPC (Fig. 1). SPPC (10 μM) rapidly increased $[Ca^{2+}]_i$, which peaked within 5-10 sec. The primary Ca^{2+} spike was succeeded by a small secondary increase that gradually returned to the prestimulation $[Ca^{2+}]_i$ level over the following 3-4 min. To explore the involvement of PTX-sensitive G proteins in SPPC-induced $[Ca^{2+}]_i$ increase, HL-60 cells were preincubated with PTX (100 ng/ml) for 24 hr. As shown in Fig. 1 (*top*), a similar trace was obtained, but $[Ca^{2+}]_i$ peak increases in response to 10 μM SPPC were significantly reduced (by 52 ± 3%, six independent experiments). Differentiation of HL-60 cells into a granulocyte-like phenotype through treatment with 0.5 mM dibutyryl cAMP for 72-96 hr changed neither the magnitude nor the PTX sensitivity of the SPPC response and did not alter the lack of responsiveness of HL-60 cells to SPP (data not shown). The inclusion of 5 mM EGTA to chelate extracellular Ca^{2+} significantly reduced the peak increase in $[Ca^{2+}]_i$ (by 34 ± 7%, three independent experiments) and eliminated the plateau phase (Fig. 1, *bottom*). This indicates that the $[Ca^{2+}]_i$ increase is derived from both intracellular and extracellular sources. When PTX-treated cells were exposed to EGTA before the addition of SPPC, the increase in $[Ca^{2+}]_i$ in response to 10 μM SPPC was almost fully abolished. These results demonstrate that the SPPC receptor-induced mobilization of internal Ca^{2+} is predominantly, if not totally, mediated by PTX-sensitive G proteins.

To determine the specificity of the action of SPPC, we performed concentration-effect experiments in HL-60 cells with SPPC, SPP, and SPH at concentrations of ≤20 μM (Fig.

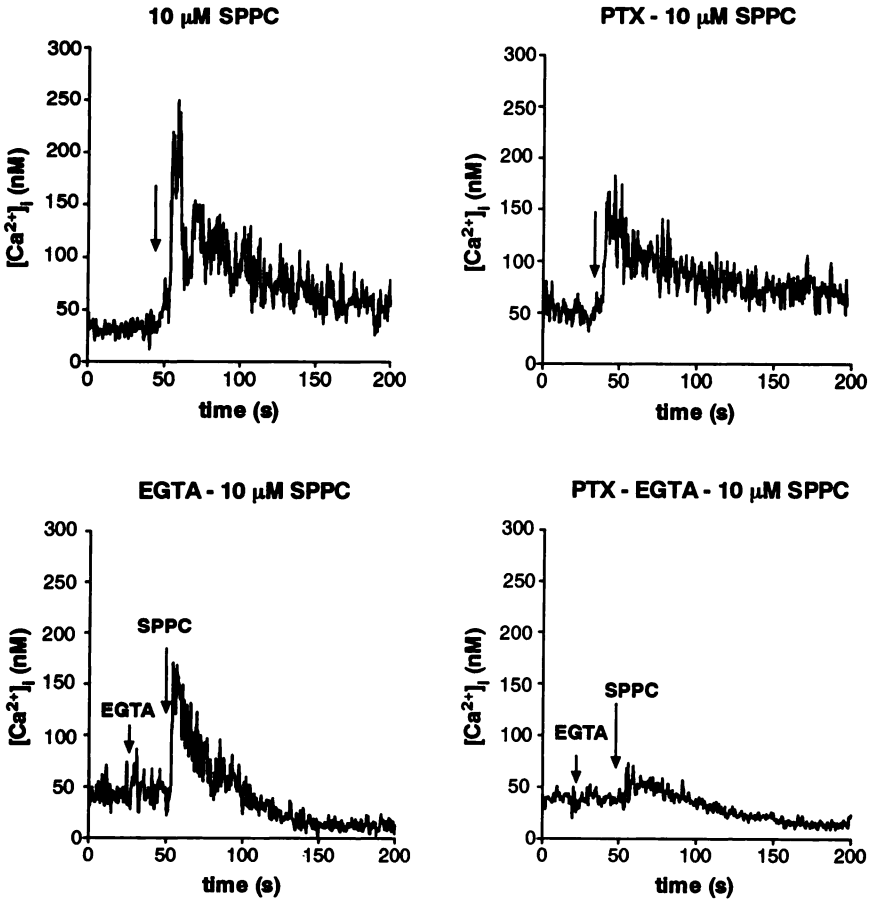

Fig. 1. Changes in $[Ca^{2+}]_i$ after the addition of SPPC in HL-60 cells showing the effects of treatment with PTX and EGTA. The influence of SPPC (10 μM) on $[Ca^{2+}]_i$ was determined in nondifferentiated HL-60 cells with and without pretreatment with PTX (100 ng/ml for 24 hr) or EGTA (5 mM for 30 sec) as indicated with the Fura-2 method (see Experimental Procedures). *Arrows*, addition of EGTA and SPPC.

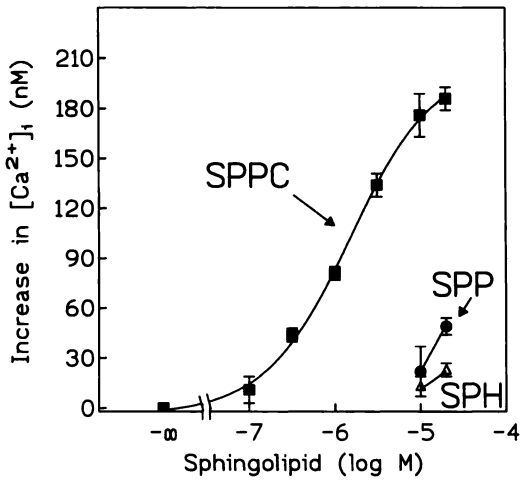

Fig. 2. Concentration-response relationship for SPPC-induced increase in $[Ca^{2+}]_i$ in HL-60 cells. $[Ca^{2+}]_i$ responses to SPPC, SPP and SPH were determined in nondifferentiated HL-60 cells at the indicated concentrations. Values are mean ± standard error of five (SPPC) or three (SPP and SPH) sets of experiments, each done in duplicate. Basal $[Ca^{2+}]_i$ level was 56 ± 7 nM. The Hill slope of the SPPC concentration-response curve is 0.86 ± 0.09.

2). Higher concentrations of SPPC, which have been reported to be cytotoxic (5), were not tested. With the absolute response measured at 20 μM SPPC as the 100% reference value, SPPC increased $[Ca^{2+}]_i$ with an apparent EC_{50} value of 1.5 ± 0.3 μM. SPP and SPH did not change or only marginally elevated $[Ca^{2+}]_i$ (i.e., <25 nM increase in $[Ca^{2+}]_i$ in response to 10 μM of either sphingolipid) in HL-60 cells. The effect of SPPC was also compared with the effect of similar concentrations of structurally related phospholipids. Lysophosphatidic acid (1 μM) did not increase $[Ca^{2+}]_i$ in HL-60 cells. Stimulation of HL-60 cells with lysophosphatidylcholine (1-2 μM) increased $[Ca^{2+}]_i$ in HL-60 cells but with a delayed onset of change apparent only after 10 sec. Thus, the SPPC-induced change in $[Ca^{2+}]_i$ is not mediated by receptors for structurally related phospholipids.

Effects of SPPC on phospholipases C and D in HL-60 cells. To investigate the signal transduction pathways activated by SPPC receptors in HL-60 cells, we first tested whether SPPC stimulates the formation of inositol phosphates in HL-60 cells loaded with [^{3}H]inositol. Activation of SPPC receptors in dibutyryl cAMP-differentiated cells with 10 μM SPPC for 30 min did not change the cellular concentration of [^{3}H]inositol phosphates (Fig. 3). In contrast, activation of formyl peptide receptors by 10 μM FMLP increased the formation of [^{3}H]inositol phosphates nearly 4-fold. SPPC did not activate phospholipase D in differentiated HL-60 cells either. Treatment of the cells with 10 μM FMLP, however, increased [^{3}H]PtdEtOH formation ~3-fold. In nondifferentiated HL-60 cells, 10 μM SPPC was also ineffective in stimulating the formation of [^{3}H]inositol phosphates or activating [^{3}H]PtdEtOH formation (three independent experiments; data not shown).

SPPC does not stimulate HL-60 cell proliferation. To determine the potential physiological role of SPPC receptors in HL-60 cells, we tested whether SPPC stimulates cell proliferation, as has been demonstrated in Swiss 3T3 cells and other cell lines (4, 5). HL-60 cells seeded at a low initial

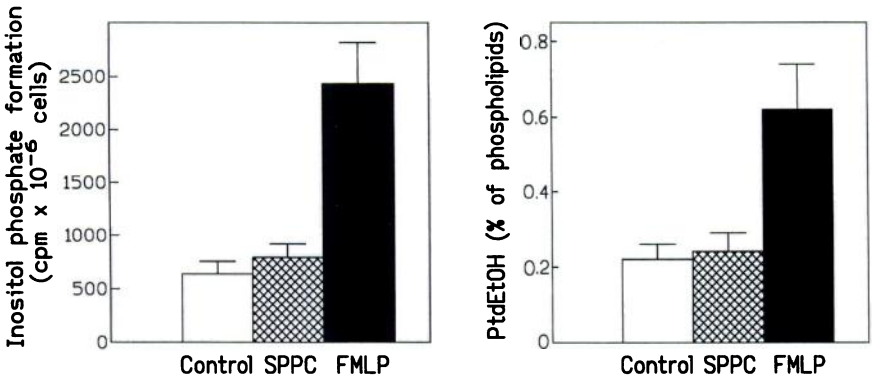

Fig. 3. Lack of effect of SPPC on phospholipase C and D activities in differentiated HL-60 cells. Formation of [^{3}H]inositol phosphates and [^{3}H]-PtdEtOH was determined in dibutyryl cAMP-differentiated HL-60 cells prelabeled with *myo*-[^{3}H]-inositol (*left*) and [^{3}H]oleic acid (*right*), respectively, in response to vehicle, 10 μM SPPC, or 10 μM FMLP, as described in Experimental Procedures. Values are mean ± standard error of three experiments, done in triplicate.

density (3×10^5 cells/ml) were incubated in defined, serum-free RPMI 1640 medium with and without SPPC or horse serum for ≤4 days. The inclusion of 10% horse serum increased HL-60 cell number by 4.5-fold at day 4. In contrast, the addition of SPPC (1 or 10 μM) neither stimulated nor inhibited basal cell growth (2.9-fold increase at day 4). Furthermore, SPPC did not induce gross morphological changes in HL-60 cells.

SPPC stimulates the production of superoxide anions in dibutyryl cAMP-differentiated HL-60 cells and human neutrophils. The formation of superoxide anions in response to activation of receptors coupled to PTX-sensitive G proteins is a well known phenomenon in dibutyryl cAMP-differentiated granulocyte-like HL-60 cells and human neutrophils (15). We therefore investigated whether SPPC stimulates the production of superoxide anion generation in these cells, as measured with the lucigenin assay (14). As summarized in Table 1, SPPC (10 μM) evoked a rapid and transient increase in chemiluminescence, indicating stimulation of the production of superoxide anions in HL-60 cells. This stimulation amounted to 35 ± 7% of that observed in response to a maximally effective concentration of FMLP (1 μM). The velocity of the chemiluminescence response to SPPC (10 μM) was approximately one third of that to FMLP (1 μM). This is probably due to the submaximal stimulation of NADPH oxidase by SPPC, as a concentration of FMLP (30 nM) that stimulated superoxide anion production by the same extent as 10 μM SPPC exhibited a similar reduced velocity as 10 μM SPPC. Pretreatment of differentiated HL-60 cells with PTX (100 ng/ml) for 24 hr reduced the SPPC response by 71 ± 12% and almost completely prevented the FMLP response. To corroborate these observations in granulocyte-like HL-60 cells, we determined whether SPPC also stimulates superoxide anion generation in human neutrophils isolated from peripheral blood. As shown in Fig. 4A, SPPC (10 μM) evoked a rapid and steep rise in chemiluminescence that was followed by a decline. The increase in chemiluminescence was concentration dependent, and half-maximal effect was noted at ~5 μM SPPC (Fig. 4B). SPPC (10 μM) activated superoxide generation in human neutrophils almost as effectively as FMLP [i.e., 82 ± 20% of that observed with 1 μM FMLP (five independent experiments)]. The velocity of respiratory burst response to SPPC (10 μM) was ~50-60% of that observed with FMLP (1 μM) (Fig. 4C). In contrast to SPPC, SPP (10 μM)

TABLE 1

Stimulation of superoxide anion generation in differentiated HL-60 cells

Superoxide anion generation was measured in dibutyryl cAMP-differentiated HL-60 cells pretreated or not pretreated with PTX (100 ng/ml for 24 hr) in the presence of 1 μM FMLP or 10 μM SPPC with the lucigenin assay as described in Experimental Procedures. Increase in chemiluminescence is given as either percent of the maximal response induced by FMLP (1 μM) or velocity, arbitrarily expressed as the slope of the chemiluminescence trace [i.e., height of the recorded trace (in mm) attained in the first 20 sec]. Results shown are mean ± standard error of five experiments, done at least in triplicate. Superoxide anion production was not stimulated by SPPC in nondifferentiated HL-60 cells.

	FMLP	SPPC
Control cells		
Percent of maximum (%)	100	35 ± 7
Velocity (mm)	36 ± 5	11 ± 3
PTX-treated cells		
Percent of maximum (%)	13 ± 5	10 ± 4

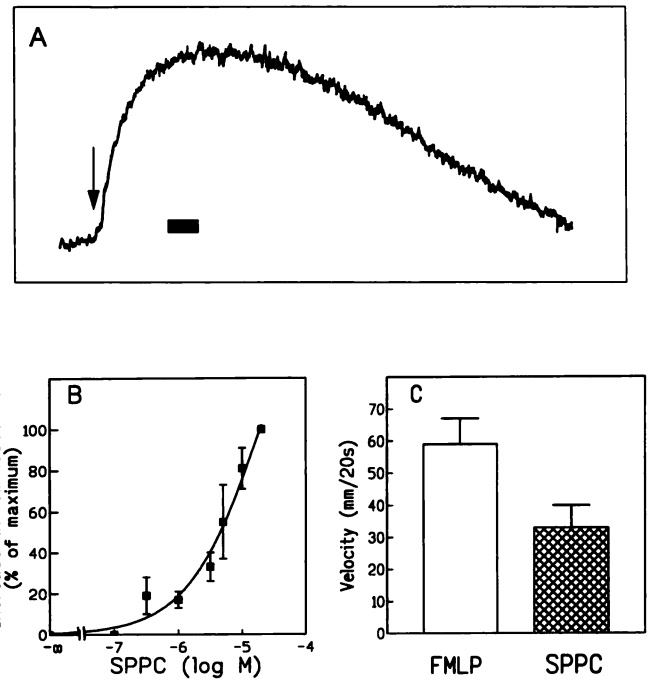

Fig. 4. SPPC-induced superoxide anion generation in human neutrophils. A, Change in chemiluminescence evoked by SPPC showing a typical response to SPPC (10 μM) detected by the lucigenin assay. *Arrow*, addition of SPPC is indicated. *Scale bar*, 20 sec. B, Concentration-response relationship for SPPC-induced stimulation of superoxide anion generation in human neutrophils. Values are mean ± standard of three experiments (neutrophils isolated from three healthy volunteers), done at least in duplicate. Response to 20 μM SPPC is taken as 100%. C, Velocity of luminescence response to 1 μM FMLP and 10 μM SPPC. Values are mean ± standard error of five separate experiments (neutrophils isolated from three healthy volunteers), done at least in triplicate.

either did not stimulate superoxide generation in differentiated HL-60 cells or only marginally (<10% of that observed with 10 μM SPPC) stimulated superoxide generation in isolated human neutrophils.

Discussion

The aim of the current study was to investigate whether SPPC activates a G protein-coupled receptor that is distinct from the SPP receptor; the results support that it does. We demonstrated that HL-60 cells, which express the PTX substrates $G_{\alpha i2}$ and $G_{\alpha i3}$ but not $G_{\alpha o}$ (8), are essentially nonresponsive to SPP and SPH, whereas SPPC rapidly and transiently increases $[Ca^{2+}]_i$ and stimulates the generation of superoxide anions in a PTX-sensitive manner. Because the structurally related phospholipids SPH, SPP, and lysophosphatidylcholine did not mimic the effects of SPPC, we conclude that HL-60 cells express a distinct G_i protein-coupled receptor for SPPC.

Because SPP, but not SPPC, was found to activate phospholipases C and D in Swiss 3T3 fibroblasts, it was suggested that SPPC may activate signaling pathways distinct from those used by SPP (5, 16). However, Swiss 3T3 cells responded to both SPPC and SPP with rapid rises in $[Ca^{2+}]_i$ that were of similar magnitude and occurred at similar sphingolipid concentrations. Thus, the important question of whether SPP and SPPC act on common or distinct receptors could not be answered based on those studies. Furthermore, the differential effects of SPPC and SPP on the phospholipases in Swiss 3T3 cells may have resulted from intracellular action of the sphingolipids or differential metabolism of the two sphingolipids, as both sphingolipids applied exogenously are rapidly taken up by cells and subject to different metabolic pathways (5, 17). In contrast, in HL-60 cells, only SPPC (not SPP) was able to elicit a rapid increase in $[Ca^{2+}]_i$ and superoxide anion generation. In addition, our study is the first report of a rapid and significant physiological response to SPPC that is not mimicked by SPP in a terminally differentiated cell (i.e., the human neutrophil).

SPPC has recently been shown to potently stimulate DNA synthesis and cell division in quiescent Swiss 3T3 fibroblasts, possibly via activation of p42 mitogen-activated protein kinase and stimulation of the DNA-binding activity of the transcription factor activating protein-1 (18, 19). PTX treatment blocked the mitogenic response, suggesting involvement of a G_i-type G protein in the action of SPPC (19). In HL-60 cells, however, SPPC did not influence cell proliferation, whereas inclusion of horse serum significantly accelerated HL-60 cell division. These observations suggest that the potency of SPPC to promote cell proliferation is cell type dependent.

Sphingosine was previously observed to inhibit the activation of respiratory burst by FMLP and other stimuli in human neutrophils, possibly via inhibition of protein kinase C (20). In contrast, SPPC is shown in the current study to activate the production of superoxide anions in both dibutyryl cAMP-differentiated HL-60 cells and human neutrophils. These findings signify the selectivity of SPPC action in HL-60 cells and human neutrophils. Neutrophils participate in the killing of microorganisms after infection, and during phagocytosis, neutrophils produce superoxide anions through reduction of oxygen, catalyzed by NADPH oxidase (15). Our study indicates that the NADPH oxidase can be activated by stimulation of the SPPC receptors and that these receptors may play a role in the phagocytosis of microorganisms. The seminal question as to which metabolite pathways lead to the generation of SPPC has yet to be answered. It is possible that, in analogy to the synthesis of FMLP by bacteria, microorganisms are the source of SPPC production. Alternatively, microorganisms may release enzymes that catalyze the generation of SPPC in the plasma membrane of mammalian cells. Recently, a novel enzyme that cleaves the *N*-acyl linkage of ceramides in sphingomyelin to produce SPPC has been isolated from the culture filtrate of a newly isolated bacterium, *Pseudomonas* sp. TK4 (21). Whether the action of such a bacterial enzyme generates SPPC in mammalian cells, including human neutrophils, should be clarified in further studies. There is no conclusive evidence of how SPPC is synthesized in mammalian cells. The formation of SPPC from sphingosine and CDP-choline through a phosphotransferase activity has not been demonstrated unambiguously (22, 23). Furthermore, SPPC does not seem to be a precursor of sphingomyelin as sphingomyelin is synthesized in mammalian cells through transfer of phosphorylcholine from phosphatidylcholine to ceramide (24). However, in analogy to phospholipase A_2, which catalyzes liberation of arachidonic acid from phospholipids, a "sphingomyelin-deacylase" has been hypothesized that might function to increase levels of SPPC in mammalian cells (1). Such enzymatic activity may be responsible for the accumulation of SPPC in the spleen, liver, and brain of patients with Niemann-Pick type A disease, who have sphingomyelinase deficiency, hepatosplenomegaly, and brain dysfunction (25).

Recently, a novel intracellular calcium channel gated by SPPC, but not by SPP, was reported in rat basophilic leukemia cells and pancreatic acinar cells (3, 26). Thus, our results suggest that SPPC has two molecular targets of action in mammalian cells (i.e., an SPPC-gated Ca^{2+}-permeable channel in the endoplasmic reticulum and a G protein-coupled receptor in the plasma membrane). The observation that SPPC strongly activates superoxide anion generation in human neutrophils further strengthens the need to identify the cellular sources of SPPC production.

Acknowledgments

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