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Sphingosylphosphocholine, a signaling molecule which accumulates in Niemann–Pick disease type A, stimulates DNA-binding activity of the transcription activator protein AP-1

ALVIN BERGER, DEAN ROSENTHAL, AND SARAH SPIEGEL*

Department of Biochemistry and Molecular Biology, Georgetown University Medical Center, Washington, DC 20007

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ABSTRACT **Sphingosylphosphocholine (SPC) is the deacylated derivative of sphingomyelin known to accumulate in neuropathic Niemann–Pick disease type A. SPC is a potent mitogen that increases intracellular free Ca^{2+} and free arachidonate through pathways that are only partly protein kinase C-dependent. Here we show that SPC increased specific DNA-binding activity of transcription activator AP-1 in electrophoretic mobility-shift assays. Increased DNA-binding activity of AP-1 was detected after only 1–3 min, was maximal after 6 hr, and remained elevated at 12–24 hr. c-Fos was found to be a component of the AP-1 complex. Northern hybridization revealed an increase in c-*fos* transcripts after 30 min. Since the increase in AP-1 binding activity preceded the increase in c-*fos* mRNA, posttranslational modifications may be important in mediating the early SPC-induced increases in AP-1 DNA-binding activity. Western analysis detected increases in nuclear c-Jun and c-Fos proteins following SPC treatment. SPC also transactivated a reporter gene construct through the AP-1 recognition site, indicating that SPC can regulate the expression of target genes. Thus, SPC-induced cell proliferation may result from activation of AP-1, linking signal transduction by SPC to gene expression. Since the expression of many proteins with diverse functions is known to be regulated by AP-1, SPC-induced activation of AP-1 may contribute to the pathophysiology of Niemann–Pick disease.**

In some forms of sphingolipidoses, disorders of sphingolipid catabolism, lyso derivatives of the parent sphingolipids are known to accumulate (1–3). Sphinogosylphosphocholine (SPC), the N-deacylated derivative of sphingomyelin, has been reported to be significantly increased (up to 40-fold) in spleen, liver, and brain of patients with Niemann–Pick type A (1A) disease who have sphingomyelinase deficiency, hepatosplenomegaly, and brain dysfunction (2–4). It has been suggested that SPC may contribute to the pathophysiology of this disease by deleteriously affecting mitochondrial function and Ca^{2+} uptake (2, 4). SPC has other diverse biological effects which could be involved in the pathophysiology of Niemann–Pick type A disease. In mouse neuroblastoma cells, SPC induces neurite outgrowth (5, 6). SPC is also an extremely potent mitogen in numerous cell types (7). In Swiss 3T3 fibroblasts, the mitogenic signaling pathways of SPC were found to be both protein kinase C-dependent and -independent, and SPC did not increase levels of sphingosine-1-phosphate (SPP), phosphatidate, inositol phosphate, or cAMP but did stimulate arachidonate release (7, 8). SPC induced Ca^{2+} release from internal stores via a ligand-gated and voltage-modulated channel with unique pharmacologic and electrophysiologic properties in permeabilized leukemia cells (9) and mobilized Ca^{2+} from internal sources in other cell types, including fibroblasts (7, 9–14). Finally, SPC has been found to stimulate a kinase that may be related to casein kinase II (15, 16).

The nuclear events that follow these early events regulated by SPC are not known. We examined the role of the transcription factor AP-1 because AP-1 proteins bind to regulatory AP-1 consensus sequences of numerous genes involved in many long-term cellular responses, including DNA synthesis (17). We found that SPC stimulated DNA-binding activity of AP-1 and transactivated a reporter gene containing AP-1 recognition sites.†

EXPERIMENTAL PROCEDURES

Lipids. SPC (Matreya, Pleasant Gap, PA) was produced by acid hydrolysis of sphingomyelin and has been reported to be a 72:28 D-*erythro*/L-*threo* mixture (18). Methanolic SPC solutions were dried under N_2, and the residue was dissolved in 0.4% bovine serum albumin (BSA; Sigma) by sonication.

Cell Culture. Swiss 3T3 fibroblasts (CCL 92; American Type Culture Collection) were cultured in 150-cm^2 plastic Petri dishes, refed every 3 days until 90% confluent, and then refed and used 5 days later when quiescent (19). Prior to treatment, medium was aspirated and replaced with 10 ml of Dulbecco's modified Eagle's medium plus transferrin (5 μg/ml) and BSA (20 μg/ml) for at least 6 hr. Cells were generally treated with vehicle (BSA) or with 10 μM SPC, a mitogenic, noncytotoxic concentration (11).

Electrophoretic Mobility-Shift Assay (EMSA). Nuclear extract was prepared as described (20). Protein was determined with a Coomassie Plus kit (Pierce). Nuclear extract (5 μg) was incubated with poly(dI-dC) (1 μg; Pharmacia/LKB) and double-stranded ^{32}P-labeled consensus oligonucleotide probe (0.3 ng, 2×10^6 cpm) with or without oligonucleotide competitors or antibodies (20). Probes were 5′-end-labeled with [γ-^{32}P]ATP (Amersham) by T4 polynucleotide kinase (New England Biolabs) and purified with Sephadex G-50 spin columns (Pharmacia). DNA–protein complexes were resolved in 0.8-mm, 6% polyacrylamide gels (acrylamide/*N,N′*-methylenebisacrylamide weight ratio, 60:1) containing 0.5× TBE (1× TBE is 90 mM Tris/90 mM boric acid/2 mM EDTA) at 200 V for 1 hr at 25°C, autoradiographed, and quantified by scanning. Figures show autoradiographs representative of two to three experiments.

Oligonucleotides and Antibodies. Oligonucleotides and sonicated salmon sperm DNA were from Stratagene. The AP-1 oligonucleotide contained an AP-1 consensus sequence (underlined) and an artificial arrangement of neighboring se-

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Abbreviations: BSA, bovine serum albumin; EMSA, electrophoretic mobility-shift assay; SPC, sphingosylphosphocholine; SPP, sphingosine-1-phosphate; TPA, "12-*O*-tetradecanoylphorbol 13-acetate" (phorbol 12-myristate 13-acetate).

*To whom reprint requests should be addressed.

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quences (21), as follows: 5′-CTAGTGATGAGTCAGCCG-GATC-3′. An affinity-purified polyclonal rabbit antibody directed against aa 128–152 of rat c-Fos, a region conserved in rats, mice, and humans (c-Fos antibody 1) (22), and a polyclonal rabbit antibody directed against a rat Fra-1-specific region (aa 178–194) were kindly provided by Michael J. Iadarola (National Institutes of Health, Bethesda, MD). A sheep polyclonal antibody directed against aa 2–17, a region conserved in v-Fos and in human and mouse c-Fos (c-Fos antibody 2), was from Cambridge Research Biochemicals. Rabbit anti-c-Jun polyclonal antibody was from Oncogene Science. Undiluted normal rabbit serum (Collaborative Research) was used as a source of nonspecific antibodies.

Western Blot Analysis. Nuclear extract (50 μg) or purified recombinant Jun (Promega) was heated for 5 min at 95°C in sample buffer and then resolved in 1.5-mm, 0.1% SDS/10% acrylamide gels with an upper stacking gel, in 1× Douglas running buffer (0.4 M glycine/50 mM Tris·HCl, pH 8.3) containing 0.1% SDS, at 20 mA for 6 hr at 25°C. Proteins were electroblotted onto nitrocellulose (Trans-Blot Cell apparatus; Bio-Rad) and equal transfer was verified with Ponceau S staining (Sigma). After destaining, nitrocellulose was blocked with 5% nonfat milk in phosphate-buffered saline (PBS)/0.05% Tween 20, incubated with anti-c-Jun polyclonal antibody or anti-c-Fos 1 or Fra-1 antibody (10 μg/ml) followed by a 1:3000 dilution of peroxidase-conjugated anti-rabbit antibody, and detected by enhanced chemiluminescence (ECL; Amersham). Following removal of antibodies with 100 mM 2-mercaptoethanol/2% SDS/62.5 mM Tris·HCl, pH 6.7, nitrocellulose blots were reprobed with different antibodies.

Northern Hybridization. After treatment with 10 μM SPC, fibroblasts were rinsed with cold PBS, total cellular RNA was extracted with Tri Reagent (Molecular Research Center, Cincinnati), and equal amounts of RNA (1 μg) were size-fractionated by electrophoresis in denaturing 1% agarose gels containing 2% formaldehyde and 1× Mops buffer (16 mM Mops/6.7 mM sodium acetate/0.8 mM EDTA) and transferred by capillary action overnight onto Duralon membranes (Stratagene). Following UV crosslinking (Stratalinker 1800; Stratagene), prehybridization and hybridization were performed at 43°C in 0.125 M NaH_2PO_4, pH 7.2/0.125 M NaCl/0.5 mM EDTA/3.5% SDS with 200 ng of a 2.1-kbp full-length cDNA probe for human c-*fos* (ATCC no. 41042) and 200 ng of a 700-bp cDNA probe (*Pst* I–*Bam*HI fragment) for cyclophilin, a constitutively expressed protein that has been shown to be invariant for expression under a variety of conditions (23). Probes (200 ng) were random-primer labeled with 100 μCi (3700 kBq) of [α-^{32}P]dCTP (Amersham) by use of a Stratagene Prime-It II kit. Prehybridization (0.5 hr) and hybridization (24 hr) were performed in a hybridization incubator at 43°C in 50% formamide/125 mM sodium phosphate, pH 7.2/0.5 mM EDTA/3.5% SDS. Posthybridization stringency washes were with 2× SSC buffer (1× SSC is 150 mM NaCl/17.1 mM sodium citrate, pH 7.0), 2× SSC/1% SDS, and finally 0.1× SSC for 60 min per wash at 65°C. Cyclophilin detection was used to control for loading and transfer efficiency. Autoradiography was for 3 days at −80°C with Amersham film and intensifying screens.

Transfection. NIH 3T3 cells (10^6) were seeded into 75-cm^2 tissue culture dishes 24 hr before transfection, and fresh medium (15 ml) was provided 4 hr before transfection. Cells were stably transfected with a calcium phosphate DNA coprecipitate containing 5 μg of 2×AP-1 LUC plasmid [two copies of AP-1 binding site (TCGAGTTGAGTCAGGGTA-ACGATTGAGTCAGGAG) located 5′ of a 72-bp region of the rat prolactin promoter linked to the luciferase gene; kindly provided by K. Chien, University of California, San Diego], 1 μg of pSV2neo plasmid conferring resistance to antibiotic G418, and 30 μg of sonicated salmon sperm carrier DNA (Stratagene) (24). Cells were washed and medium was changed 24 hr after transfection, and selection with G418 (1 mg/ml; GIBCO/BRL) was initiated after 24 hr. Pooled clones were used for transactivation experimentation after 1 month of culture. Cells were seeded into 10-cm^2 tissue culture dishes, and after 48 hr, when 90% confluent, cells were incubated in medium with 0.5% calf serum for 18 hr, and then treated with SPC or BSA for 6 hr. Luciferase activity in 3–6 μg of cell lysate (10 μl), expressed as light units/10 sec per μg of protein, was measured on a Monolight 2001 luminometer (Analytical Luminescence Laboratory, San Diego) with lysis and assay buffers from Promega.

RESULTS

SPC Induces DNA-Binding Activity of AP-1. Like serum and phorbol 12-myristate 13-acetate ("12-*O*-tetradecanoylphorbol 13-acetate," TPA), SPC induced the appearance of a distinct DNA–protein complex detected by EMSA (Fig. 1). The radiolabeled complex was eliminated or reduced with a 10-fold excess of unlabeled AP-1 probe, but not with AP-2, AP-3, NF1/CTF, or Sp-1 oligonucleotides or salmon sperm DNA, demonstrating that binding was specific for AP-1 sequences

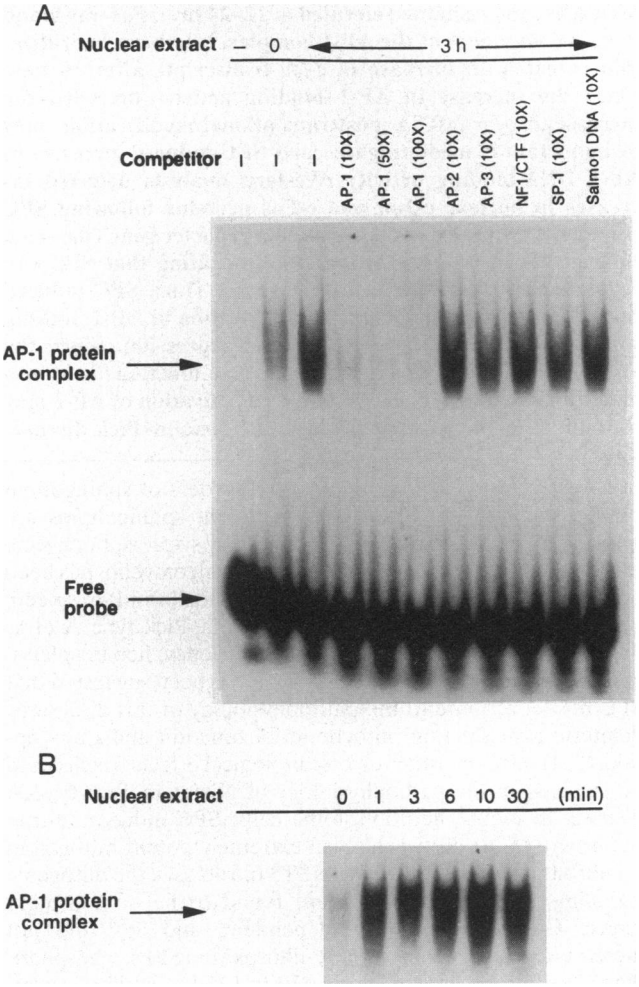

FIG. 1. SPC rapidly stimulates AP-1 DNA-binding activity. (*A*) EMSA performed with nuclear extracts (5 μg) prepared from quiescent Swiss 3T3 fibroblasts treated with vehicle (0) or with 10 μM SPC for 3 hr. Incubation mixtures contained 0.3 ng of ^{32}P-labeled AP-1 probe in the presence or absence (−) of extract and competitor DNA (10-, 50-, or 100-fold molar excesses of unlabeled AP-1 probe; oligonucleotides containing binding sites for AP-2, AP-3, NF1/CTF, or Sp1; or salmon sperm DNA). (*B*) Time course for SPC-induced AP-1 DNA-binding activity in fibroblasts treated with vehicle (0) for 15 min or with 10 μM SPC for the indicated times.

(Fig. 1*A*). There was a lesser reduction with 10-fold excesses of unlabeled probes containing either a T → G mutation in the first base of the AP-1 consensus sequence or an A → C mutation in the last base of the consensus sequence, further demonstrating binding specificity (data not shown). DNA-binding activity of AP-1 was stimulated extremely rapidly, increasing significantly after only 1–3 min (Fig. 1*B*). Addition of SPC directly to nuclear extract did not affect AP-1 DNA-binding activity (data not shown). Binding activity was maximal after 6 hr (18-fold) and remained elevated for 12–24 hr. After 1–3 hr of treatment with SPC, the induction was equivalent to that achieved with 10% calf serum (8- to 10-fold). Vehicle-treated controls did not show any stimulation in AP-1 DNA-binding activity and levels were constant over a period of many hours.

Protein Components of the AP-1 Complex. Preincubation of nuclear extract with c-Fos antibody 1 produced a supershifted band in EMSA, whereas preincubation with c-Fos antibody 2 eliminated binding (Fig. 2*A*). These results suggest that c-Fos was present in the complex. Although c-Fos antibody 1 is directed against a region of the DNA-binding domain of c-Fos, it is very well established that this antibody produces a specific supershifted band when added to the AP-1 complex induced in many cell types (20, 22). c-Fos antibody 2 may have eliminated binding by inducing an unfavorable protein conformation for DNA interactions. Preincubation with unrelated rabbit antiserum did not supershift or disrupt the complex. SPC treatment for 30 min also induced a transient increase in

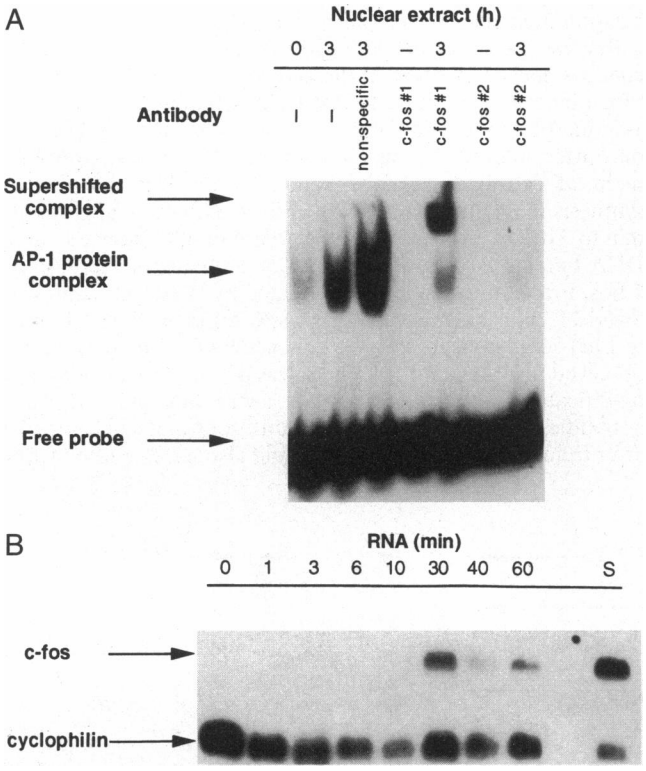

FIG. 2. c-Fos, a component of the protein/AP-1 complex (*A*), is transcriptionally activated by SPC (*B*). (*A*) EMSA with nuclear extracts (5 μg) prepared from quiescent Swiss 3T3 fibroblasts treated with vehicle (0) for 3 hr or with 10 μM SPC for 3 hr. Presence of c-Fos was demonstrated as a supershifted band following incubation of extract with 1 μl of c-Fos antibody 1 (see *Experimental Procedures*) for 15 min at 25°C. No shift occurred with preimmune rabbit serum (nonspecific). DNA–protein binding was eliminated by incubation of nuclear extract with 5 μg of c-Fos antibody 2. (*B*) Northern blot analysis of the transient increase in c-*fos* mRNA in cells treated with SPC for various times. Lane 0, cells treated with vehicle for 30 min; Lane S, cells treated with 10% calf serum for 30 min.

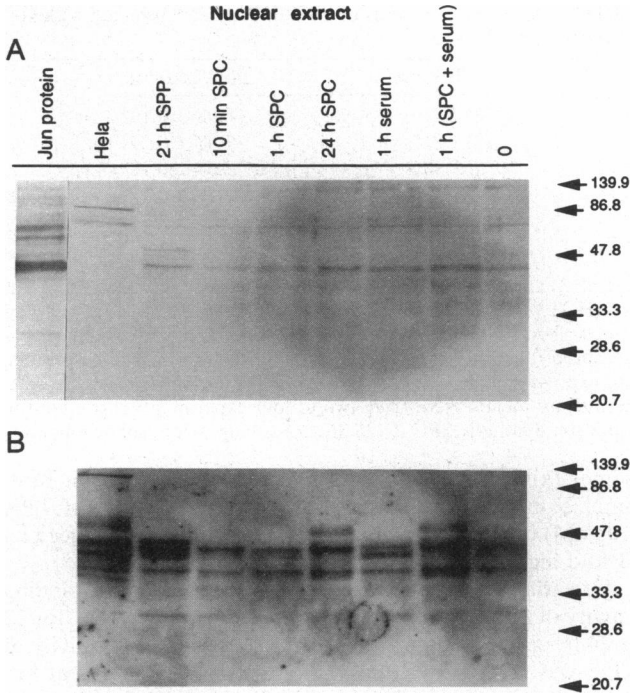

FIG. 3. Western blot analysis of c-Jun and c-Fos in nuclear extracts from cells stimulated with SPC. Quiescent Swiss 3T3 fibroblasts were treated with vehicle (0) for 1 hr or with 10 μM SPP, 10 μM SPC, or 10% calf serum alone or with SPC for the indicated times. Nuclear extracts (50 μg) from these cells and from HeLa cells were immunoblotted with c-Jun polyclonal antibody (*A*) or c-Fos antibody 1 (*B*). Arrows, positions of prestained markers (kDa).

c-fos mRNA (Fig. 2*B*), which then rapidly decreased and returned to basal levels by 2 hr. SPC was not as potent as 10% serum in increasing c-*fos* mRNA after 30 min (Fig. 2*B*).

Western analysis with c-Jun antibody detected a major band with the same mobility as the 40-kDa human recombinant Jun protein (Fig. 3*A*). Relative to unstimulated cells, Jun was increased in cells treated with SPC for 24 hr, or with serum for 1 hr, but not in cells treated with SPC for 10 min or 1 hr. For comparative purposes, we evaluated the effects of SPP, which is structurally similar to SPC but lacks the choline esterified to the 1-phosphate group. In cells treated with SPP for 21 hr, there was an increase in the 40-kDa c-Jun species, as well as in a species of ≈48 kDa (Fig. 3*A*). HeLa cells grown in 10% calf serum were included to evaluate cell type-specific differences. As previously reported (25), the two immunopositive c-Jun bands in HeLa cells had apparent molecular masses of 70 and 87 kDa. c-Fos antibody 1 detected a series of c-Fos proteins ranging from 37 to 50 kDa in 3T3 cells following SPC stimulation (Fig. 3*B*). The detection of the 37-kDa species may reflect the known crossreactivity of c-Fos antibody 1 with Fra proteins. There was a marked increase in c-Fos (50 kDa) in cells treated with SPC for 24 hr or SPP for 21 hr. Relative to unstimulated cells, increases were also detected in 33-kDa Fra-1 species in cells treated with SPC for 1 hr and in 33- and 35-kDa species in cells treated with SPC for 24 hr, or serum for 1 hr (data not shown).

SPC Transactivates a Reporter Gene Through the AP-1 Recognition Site. To determine whether the increased DNA-binding activity of AP-1 induced by SPC was correlated with transcriptional activation, we utilized cells stably transfected with an AP-1-responsive promoter–luciferase construct. After 6 hr of treatment with 10 μM or 20 μM SPC, there was a 2.3-fold increase in luciferase reporter activity (Table 1). A larger increase (2.8-fold) was achieved with chemically synthesized (2*S*, 3*R*, 4*E*)-SPC (D-*erythro*), the naturally occurring

Table 1. Promoter activities of NIH 3T3 cells transfected with 2XAP-1 LUC plasmid

Treatment	Fold increase
1 μM SPC	0.98 ± 0.15
5 μM SPC	1.07 ± 0.21
10 μM SPC	2.34 ± 0.13
20 μM SPC	2.36 ± 0.17
10 μM synthetic SPC	2.80 ± 0.04
100 nM TPA	10.76 ± 0.97
10% calf serum	11.32 ± 1.57

NIH 3T3 cells were stably transfected with the 2xAP-1 LUC plasmid as described under *Experimental Procedures.* Luciferase activity was measured 6 hr after treatment with agonists, normalized to protein, and expressed as fold stimulation relative to cells treated with BSA. Results are means ± SE from two to four experiments carried out in triplicate. Synthetic SPC is (2*S*,3*R*,4*E*)-sphingosylphosphocholine.

isomer (kindly provided by R. R. Schmidt, Universitat Konstanz, Konstanz, Germany). SPC was not as potent as TPA (100 nM) or calf serum (10%) in this assay; both produced an 11-fold increase.

Activation of Related Transcription Factors. DNA-binding activity of AP-2 was increased in cells treated with SPC for 12 or 24 hr or serum for 1 hr (Fig. 4*A*). DNA-binding activity of AP-3 was increased following 1 hr of serum treatment but minimally affected by SPC (Fig. 4*B*). Reduction in binding with 10-fold and 50-fold molar excesses of unlabeled AP-2 and AP-3 probes confirmed binding specificity (Fig. 4*C*).

DISCUSSION

Previous work has shown that SPC is a potent mitogen for various cells, including 3T3 fibroblasts (7). SPC alone was much more mitogenic than other known growth factors, such as insulin and epidermal growth factor. The mitogenic effect of SPC appeared to be very specific, since SPC was more potent than other lysophospholipids and structurally related sphingolipids examined (including ceramide, sphingosine, and SPP) (7). Similarly, in EMSAs and transfection assays, activity of SPC was reduced by N-acetylation and by dehydrogenation of the 4*E* double bond (data not shown). It is unlikely that SPC activates AP-1 binding activity via metabolism to other products, since exogenous SPC is readily taken up by cells and metabolized slowly after uptake in Swiss 3T3 fibroblasts (7).

The marked elevation in AP-1 DNA-binding activity within 1–3 min after SPC stimulation, the rapid increase of c-*fos* mRNA levels (30 min), the sustained elevation of AP-1 DNA-binding activity for up to 24 hr, and the ability of SPC to transactivate a reporter gene through an AP-1 recognition site are all consistent with a role of AP-1 in the transcriptional activation of genes necessary for G_0–G_1 transition and entry of cells into S phase. That serum and SPC enhanced DNA-binding activities of AP-1, AP-2, and AP-3 with different magnitudes and time courses suggests that SPC may stimulate mitogenesis through signaling pathways different from those mediated by serum. It is also noteworthy that SPC was significantly less effective than serum in stimulating AP-1 promoter activity in reporter assays.

AP-1, which consists of homo- or heterodimers of *jun* or *fos* gene products, modulates cell growth by binding to the AP-1 recognition sequence TGANTCA or TGANATCA (26) in regulatory regions of several genes, including c-*jun*, c-*fos*, DNA polymerase α, and human H4 histone, in concert with other factors (17). The importance of AP-1 in controlling cell proliferation is evidenced by inhibition of cell cycle progression when Fos and Jun synthesis and function are impaired (27) and by malignant cell transformation when expression of these proteins (including Fra-1) is deregulated (28).

Posttranslational changes, such as phosphorylation of AP-1, are known to affect the stability and conformation of the dimer complex and to influence DNA-binding activity and transcriptional activation of AP-1 (29). Jun (29, 30) and Fos (31) can be phosphorylated by various kinases at sites that both positively and negatively regulate transcriptional activity. Specific phosphatases may also regulate AP-1 activity by dephosphorylation near the carboxyl terminus (32).

Several lines of evidence suggest that such posttranslational changes may be involved in the activation of AP-1 by SPC. (*i*) SPC induced an increase in AP-1 DNA-binding activity after 1–3 min that preceded the increase in c-*fos* mRNA (observed only after 30 min). Consistent with this observation, pretreatment of cells with cycloheximide, an inhibitor of protein synthesis (33), for 10 min prior to the addition of SPC for 10 min to 3 hr, failed to inhibit the increase in SPC-induced AP-1 DNA-binding activity in EMSA. (*ii*) The lack of increase in c-Fos, Fra-1, and c-Jun levels detected by Western analysis in Swiss 3T3 fibroblasts treated with SPC for short times (10 min to 1 hr) could indicate that the enhanced AP-1 binding activity observed in EMSA at these times was due to posttranslational modifications (29). (*iii*) Anti-c-Fos, anti-c-Jun, and anti-Fra-1 antibodies detected proteins with different molecular masses after treatment with SPC, which could also have resulted from

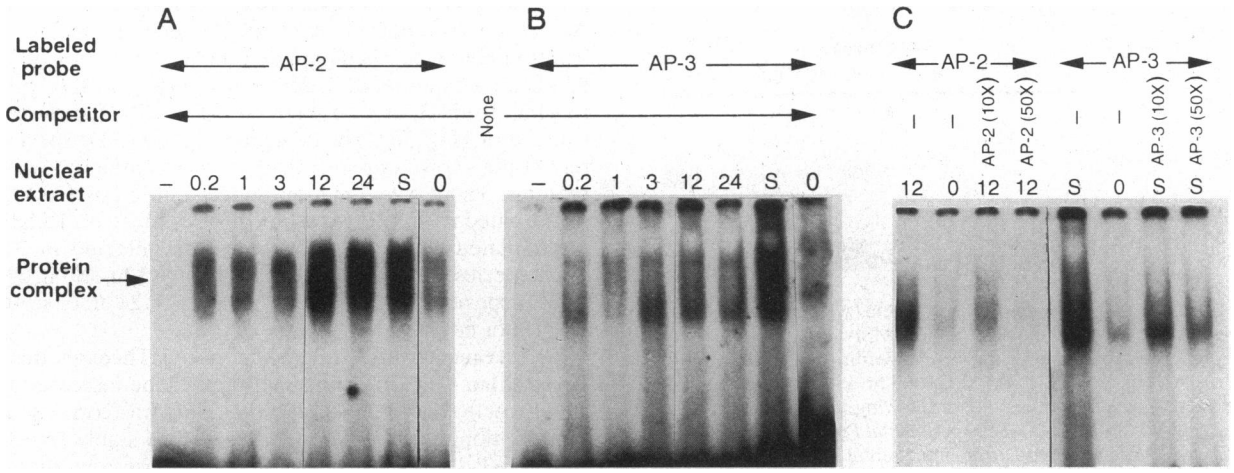

FIG. 4. Stimulation of AP-2 and AP-3 DNA-binding activity. Nuclear extracts (5 μg) were prepared from quiescent Swiss 3T3 fibroblasts treated with 10 μM SPC for the indicated times (hr) or for 1 hr with 10% calf serum (S). There was increased AP-2 binding activity in response to 12- or 24-hr SPC treatment (*A*) and increased AP-3 binding activity in response to S, but a relative lack of effect of SPC (*B*). Competitive inhibition with unlabeled AP-2 and AP-3 probes demonstrated binding specificity (*C*). Lanes 0, extracts from cells treated with vehicle alone.

differential posttranslational phosphorylation of respective proteins (17, 22, 31, 34). Experiments in which nuclear extract was pretreated with alkaline phosphatase (35) for 10 min prior to EMSA resulted in an upward shift in the DNA–AP-1 complex, which also indicates that the complex contained nuclear phosphoproteins (data not shown).

Posttranslational changes in Fos and Jun may occur by protein kinase C-dependent (17) and -independent (16) pathways. SPC was reported to induce protein phosphorylation in Jurkat T cells via a kinase that was distinct from protein kinase C but possibly related to casein kinase II (15, 16). Casein kinase II has been reported to activate c-*fos* expression via the serum-response-element pathway (36, 37).

The transient increase in c-*fos* mRNA in response to SPC treatment showed a similar pattern to that observed previously with numerous growth-promoting agents (38, 39), including TPA (34). Increases in c-*fos* mRNA could arise due to either an enhanced stability of c-*fos* transcripts (40) or an increase in transcription. The predominant transcriptional regulation pathway of c-*fos* proceeds via the binding of serum response factor–ternary complex factor to the serum response element (41); however, other pathways are also important (28, 30). SPC-induced phosphorylation of Fos and Jun that enhance binding to AP-1 sites in the c-*fos* promoter could be a mechanism for transcriptional activation of c-*fos* by SPC. Another mechanism of activation may be Ca^{2+}-induced transcriptional enhancement (42), since SPC is known to transiently increase intracellular Ca^{2+} in fibroblasts (7) and other cell types (10, 13). Additionally, in fibroblasts (7, 8) and thyroid FRTL-5 cells (14), SPC was found to release arachidonate, and in rat aortic smooth muscle cells, arachidonate has been implicated in stimulating transcription of c-*fos* as well as c-*jun* (43).

The differential induction kinetics of the various *jun* and *fos* genes result in the formation of different AP-1 complexes following cell stimulation (17). We detected c-Fos in the AP-1 complex by EMSA supershift experiments. By Western analysis, we demonstrated that SPC increased levels of c-Fos, c-Jun, and Fra-1. Jun or Jun-related proteins must also be present in the DNA–AP-1 complex, since Fos–Fos homodimers are not known to form *in vivo* or *in vitro* (17). Fra-1 could also be a component of the AP-1 complex, since newly synthesized Fra-1 rapidly dimerizes with c-Jun after serum stimulation in fibroblasts (44).

In addition to SPC, we have recently shown that other structurally related sphingolipids, sphingosine and SPP, also enhance AP-1 DNA-binding activity in 3T3 cells (20), despite some differences in signaling pathway (7). AP-1 could thus be a convergence point for diverse sphingolipid signaling pathways culminating in DNA synthesis. The initial, rapid increase in AP-1 binding activity induced in response to SPC could be important in G_0–G_1 transitions, whereas the sustained increase in AP-1 binding activity could be important in G_1–S transitions. The posttranslational mechanisms by which SPC rapidly increases AP-1 binding activity are an important area of investigation. What kinases might be involved, and what might be the role of SPC-induced increases in intracellular Ca^{2+} via channels with unique pharmacologic and electrophysiologic properties (9)? The activation of AP-1 by SPC may be important in the pathophysiology of Niemann–Pick type A disease. Transcriptional activation by AP-1 is now known to participate in diverse cellular processes other than stimulation of cell growth. For example, AP-1 regulates levels of β-amyloid precursor protein and may contribute to brain dysfunction in Alzheimer disease (45). SPC is increased in neuropathic Niemann–Pick type A disease, but not in non-neuropathic Niemann–Pick type 1S disease (2–4). SPC may also be increased under conditions in which phospholipase activity is greatly increased, such as during myocardial ischemia, where other lyso lipids are known to accumulate (46).

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