Signal transduction of somatostatin in human B lymphoblasts

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Somatostatin (SST) and somatostatin receptors (SSTR) are widely distributed in lymphoid tissues. Here, we report on the stimulatory effects of SST in Epstein-Barr virus-immortalized B lymphoblasts. By RT-PCR, we demonstrated the exclusive expression of the somatostatin receptor isoform 2A (SSTR2A) in B lymphoblasts. Addition of SST rapidly increased the cytosolic free calcium concentration ([Ca2+]i) maximally by about 200 nM, with an EC50 of 1.3 nM, and stimulated the formation of inositol phosphates. Furthermore, SST increased binding of guanosine 5′-O-(3-thiotriphosphate) by 50% above basal. These effects were partly inhibited by pertussis toxin (PTX), which indicates the involvement of PTX-sensitive G proteins. We provide further evidence that Go13ε, a PTX-insensitive G protein confined to lymphohematopoietic cells, is involved in the otherwise unusual coupling of SSTR2A to phospholipase C activation. In addition, SST activated extracellular regulated kinases and induced a 3.5-fold stimulation of DNA synthesis and a 4.4-fold stimulation of B lymphoblast proliferation, which was accompanied by an enhanced immunoglobulin formation. Thus SST exerts a growth factor-like activity on human B lymphoblasts.

G protein; immunoglobulin formation; MAP kinase; pertussis toxin; phospholipase C

B lymphocytes are the principal mediators of adaptive humoral immunity. The differentiation and selection processes from quiescent B lymphocytes to antibody-secreting plasma cells and memory cells are governed by specific antigen receptors and a complex network of modulating signals in a specialized lymphoid environment (10). These modulating signals are generated by selective cytokines, B cell coreceptors, and cell adhesion molecules confined to the lymphoid system. In addition, hormones and neurotransmitters that regulate many different physiological systems are also involved in B cell control (9). Such agonists include platelet-activating factor (PAF; Ref. 28), lysophosphatidic acid (LPA; Ref. 44), vasoactive intestinal polypeptide (VIP; Refs. 17 and 18), and catecholamines (26), to name but a few.

Somatostatin (SST), a cyclic tetradecapeptide, was first described as a potent inhibitor of growth hormone secretion (39). Subsequent studies have shown that SST has widespread physiological functions in hormone release, regulation of exocrine secretion, modulation of neural activity, and the inhibition of tumor growth (reviewed in Refs. 34, 39, and 46).

SST binding sites were first detected on circulating human blood lymphocytes (5) but also on many lymphoid tissues (42, 43). Somatostatin-receptor (SSTR)-based imaging has been widely used for the diagnosis of malignant lymphomas and hyperplastic or granulomatous nonmalignant lymph nodes (31, 42, 43).

Despite this widespread distribution in lymphoid tissues, only limited information on the physiological roles of SST and SSTRs in the immune system exists (reviewed in Refs. 53 and 54). Several groups of investigators have used lymphocytes to study SST effects on intracellular effector systems, including adenyl cyclase and the Na+/H+ exchanger (23, 34). Both anti-proliferative and growth-promoting activities of SST have been reported (27, 36, 37). More recent reports have shown that SST regulates T cell interferon-γ and interleukin (IL)-2 release (7, 11). However, for the B cell system, such functional data on the role of SST and its receptors are lacking.

Five different human SST receptor subtypes (SSTR1–SSTR5) have been cloned (16, 33, 34, 46, 58), and splice variants of SSTR2 exist (35). All SSTRs belong to the family of G protein-coupled or heptahelical receptors but differ with respect to their tissue-specific distribution and pharmacological properties (16, 33).

The predominantly inhibitory cellular actions of SST are mediated by multiple effector pathways (23, 33, 34), including the inhibition of adenyl cyclase (19) and voltage-dependent Ca2+ channels (25), a reduced mobilization of intracellular Ca2+ (19), and the attenuation of Na+/H+ exchange activity. Furthermore, SST stimulates voltage-dependent K+ channels (59) and protein tyrosine phosphatases (6), the activation of

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which coincides with an antiproliferative effect via proapoptotic pathways (34). SST has also been reported to activate phospholipase A2 and the MAP kinase cascade (34). In some cell lines, predominantly on overexpression of SSTRs, SST stimulates phospholipase C (PLC) activity (2, 8, 29, 47, 51).

Most effects of SST are transmitted via pertussis toxin (PTX)-sensitive G proteins, although partially PTX-insensitive effects including activation of PLC have been reported (8, 48, 51). B lymphoblasts express the PTX-sensitive G proteins Go12 and Go13 but not Go11 and Goq (44). G proteins of the Goq family are predominantly involved in activation of PLCβ isoforms (40), and Goq and Go11 are expressed in B lymphoblasts. Highly restricted to lymphohematopoietic cells, an additional Goq class protein exists, Go16 (and its murine homolog Go13; Refs. 3 and 57), which is able to link "promiscuously" numerous heptahelical receptors to PLC activation, a peculiarity that is not observed with other Goq class proteins (32, 57).

Here, we investigated the effects of SST on proliferation and immunoglobulin formation of human B lymphoblasts, and we characterized the early signal transduction of SST in these cells. We report the novel observation that SST stimulates PLC activity and increases [Ca\textsuperscript{2+}]; in B lymphoblasts, events that most likely involve the coupling of SSTR2A to Go16 and PLCβ2. These stimulatory effects of SST on early signal transduction are accompanied by an increased cell proliferation and immunoglobulin formation.

MATERIALS AND METHODS

Materials. Fura 2-AM was purchased from Molecular Probes (Eugene, OR). [3H]-methylthymidine was bought from Hartmann Analytics (Braunschweig, Germany). [35S]-guanosine 5’-O-(3-thiotriphosphate ([35S]GTP\textsuperscript{S}) (specific activity 1,200–1,400 Ci/mmol) was from DuPont-NEN (Bad Homburg, Germany), and myo-[3H]-inositol was from American Radiolabeled Chemicals (St. Louis, MO). Somatostatin (SST-14) and PAF were purchased from Calbiochem (Bad Soden, Germany), and 12-O-tetradecanoylphorbol 13-acetate (TPA) was from Sigma (Deisenhofen, Germany). Primary and secondary antibodies for immunoglobulin ELISA were purchased from Tago Immunologicals (Burlingame, CA). Unlabeled nucleotides were from Boehringer Mannheim, and PTX was from List Biological Laboratories (Campbell, CA). The phosophspecific p42/44 MAP kinase antibody was from New England Biolabs (Schwalbach, Germany), and the polyclonal anti-ERK1/ERK2 antisera was from Santa Cruz Biotechnology (Heidelberg, Germany). Reverse transcriptase (RT; Superscript) was from Life Technologies (Eggenstein, Germany). Taq polymerase, RNasin, RNase-free DNase, and restriction enzymes were purchased from Fermentas (St. Leon-Rot, Germany), and Pfu-Taq polymerase was from Promega (Heidelberg, Germany).

B lymphoblast cell lines and cell culture. Human B lymphoblast cell lines were derived from peripheral blood lymphocytes and immortalized with Epstein-Barr virus as described (44, 48). They were cultured in RPMI 1640 medium containing 2 mM L-glutamine, which was supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, and 10% FCS (all from Life Technologies). If not indicated otherwise, cells were routinely subcultured into fresh RPMI 1640 medium with 10% FCS at a density of 10\textsuperscript{6} cells/ml 1 day before the experiment.

Detection of SSTRs by reverse transcription-polymerase chain reaction (RT-PCR). Lymphoblast RNA was prepared and reverse transcribed using Superscript reverse transcriptase as described (44). Before reverse transcription, RNA was treated with RNase-free DNase according to the manufacturer’s instructions. PCR primer sequences were taken from the published sequence for human SST (58). The reverse primer sequence was modified to 5’-TCACCATGATCTGTTCCTTGCC-3’. PCR amplicons flanked by these primers contained at least one single restriction site, and the specificity of the synthesized PCR products was confirmed by restriction analysis. To discriminate the splice variant SSTR2A from SSTR2B, RT-PCRs were repeated using the oligonucleotide primers 5’-CTCCGTCCTCCATGGCCATGC-3’ and 5’-GGTAATTGCTTATAGAGAATAATA GG-3’. The specific amplicons generated with these primers comprise 686 base pair (bp) for SSTR2A transcripts and for genomic DNA. In SSTR2B, a fragment of 341 bp is removed by alternative splicing, resulting in an amplicon of only 345 bp flanked by these oligonucleotide primers (38). PCRs were carried out in reaction volumes of 50 µl containing 200 µM of each dNTP, 10 pmol of each oligonucleotide primer, cDNA corresponding to 100 ng RNA, and 2.5 U Taq polymerase in reaction buffer supplied by the manufacturer. The amplification profile involved denaturation at 94°C (30 s), annealing at 55°C (45 s), and extension at 72°C (90 s) for 35 cycles. A negative control containing 100 ng of RNA instead of cDNA was included in each experiment. Because all SSTRs lack introns, genomic DNA was used as positive control. PCR products were analyzed by electrophoresis on 1.5% agarose gels and stained with ethidium bromide. Primer sequences were derived from the following GenBank entries: M81829 (SSTR1), M81830 (SSTR2A), L13033 (SSTR2B), AF184174 (SSTR2B), XM_009963 (SSTR3), NT_011387 (SSTR4), and NT_010552 (SSTR5).

Measurement of cytoplasmic free Ca\textsuperscript{2+}. Changes in intracellular free Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]), were measured, as described in detail (44, 48), using the calcium-sensitive fluorescent dye fura 2. One day before the experiment, B lymphoblasts were subcultured at a density of 2–5 × 10\textsuperscript{6} cells/ml in RPMI 1640 medium containing antibiotics and 0.5% FCS. Dye loading, measurements of fluorescence, and calibration of emission ratios (λ = 495 nm) after rapid alternating excitation wavelengths from λ = 340 to 380 nm were performed, exactly as described, in a Perkin-Elmer LS5B spectrometer (44, 48). For each experiment, ~1 × 10\textsuperscript{6} dye-loaded cells were measured at 37°C in HEPES-buffer containing 1 mM CaCl\textsubscript{2} (44, 48). For experiments in Ca\textsuperscript{2+}-free medium, EGTA (5 mM final) was added ~5 s before addition of agonist.

Analysis of inositol phosphate formation. Formation of inositol phosphate (IP) was quantified in lymphoblasts grown at a density of 2 × 10\textsuperscript{6} cells/ml and labeled with 5 μCi/ml myo-[3H]inositol in serum- and inositol-free RPMI 1640 medium as described (44, 48). Lymphoblasts were stimulated, and water-soluble IP were fractionated by anion-exchange chromatography on AG 1-X8 formate resin (Bio-Rad, Munich, Germany) as detailed previously (44). GTP-S binding assay. G-protein-induced activation of G proteins was determined by measurement of [35S]GTP\textsuperscript{S} binding to digitonin-permeabilized lymphoblasts as described (44, 48, 56). Single measurements were conducted on 1 × 10\textsuperscript{6} lymphoblasts for 10 min at 30°C. Nonspecific binding was defined as the fraction of bound [35S]GTP\textsuperscript{S} not com...
peted for by 10 μM unlabeled GTPγS. Measurements were carried out in triplicate.

Cloning, sequencing, and expression of Go subunits and SSTR2 in COS-7 cells. The cDNAs encoding the coding sequences of the human Goα, Go11, Go16, and SSTR2 were cloned by RT-PCR from B lymphoblast RNA using the oligonucleotide primers 5'-CCACCGCACATGGCGCCGTCG-3' and 5'-GCGGCTCTGGGTCAGAATGC-3' for the amplification of Goα16 (accession no. NM_002068), 5'-GAAGATGAGCTTCCGGATCTCAGG-3' and 5'-CAGGACCATTTAGACAGATTGTTACTTCG-3' for Go11 (accession no. AF011497), 5'-GTTTTCCTTCTTCACACCCCTTGG-3' and 5'-GAAGACTTTGAATATAAAACAAGG-3' for Go14 (accession no. XM_005478), and the oligonucleotides 5'-AAAGCAGCCATGGACATGGCG-3' and 5'-CCCCAGCAGATTCCGACTGG-3' for the amplification of SSTR2 (accession no. M81830), respectively. Reverse transcription was performed as described above. For PCR amplification, PfuTurq (Promega) was used according to the manufacturer's instructions. PCR products were cloned into pGEM-T easy vector (Promega), sequenced, and further subcloned into the mammalian expression vector pcDNA3.1 + (Invitrogen). Human brain RNA was used for the amplification of Go14. The PLCβ2 expression vector was a kind gift of Dr. M. Simon (Pasadena, CA).

COS-7 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 μM penicillin, 100 μg/ml streptomycin, and 10% FCS at 37°C. For transient transfection, ~3 × 10^4 COS-7 cells were plated onto each well of a 24-well dish 1 day before the transfection. Cells were transfected using the transfection reagent Effectene (Qiagen, Hilden, Germany) according to the manufacturer's instructions. For each well, a total of 0.2 μg of DNA was mixed with 1.6 μl of the enhancer reagent and 5 μl of the transfection reagent in 60 μl of transfection buffer (all supplied by the manufacturer). Each component in the cotransfection experiment was represented by 0.06 μg of DNA, and the total amount of DNA was supplemented to 0.2 μg/well with empty pcDNA3.1 + vector DNA if necessary. This procedure resulted in a transfection efficacy of ~70%, as estimated by cotransfection assays using an enhanced green fluorescence protein vector. After transfection (24 h), cell culture medium was replaced with inositol-free DMEM supplemented with 7 μCi/ml myo-[3H]inositol and the cells were incubated for another 24 h. The label medium was replaced with inositol-free DMEM containing 20 mM LiCl, and cells were stimulated by addition of 100 nM SST for 1 h. Cell lysis and separation of total IP were performed as described (32).

Measurement of MAP kinase activity. B lymphoblasts (~1 × 10^7) were incubated in the presence or absence of SST (100 nM) for 2 min at 37°C, rapidly spun down, and lysed exactly as described (44). MAP kinase activity was determined by immunoblotting with an antibody that recognizes the phosphorylated, and thereby activated, MAP kinase isoforms (44). Aliquots of these lysates were analyzed in a second control Western blot for equal expression and handling of ERK1/ERK2 proteins during preparation with an anti-ERK1/ERK2 antiserum.

Determination of cell proliferation, thymidine incorporation, and immunoglobulin synthesis. Cells were seeded at an initial density of 2 × 10^4/ml in serum-free RPMI 1640 medium, stimulated with SST, and propagated for 4 days (44). Cells were counted daily using a CASY cell analyzer system (Schafer, Reutlingen, Germany). For determination of DNA synthesis, B lymphoblasts were subcultured in 24-well dishes under identical experimental conditions as described above for analysis of cell proliferation. They were propagated for two days and prepsulted with 1 μCi of [3H]methylthymidine for 18 h. The incorporation of radioactivity was determined exactly as described (44). Supernatants of SST-stimulated and control cells from the proliferation assay described above were harvested, and immunoglobulin concentrations were quantified by ELISA as described (44).

Statistical analysis and presentation of data. Data were analyzed by two-tailed Student's t-tests and regarded significantly different at P < 0.05. If not indicated otherwise, all experiments were performed in triplicate using at least two different cell lines. Data represent means ± SE if not indicated otherwise.

Expression of SSTR type 2A transcripts in B lymphoblasts. In the first series of experiments, we examined the expression of the various SSTR isoforms in human B lymphoblasts by RT-PCR using established oligonucleotide primers (22), which do not flank intron sequences. By using human genomic DNA as control, all five SSTRs were amplified (Fig. 1A), and the specificity of the PCR fragments was confirmed by restriction analysis (not shown). In RT-PCR studies, we only detected mRNA transcripts encoding for the isoform SSTR2 (Fig. 1A). To exclude fortuitous amplification of potentially contaminating genomic DNA in RT-PCRs, we included controls of the RNA without reverse transcription. Furthermore, all RNA specimens were treated with RNase-free DNase before reverse transcription. No PCR amplicons were detected in these samples.

SSTR2-specific transcripts were identified in all RNA samples from six different B lymphoblast cell lines (Fig. 2B). However, when we examined RNA from quiescent peripheral blood lymphocytes of different individuals, a faint SSTR2-specific band was only observed in one of five samples (Fig. 2B). This observation confirms previous reports (52) and suggests that SSTR2 expression increases on lymphoblastic differentiation (52, 53).

For SSTR2, two splice variants have been identified (38), with SSTR2A being the wild-type receptor. In SSTR2B, a cryptic intron of 341 bp, located at the 3'-end of the open reading frame, is removed by alternative splicing. Thus the COOH-terminal sequence of SSTR2B varies from SSTR2A (38). To distinguish between these variants, we performed RT-PCR studies with oligonucleotide primers that flank the cryptic intron. In RNA samples from B lymphoblasts and peripheral blood lymphocytes, we detected only long transcripts encoding SSTR2A (Fig. 2C). Thus we conclude that SSTR2A is the only known SSTR present on human B lymphoblasts.

Effects of SST on [Ca2+]i, IP formation, and binding of GTPγS. Having demonstrated the expression of SSTR2A-specific transcripts in B lymphoblasts, we examined the effects of agonist stimulation on different effector systems. In the presence of 1 mM extracellular Ca2+, basal [Ca2+]i, amounted to 111 ± 13 nM (n = 31). On addition of 100 nM SST, [Ca2+]i, increased by 191 ±
Fig. 1. Expression of somatostatin (SST) receptor (SSTR) isoforms in human B lymphoblasts. A: transcription of specific mRNA for SST receptor isoforms 1–5 was examined by RT-PCR. Lanes 1-5 show positive controls for the SSTR1–SSTR5 obtained with genomic DNA (left). Only SSTR2-specific transcripts were detected in RNA from human B lymphoblasts by RT-PCR (right). The sizes of the HaeIII-digested marker FX174 are indicated at left in base pairs (bp). The predicted sizes for the specific PCR products are SSTR1, 233 bp; SSTR2, 284 bp; SSTR3, 222 bp; SSTR4, 321 bp; and SSTR5, 223 bp. Ethidium bromide-stained 1.5% agarose gel. B: occurrence of SSTR2-specific transcripts in RNA preparations from 6 different B lymphoblasts (LB1–LB6) and from 5 different peripheral blood lymphocyte preparations (LY1–LY5). Arrow indicates position of SSTR2-specific amplicons (284 bp). Numbers at left refer to marker positions of AluI-digested pBR322 DNA. C: discrimination between alternatively spliced SSTR2-specific transcripts. PCR amplicons shown left were generated with oligonucleotide primers used for the experiment in A, which do not differentiate between splice variants SSTR2A and SSTR2B (open arrow). The oligonucleotide primers used at right encompass the sequence fragment of SSTR2, which is deleted in SSTR2B by alternative splicing. Predicted PCR amplicons for SSTR2A and SSTR2B are 686 and 345 bp, respectively (indicated by bold arrows). Numbers at left refer to marker positions of AluI-digested pBR322 DNA. N, negative control; RNA, use of RNA which was not reverse transcribed; DNA, genomic DNA used for positive controls. In LB2 and LY3, RNA preparations are the same as in B.

26 nM above baseline values (Fig. 2A). SST-evoked Ca\(^{2+}\) signals consisted of an initial peak followed by a sustained plateau (Fig. 3A). When extracellular Ca\(^{2+}\) was chelated by addition of EGTA (5 mM) before stimulation with the agonist, SST-induced Ca\(^{2+}\) increases were markedly reduced and amounted to 45 ± 12 nM above basal (Fig. 3B). Thus SST evoked both Ca\(^{2+}\) influx and mobilization from intracellular stores. The increases in [Ca\(^{2+}\)]\(_i\) were concentration dependent in the range of 10\(^{-10}\) to 10\(^{-6}\) M SST (Fig. 3C), and the EC\(_{50}\) for SST-induced Ca\(^{2+}\) signals was 1.3 ± 0.9 nM. Maximum changes in [Ca\(^{2+}\)]\(_i\) were observed at 10\(^{-7}\) M SST. Because SSTRs couple to PTX-sensitive G\(_i\) and G\(_o\) proteins, we examined the effects of PTX (50 ng/ml; 16 h) on SST-induced [Ca\(^{2+}\)]\(_i\) transients. PTX treatment resulted in a distinct inhibition of SST-stimulated Ca\(^{2+}\) signals by 71 ± 12%. (Figs. 2A and 3A), which indicates that transmembrane signaling of SSTR2A in human B lymphoblasts involves PTX-sensitive G proteins.

Ca\(^{2+}\) mobilization from intracellular stores is frequently caused by the generation of inositol 1,4,5-trisphosphate (IP\(_3\)). Hence, we analyzed the effect of SST on IP levels in B lymphoblasts. Addition of 100 nM SST to B lymphoblasts resulted in an increased formation of IP\(_3\) (Fig. 2B), as well as inositol 4-monophosphate plus inositol 4,5-biphosphate (IP\(_2\) + IP\(_1\)). At 1 min after stimulation, IP\(_3\) and (IP\(_2\) + IP\(_1\)) levels amounted to 162 ± 16% (Fig. 2B) and 133 ± 10% (not shown), respectively. Treatment of B lymphoblasts with 50 ng/ml PTX for 16 h resulted in a strong inhibition of SST-evoked IP formation, and markedly reduced increases in IP\(_3\) (Fig. 2B) and (IP\(_1\) + IP\(_2\)) levels (not shown) were observed.

To further demonstrate the involvement of G proteins in SST actions, we analyzed the SST-induced binding of the stable GTP analog \([\text{35S}]\text{GTP}\gamma\text{S}\) to permeabilized lymphoblasts. SST (100 nM) increased \([\text{35S}]\text{GTP}\gamma\text{S}\) binding by 42 ± 12%, and this effect was completely blunted in cells pretreated with PTX (Fig. 3C).
4). Again, these experiments suggest that SSTR2A relays signals via PTX-sensitive G proteins. However, it is important to note that the complete inhibition of SST-induced \([\text{35S}]{\text{GTP}^*}\)_S binding by PTX does not preclude an activation of PTX-insensitive G proteins by SST (56). PTX-sensitive G proteins are the most abundant class of \(G\) subunits. Thus binding of \([\text{35S}]{\text{GTP}^*}\)_S to other \(G\) proteins may escape detection in this assay (56) and most likely explains the only partial PTX-sensitivity of the SST-induced \(Ca^{2+}\) signals described above.

Expression of transcripts encoding \(G\) subunits of the \(G_{\alpha_q}\) family and reconstitution of SSTR2A-mediated IP generation. Our data presented so far suggest that both PTX-sensitive and PTX-insensitive G proteins are involved in SST-stimulated IP generation, which is in accordance with results of other groups (2, 8, 47). The PTX-sensitive fraction of SST-stimulated IP formation is attributable most likely to the well-known activation of \(G_{\alpha_i}\) proteins by SSTRs (23, 34). This results in the liberation of \(G_{\beta\gamma}\) subunits, which in turn activate phospholipase C \(\beta\) (PLC\(\beta\)) isoforms (40). However, because \(G_{\alpha_i}\) proteins are expressed ubiquitously, addition of SST should result in IP generation in all cells expressing SSTR2A, which is obviously not the case. Therefore, we wondered which components of the signaling cascade in lymphoblasts contribute to their peculiar behavior. Interestingly, the expression patterns of \(G_{\alpha_{16}}\) and PLC\(\beta_2\), two proteins involved in IP generation, are restricted to lymphohematopoietic cells (3,
Fig. 4. Effect of SST upon $[^{35}S]$GTP$_S$ binding to permeabilized human B lymphoblasts. B lymphoblasts were grown for 16 h in the presence (right) or absence (left) of 50 ng/ml PTX. Subsequently, lymphoblasts were permeabilized with digitonin and stimulated with 100 nM SST (solid bars) for 1 min, and the specific binding of $[^{35}S]$GTP$_S$ was measured for 10 min. Control cells (shaded bars) remained nonstimulated. Data represent means ± SE of measurements performed in triplicate from 1 experiment representative of 4 independent experiments. *Significant difference at $P < 0.05$.

40). Studies with other $G$ protein-coupled receptors expressed in blood cells suggested that a basal activation of a $G_q$ family protein, most likely $G_{16}$, is required for activation of PLC$\beta_2$, which is then further potentiated by free $G_\beta\gamma$ subunits (4). Furthermore, $G_{16}$ is able to couple nonspecifically to a wide variety of different heptahelical receptors (32). By RT-PCR, we detected $G_{16}$-specific transcripts together with transcripts encoding the widely expressed $G_q$ family members $G_{14}$ and $G_{11}$ in human B lymphoblasts (Fig. 5, A–C). The identities of these transcripts were verified by cloning and sequencing. Transcripts of the fourth member of this $G_q$ family, $G_{14}$, were not detectable in B lymphoblasts but were present in RNA from human brain (Fig. 5D).

To test the hypothesis that PLC$\beta_2$ and $G_{16}$ contribute to SST-mediated IP generation in B lymphoblasts, we reconstituted these components transiently in COS-7 cells. Native COS-7 cells express neither SSTR2 nor $G_{16}$, as revealed by RT-PCR (data not shown). In accord, addition of SST (100 nM) to nontransfected COS-7 cells did not result in an increase in IPs (Fig. 6A). Sole expression of SSTR2A resulted in a significant but modest stimulation of IP formation on addition of SST to $130 ± 4.5\%$ ($P < 0.05; n = 3$). However, expression of SSTR2A together with $G_{16}$ led to distinct SST-stimulated increases in IP formation to $193 ± 12\%$. Coexpression of PLC$\beta_2$ together with SSTR2A resulted in higher basal IP levels but did not stimulate IP generation ($135 ± 7.4\%$) above the values observed in cells expressing SSTR2A only (Fig. 6A). Maximum SST-stimulated increases in IP formation were observed in COS-7 cells that expressed SSTR2A together with $G_{16}$ and PLC$\beta_2$ (234 ± 2% of nonstimulated controls; $P < 0.05; n = 3$). These results suggest that $G_{16}$ and PLC$\beta_2$ are efficient mediators of SST-stimulated IP formation. In a final series of experiments, we tested whether other members of the $G_q$ family can substitute for $G_{16}$ in this pathway. The widely expressed $G_\alpha$ subunits $G_{14}$ and $G_{11}$ were cloned and sequenced from human B lymphoblasts. $G_{14}$, which was not detectable in B lymphoblasts, was cloned from human brain. Again, expression of $G_{16}$ together with PLC$\beta_2$ mediated marked SST-stimulated increases in IP levels ($290.3 ± 20.3\%$ of nonstimulated controls; $n = 3$ independent experiments; $P < 0.01$, Fig. 6B). However, in the presence of $G_\alpha_q$ and $G_{11}$, SST stimulation resulted in only modest increases in IP levels ($129.2 ± 0.6\%$ and $138.5 ± 2.4\%$ for

Fig. 5. Expression of specific transcripts for $G_\alpha$ proteins of the $G_q$ family in human B lymphoblasts. RT-PCR experiments with specific oligonucleotide primers that encompass the entire open reading frames of $G_{16}$ (A), $G_{14}$ (B), $G_{11}$ (C), and $G_{14}$ (D). Arrows indicate the expected positions of the amplicons. Numbers indicate the positions of the DNA size markers from pBR322 DNA digested with $AluI$ (maximum 908 bp) or $MvaI/ApaLI$ (maximum 2,614 bp). M, marker; N, negative control; LB1 and LB2, RT-PCR based on RNA from 2 different lymphoblast cell lines; Br, brain; Fb, fibroblast.
SST-mediated IP formation in the presence of Ga subunits of the Gaq family and PLCβ2. A: COS-7 cells were transiently transfected with expression vectors for the indicated proteins and formation of total IPs was analyzed on stimulation with SST (100 nM). Shown are means ± SE of measurements performed in quadruplicates from 1 experiment representative of 3 independent experiments. B: COS-7 cells were transiently transfected with vectors encoding SSTR2, PLCβ2, and the indicated Ga protein. The formation of total IPs on stimulation with 100 nM SST was measured as in A. Data represent means ± SE from 3 independent experiments with triplicates. *Significant difference at \( P < 0.05 \). **Significant difference at \( P < 0.01 \).

Ga\(_q\) and Ga\(_{11}\), respectively. Interestingly, SST-mediated PLCβ2 stimulation was also very efficient in the presence of Ga\(_{14}\) (478.0 ± 24.2%; \( n = 3 \), Fig. 6B), an observation without obvious relevance for B lymphoblasts. Taken together, these results suggest, but do not prove, that Ga\(_{16}\) and PLCβ2 are responsible for the ability of SSTR2A to stimulate increases in IP levels in B lymphoblasts, a property not observed in other cell types.

**MAP kinase activation, DNA synthesis, cell proliferation, and synthesis of immunoglobulins.** The experiments presented so far provided evidence for stimulatory actions of SST in B lymphoblasts, as shown for IP formation and G protein activation. Next, we studied whether SST affects typical cellular functions of B lymphoblasts, i.e., proliferation and immunoglobulin synthesis. Furthermore, we investigated whether SST stimulates additional signaling pathways in these cells and examined the effects of SST on MAP kinase activation. Available evidence suggests that MAP kinase activation by heptahelical receptors that couple to PTX-sensitive G proteins involves a complex array of adaptor proteins, GTPases, and kinases (for review, see Ref. 45). Liberated G\(\alpha\gamma\) subunits from activated PTX-sensitive G proteins are thought to initiate this pathway (45). As shown in Fig. 7, SST (100 nM; 2 min) induced an increase in phospho-ERK1/2, which predominantly affected p42ERK2. This activation was stronger than that induced by PAF (100 nM; 2 min), another well-characterized B lymphocyte activator (28). SST-induced MAP kinase activation varied between three- and sevenfold compared with the nonstimulated controls, as determined by image analysis. Treatment of B lymphoblasts with PTX (50 ng/ml; 16 h) completely blocked SST-induced MAP kinase activation (not shown).

For the analysis of B lymphoblast proliferation, cells were seeded at a density of \(-2 \times 10^5\) cells/ml in serum-free medium. After \( \sim 24\)–36 h, we observed a marked increase in cell number of SST-stimulated cells above that of nonstimulated controls (Fig. 8A). This difference reached its maximum on day 4, and cell numbers of SST-stimulated cells were 4.4 ± 1.5-fold higher (\( n = 6 \) independent experiments with 3 different cell lines) compared with nonstimulated controls. There was a considerable variation in this growth-promoting effect, ranging from 1.2-fold to 11.4-fold increases depending on cell line and experiment. Interestingly, PTX inhibited the SST-stimulated B lymphoblast proliferation only modestly by 22 ± 5% (\( n = 4 \); not shown).

In accordance with the growth-promoting action of SST, \[^3H\]thymidine incorporation into SST-stimulated B lymphoblasts was 3.5 ± 1.2-fold higher (\( n = 6 \) independent experiments with 3 different cell lines) than that of nonstimulated controls (Fig. 8B).

In parallel to these proliferation assays, supernatants were harvested and used for the quantification of immunoglobulin concentrations by ELISA. Immunoglobulin formation increased significantly 4.8 ± 1.2-fold (\( n = 6 \) independent experiments with 3 different cell lines) above nonstimulated controls. Again, we observed a considerable variation in the range of 2.4- to 9.2-fold depending on cell line and experiment.

**DISCUSSION**

SST is a widely expressed neuropeptide that affects numerous organ systems and cell types in a prevalingly inhibitory fashion. Increasing evidence, predominantly from binding, nuclear imaging, and expression studies, suggests that SST also plays a role in the immune system (53, 54). On the basis of observations that SST can evoke Ca\(^{2+}\) signals in human B lymphoblasts (48), we investigated this signaling pathway in more detail. Specifically, we tried to unravel which components of the B lymphoblast signaling network are responsible for this unusual effector activation by SST. A second and independent focus of this work was
to elucidate the significance of SST for B lymphoblast function, and we examined two typical B cell responses, proliferation and immunoglobulin formation. Our results suggest that SST acts as a B cell growth factor. Both issues, early effector activation and cell proliferation, deserve a more detailed discussion. Finally, we have to address the question of potential physiological roles for SST in B cell regulation, an issue which will remain mostly speculative.

**SSTR expression and early signaling.** The only SSTR isoform we detected in B lymphoblasts is SSTR2A, which is the predominant SSTR in nonneural tissues. Although this notion is drawn from RT-PCR studies, it is in accord with other expression, binding, and imaging studies (52, 53). SSTR2A is a typical heptahelical receptor that couples to heterotrimeric G proteins. This is also true for B lymphoblasts, as shown here by an increased GTP\(\gamma\)S binding on SST stimulation. Direct biochemical interaction studies provided evidence for coupling of Go\(_{13}\) and Go, to SSTR2 (24). Because B lymphoblasts lack Go, (44), which is predominantly expressed in neural tissues, SSTR2 most likely couples to Go\(_{i}\) subunits in this cell type. This notion is further corroborated by the inhibitory effects of PTX on SST-stimulated GTP\(\gamma\)S binding, IP formation, increases in [Ca\(^{2+}\)], and MAP kinase activation. Of note, these effects of PTX varied considerably between different effector systems. Whereas SST-stimulated MAP kinase activation and GTP\(\gamma\)S binding were totally blocked and Ca\(^{2+}\) signals were inhibited by ~70%, PTX diminished SST-induced lymphoblast proliferation by only 20%. Because PTX-sensitive G proteins are most abundantly expressed in mammalian cells, and because Go proteins have a considerable rate of spontaneous GDP/GTP exchanges, a concomitant activation

![Image](http://ajpcell.physiology.org/)

**Fig. 7.** Effect of SST on activation of mitogen-activated (MAP) kinase. B lymphoblasts were stimulated for 2 min with 100 nM SST, 100 nM platelet-activating factor (PAF), or for 15 min with 250 nM of the phorbol ester TPA or remained nonstimulated (control). A: equal amounts of cell lysates were analyzed by immunoblotting, and activated MAP kinases were detected with a phosphospecific ERK1/ERK2 kinase antibody that recognizes p42 and p44 isoforms. B: control Western blots with aliquots from lysates used in A. Immunostaining was performed with an antibody that recognizes both ERK isoforms. Arrows indicate positions of molecular weight standards. Experiments are representative of 3 others.

**Fig. 8.** Effect of SST on B lymphoblast proliferation, DNA synthesis, and immunoglobulin formation. A: B cells were seeded at 2–3 \(\times\) 10\(^5\) cells/ml on day 0. On day 1, either SST (\(\bullet\)) or buffer (○) was added (arrow). Aliquots were counted daily. Data represent means ± SD from 1 representative experiment, replicated with 4 different cell lines. B: lymphoblasts were seeded on 24-well culture dishes as described for A. On day 2, cells were prepulsed with 2 \(\mu\)Ci of \(^{3}H\)methylthymidine for 16 h. Values are means ± SE from 1 representative experiment with measurements performed in triplicate. *Significant difference from controls at \(P < 0.05\).
of PTX-insensitive G proteins most likely escapes gross binding studies as performed here by GTPγS binding to whole cells, a method which has even been optimized for the analysis of PTX-sensitive G proteins (56). Thus our results from GTPγS binding analyses do not exclude a simultaneous activation of, for example, Goq class G proteins, which is in accord with the partial PTX-insensitivity of SST-stimulated Ca²⁺ signals observed here. At this point, we have to consider further that PTX is a highly sensitive but not an entirely specific tool for the analysis of Goα-dependent signaling pathways, especially if immune cells are concerned. There is evidence that long-term treatment with PTX stimulates proliferation of T and B lymphocytes (20), an effect that has been attributed to the B oligomer of the toxin and does not involve ADP ribosylation (20). Although we have verified in previous studies that our PTX treatment conditions result in a complete modification of Goq proteins in B lymphoblasts (48), we also observed that long-term treatment with the toxin inconsistently evoked minor increases in cell number (44), which could also explain the limited effect of PTX on SST-stimulated B lymphoblast proliferation shown here.

**SSTR2A-mediated Ca²⁺ signals in B lymphoblasts.** Although there is evidence from one tumor cell line that SSTR2 can activate Ca²⁺ channels (50), in most cell types SSTR2 stimulation is associated with decreased Ca²⁺ signals caused by an inhibition of Ca²⁺ channels or an activation of K⁺ channels (25, 33, 34, 59). The original tracings shown in Fig. 3 suggest the contribution of both Ca²⁺ influx and Ca²⁺ liberation from intracellular stores to the generation of Ca²⁺ signals in SST-stimulated B cells. Concomitantly, we observed an activation of PLC by SST. To our knowledge, the human B lymphoblast is the only nontransfected cell type for which a direct activation of PLC by SSTR2 stimulation has been observed so far. Hence, we wondered which mechanisms confined to B lymphoblasts contribute to this unusual PLC activation. Lymphohematopoietic cells specifically express PLCβ2 and Go16, a Goq class G protein (3, 40). Go16 can couple promiscuously to a wide variety of heptahelical receptors, including the 5-HT1A receptor, the formyl peptide receptor, the μ-opioid receptor, the β2-adrenergic receptor, the M₄-muscarinic acetylcholine receptor, the LTB4 receptor, and the P₂U purinoreceptor (4, 14, 32). Baltensperger and Porzig (4) provided evidence that heptahelical receptors that couple to PTX-sensitive G proteins in lymphohematopoietic cells require a basal activation of a Goq class G protein, e.g., Go16, for efficient activation of PLC. Free Gβγ subunits arising from concomitant Goα activation then further potentiate PLC activity in a PTX-sensitive manner (4). However, sole free Gβγ dimers were unable to mediate PLC activation in the absence of activatable Go16 in this system (4). Interestingly, Go16 activation has also been related to cell growth stimulation of hematopoietic cells by heptahelical receptors (15).

To test whether the above described dual activation model explains SSTR2A-mediated PLC activation in lymphoblasts, we conducted a series of reconstitution experiments. RT-PCR studies indicated that B lymphoblasts express transcripts for the Goα class proteins Go16, Goq and Go11 but not Go14 (Fig. 5). Transfection of SSTR2A into COS-7 cells, which lack endogenous SSTRs, resulted in a modest increase in IP levels on stimulation with SST. This ~30% increase in IP levels is in perfect accordance with the small effects observed on expression and stimulation of SSTR2 in COS-7 cells (2, 51), CCL39 cells (47), and FcεR1 pituitary cells (8). However, coexpression of SSTR2A together with Go16 and PLCβ2 resulted in pronounced increases in IP levels on stimulation with SST. A second series of experiments revealed that the widely expressed Goq class proteins Go11 and Goq cannot sufficiently substitute for Go16 to mediate SST-stimulated PLCβ2 activation. Interestingly, Go14, a Goq class protein with restricted expression (e.g., kidney and brain; 57) shared the ability of Go16 to relay SSTR2A activation to PLCβ2 stimulation. This feature of Go14 deserves further investigation but does not contribute to the B lymphoblast signal transduction discussed here.

Taken together, the data presented here support, but do not ultimately prove, a scenario that the dual activation of Go16 and Goq proteins by SST in B lymphoblasts causes PLCβ2 activation and Ca²⁺ transients. Such a dual activation mechanism for PLC stimulation appears to occur frequently in B lymphoblasts, as can be inferred from the only partial PTX sensitivities of LPA and PAF in other studies (44, 48).

In contrast to SSTR2A, SSTR3 can activate PLCβ isoforms in nonlymphohematopoietic cells, as has been shown for PLCβ3 activation in intestinal smooth muscle cells (29), which indicates subtype-specific effector activation.

**Somatostatin-induced B lymphoblast proliferation.** The second novel observation of this report is an augmented B lymphoblast proliferation on stimulation with SST, which was further accompanied by an increase in immunoglobulin secretion.

In most cell types, mainly antiproliferative effects of SST have been observed (6, 33, 39). For the immune system, early studies reported growth-inhibiting effects of SST in activated T lymphocytes (27, 37). In B lymphocytes, biphasic effects of SST have been described: SST at 10⁻⁹ to 10⁻⁶ M exhibited a modest inhibitory effect on lymphocyte DNA synthesis; however, at 10⁻⁷ M SST-induced a twofold increase in DNA synthesis (36). Other reports demonstrate that SST potentiates the proliferation of mitogen-activated Jurkat and peripheral blood mononuclear cells (7, 21). In the case of Jurkat cells, this is caused by an increased IL-2 secretion mediated by SSTR3 activation (7).

In our in vitro B cell model, SST stimulates proliferation and immunoglobulin synthesis and may be regarded as a B cell growth factor. The observed effects resemble those of PAF and LPA, both in magnitude and time course of the signals (38, 44, 48). Furthermore, our data and results from others indicate that the expression of SSTR2 on lymphocytes is developmentally regulated (52, 53). Quiescent peripheral lym-
phocytes lack SSTR2 or express transcripts only at low levels, whereas all B lymphoblast lines investigated here expressed these mRNA transcripts. A recent report describes a similar developmental regulation in thymocytes and provides evidence that SSTR2 activation is involved in thymic maturation and thymocyte proliferation (49). This study also examined the effects of SST on splenocyte proliferation. Whereas SST increased the proliferation of total splenocytes, it inhibited growth of purified splenic T cells and T lymphocytes (49). Thus effects of SST on different lymphocyte populations depend on the cellular context and may be mediated by the secretion of additional auto- and paracrine factors.

At present, we do not know the exact sequence of events coupling SSTR2A activation in B lymphoblasts to enhanced proliferation and immunoglobulin secretion. Current evidence suggests that sole activation of PLCγ2 is not sufficient for stimulation of proliferation (30), and additional effector systems are probable candidates for this effect. Free Gβγ subunits, liberated from activated Goi proteins, are frequently involved in the initiation of growth signals, e.g., by activating the MAP kinase cascade (45). For lymphohematopoietic cells, evidence exists that Go16 also contributes to growth regulation (15). Hence, inactivation of Go16 in erythroleukemia cells is accompanied by a decreased proliferation rate (15). Both signaling pathways may also contribute to SSTR2A-induced B cell proliferation.

Given the biphasic and context-dependent effects of SST on lymphocyte proliferation in the literature (7, 27, 36, 37, 49), it is also conceivable that SST stimulates the secretion of autocrine growth factors in B lymphoblasts, which in turn stimulate lymphoblast proliferation. For T lymphocytes, an increased IL-2 secretion on SSTR3 activation has been reported, which subsequently stimulates T cell proliferation (7). Both signaling pathways may also contribute to SSTR2A-induced B cell proliferation.

Somatostatin and the immune system. Immortalized B lymphoblasts are a frequently employed model system for differentiated antibody-secreting B cells (28, 44). However, some caution should be taken when extrapolating data derived from such cell lines. Based primarily on these in vitro studies, it is only possible to speculate on the precise physiological roles of SST for B cells in the immune system.

SSTRs are widely expressed in lymphoid tissues (31, 42, 43), and both peptidergic nerves and autocrine SST secretion are potential sources for SST in the immune system (1, 12, 13, 49). High levels of SST binding sites are found in germinal centers of Peyer’s patches, tonsils, appendix, colonic lymphoid tissue, and on thymocytes (41, 49). In quiescent blood lymphocytes, mRNA encoding for SSTRs is found at low levels only (52). On lymphoblastic differentiation and proliferation, beginning at early differentiation stages, B and T cells start to express SSTRs, with SSTR2 being the predominant SSTR isoform (52, 53), which is also confirmed in our study for B lymphoblasts. SSTR2 transcripts are also found in plasma cells (53). Messenger RNAs for SSTR3, SSTR4, and SSTR5 have been detected in a few lymphocyte cell lines, whereas SSTR1 mRNA was undetectable (7, 52, 53).

There is evidence for autocrine SST secretion in lymphoid tissues, including thymus and spleen (1, 12, 13, 49). Lipopolysaccharide, interferon-γ (IFN-γ), and IL-10 stimulate SST expression in the spleen (55). On the other hand, SST inhibits IFN-γ release by granuloma cells, splenocytes, and T cells (11), which suggests complex immunoregulatory circuits.

SST has been reported to stimulate IL-2 secretion and T cell proliferation in Jurkat cells via SSTR3 (7), which resembles the findings of this study. Conversely, other studies show antiproliferative effects of SST in the immune system (27, 37). With respect to B cell physiology, SST has been reported to be ineffective in stimulating immunoglobulin secretion of plasma cells, whereas vasoactive intestinal peptide markedly increased immunoglobulin production (17, 18).

Emerging evidence suggests that these varying and contradicting effects of SST in the immune system depend on the context and developmental stage of the cells investigated. Results from a recent report support that notion. Hence, SST stimulates fetal thymocyte growth and maturation under organ culture conditions (49). Similarly, total splenocyte proliferation increases in the presence of SST (49). However, repeating these experiments with purified thymocytes or splenic T lymphocytes revealed an antiproliferative effect of SST (49).

B lymphocyte proliferation and immunoglobulin formation are regulated in complex network of antigen activation and accessory stimuli, e.g., cytokines, adhesion molecules, hormones, and neurotransmitters (9, 10, 12), that govern the fate of the respective B lymphoblast population to proliferation, immunoglobulin synthesis, or apoptosis. SST obviously contributes to this network. With the availability of more isotype-specific SSTR agonists and antagonists, we now have the tools to challenge the in vivo significance of these in vitro results.

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