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 Gₛ₁₆, 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 adenylyl 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 adenylyl 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 Go₅₃ but not Go₁₁ and Go₆ (44). G proteins of the Go₂₂ family are predominantly involved in activation of PLCβ isoforms (40), and Go₆ and Go₁₁ are expressed in B lymphoblasts. Highly restricted to lymphohematopoietic cells, an additional Go₉₂ class protein exists, Go₁₆ (and its murine homolog Go₁₅; Refs. 3 and 57), which is able to link "promiscuously" numerous heptahedral receptors to PLC activation, a peculiarity that is not observed with other Go₉₂ 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 \([Ca^{2+}]_i\) in B lymphoblasts, events that most likely involve the coupling of SSTR2A to G proteins of the Gq family (32, 57), which is able to discriminate the splice variant SSTR2A from SSTR2B, RT-PCR products were repeated using the oligonucleotide primers 5′-CTCCGTCTCCATGGCCATGC-3′ and 5′-GGTAAT-GCCTATACAGAATAAATA GG-3′. The specific amplification products 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 μU 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.

MATERIALS AND METHODS

Materials. Fura 2-AM was purchased from Molecular Probes (Eugene, OR). [3H]-methylthymidine was bought from Hartmann Analytics (Braunschweig, Germany), and [35S]GTP \(_{\gamma}\) from DuPont-NEN (Bad Homburg, Germany), and myo-[3H]-inositol was from American Radiolabeled Chemicals (St. Louis, MO). Somatostatin (SSTR-14) and PAF were purchased from Calbiochem (Bad Soden, Germany), and 12-\((\text{SST-14})\) and PAF were purchased from Boehringer Mannheim, and PTX was from List Biological Laboratories (Campbell, CA). The phosphospecific p42/p44 MAP kinase antibody was from New England Biolabs (Schwalbach, Germany), and the polyclonal anti-ERK1/ERK2 antiserum was from Santa Cruz Biotechnology (Heidelberg, Germany). Reverse transcriptase (RT; Superscript) was from Life Technologies (Eggenstein, 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 10⁶ 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 report by Kubota et al. (22). According to the published sequence for human SSTR2 (58), the reverse primer sequence was modified to 5′-TACAGTATCCTGGTCTGTGC-3′. PCR amplions 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′-CTCCGTCTCCATGGCCATGC-3′ and 5′-GGTAAT-GCCTATACAGAATAAATA GG-3′. The specific amplification products 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 μU 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.
petitioned for by 10 μM unlabeled GTPγS. Measurements were carried out in triplicate.

Cloning, sequencing, and expression of Gα subunits and SSTR2 in COS-7 cells. The cDNAs encoding the coding sequences of the human Gαq, Gα11, Gα16, and SSTR2 were cloned by RT-PCR from B lymphoblast RNA using the oligonucleotide primers 5′-CCACGGCCACATGCGCGCTCG-3′ and 5′-TGGGGGCTGGTCTAAGTGG-3′ for Gαq (accession no. NM_002072), 5′-GGAAGATGGACTCCTGAGTCATGCTCG-3′ and 5′-CAGGCACAATTAGACCAATGTGTTCTCC-3′ for Gα11 (accession no. NM_002072), 5′-GGCCGGAGCATGCTCTGG-3′ and 5′-GGAATAATGTCAGGGAGGAGATG-3′ for Gα16 (accession no. AF011497), 5′-GTTCTTTCTTTCCACACCCTGTTG-3′ and 5′-GAAGACTTCTGCAAATAAAACAAGG AG-3′ for Gα14 (accession no. XM_005478), and the oligonucleotides 5′-AAAGCAGCCATGGACATGGCGG-3′ and 5′-CCCCAAGCATGGGACGAGTTCT-3′ for the amplification of SSTR2 (accession no. M81830), respectively. Reverse transcription was performed as described above. For PCR amplification, Pfu Turbo (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 Gα14. The PCR expression vector was a kind of gift from 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 × 10⁴ 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 transfection 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 incubated for another 24 h. The incorporation of radioactivity was determined for analysis of cell proliferation. They were propagated for two days and preincubated 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.

RESULTS

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). However, 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⁺], 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⁺], amounted to 111 ± 13 nM (n = 31). On addition of 100 nM SST, [Ca²⁺] increased by 191 ±
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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 amplification (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 Ca2+ signals consisted of an initial peak followed by a sustained plateau (Fig. 3A). When extracellular Ca2+ was chelated by addition of EGTA (5 mM) before stimulation with the agonist, SST-induced Ca2+ increases were markedly reduced and amounted to 45 ± 12 nM above basal (Fig. 3B). Thus SST evoked both Ca2+ influx and mobilization from intracellular stores. The increases in [Ca2+]i were concentration dependent in the range of 10–10 to 10–6 M SST (Fig. 3C), and the EC50 for SST-induced Ca2+ signals was 1.3 ± 0.9 nM. Maximum changes in [Ca2+]i were observed at 10–7 M SST. Because SSTRs couple to PTX-sensitive Gi and Go proteins, we examined the effects of PTX (50 ng/ml; 16 h) on SST-induced [Ca2+]i transients. PTX treatment resulted in a distinct inhibition of SST-stimulated Ca2+ signals by 71 ± 12%. (Figs. 2A and 3A), which indicates that transmembrane signaling of SSTR2A in human B lymphoblasts involves PTX-sensitive G proteins.

Ca2+ mobilization from intracellular stores is frequently caused by the generation of inositol 1,4,5-trisphosphate (IP3). 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 IP3 (Fig. 2B), as well as inositol 4-monophosphate plus inositol 4,5-bisphosphate (IP2 + IP1). At 1 min after stimulation, IP3 and (IP2 + IP1) 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 IP3 (Fig. 2B) and (IP1 + IP2) 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 [35S]GTPγS to permeabilized lymphoblasts. SST (100 nM) increased [35S]GTPγS binding by 42 ± 12%, and this effect was completely blunted in cells pretreated with PTX (Fig.

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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 [35S]GTP\(\gamma\)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\(\alpha\) subunits. Thus binding of [35S]GTP\(\gamma\)S to other G\(\alpha\) 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\(\alpha\) 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\(\beta\)) isofoms (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,
Studies with other G protein-coupled receptors expressed in blood cells suggested that a basal activation of a Goq family protein, most likely Go16, is required for activation of PLCβ2, which is then further potentiated by free Gβγ subunits (4). Furthermore, Go16 is able to couple nonspecifically to a wide variety of different heptahelical receptors (32). By RT-PCR, we detected Go16-specific transcripts together with transcripts encoding the widely expressed Goq family members Goq and Go11 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 Goq family, Go14, were not detectable in B lymphoblasts but were present in RNA from human brain (Fig. 5D).

To test the hypothesis that PLCβ2 and Go16 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 Go16, 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 Go16 led to distinct SST-stimulated increases in IP formation to 193 ± 12%. Coexpression of PLCβ2 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 Go16 and PLCβ2 (234 ± 2% of nonstimulated controls; P < 0.05; n = 3). These results suggest that Go16 and PLCβ2 are efficient mediators of SST-stimulated IP formation. In a final series of experiments, we tested whether other members of the Goq family can substitute for Go16 in this pathway. The widely expressed Gα subunits Gαq and Gα11 were cloned and sequenced from human B lymphoblasts. Gα14, which was not detectable in B lymphoblasts, was cloned from human brain. Again, expression of Go16 together with PLCβ2 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 Gαq and Gα11, SST stimulation resulted in only modest increases in IP levels (129.2 ± 6.0% and 138.5 ± 2.4% for
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 \(\sim 2 \times 10^5\) 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 \(4.4 \pm 1.5\)-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 \(22 \pm 5\%\) \((n = 4\); not shown).

In accordance with the growth-promoting action of SST, \([3H]\)thymidine incorporation into SST-stimulated B lymphoblasts was \(3.5 \pm 1.2\)-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 \(4.8 \pm 1.2\)-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 prevailingly 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

Fig. 6. SST-mediated IP formation in the presence of Ga subunits of the Ga\(\alpha\) family and PLC\(\beta\)2. A: COS-7 cells were transiently transfected with expression vectors for the indicated proteins and formation of total IPs on stimulation with SST (100 nM) was analyzed on stimulation with SST (100 nM). Shown are means \(\pm\)SE of measurements performed in quadruplicates from 1 experiment representative of 3 independent experiments. B: COS-7 cells were transiently transfected with vectors encoding SSTR2A, PLC\(\beta\)2, and the indicated Ga protein. The formation of total IPs on stimulation with 100 nM SST was measured as in A. Data represent means \(\pm\)SE from 3 independent experiments with measurements performed as triplicates. *Significant difference at \(P < 0.05\). **Significant difference at \(P < 0.01\).
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 GTPγS binding on SST stimulation. Direct biochemical interaction studies provided evidence for coupling of Go13 and Gαo to SSTR2 (24). Because B lymphoblasts lack Gαo (44), which is predominantly expressed in neural tissues, SSTR2 most likely couples to Gαi subunits in this cell type. This notion is further corroborated by the inhibitory effects of PTX on SST-stimulated GTPγS binding, IP formation, increases in [Ca2+]i, and MAP kinase activation. Of note, these effects of PTX varied considerably between different effector systems. Whereas SST-stimulated MAP kinase activation and GTPγS binding were totally blocked and Ca2+ 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 Gαi proteins have a considerable rate of spontaneous GDP/GTP exchanges, a concomitant activation

![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 x 10^5 cells/ml on day 0. On day 1, either SST (●) 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 μCi of [3H]methylthymidine for 16 h. Values are means ± SE from 1 representative experiment with measurements performed in triplicate. C: supernatants from experiments in A were harvested on day 4 and IgM levels were quantified. Values are means ± SE from 1 representative experiment with measurements performed in triplicate. *Significant difference from controls at P < 0.05.](http://ajpcell.physiology.org/)

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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 nontransformed 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 Goα16, a Goq class G protein (3, 40). Goα16 can couple promiscuously to a wide variety of heptahedral receptors, including the 5-HT1A receptor, the formyl peptide receptor, the μ-opioid receptor, the β2-adrenergic receptor, the M2-muscarinic acetylcholine receptor, the LTB4 receptor, and the P2U purinoreceptor (4, 14, 32). Baltensperger and Porzig (4) provided evidence that heptahedral receptors that couple to PTX-sensitive G proteins in lymphohematopoietic cells require a basal activation of a Goq class G protein, e.g., Goα16, 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 Goα16 in this system (4). Interestingly, Goα16 activation has also been related to cell growth stimulation of hematopoietic cells by heptahedral 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 Goq class proteins Goα16, Goq, and Goα11 but not Goα14 (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 F4C1 pituitary cells (8). However, coexpression of SSTR2A together with Goα16 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 Goα11 and Goq cannot efficiently substitute for Goα16 to mediate SST-stimulated PLCβ2 activation. Interestingly, Goα14, a Goq class protein with restricted expression (e.g., kidney and brain; 57) shared the ability of Goα16 to relay SSTR2A activation to PLCβ2 stimulation. This feature of Goα14 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 Goα16 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 10−8 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-
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 Gq 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). Furthermore, a SST-triggered release of autocrine B cell growth factors could alternatively explain the different inhibitory potencies of PTX on SST-stimulated “early signals” and proliferation observed here. Future experiments are needed to elucidate the mutual contribution of these signaling pathways for SST-stimulated B cell proliferation.

Somatostatin and the immune system. Immortalized B lymphoblasts are a frequently employed model system for differentially 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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REFERENCES


