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Somatostatin-Dependent Adenylyl Cyclase Activity in Nonactivated and Mitogen-Activated Human T Cells: Evidence for Uncoupling of sst3 Receptor From Adenylyl Cyclase

Nathalie Giannetti,[1] Branka Horvat,[2] Nicole Gautier,[3] Christelle El Ghamrawy,[4] Chantal Rabourdin-Combe,[2] Alain Enjalbert,[5] and Slavica Krantic[4]*

[1]Laboratoire de Neurosciences, UFR des Sciences de Franche-Comté, 25030 Besançon Cédex, France
[2]Laboratoire de Biologie Moléculaire et Cellulaire, UMR 49, Ecole Normale Supérieure, 69364 Lyon, Cédex 07, France
[3]BGBP, UMR 5558, Université Claude Bernard Lyon 1 (bât 741), 69622 Villeurbanne Cédex, France
[4]INSERM Unité 407, Communication Cellulaire en Biologie de la Reproduction, Faculté de Médicine Lyon-Sud, 69921 Oullins, France
[5]ICNE, UMR 6544, Faculté de Médecine Secteur Nord, 13916 Marseille, Cédex 20, France

Abstract Neuropeptide somatostatin (SRIF) has been shown to modulate interleukin-2 (IL-2) secretion by mitogen-activated T cells. In this study, we further analyzed the transduction pathways underlying SRIF actions on human Jurkat T cells and compared SRIF signaling between nonactivated and mitogen-activated cells. SRIF effects on adenylyl cyclase activity in the absence and presence of mitogens were addressed by using three different analogs: SRIF14, SRIF28, and SMS 201–995. In semipurified membrane preparations obtained from nonactivated cells, all analogs inhibited adenylyl cyclase. However, in membrane preparations obtained from mitogen-activated cells, the maximal inhibition of adenylyl cyclase mediated by SRIF14 and SRIF28 equaled only one third of that measured in the absence of mitogens, whereas SMS 201–995 was completely inactive. To assess the relevant mechanisms associated with different effects of SRIF on adenylyl cyclase activity in nonactivated and mitogen-activated T cells, we performed binding assays by using iodinated SRIF as a radioligand. These experiments suggested that both the number of receptors and their affinities were almost identical in either nonactivated or activated cells. RT-PCR analysis of the pattern of SRIF receptor expression showed that nonactivated as well as activated Jurkat cells expressed only mRNA corresponding to the sst3 receptor subtype. Altogether, these data point to a functional activation-associated uncoupling of sst3 receptors from adenylyl cyclase in human T cells, indicating a T-cell activation-induced alteration in the sst3 receptor transduction pathway. J. Cell. Biochem. 72:221–231, 1999.

Key words: somatostatin; receptor subtypes; mitogenic activation; Jurkat T cells; adenylyl cyclase

The somatostatin (SRIF) neuropeptide family comprises a few peptides issued from different posttranslational processings of preprosomatostatin mRNA encoded by a single gene. Only two of these peptides, somatostatin 14 (SRIF14) and its N-terminus extended form built up of 28 amino acids (SRIF28), are biologically active and are the major SRIF peptides found in the periphery and the central nervous system (CNS). The relative proportions of SRIF14 and SRIF28 differ among various somatostatin-producing tissues [Reisine and Bell, 1995]. However, SRIF14 and SRIF28 display overlapping physiological functions.

SRIF inhibits numerous endocrine secretions, acting either as a neurohormone (e.g., in the pituitary where it inhibits growth hormone, prolactin, and thyrotropin secretion) or a hormone (e.g., in the pancreas, where it inhibits glucagon and insulin release [Reichlin, 1987]). In the CNS, SRIF acts as a neurotransmitter within discrete somatostatinergic neuronal

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*Correspondence to: Dr. Slavica Krantic, Laboratoire de Communication Cellulaire en Biologie de la Reproduction, INSERM 407, Faculté de Médicine Lyon-Sud, BP12, 69921 Oullins, France. E-mail: krantic@lyon151.inserm.fr

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pathways [Epelbaum, 1986]. Neuromodulatory actions of SRIF are generally recognized as well. For example, SRIF modulates neurotransmitter (including serotonin, acetylcholine, glutamate, and γ-aminobutyric acid (GABA) and neurohormone (e.g., Growth hormone-releasing hormone, GHRH) release [Reisine and Bell, 1995]).

SRIF is also one of the plethora of biochemical messengers (neurotransmitters, neurohormones, hormones, cytokines, and growth factors) shared by CNS, endocrine, and immune systems, allowing them to modulate reciprocally each other's functions in what are called neuro-endocrine-immune interactions [Besedovsky and Del Rey, 1996]. The particular immunomodulatory actions of SRIF include both stimulation [Nio et al., 1993; Cardoso et al., 1998] and inhibition [Muscettola and Grasso, 1990] of cytokine secretions by T cells. In addition, SRIF decreases production of several immunoglobulins (IgA, IgE, and IgG4) by B cells [Scicchitano et al., 1988; Kimata et al., 1993]. In accordance with the physiological roles of SRIF in the immune system, both SRIF14 and SRIF28 have been identified in lymphoid organs [Aguila et al., 1991], and at least SRIF14 is produced by lymphocyte cultures in vitro [Aguila et al., 1996].

These diverse biological actions of SRIF are mediated by five (sst1–sst5) G protein-coupled receptor subtypes which are encoded by five different genes. All five SRIF receptors have been identified throughout the CNS, endocrine and exocrine glands, and immune organs [reviewed in Reisine and Bell, 1995]. Both SRIF14 and SRIF28 recognize five SRIF receptors with similar affinities, but SRIF28 binds with slightly better affinity to sst5 receptors. No receptor-selective ligands, able to discriminate a given SRIF receptor subtype from others, are yet available. However, two subfamilies of SRIF receptors were distinguished based on their affinity for existing SRIF analogs. Thus, SMS 201–995, MK 678, and RC 160 distinguish sst2/sst3/sst5 from sst1/sst4, since they bind to the sst2/sst3/sst5 subfamily with subnanomolar affinity and are 1,000-fold less efficient on the sst1/sst4 subfamily of receptors [Patel and Srikant, 1994].

After expression of the cloned receptors in heterologous cell lines, five SRIF receptors activate multiple transduction effectors such as phospholipase C, tyrosine phosphatases, and ionic channels. Additionally, all of them inhibit adenylyl cyclase activity, thus leading to a subsequent decrease of intracellular cAMP content and inhibition of cAMP transduction pathway [Florio and Schettini, 1996].

cAMP regulates the immune response in a complex manner. For example, it modulates antibody production by B cells [Kammer, 1988] and inhibits antigen-induced histamine release from basophiles as well as T-cell cytotoxic response [Kammer, 1988]. In the particular case of T lymphocytes, cAMP plays the role of a molecular switch in lymphocyte differentiation [Lalli et al., 1996] and in determining the Th1/Th2 pattern of cytokine secretion [Haraguchi et al., 1995]. In addition, cAMP inhibits clonal T-cell expansion by inhibiting the proliferation of these cells, hence the hypothesis that cAMP might be involved in the arrest of immune response [Kammer, 1988; Fratelli et al., 1989; Gonsalkorale et al., 1993; Tamir and Isakov, 1994]. This consensus is derived from the observed cAMP-mediated inhibition of interleukin-2 (IL-2) production in T lymphocytes [Lingk et al., 1990; Paliogianni et al., 1993]. IL-2 is in vivo the predominant T-cell growth factor regulating cell proliferation in an autocrine/paracrine manner [Alcover et al., 1987]. It is secreted in response to antigenic or mitogenic stimulation of T-cell receptor (TcR). Nevertheless, the molecular mechanisms by which the extracellular signals regulating intracellular cAMP levels interfere with TcR signaling are yet poorly understood [Tamir and Isakov, 1994; Lalli et al., 1996].

We recently reported that SRIF14 increases IL-2 secretion via adenylyl cyclase inhibition in the mitogen-activated human Jurkat T-cell line, but that it is unable to trigger IL-2 secretion in nonactivated cells [Cardoso et al., 1998]. It has been previously documented that both nonactivated and mitogen-activated Jurkat cells express SRIF receptors [Sreedharan et al., 1989; Cardoso et al., 1998] and functional adenylyl cyclases [Fratelli et al., 1989; Aune et al., 1993; Cardoso et al., 1998]. This study analyzes the mechanisms of differential actions of SRIF on IL-2 secretion by nonactivated and mitogen-activated Jurkat cells, and suggests that mitogen activation triggers modulation of receptor coupling to adenylyl cyclase.

MATERIALS AND METHODS

Chemicals

[^{125}I]Tyr^{1}-SRIF14 (2,200 Ci/mmol) was purchased from NEN (Du Pont de Nemours, France). [α^{32}P]ATP (30 Ci/mmol) and [^{3}H]cAMP

(40 Ci/mmol) were from Amersham (Amersham, U.K.). SRIF14 and SRIF28 were obtained from Peninsula Laboratories (San Carlos, CA). SMS 201–995 was a kind gift from Novartis (Basel, Switzerland). Creatine kinase, creatine phosphate, ATP, and GTP were from Boehringer Mannheim (Indianopolis, IN). All other chemicals were purchased by Sigma (L'Isle d'Abeau, France).

Cell Culture and Mitogen Stimulation

Jurkat cells (clone J77.6.8) were grown in RPMI-1640 medium (Gibco, Cergy-Pontoise, France) supplemented with 10% (w/v) heat-inactivated fetal calf serum (FCS) (Boehringer, Meylan, France), 10 mM HEPES, 2 mM L-glutamine, and 100 U/ml gentamycin in a 5% CO_2 humidified atmosphere at 37°C.

Jurkat-cell suspensions (0.5×10^6 cells/ml) were either left untreated (control group corresponding to nonactivated cells) or incubated with a combination of phorbol ester, TPA (16 nM, Sigma, l'Isle d'Abeau, France), and lectin PHA-L (from Phaseolus vulgaris) (2 µg/ml, Sigma) for 24 h (mitogen-activated cell group). Only cell batches in which viability exceeded 90% (as determined by trypan blue exclusion test) were used for further experiments.

Adenylyl Cyclase Assay on Semipurified Membrane Preparations

Cells from nonactivated and mitogen-activated experimental groups were sedimented by quick centrifugation (1,000*g*, 5 min, 25°C) and washed three times in cell culture medium. After the last wash, the cell pellets were homogenized by 10 hand strokes in 1 mM TRIS-HCl buffer, pH 7.2, supplemented with 10 mM EGTA and 3% (w/v) sucrose, and centrifuged 3 min at 1,800*g* (4°C). Supernatants were centrifuged again (15 min, 28,000*g*, 4°C); resulting pellets were resuspended and frozen in the same buffer containing 10 mM EGTA and 10% (w/v) sucrose.

Male Wistar rats (200–250 g) were sacrificed by decapitation in accordance with the European Communities Council Directive of November 24, 1986. Striatum and olfactory bulb were dissected on ice and then homogenized and centrifuged as described for Jurkat cells in order to prepare the semipurified plasma membrane samples.

Adenylyl cyclase activity was estimated by measurement of [^{32}P]ATP conversion to [^{32}P]cAMP [Enjalbert et al., 1986]. For basal adenylyl cyclase activity determination, the final incubation medium (50 µl) contained 50 mM TRIS-maleate buffer (pH 7.2), 1.5 mM $MgSO_4$, 1 mM cAMP, 5 mM creatine phosphate, 0.1 mg/ml creatine kinase, 0.15 mM ATP, 0.01 mM GTP, 10 mM theophyline, 1 µCi [^{32}P]ATP, and 0.001 µCi of [^{3}H]cAMP. In other experiments, forskolin (10^{-5} M), a diterpene that stimulates basal adenylyl cyclase activity by direct targeting of the enzyme catalytic domain, SRIF analogs (10^{-12}–10^{-6} M), or both were added to the incubation medium. The reaction was initiated by addition of 10 µl homogenate and performed under conditions of linear [^{32}P]cAMP production (37°C, 30 min). The incubation was terminated on ice, by addition of an excess of ATP (1 mM) and cAMP (1 mM). [^{32}P]cAMP was separated from [^{32}P]ATP by two-step elution, as previously reported [Salomon et al., 1973]. Recovery of [^{3}H]cAMP on individual columns varied from 70–80%. Adenylyl cyclase activity was expressed in pmol of [^{32}P]cAMP (corrected for elution efficiency of each individual column determined on the basis of [^{3}H]cAMP recovery) formed per milligram of protein over 30 min. In some experiments the results were expressed as a percentage of forskolin-stimulated adenylyl cyclase activity (i.e., activity measured in the presence of forskolin and absence of SRIF analogs), considering the average of forskolin-stimulated values as 100%. The measurements of adenylyl cyclase activities corresponding to these reference values were repeated 3–5 times in every (n = 3–6) independent experiment; each repetition was performed in triplicate.

[^{125}I]Tyr^{1}SRIF14 Binding Assay

Cells were washed and resuspended in a small volume of 50 mM TRIS-HCl buffer, pH 7.4, transferred to a glass-glass tissue grinder and broken by 20 hand strokes. Homogenates were centrifuged for 3 min at 8,000*g* (4°C); pellets were discarded while supernatants were recentrifuged for 15 min at 28,000*g* (4°C). Resulting pellets were resuspended in an incubation buffer consisting of 50 mM TRIS-HCl buffer, pH 7.4, supplemented with 5 mM $MgCl_2$, 0.2 % (w/v) of bovine serum albumin (BSA), and 0.05 % (w/v) bacitracin. Aliquots of these semipurified membrane preparations were frozen and kept at -80°C until used.

[^{125}I]Tyr^1-SRIF14 binding assay was performed as previously reported [Krantic et al., 1992; Cardoso et al., 1998]. Aliquots of semipurified membrane preparations (40–80 µg of pro-

teins) were incubated with [^{125}I]Tyr^1-SRIF14 (35 pM) for 90 min at 25°C. This ^{125}I-Tyr^1-SRIF14 concentration was chosen since it corresponds to a half-K_d value reported for SRIF binding sites in Jurkat cells [Cardoso et al., 1998]. The nonspecific component of total binding was determined in the presence of 1 µM SRIF14 and represented 30–40% of the total binding.

Results were expressed in fmol of [^{125}I]Tyr^1-SRIF bound per milligram of protein. Protein concentration was determined according to the method of Lowry et al. [1951].

RT-PCR Analysis of Jurkat T-Cell SRIF Receptors

Total cellular RNA was extracted from Jurkat cell pellets by using RNA^{NOW} (Biogentex, Ozyme, Montigny-le-Brotonneux, France) according to the manufacturer's instructions. An aliquot of each sample (10 µg/5 µl) was treated with 0.1 µl (0.75 U) of DNA-se (Pharmacia Biotech, Uppsala, Sweden) for 10 min at 37°C in order to exclude any genomic DNA contamination. cDNA was synthesized (1 h, 42°C) from 10 µg of cellular RNA by using Moloney monkey leukemia virus reverse transcriptase (200 U), as previously described [Frohman et al., 1988; Cardoso et al., 1998]. To ascertain that cDNA was not contaminated by genomic DNA, reverse transcription for each sample was also performed in the absence of Moloney monkey leukemia virus reverse transcriptase.

One tenth (2 µl) of the first strand of cDNA synthesis reaction was added to PCR buffer (100 mM Tris) containing 0.2 mM dNTP, 1.25 U Taq DNA polymerase (Promega, Charbonnière, France), in a total volume of 50 µl. Concentration of $MgCl_2$ in the reaction mixture was 2.5, 4, 1.5, 1.5, 1, and 4 mM for sst1–sst5 and β-actin, respectively. Primers for SRIF receptors were added in a final concentration 0.4 µM and have been previously described: for sst1 [Kubota et al., 1994]; for sst2 [Rohrer et al., 1993]; and for sst3, sst4, and sst5 [Miller et al., 1995]. Amplification of cDNA encoding human β-actin by using the sense primer 5'-CTAGAAGCATTTGCGGTGGACGATGGAGGG-3' and the antisense primer 5'-TGACGGGGTCACCCACACTGTGCCCATCTA-3' (Stratagene, Cambridge, UK) was used as a positive control of reverse transcription. Expected amplification products were the following lengths (bp): 233, 414, 222, 321, 154, and 661 for sst1–sst5 receptors and human β-actin, respectively. After initial denaturation (150 sec, 94°C), the samples were subjected to 40 cycles (for sst1–sst5 receptors) or 25 cycles (for human β-actin) of amplification including denaturation (60 sec, 94°C), hybridization (60 sec: 65°C for sst1, sst3, and sst4; 60°C for sst2 and sst5; and 67°C for β-actin, respectively), and elongation (75 sec, 72°C). Final elongation was achieved at 72°C for 5 min. The absence of reactive contamination by genomic DNA or cDNA was checked by PCR amplifications systematically carried out in parallel by replacing cDNA by water.

Data Analysis

Pharmacological parameters such as concentration necessary to inhibit 50% of maximal response (IC_{50}), inhibition constant (K_I), maximal inhibition measured at the greatest concentration of analogs tested (I_{max}), and maximal binding capacity (B_{max}) were estimated by computer-assisted nonlinear regression analysis program (EBDA-LIGAND), using the modified method of McPherson [1983].

Results were expressed as means ± SEM. The experiments were run in triplicates and repeated 3–6 times. Statistical analysis consisted of evaluation of experimental differences by one-way analysis of variance (ANOVA), using a Systat (SPSS Inc.) computer program. Differences were considered significant for the *P*-value of F-statistics less than 0.05.

RESULTS

Effects of Somatostatin on Adenylyl Cyclase Activity

We first determined the adenylyl cyclase activity in membrane preparations obtained from nonactivated and mitogen-activated Jurkat cells. The average of basal adenylyl cyclase activity (i.e., in the absence of drugs) was 34 ± 8 (n = 4, n corresponding to the number of independent experiments) pmols of cAMP produced over 30 min per mg of proteins (pmol cAMP min^{-30} mg^{-1} protein) in membranes obtained from nonactivated cells and 30 ± 4 (n = 6) pmol cAMP min^{-30} mg^{-1} protein in membranes originating from cells previously activated with TPA/PHA (Fig. 1). When 10^{-5} M forskolin was added to the incubation medium, adenylyl cyclase activity reached 182 ± 38 (n = 3) pmol cAMP min^{-30} mg^{-1} protein and 144 ± 40 (n = 5) pmol cAMP min^{-30} mg^{-1} protein in membrane preparations from nonactivated and mitogen-activated cells, respectively. The forskolin concentration used (10^{-5} M) corresponds to the concentration routinely used in human T

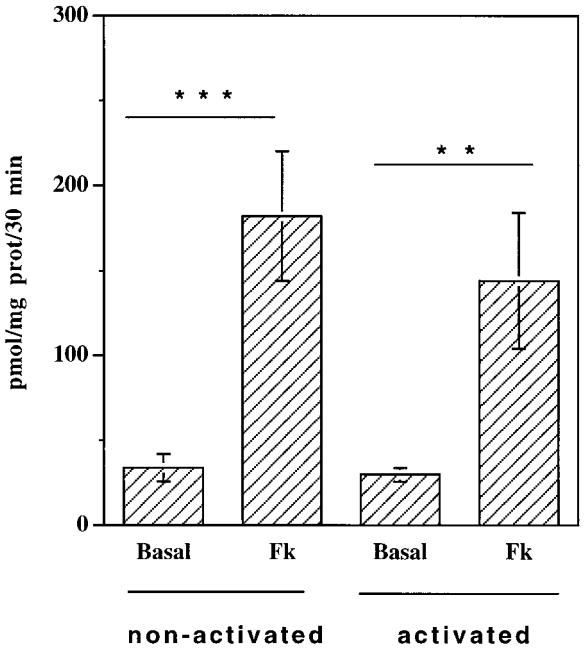


Fig. 1. Effect of forskolin (Fk, 10^{-5} M) and mitogens (PHA, 2 μg/ml; TPA, 16 nM) on cAMP production in membrane preparations obtained from nonactivated or activated cells. Each bar corresponds to the mean ± SEM obtained from triplicates of 3–6 different experiments. $^{**}P < 0.01$, $^{***}P < 0.001$ as determined by one-way ANOVA.

cells [Seamon et al., 1981; Minakuchi et al., 1990; Krantic et al., 1997]. Independently of the activation state of Jurkat cells, forskolin increased adenylyl cyclase activity 4–5- fold over the basal level (Fig. 1). The adenylyl cyclase activation measured in the presence of identical forskolin concentration in some rat brain structures (e.g., olfactory bulb) is similar to that seen in our experiments [Olianas and Onali, 1996]. However, in rat brain striatum (usually taken as a reference in the literature), it is at least twice that determined here. To ascertain that the chosen experimental conditions were optimal, we assayed adenylyl cyclase stimulation by 10^{-5} M forskolin in these rat brain structures. The values obtained for positive controls (Table I) are in perfect agreement with those reported previously [Seamon et al., 1981; Onali et al., 1985; Minakuchi et al., 1990; Olianas and Onali, 1996; Krantic et al., 1997].

The basal adenylyl cyclase activity was relatively low in both types of membrane preparations used (i.e., obtained from cells treated or not with mitogens). Indeed, in the absence of forskolin, a detectable ATP to cAMP conversion was near the inferior limit of the assay sensitivity (exceeding blank values only 2–3-fold). Consequently, it was reasonable to assume that the

TABLE I. Forskolin-Induced Adenylyl Cyclase Activation

	Adenylyl cyclase activity pmol of cAMP produced over 30 min per mg of proteins		
	Basal	Forskolin (10 μM)	Fold-increase over basal
Jurkat cells	34 ± 8	182 ± 38*	4–5
Striatum	66 ± 2	839 ± 82*	11–13
Olfactory bulb	116 ± 4	703 ± 5*	4–6

*$P < 0.001$ vs. basal activity.

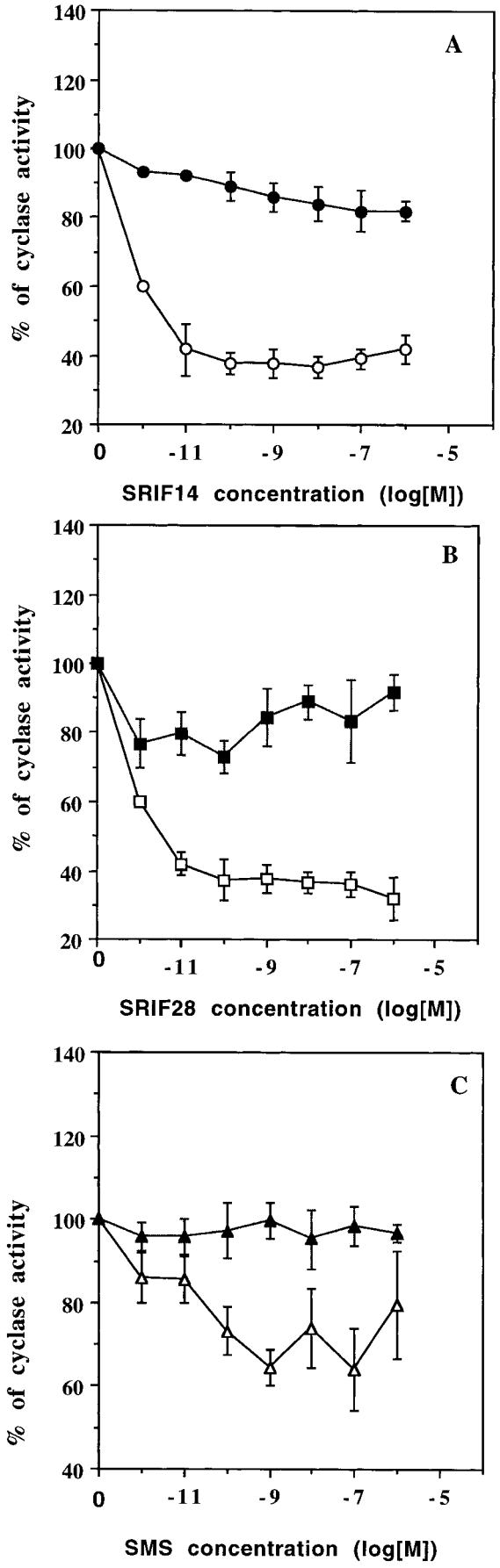
actions of compounds such as SRIF, expected to further lower adenylyl cyclase activity, would be difficult to measure. All further experiments were therefore performed on forskolin-stimulated membrane preparations.

We then analyzed the effects of SRIF analogs SRIF14, SRIF28, and SMS 201–995 on adenylyl cyclase activity in membrane preparations obtained from nonactivated and mitogen-activated Jurkat cells. All SRIF analogs tested significantly decreased the adenylyl cyclase activity in preparations obtained from nonactivated cells in a dose-dependent manner (Fig. 2A–C). This decrease was very similar for SRIF14 and SRIF28, since the maximal inhibition of 60% was reached at 10^{-10} M for both analogs (Fig. 2A,B). For SMS 201–995, the maximal inhibition was about 2-fold lower (30%) and was reached at greater concentrations (10^{-9} M) (Fig. 2C). Estimation of the concentrations of analogs necessary to elicit a half-maximal inhibition (IC_{50}) revealed almost identical IC_{50} for SRIF14 and SRIF28 in nonactivated cells (2.2 ± 0.4 pM and 2.0 ± 0.3 pM, respectively). For SMS 201–995, IC_{50} value equaled 38 ± 24 pM.

In semipurified plasma membranes obtained from mitogen-activated cells, endogenous SRIF still inhibited the adenylyl cyclase activity significantly (I_{max} = 18 ± 3%, and I_{max} = 20 ± 2% at 10^{-6} M for SRIF14 and SRIF28, respectively) but to only one third of the inhibition seen in nonactivated cells (Fig. 2A,B). The SMS 201–995-dependent inhibition of adenylyl cyclase activity observed in nonactivated cells was lost in activated cells (Fig. 2C).

Somatostatin Binding in Nonactivated and Activated Jurkat Cells

Both nonactivated [Sreedharan et al., 1989] and mitogen-activated [Cardoso et al., 1998] Jurkat cells bind radioactive SRIF in very simi-



lar manner, thus suggesting that these cells display identical plasma membrane receptors independent, of their activation state. In order to assess if SRIF-dependent modulation of adenylyl cyclase was related to the level of receptor-protein expression in our experimental conditions, we studied the number and the affinity of these receptors in Jurkat cells activated or not with mitogens. To do that we performed a competition between ^{125}I-Tyr^{1}-SRIF14 and nonradioactive SRIF14 for binding on plasma membrane preparations obtained from either nonactivated or mitogen-activated cells. This radioligand binds with subnanomolar affinity all five cloned SRIF receptors. The analysis of SRIF14 inhibition of ^{125}I-Tyr^{1}-SRIF14-specific binding yielded an almost identical number of binding sites in membrane preparations studied (B_{max} = 42 ± 3 fmol per mg prot and B_{max} = 38 ± 5 fmol per mg prot in preparations obtained from nonactivated and activated cells, respectively). In addition, two binding sites characterized with K_{I1} of 31 ± 11 pM and K_{I2} of 6 ± 2 nM were identified in membrane preparations of nonactivated cells (Fig. 3). Similarly, in the preparations obtained from activated Jurkat cells, two binding sites were revealed: one with K_{I1} of 14 ± 8 pM and the other with K_{I2} of 7 ± 4 nM (Fig. 3).

Somatostatin Receptor Expression in Nonactivated and Activated Jurkat Cells

Differential effects of SRIF on adenylyl cyclase observed in nonactivated and activated Jurkat cells might be associated with the different expression pattern of SRIF receptor subtypes before and after activation. Indeed, a possible differential receptor expression could remain undetectable in binding studies performed with nonselective ligand (see above). Therefore, we analyzed by RT-PCR the expression of five known human SRIF receptors on cells treated or not with mitogens.

Fig. 2. Effects of increasing concentrations of SRIF analogs SRIF14 **(A)**, SRIF28 **(B)** and SMS201-995 **(C)** on adenylyl cyclase activity in membrane preparations obtained from nonactivated (open symbols) and from mitogen-activated (solid symbols) cells. In the absence of SRIF and in the presence of 10^{-5} M forskolin, the adenylyl cyclase activity was 182 ± 38 (mean ± SEM) pmol min^{-30} mg^{-1} protein in membrane preparations of nonactivated cells, and 140 ± 40 pmol min^{-30} mg^{-1} protein in membrane preparations from mitogen-activated cells. These values were taken as a reference (100%) value. The effects of SRIF analogs are expressed as percentage of reference value. All analogs were tested in three different experiments performed in triplicate.

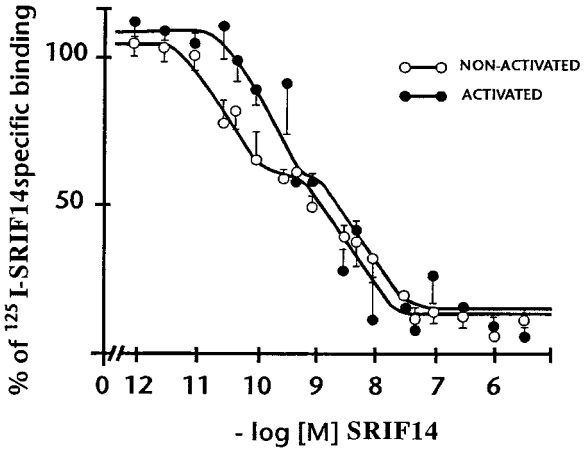


Fig. 3. SRIF14 inhibition of ^{125}I-Tyr^{1}-SRIF14-specific binding in membrane preparations obtained from nonactivated (open symbols) and mitogen-activated (solid symbols) cells. Data are the mean + SEM of two independent experiments, each performed in triplicate. The specific binding measured in the absence of nonradioactive competitor (SRIF14) was taken as a reference (100%) value. This reference value for specific binding was 21 + 3 fmol mg^{-1} protein; it represented 55% of total ^{125}I-Tyr^{1}-SRIF14 binding measured at 35 pM radioligand concentration.

According to our previous study [Cardoso et al., 1998], the analysis of PCR products amplified with primers specific for sst3 demonstrated that the 222-base-pair fragment was present in cDNA reverse transcribed from mRNAs isolated from mitogen-activated Jurkat cells (Fig. 4). In addition, we show here that the same primers amplified the 222-base-pair fragment on cDNA obtained from nonactivated Jurkat cells as well (Fig. 4). Since this gene contains no introns [Reisine and Bell, 1995], the size of amplified products obtained with cDNA used as a template was identical to the size of the fragments amplified from genomic DNA of Jurkat cells. No amplification product was obtained using as a template the cDNA from either nonactivated or activated Jurkat cells with primers specific for sst1, sst2, sst4, and sst5. However, these primers amplified the fragments of the expected size from genomic DNA (Fig. 4). In addition, the specific primers for the five SRIF receptors used in the present study amplified the fragments of expected size for each respective receptor when the mRNA from fetal and adult human brains (Clontech, Palo Alto, CA) was used as a template for reverse transcription (data not shown). The five SRIF receptors have been previously described in human brain [Rohrer et al., 1993; Panetta and Patel, 1994]. In contrast, no amplification occurred when RT-PCR was performed in the absence of Moloney monkey leukemia virus reverse transcriptase (Fig. 4).

DISCUSSION

In the present study we report on an activation-associated modulation of SRIF receptor signaling in Jurkat T cells. Direct assessment of SRIF receptor coupling to adenylyl cyclase in semipurified membrane preparations performed here indicated that T-cell activation decreased efficiency of SRIF analogs to inhibit adenylyl cyclase activity, without altering receptor expression at either protein or mRNA level.

To our knowledge, this is the first report on activation-associated uncoupling of G_i-protein linked receptors from the adenylyl cyclase transduction pathway in T cells. The only other previously published work reported that exposure of human T-lymphocytes to PHA prevents the agonist-induced β-adrenergic receptor uncoupling from G_s-protein, which physiologically occurs in resting cells [Carlson et al., 1994].

The experimental approach used was validated by testing the capacity of adenylyl cyclase to be stimulated with forskolin in both experimental conditions studied. In semipurified membrane preparations from either nonactivated or activated cells, the observed forskolin stimulation of adenylyl cyclase activity is in perfect agreement with those previously reported in the literature [Seamon and Wetzel, 1984; Krantic et al., 1997]. Such functional testing of adenylyl cyclase activity made it possible to exclude an impairment of enzyme integrity during the procedure of membrane preparations. This control was indeed necessary, since the basal adenylyl cyclase activities were relatively low and could suggest enzyme integrity disruption. However, the basal adenylyl cyclase activities that we could measure in the preparations obtained from both nonactivated and mitogen-activated cells are of the same order of magnitude as those previously documented [Mary et al., 1987; van Tits et al., 1991]. Moreover, the basal adenylyl cyclase activities determined here were almost identical either in the presence or in the absence of mitogens, as expected from already reported data for Jurkat cells and human T-lymphocytes [Mary et al., 1987; van Tits et al., 1991].

Our data concerning adenylyl cyclase inhibition by SRIF analogs used are in agreement with the functional involvement of sst3 receptors (as suggested by RT-PCR data) in SRIF actions in nonactivated cells. Indeed, as expected for sst3 receptor [Patel and Srikant,

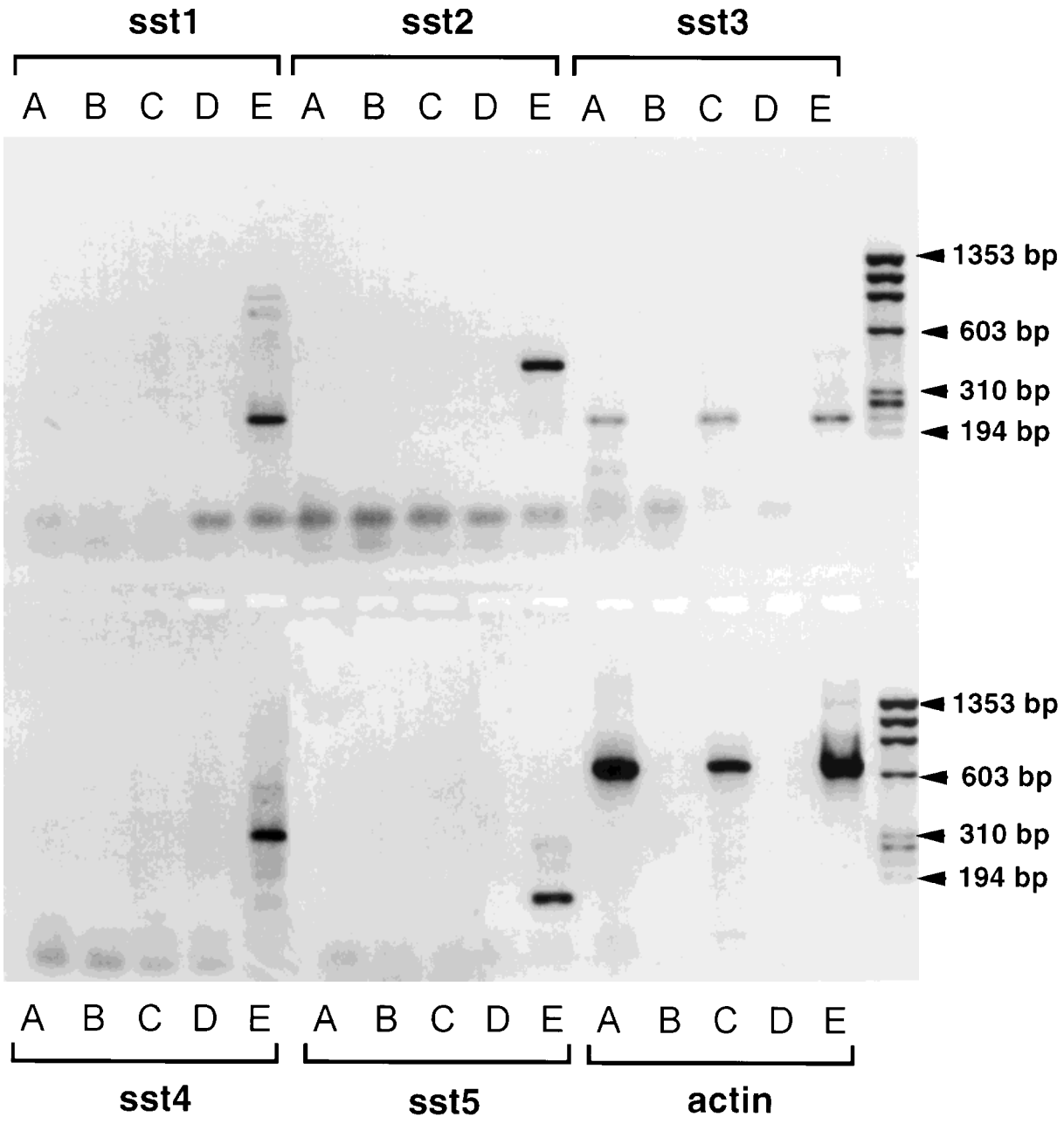


Fig. 4. RT-PCR analysis of SRIF receptor expression in nonactivated (**lanes A and B**) and mitogen-activated (**lanes C and D**) Jurkat cells. RT-PCR was carried out on cDNA prepared from RNA isolated from Jurkat cells in the presence (lanes A and C) or absence (lanes B and D) of reverse transcriptase. Primers specific for sst1–sst5 receptors and beta-actin were used for the amplification. Genomic DNA (**lane E**) was amplified in parallel to serve as a positive control for the size of the amplified segments.

1994] SRIF14, SRIF28, and SMS 201–995 display a similar efficiency in inhibiting adenylyl cyclase in membrane preparations obtained from nonactivated cells. Moreover, the efficiency of all analogs tested in the inhibition of adenylyl cyclase are in good agreement with the subnanomolar affinity of "high-affinity" receptor identified in binding studies. However, the "low-affinity" sites identified by us [present study; Cardoso et al., 1998] and others [Sreedharan et al., 1989; Hiruma et al., 1990] still remain to be characterized.

We further show that after mitogen stimulation, SRIF14 and SRIF28 are less efficient, while SMS 201–995 becomes inefficient in the modulation of adenylyl cyclase activity. Our receptor assays indicated that both nonactivated and activated Jurkat cells have an identical num-

ber of binding sites. Hence, the observed decrease in SRIF analogs efficiencies cannot be attributed to a decrease in the number of SRIF receptors that are able to inhibit adenylyl cyclase after mitogen activation. The RT-PCR analysis performed on whole RNA extracts from mitogen-activated Jurkat cells demonstrated the expression of a single, sst3 SRIF receptor. A decreased capacity of SRIF analogs to inhibit adenylyl cyclase in membrane preparations obtained from mitogen-activated cells is thus not a consequence of a hypothetical mitogen-associated induction of another receptor subtype(s) such as sst1/sst4 with low affinity for SMS 201-995 [Patel and Srikant, 1994]. Altogether, these data are therefore compatible with a mitogen-associated decrease in the efficiency of sst3 receptor coupling to adenylyl cyclase. However, we cannot exclude the existence of other SRIF receptors, not characterized as yet, whose expression could be modulated during T-cell activation, contributing to the observed effects.

Mitogen-activated cells produce IL-2 that could theoretically affect SRIF actions on adenylyl cyclase. Therefore, effects of IL-2 could provoke different SRIF actions on adenylyl cyclase activity in nonactivated and activated cells. Nevertheless, this possibility is less likely because: 1) IL-2 is absent in our assays, since we measured adenylyl cyclase activity on membrane preparations and not in whole cells; and 2) IL-2 has been reported to inhibit adenylyl cyclase [Farrar and Beckner, 1986; Mary et al., 1987] and one would expect the potentialization of SRIF-dependent adenylyl cyclase inhibition rather than the abolishment that we observed.

The expression and binding characteristics of other members of the superfamily of G-protein-coupled receptors have previously been reported to be differentially affected during T-cell activation. For example, mitogen activation of T cells does not affect the binding characteristics (i.e., affinity and number) of β-adrenergic receptors [Carlson et al., 1994]. Conversely, mitogens do induce an increase in the receptor number of muscarinic acetylcholine receptors in T cells [Strom et al., 1981]. The only previously published study on the activation-dependent modulation of mRNA expression concerns substance P receptors; it was performed on rat macrophages [Bost et al., 1992].

The absence of activation-associated receptor alteration (at either mRNA or protein levels, as shown here by RT-PCR and binding studies, respectively), taken together with the decreased efficiency of SRIF analogs to inhibit adenylyl cyclase in the presence of mitogens, therefore strongly suggests a functional uncoupling of the sst3 receptor subtype from adenylyl cyclase during T-cell activation. This conclusion is in agreement with the previously reported data according to which sst3 (together with sst2 and sst5) receptors are desensitizable in contrast to sst1 and sst4 receptors that are not [Yasuda et al., 1992; Rens-Domiano et al., 1992; Raynor and Reisine, 1992]. Further studies are now needed in order to elucidate the mechanisms underlying the sst3 receptor uncoupling. They will certainly show whether these mechanisms involve receptor phosphorylation by G-protein receptor kinases (GRK1–GRK6) and/or second-messenger-activated kinase (protein kinase A and protein kinase C). In this light, cross-talk between the adenylyl cyclase pathway and protein kinase C has already been reported in Jurkat cells [Bihoreau et al., 1991]. Additionally, transcriptional and/or translational modification of G-proteins themselves might also be involved in the observed sst3 receptor uncoupling from adenylyl cyclase. Relevantly, an activation-dependent regulation of specific members of the G-protein family expressed in T cells has recently been reported [Lippert et al., 1997].

In conclusion, the physiological advantage of altering receptor "sensitivity" to neuro-endocrine-immunomodulatory signals in the course of mitogen/antigen activation of T cells remains to be elucidated. One hypothesis is that it would be related to the fine-tuning of immune response in order to achieve an orchestrated actions of CNS, endocrine, and immune systems.

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