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Distribution and second messenger coupling of four somatostatin receptor subtypes expressed in brain

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The mRNA distribution in the brain and the coupling to cellular effector systems of four somatostatin receptors (SSTR1–4) was studied. All four SRIF receptor subtypes were expressed in cortex and hippocampus. In addition, SSTR1 mRNA was relatively abundant in the spinal cord whereas SSTR2 mRNA was also present in the striatum. The SSTR3 gene was predominantly expressed in the olfactory bulb and in the cerebellum. Conflicting results about the effector coupling of SSTR1–3 have been published previously. We have stably expressed human SSTR1–4 in HEK 293 human embryonal kidney cells. Agonist binding to the receptor subtypes, including the recently cloned SSTR4, inhibited the formation of forskolin-induced cAMP. Is is concluded that, in an appropriate cellular environment, all four receptor subtypes can functionally couple to the inhibition of adenylyl cyclase.

SRIF receptor; Brain; Adenylyl cyclase; cAMP; G-protein

1. INTRODUCTION

Somatostatin (somatotropin release inhibiting factor, SRIF) is an important regulator of endocrine and exocrine secretion. It acts on many different cell types by inhibiting the secretion of hormones including growth hormone (GH), insulin, glucagon, gastrin, and secretin [1–3]. In addition, SRIF was shown to act as a neurotransmitter [4–6] and to display neurotrophic properties on sympathetic, dorsal root ganglion and motor neurons [7,8].

The regulatory effects of SRIF are mediated by high-affinity membrane receptors on the target tissues which are coupled to GTP-binding proteins (G-proteins) [9–12]. Pretreatment of SRIF receptor positive cells with pertussis toxin (PTX) abolished their ability to interact with cellular effector systems like the adenylyl cyclase system indicating the involvement of PTX-sensitive G-proteins [13,14].

We have recently used an expression-cloning strategy to isolate a complementary DNA (cDNA) encoding a rat somatostatin receptor [15]. Based on the distribution of the cloned receptor and on the excellent correlation between the ability to displace radioligand binding from receptors expressed in COS-1 cells and their inhibitory activity on GH release from pituitary cells, we concluded that we had cloned the cDNA for the SRIF receptor type responsible for the inhibition of growth hormone release from the pituitary cells.

Two other groups have independently isolated SRIF receptor genomic and cDNA clones based on their sequence similarity with monoaminergic receptors [16–18]. SRIF receptors contain seven putative membrane spanning regions and display sequence and structural homology to the family of G-protein coupled receptors, in particular to the monoaminergic receptors.

A total of five SRIF receptor subtypes [19–24] and one splice variant of our rat clone [25] have recently been cloned from various species. This process was greatly facilitated by the absence of introns in the coding regions of the receptor genes and by the extremely high sequence conservation between the SRIF receptor subtypes.

While various nomenclatures have been introduced by different authors, the one proposed by Bell and Reisine [26] seems most widely accepted and is used in this report with the exception that SSTR4, based on the submission date, refers to the clones isolated by Bruno et al. [20] and Rohrer et al. [24] and SSTR5 to the clone described by O'Carroll et al. [22].

Here, we describe the distribution of the mRNAs encoding receptors SSTR1, 2, 3 and 4 in the brain. In addition, we demonstrate that all four receptor subtypes inhibit the formation of cAMP when stably expressed in HEK 293 human embryonal kidney cells.

2. MATERIALS AND METHODS

2.1. *Materials*

Culture media and serum were obtained from Gibco BRL The somatostatin analogues Octreotide (SMS 201–995, Sandostatin), Tyr^3-Octreotide (SDZ 204–090) and SRIF-14 were synthetized at Sandoz Pharma (Basle, Switzerland) All other chemicals were obtained from commercial sources. Iodination of Tyr^3-Octreotide and Tyr^{11}-SRIF-14

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was performed by the chloramine-T method as described [27]. Oligonucleotides were synthesized on a 380A Applied Biosystems synthesizer.

2.2. *RNA isolation and Northern blot analysis*

Total cellular RNAs were purified from frozen tissues by the guanidinium thiocyanate/ acid phenol method [28]. Poly(A)$^+$ RNA was enriched by two passages over an oligo(dT) cellulose column as described [29]. For Northern blot analysis, the RNAs were fractionated on a 1% agarose gel containing 2.2 M formaldehyde and transferred to Hybond N membranes (Amersham) by pressure blotting After UV crosslinking the RNAs were hybridized to ^{32}P-labelled single stranded DNA probes generated by an asymmetric PCR (mSSTR1, 2, 3) or to a riboprobe (mSSTR4).

Plasmids clones for the mouse SSTR1, 2 and 3 genes were kindly provided by Dr. Graeme I. Bell, Chicago [16,23]. For the generation of single stranded DNA probes anti-sense primers corresponding to sequences located between the putative transmembrane regions IV and V of the SSTRs were designed. The primer sequences were CCGTGCCATCGCTGTTGGCT (mSSTR1), TCCTGCCCCACTGGTTGCTC (mSSTR2) and GCATGTGGCAGGTGCTCATG (mSSTR3). After linearization of the clones mSSTR1, 2 and 3 with *Pst*I, *Mme*I and *Nco*I, respectively, 30 cycles of ^{32}P-labelled run-off replications were performed as described [30] to generate single-stranded anti-sense DNA.

To synthesize a mouse SSTR4 receptor hybridization probe from the corresponding region of this receptor, a fragment of the mouse SSTR4 gene was amplified by PCR. Primer sequences (TGTCAACCATGTGTCCCTCA and TAATACGACTCACTATAGGGAGCAGGCAGGTTCTGCTTGCA) were designed based on the published rat SSTR4 sequence [20]. The second oligonucleotide had as 5′-addition the T7 promotor sequence (21 base pairs) The PCR reaction (50 μl) contained 10 mmol/l Tris-HCl, pH 8.3, 1.5 mmol/l $MgCl_2$, 50 mmol/l KCl, 0.1 mg/ml gelatin, 0.2 mmol/l dNTPs, 10pmol of each primer, 100ng of mouse genomic DNA and 2.5 units *Taq* polymerase. 34 PCR cycles were performed using a PREMIII (LEP Scientific) thermocycler (1 min 94°C, 30 s 63°C, 30 s 72°C) A fragment of the expected size (280 bp) was obtained, gel purified (Qiaex, Diagen) and cycle-sequenced (Promega TaqTrack sequencing kit). The amplification product displayed highest sequence homology to the rat SSTR4 gene (data not shown) and was less homologous to the other SSTR subtypes. A [^{32}P]-labelled riboprobe was synthetized from the amplified mSSTR4 gene fragment by in vitro transcription with T7 RNA polymerase. The probe hybridized to two RNAs of the expected size for rat SSTR4 mRNAs (2.4 and 6.0 kb; [20]), thus ensuring that we had amplified a portion of the mouse SSTR4 gene.

Hybridization was performed at 55°C (DNA probes) or 65°C (riboprobes) in 0 5 M $NaHPO_4$ pH 7.2, 7% (w/v) SDS, 1 mM EDTA, 50% (v/v) formamide [31] for 16 to 24 h. The membranes were washed twice at room temperature in 2 × SSC, 0.1% SDS for 15 min and twice for 30 min in 0.1 × SSC, 0.1% SDS at 65–75°C. Kodak X-OMAT AR films were exposed to the blots at −80°C with intensifying screens for 2 to 6 days. Before subsequent hybridizations the probes were removed by washing the filters with boiling 0.1% (w/v) SDS.

2.3. *In situ hybridization*

The oligonucleotides specific for SSTR1 (GCCTCCAGACTCCAGATTCTCGGGCTGGAAGTCGTCCACG, [16]), SSTR2 (CGCGTTGCTTGTCATGTCGTAGTATGGCTCTGTCTGGTTG, [15]), SSTR3 (TTCTTCATCCTCCTCCTCTTCAGTCTTCTCTGGAGGTCCC, [23]) and SSTR4 (GCAGCGGATTGCCGTGGAGAGCAGCCGAACGCTGAACCAA and GCAATGGAGGGCATATGCATCCTGGGCCACCTCTGCTTTT, [20]) were labelled at their 3′ ends with terminal desoxynucleotidyltransferase (TdT, Boehringer, Mannheim) and [^{32}P]dATP (3,000 Ci/mmol, Amersham). The labelling reactions were carried out by incubating 2 pmol of oligonucleotide for 2 h at 37°C in a 30 μl volume containing 16 pmol of labelled dATP and 25 units of TdT in 100 mM sodium cacodylate, pH 7.2, 2 mM $CoCl_2$, and 0.2 mM dithiothreitol (DTT). The probes were purified by chromatography through a NACS PREPAC column (BRL) according to the manufacturer's instructions.

Cryostat sections (10–20 μm) were air-dried, fixed for 20 min at room temperature in a freshly prepared solution containing 4% (w/v) paraformaldehyde in PBS (2.6 mM KCl, 1 4 mM KH_2PO4, 136 mM NaCl, 8 mM Na_2HPO_4) The slides were then washed once in 3 × PBS, twice in 1 × PBS, for 5 min each and incubated in a freshly prepared solution of predigested pronase at a final concentration of 24 U/ml in 50 mM Tris-HCl. pH 7.5, 5 mM EDTA for 10 min. The proteolytic activity was stopped by a 30 s immersion in 1 × PBS containing 2 mg/ml glycine. The slides were rinsed twice in PBS for 2 min each, and dehydrated for 2 min each in a graded series of ethanol (60%, 80%, 95%, 100%). Tissue sections were dried at room temperature.

The probe was diluted to a final concentration of 1–2 × 10^7 cpm/ml in buffer containing 50% formamide, 600 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 × Denhardt's solution (0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% BSA), 500 μg/ml yeast tRNA and 10% dextran sulfate Each section was covered with 60 μl of the hybridization solution and a Nescofilm coverslip was placed on top to prevent evaporation. The slides were placed in humid boxes and incubated at 37°C for 17 h. Following hybridization, the coverslips were removed by flotation in a solution containing 600 mM NaCl, 20 mM Tris-HCl, pH 7.5, and 1 mM EDTA. The slides were subsequently washed at 60°C for 4 h in the same buffer (with 4 changes). Then the tissues were dehydrated by immersion in 70% and 95% ethanol containing 0.3 M ammonium acetate pH 7.0, 2 min each and air dried. Hybridization signal were detected by autoradiography on β-max film (Amersham, UK). Exposure time ranged from 6 h for the SSTR3 oligonucleotides to 12 days for the other oligos. Sections were stained with 1% Cresyl violet in order to identify brain structures according to Paxinos and Watson [32].

2.4. *Cell culture*

HEK 293 human embryonal kidney cells (American Type Culture Collection) were grown in minimal essential medium (MEM, Gibco, No 041-01090) supplemented with 10% (v/v) fetal calf serum and 2 mM L-Glutamin For transfected cell lines 500 μg/ml G418 (active drug; Gibco BRL) was added to the medium.

2.5. *Expression of human SSTR subtypes in HEK 293 cells*

Four human SRIF receptor subtypes, SSTR1–4 were stably expressed in HEK 293 human embryonal kidney cells. Clones for the human SSTRs 1, 2 and 3 were kindly provided by Dr. Graeme I. Bell, Chicago [16,19] The SSTR coding sequences were subcloned into the vector pcDNAI (Invitrogen) containing the cytomegalovirus (CMV) promotor The human SSTR4 coding region cloned into a similar CMV-based expression vector (pCMX) was kindly provided by Dr. R. Schüle, Sandoz, Basle 20 μg of the constructs for hSSTR2, 3 and 4 were cotransfected with 1 μg pMC1neo polyA (Stratagene) as described [33] Cells expressing hSSTR2, 3 and 4 could be obtained by this cotransfection method. We failed to generate SSTR1-expressing cells using this procedure. Therefore, a 3.3 kb *Sca*I fragment of pcDNAI containing the CMV promotor sequence together with the hSSTR1 coding sequence and the polyadenylation signal was inserted into the *Hin*cII site of pOG45 (Stratagene), a vector containing a neomycin gene cassette on the same unit. Using this construct we readily obtained cell clones expressing high levels of hSSTR1.

The selection with the neomycin analog G418 (500 μg/ml) was started after 72 h and continued for three weeks. Individual cell clones were isolated, propagated and tested for specific binding of radiolabelled SRIF-14. For each receptor subtype three individual cell clones which revealed specific, high-affinity [^{125}I]Tyr^{11}-SRIF-14 binding, were selected for further analyses.

2.6. *Receptor binding assays*

SRIF receptor assays were performed as described previously [15]. HEK 293 cells stably expressing SSTR subtypes 1–4 were grown to confluency in 24 well plates. For receptor binding studies, cells were washed twice with 10 mM HEPES, pH 7 6, containing 5 mM $MgCl_2$,

20 μg/ml bacitracin and 0.5% (w/v) bovine serum albumin The cells were incubated with 30,000 cpm of either [^{125}I]Tyr^{11}-SRIF-14 or [^{125}I]Tyr^{3}-SMS 201–995 (specific activity 2,000 Ci/mmol) in 300 μl HEPES buffer for one hour at room temperature. The incubation was terminated by washing the cells twice with cold HEPES buffer. Cells were detached with 10% (w/v) SDS and the radioactivity bound to the cells was determined in an LKB gamma counter (80% counting efficiency). Non-specific binding of the radioligand was defined as binding in the presence of either 1 μM SRIF-14 or SMS 201–995. Competition binding experiments were performed by incubating the cells with increasing concentrations of unlabelled SRIF-14. Binding curves were generated from triplicate determinations using the computer fitting programme of De Lean [34].

2.7. *Adenylyl cyclase assay*

Cells grown to confluency in 24-well plates were labelled with [^{3}H]adenine (2 μCi/ml) for 4 h in the presence or absence of pertussis toxin (100 ng/ml, Sigma). The cells were then washed twice in HEPES-buffered salt solution (HBS; 130 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 0.9 mM NaH_2PO_4, 25 mM glucose, 20 mM HEPES, pH 7.4) and incubated in the same buffer supplemented with 1 mM isobutyl-methylxanthine (IBMX). Adenylyl cyclase was stimulated with 3 μM forskolin in the presence of different concentrations of SRIF-14 or SMS 201–995. After 15 min of incubation, cells were extracted with 5% trichloracetic acid. [^{3}H]ATP and [^{3}H]cAMP were separated by sequential chromatography on Dowex and Alumina columns as described [35]. Data are plotted as cAMP/ATP ratios normalized to the response obtained with forskolin alone (100%).

3. RESULTS

3.1. *Brain distribution of SRIF receptor mRNAs*

The mRNA distribution in the brain of somatostatin receptor subtypes SSTR1–4 was studied by Northern blotting and in situ hybridization (Fig. 1; Fig. 2). For Northern blot analyses on mouse tissues receptor specific probes were generated. Under high stringency conditions, these probes hybridized to single RNAs of different size for each receptor subtype indicating the absence of cross hybridization (Fig. 1). All four receptor subtypes are expressed in hippocampus and cortex. We also detected SSTR1 mRNA in spinal cord and substantia nigra. In addition to hippocampus and cortex, SSTR2 mRNA was present in spinal cord, substantia nigra, and striatum. SSTR3 mRNA was relatively abundant in the olfactory bulb and in the cerebellum.

The SSTR4 probe hybridized to two rat mRNAs of about 2.4 and 6.0 kb (not shown), but on mouse RNAs it hybridized only to a single mRNA species of about 6.0 kb (Fig. 1). SSTR4 mRNA was present at rather low levels throughout the mouse brain but could be readily detected in hippocampus and cortex (Fig. 1). We have not yet investigated the nature of the two SSTR4 mRNA species present in rat tissues. A splice variant of the SSTR2 RNA has been decribed which is also significantly more abundant in rat than in mouse tissues [25]. SSTR2 receptor probes label two bands in blots with rat RNAs [15] but only one band in blots with mouse RNAs (Fig. 1).

In situ hybridization on rat mesencephalon sections revealed a distribution of SSTR subtype mRNAs in the rat brain which was in agreement with the Northern

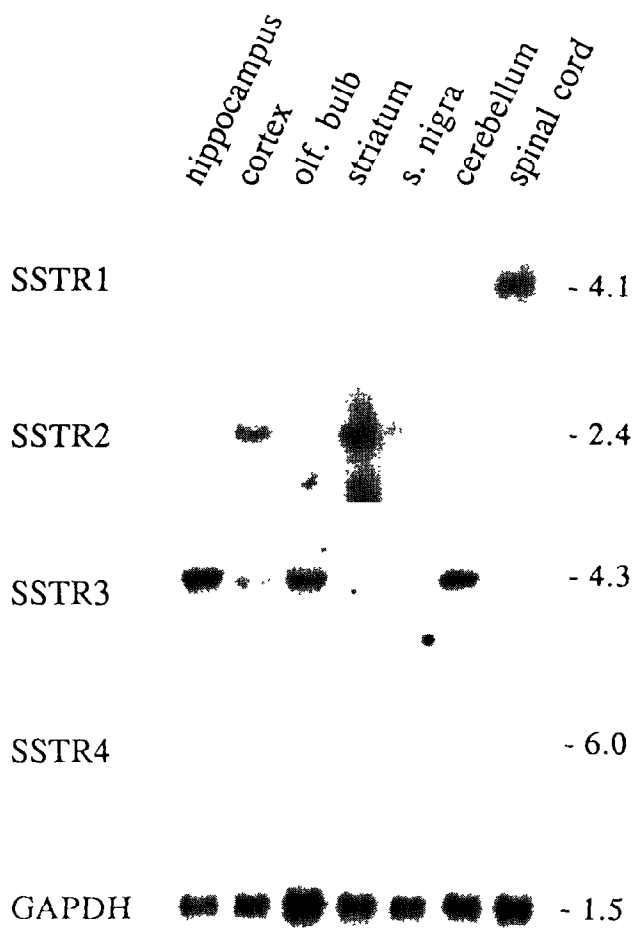

Fig. 1. RNA blot analysis of SSTR 1–4 gene expression in the mouse brain. Per lane 15 μg total RNAs prepared from tissues of 60-day-old BALB/c mice were loaded and hybridized to ^{32}P-labelled receptor subtype specific probes. Three blots were prepared using the same RNA preparation. After the SSTR1 hybridization the filter was stripped and rehybridized with the SSTR4 probe. To compare the amounts of RNA all filters were subsequently rehybridized with a probe for glyceraldehyde-3-phosphate-dehydrogenase (GAPDH, [48]). An example for a GAPDH hybridization is shown (4 h exposure). Sizes of hybridizing RNAs are given in kb.

blot analysis on mouse brain tissues (Fig. 2). The oligonucleotides specific for the four SRIF receptor subtypes hybridized to different layers of the cerebral cortex. The highest densities of SSTR-1 signals were found in layers V–VI of the cerebral cortex, in the granular layer of the dentate gyrus, and in the amygdala (Fig. 2a). Signals for SSTR2 were found in the frontal cerebral cortex (layers IV, V and VI), in the CA1 area of the hippocampus, in the central grey, in the granular cell layer of the dentate gyrus and in the amygdala (Fig. 2b). High levels of SSTR3 hybridization were found in the amygdala, in the granular layer of the dentate gyrus and in the red nucleus (Fig. 2c). The highest densities of SSTR4 mRNAs were found in the pyramidal layer and the CA1 area of the hippocampus (Fig. 2d).

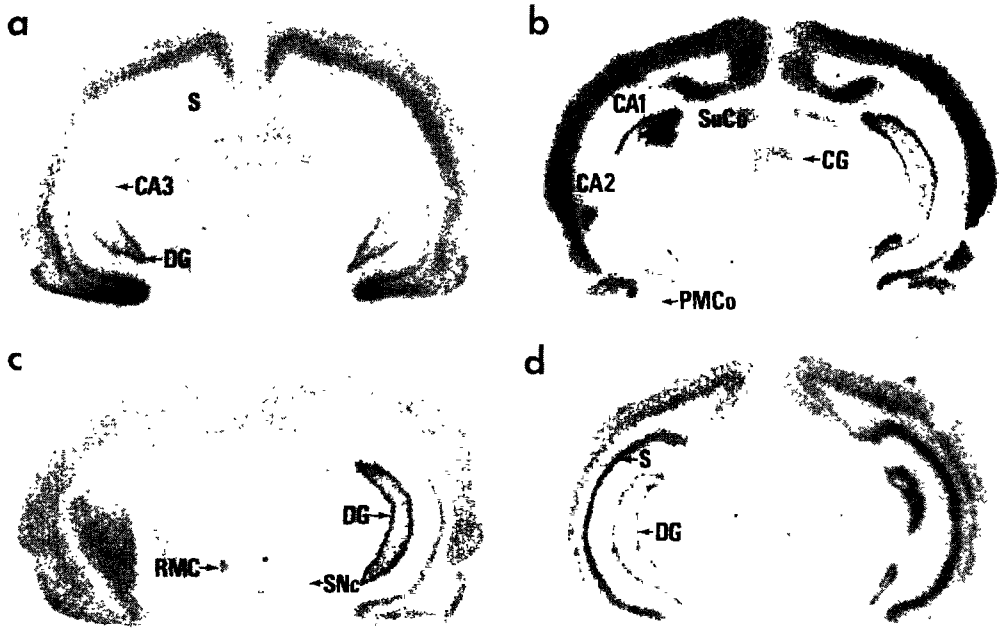

Fig. 2. Distribution of SSTR1 (a), SSTR2 (b), SSTR3 (c) and SSTR4 (d) hybridization signals in mesencephalon sections of adult rat brain in situ Dark areas indicate intense hybridization. Abbreviations: CA1, CA2, CA3: fields CA1, 2, and 3 of Ammon's horn, respectively; CG: central grey; DG: dentate gyrus; PMCo: posteromedial cortical amygdaloid nucleus; RMC: red nucleus magnocellular; SuCo: superior colliculus; S: Subiculum, SNc: substantia nigra, compact

3.2. *Expression of SRIF receptor subtypes in HEK 293 cells*

Human somatostatin receptor subtypes (hSSTR1–4) were stably expressed in HEK 293 human embryonal kidney cells. To determine the binding properties of SRIF analogs to SSTR1–4, competition binding-experiments were performed using [^{125}I]Tyr^{11}-SRIF-14 as specific radioligand. [^{125}I]Tyr^{11}-SRIF-14 exhibited high-affinity binding to all four receptor subtypes. Representative competition curves are shown in Fig. 3. The C specific binding of [^{125}I]Tyr^{11}-SRIF-14 was displaced by SRIF- 14 in a monophasic manner which is characteristic for binding to a single class of receptors. C The inhibitory concentrations for half maximal C inhibition (IC_{50}) were calculated to be $3.6 \pm 0.8 \times 10^{-10}$ M, $2.2 \pm 0.4 \times 10^{-10}$ M, $3.5 \pm 0.9 \times 10^{-10}$ M and $1.5 \pm 0.2 \times 10^{-9}$ M, (n = 3–6) for SSTR 1–4, respectively. As expected from previous studies the SRIF analogue SMS 201–995 displayed high-affinity binding only to cells expressing SSTR2 [15,23,24,36].

Very low specific binding of both [^{125}I]Tyr^{11}-SRIF-14 and [^{125}I]Tyr^{3}-SMS 201–995 could be determined on untransfected HEK 293 cells, consistent with the observation that 293 cells naturally express low levels of SSTR2 receptors [37]. However, this binding comprised less than 5 percent of the total [^{125}I]Tyr^{11}-SRIF-14 binding to the transfected cell lines.

3.3. *Second messenger coupling of SRIF receptors*

The ability of SRIF-14 and SMS 201–995 to interfere with the forskolin-stimulated rise in cAMP levels was studied in intact HEK 293 cells expressing human SSTR1–4. A concentration of forskolin was chosen (3 μM) which allows to detect both the stimulation and the inhibition of adenylyl cyclase [38]. In HEK 293 cells stably transfected with hSSTR1, 2, 3, or 4, forskolin stimulated cellular cAMP levels to more than ten times the basal levels. The cAMP formation was inhibited by SRIF-14 in a dose-dependent manner (Fig. 4). The maximal SRIF-14 mediated inhibition of cAMP formation was at least 70% for all four receptor subtypes. The somatostatin analog SMS 201–995, which displays high affinity for SSTR2 and a moderate affinity to SSTR3 [23,36] but low affinity for SSTR1 and SSTR4 [16,24], also inhibited the forskolin-induced cAMP formation in cells expressing SSTR2 and SSTR3 as expected.

Occasionally, SRIF-14 and SMS 201–995 slightly inhibited the forskolin-induced cAMP formation in untransfected HEK 293 cells to about 10 percent of the maximal response. This indicates that the strong inhibition of the cAMP formation seen in the transfected cells is mediated through the SRIF receptor subtypes overexpressed in the HEK 293 cells. The slight effect of nanomolar concentrations of SMS 201–995 in cells expressing SSTR1 and 4 may be related to the low SSTR2 background in these cells (Fig. 4).

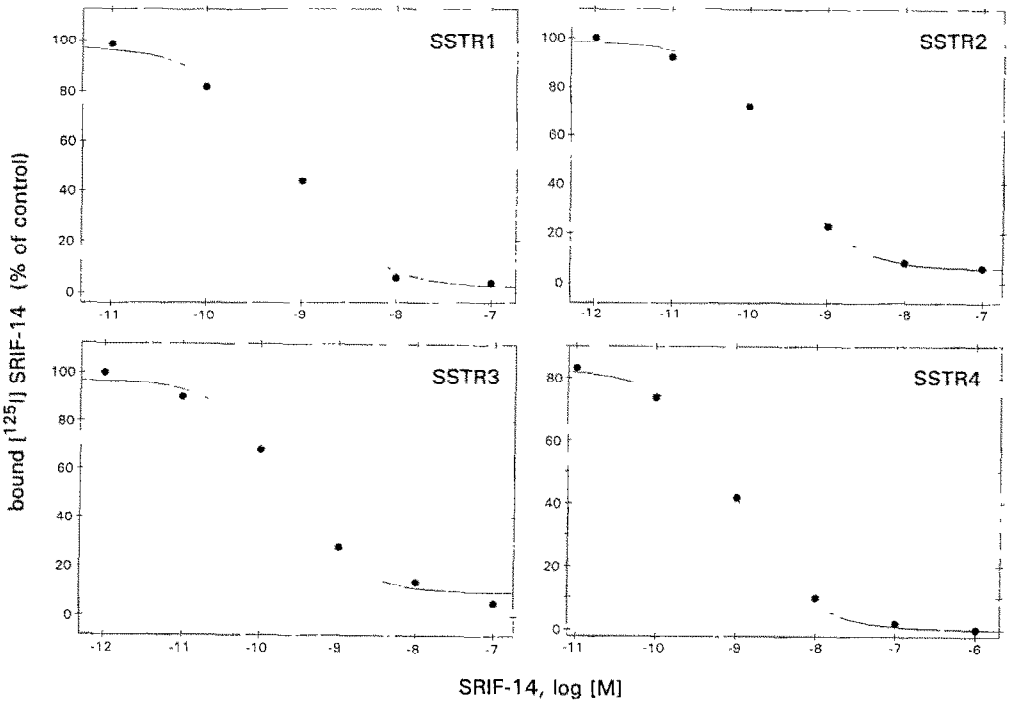

Fig. 3. Expression of SRIF receptor subtypes in HEK 293 cells Concentration dependence for the inhibition of ^{125}I-Tyr^{11}-SRIF-14 binding by unlabelled SRIF-14. Results are expressed as percentage of maximal specific binding in the absence of competitor. The non-specific binding was less than 20% of the total binding.

Pretreatment of the cells with pertussis toxin abolished the ability of the activated receptors to inhibit cAMP formation indicating that all four receptors inhibit adenylyl cyclase activity via pertussis toxin-sensitive G-proteins (Fig. 4).

4. DISCUSSION

The four somatostatin receptor subtypes SSTR1–4 studied are expressed in an overlapping, but distinct distribution in the rodent brain. The distribution of SSTR1–4 mRNAs as studied by Northern blot analysis and in situ hybridization is generally consistent with that of SRIF binding sites determined in autoradiographic studies [39–41]. A major exception was observed for the cerebellum. SRIF receptors are believed to be not expressed in the adult cerebellum but are transiently expressed during development [41–43]. However, SSTR3 mRNA was relatively abundant in adult cerebellum (Fig. 1). This apparent discrepancy between occurence of SSTR3 mRNA and receptor protein expressed in the cerebellum requires further analysis.

At present, it is difficult to correlate specific SRIF functions with the different receptor subtypes. Our previous conclusions that inhibition of GH release is mediated by the SSTR2 receptor type [15] is consistent with the characteristics of this receptor described here. In addition to its effects on the inhibition of hormone release SRIF acts as a neurotransmitter and may play a role in cell growth and in the development of the nervous system. SRIF has been reported to prevent naturally occuring motoneuron cell death in the embryonal spinal cord [7]. Interestingly, SSTR1 mRNA and, to a lower extent, SSTR2 mRNA could be detected in the spinal cord (Fig. 1). Conceivably, the neurotrophic actions of SRIF on these cells are mediated by SSTR1 or 2. In rodent epilepsy models, an anticonvulsant effect of somatostatin has been discussed [44]. The seizure activity caused by intrahippocampal injections of kainic or quinolinic acid, two excitatory amino acids acting on glutamate receptors, could be inhibited by local administration of SRIF or its analogue SMS 201–995. The expression of the SMS 201–995 sensitive receptor subtype SSTR2 in the hippocampus suggests that the anticonvulsant actions of SRIF may be mediated through SSTR2.

SRIF receptors which have been shown to belong to the family of G-protein coupled receptors, act on various cellular effector systems such as adenylyl cyclases [14], ion channels [4] or phosphatases [45]. In order to characterize the cellular effector systems of the cloned SSTR subtypes, human SSTR1–4 were stably expressed in HEK 293 cells. Agonist binding to all four receptors inhibited the forskolin-induced cAMP formation in cells expressing SSTR1–4. The inhibition was pertussis toxin-sensitive indicating the coupling of these receptors to pertussis toxin-sensitive G-proteins. Rat SSTR2 and human and mouse SSTR3 have previously been de-

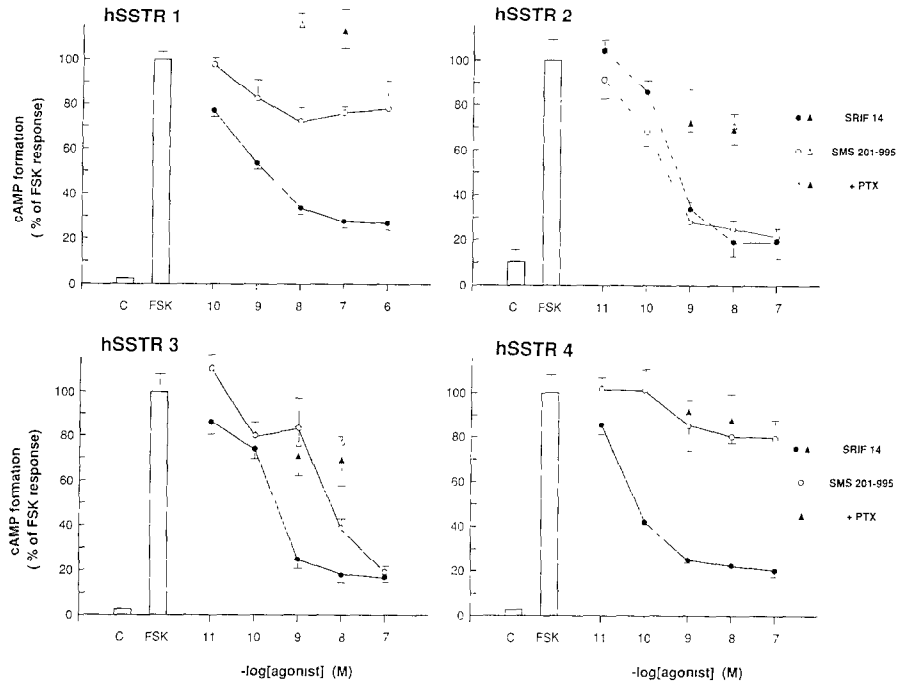

Fig. 4. Inhibition of forskolin-induced cAMP formation by agonist binding to SRIF receptors expressed in HEK 293 cells. The results of typical experiments are shown as mean ± S.E M. for duplicate or triplicate determinations c control (basal cAMP level), Fsk: forskolin, PTX: pertussis toxin.

cribed to mediate the inhibition of adenylyl cyclase [19,23,46]. In the present study we demonstrated that hSSTR1 and the recently cloned hSSTR4 inhibit adenylyl cyclase activity. In contrast, Reisine and co-workers reported that human SSTR1, after expression in CHO and COS cells, and human SSTR2, after expression in COS, CHO and HEK 293 cells, do not show this inhibitory effect on cAMP formation [36,37]. In agreement with this we did not find coupling of SSTR1 to adenylyl cyclase after stable transfection in CHO cells (data not shown). The negative coupling of SSTR2 to adenylyl cyclase is further confirmed by the fact that SRIF receptors in AR4–2J cells can inhibit adenylyl cyclase via pertussis toxin-sensitive G-proteins [47]. This pancreatic acinar cell line predominantly expresses SSTR2 ([15]; Kaupmann et al., unpublished). In addition, we observed negative coupling of the SSTR2 receptors to adenylyl cyclase in Neuro2A cells, which express this receptor subtype selectively (data not shown).

The cloning of a fifth SRIF receptor expressed in the pituitary has recently been published [22]. The DNA sequence, the binding properties, and second messenger coupling (inhibition of adenylyl cyclase) of this receptor are closely related to SSTR2.

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