The tetradecapeptide somatostatin (SRIF) is a hormone release-inhibiting substance that mediates diverse effects in brain and peripheral organs via specific receptors. A cDNA encoding a rat SRIF receptor was identified by use of degenerate oligonucleotide primers and polymerase chain reaction amplification of cDNA prepared from transcripts expressed in rat brain. The complete cDNA encodes a protein of 391 amino acids with seven potential transmembrane domains. Expression of the cDNA product in transfected COS-7 cell lines provides the same high affinity of binding to [\( ^{125} \text{I} \)-Tyr\(^{1} \)]SRIF-14 as that of rat cerebral cortex tissues. However, the binding of [\( ^{125} \text{I} \)-Tyr\(^{11} \)]SRIF-14 to cloned rat SRIF receptor is not displaced by MK678, a SRIF analog that partially displaces [\( ^{125} \text{I} \)-Tyr\(^{11} \)]SRIF-14 binding sites in membranes of rat cerebral cortex. Northern analysis and in situ hybridization indicate that mRNA (4.0 kilobases) for cloned rat SRIF receptor is preferentially expressed in rat brain regions such as cerebral cortex and hippocampus with no detectable expression in most peripheral organs. This pattern contrasts with the exclusive peripheral expression of a recently cloned human SRIF receptor.

The cDNA probe of rat receptor detects mRNA from mouse brain but not from human cerebral cortex and cerebellum.

**MATERIALS AND METHODS**

PCR—PCR to amplify cDNA encoding a potential G protein-linked receptor was performed as described previously (22). First strand cDNA for PCR reaction was generated from RNA of whole rat brain using murine leukemia virus reverse transcriptase (Bethesda Research Laboratories). The 25-μl reaction contained 1 μg of poly(A)\(^+\) RNA, 10 pmol of oligo(dT), 10 units of RNasin (Promega), 300 units of reverse transcriptase, and 0.5 mM dNTP. Following incubation at 37 °C for 1 h, 1 μl of reaction was amplified in a 25-μl reaction containing 50 mM KCl, 10 mM Tris (pH 8.3), 1.5 mM MgCl\(_2\), 200 mM dNTP, 10 mM dithiothreitol, 10 μM Tau1 polymerase (Bethesda Research Laboratories), and 10 pmol of both the reverse and forward primers. A nested PCR strategy was adopted in this study that employed two sets of degenerate oligonucleotide primers designed from cloned mammalian tachykinin receptors (23) and a Drosophila tachykinin receptor (24). The first set of primers corresponds to residues in TM2 and TM7 and the second set to residues in TM3 and TM6 that are conserved in all known tachykinin receptors. These primers were used in the first PCR reaction with total rat brain cDNA as a template. The first forward primer is ATG C/(A)GN ACN AA (encoding MRTVTN in TM2), and the first reverse primer is C/(A)GN ACN AT (encoding DRmA1 in TM3) and contains a BamHI site (underlined). The presence of restriction sites facilitated subsequent subcloning of amplification products. The second PCR was performed under conditions similar to the first except for 1 μl of product was then withdrawn and used as a template for a second PCR reaction using two additional oligonucleotide primers; the second forward primer is GCAG GGATCC AAA/(G)ICA T/(A)NGC A/(G)AA (encoding FAICW in TM7). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The abbreviations used are: SRIF, somatostatin; BR2, rat somatostatin receptor; SSRT1, human somatostatin receptor; PCR, polymerase chain reaction; TM, transmembrane domain; G protein, GTP-binding protein; PBS, phosphate-buffered saline.

(7, 8). Diminution of SRIF receptor density is reported in Alzheimer’s disease (9), whereas large numbers of high affinity SRIF binding sites occur in most pancreatic endocrine tumors and some meningiomas (10, 11).

Receptor binding sites have been characterized for SRIF in cerebral cortex and peripheral tissues (12–16). Apparent receptor subtypes have been discriminated with the drug MK678 (17). SRIF mediates its various functions through receptors that link to GTP-binding proteins (G protein, providing inhibition of adenylyl cyclase, reduction in calcium currents, or increases in potassium channel conductance (18–20). In a search for novel G protein-coupled receptors from mammalian brain using degenerate oligonucleotide primers in polymerase chain reaction (PCR), we have identified a SRIF receptor in rat brain. Functional expression studies show that this receptor represents a subtype with high affinity for [\( ^{125} \text{I} \)-Tyr\(^{11} \)]SRIF-14 but which is insensitive to the SRIF analog MK678. The sequence of the rat SRIF receptor closely resembles a very recently cloned human homolog (21), but tissue-specific expression of the rat SRIF receptor differs dramatically from the human homolog.
that annealing was performed at 47 °C, and the sample was amplified for 35 cycles. The final reaction was extracted with phenol/chloroform, precipitated with ethanol, and then digested with BamHI and SalI. The PCR product was electrophoresed on a 1% agarose gel and 400-base pair fragments (the size expected based on the known tachykinin receptors) cut from the gel and subcloned directly into the pBluescript vector. Dideoxynucleotide sequencing was then performed by the chain-termination method with Sequenase version 2 (United States Biochemical Corp.).

cDNA Cloning and Expression in Transfected Cell Line—The subcloned PCR fragment with sequence characteristics found in G protein-coupled receptors was labeled by the random-primed incorporation of [32P]dCTP and used as a probe to screen a rat brain cDNA library (provide by Dr. M. Chinkers, Vollum Institute, Oregon Health Sciences University). Five clones were obtained by screening 10^8 recombinants under high stringency hybridization (50% formamide, 5 × SSC, 5 × Denhardt’s, 25 mM sodium phosphate, 0.1% sodium pyrophosphate, 100 μg/ml yeast tRNA, and 1% SDS at 42 °C) and washing (0.2 × SSC at 55 °C). Restriction mapping and partial sequencing of these clones revealed that two were identical and the remainder differed in the length of 5'-untranslated sequences. BR2, the longest cDNA, was inserted into the pBluescript vector and sequenced as described above. This clone contained the expression vector pCIS-2 (Genentech) for transient expression in monkey tumor COS-7 cells or into pcDNA.neo vector for stable expression in human embryonic kidney 293 cells. A calcium phosphate method for transfection was employed as described previously (21). About 15 μg of plasmid DNA were used in each 90-mm plate for transfection. At 48-72 h after transfection, cells were rinsed with cold PBS buffer and scraped off the culture plates for receptor binding assay. For stable expression in human kidney 293 cells, 100 μg/ml genetin (G418) was added to minimal essential medium plus 10% fetal bovine serum to select 293 cells with stably expressed BR2 receptor.

Binding Assay—The cerebral cortex from adult Sprague-Dawley rat brain and cultured cells was homogenized in PBS solution and crude membranes obtained by centrifugation at 20,000 × g for 20 min at 4 °C. The membranes were resuspended in 50 mM Tris-HCl (pH 7.5), 5 mM MgCl₂, and 0.1% bacitracin. The microsomal membrane proteins (150 μg) were incubated for 30 min at 22 °C with [35S]-Tyr)SRIF-14 (Amersham Corp., 2200 Ci/mmol; 55,000 cpm) in a buffer containing 50 mM Tris-HCl (pH 7.5), 5 mM MgCl₂, 0.1% bovine serum albumin, and 0.1% bacitracin in a total volume of 200 μl. Different concentrations of unlabeled SRIF-14 and the SRIF analog MK678 (provided by Dr. T. Reins) were added to displace specific binding. The binding reaction was terminated by vacuum filtration over Whatman GF/C glass fiber filters which had been pre-soaked in 0.5% polyethyleneimine (w/v) and 0.1% bovine serum albumin. The filters were washed with 12 ml of cold Tris buffer (pH 7.5) and assayed in a γ counter (90% efficiency).

Northern Analysis—Total RNA from rat brain and peripheral organs was extracted by the acid guanidinium isothiocyanate-phenol-chloroform method (26). RNA from cerebral cortex and cerebellum of a normal human brain obtained at the time of autopsy from mouse brain were also prepared with the same method. RNA species were separated on denaturing gels, transferred to nylon supports, and hybridized as described previously (22, 24). Twenty μg of total RNA from each tissue sample were loaded onto the denatured gel, and the amount of RNA in the blot verified with staining with 0.1% methylene blue in 0.3 M sodium acetate (pH 5.2). Probes were generated by random priming of the BR2 cDNA (0.5 kb) in the presence of [32P]dATP. The (3 × 10⁶ cpm/ml) was hybridized on nylon membranes in hybridization solution (50% formamide, 5 × SSC, 50 mM sodium phosphate (pH 7.4)) for 1 h Denhardt’s solution, 0.1 mg/ml yeast tRNA, 0.1 mg/ml sonicated denatured salmon sperm DNA, 10 mM dithiothreitol) and then applied to sagittal or coronal sections on slides that were incubated overnight at 55 °C. After hybridization, slides were rinsed with 2 × SSC for 10 min and then treated with RNase A 20 μg/ml in 2 × SSC for 30 min at 37 °C. Subsequently, sections were rinsed in 2 × SSC, washed with 0.1 × SSC at 35 °C for 30 min, and finally dehydrated in a graded alcohol series and air-dried. Sections were exposed to Kodak XAR-5 x-ray film for 14 days, and films were subsequently developed.

RESULTS
cDNA Cloning and Sequence—PCR amplification of cDNA prepared from total rat brain mRNA with primers representing amino acid sequences found in the known tachykinin receptors resulted in a 400-base pair band. This size is consistent with that expected from one of the tachykinin receptors. Fragments of this size were isolated and subcloned. Restriction analysis and nucleotide sequencing show that the majority of the subcloned products represents the neuromedin K receptor (23). One product, however, has a unique sequence containing many of the characteristics of a G protein-coupled receptor. This product was used as a probe to identify a full-length cDNA from rat brain cDNA library. The sequence of the longest clone, BR2, contains one long open reading frame encoding a protein of 391 amino acids and approximate molecular mass of 43.4 kDa (Fig. 1). Hydropathy analysis of the derived amino acid sequence indicates the presence of seven hydrophobic regions with the potential to represent transmembrane domains separated by stretches of hydrophilic residues (data not shown), a structure consistent with that of a G protein-coupled receptor. The amino- and carboxyl-terminal regions of BR2 also show structural characteristics of G protein coupled receptors, including asparagine (Asn⁶), Asn¹⁶, and Asn²⁸) residues that are potential N-glycosylation sites, 7 serine (Ser) and 3 threonine (Thr) residues in the carboxyl terminus that may serve as possible phosphorylation sites (28). Thr⁷² and Ser²⁰⁹ are located in the regions of intracellular loops II and III, respectively, and may serve as putative phosphorylation sites for cAMP-dependent protein kinase (29). BR2 also contains residues in potential transmembrane domains whose positions are conserved in most G protein-coupled receptors; for example, an acidic residue within TM2, the Arg-Ser-Ser sequence in the second intracellular loop, and cysteine residues in the first and second extracellular loops which may form a structurally important disulfide bond (30). Surprisingly, BR2 does not appear to be related to the tachykinin receptors. Within transmembrane domains (from residues 57 to 320), BR2 shares little homology to the tachykinin receptors (about 25% amino acid identity) as well as most other G protein coupled receptors.

Functional Expression—The short length (25 residues) of the cytoplasmic domain between TM5 and TM6 in the BR2 cDNA may make it a poor peptide receptor, suggesting that BR2 cDNA may encode a peptide receptor. We first attempted to identify BR2 protein by expression in Xenopus oocytes of RNA transcribed in vitro from the cDNA (22, 24). No responses (voltage-clamp at -60 mV) were observed with thyrotropin releasing hormone, gonadotropin-releasing hormone, angiotensin II, vasooactive intestinal polypeptide, SRIF,

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14, bradykinin, vasopressin, oxytocin, neuropeptide Y, cholecystokinin-8, pentagastrin, galanin, baclofen, or opioid peptides (each 100 nM to 10 μM). Since activation of chloride currents of Xenopus oocytes reflects activation of phosphoinositide turnover, the unsuccessful identification of BR2 using Xenopus oocytes suggested that this receptor may couple to adenyl cyclase rather than phosphoinositide turnover. Very recently Yamada et al. (21) cloned cDNAs for two human SRIF receptors and their mouse homologs. BR2 cDNA possesses 97.5% amino acid identity to one of the cloned human SRIF receptor, SSTR1, and 98.7% identity to its mouse homolog. There is less than 45% identity between the amino acid sequences of BR2 and the other cloned human SRIF receptor, SSTR2. Fig. 1B shows a few amino acid residues that differentiate BR2 and SSTR1 sequences, most of which are near the NH2 terminus of receptors.

To confirm that cloned BR2 cDNA encodes a rat SRIF receptor, we transfected monkey tumor COS-7 cells with BR2 cDNA. BR2 receptor expressed in COS-7 cells binds [125I-Tyr11]SRIF-14 saturably with unlabeled SRIF-14 displaying an IC50 of 0.5 nM (Fig. 2A). The cloned BR2 receptor has also been stably expressed in human fetal kidney cell line 293, and a similar binding affinity to [125I-Tyr11]SRIF-14 closely resembles rat cerebral cortex SRIF receptors (Fig. 2B). The IC50 for unlabeled SRIF is similar for both cloned BR2 receptor and rat brain cortex. MK678, an analog of SRIF, does not displace [125I-Tyr11]SRIF-14 binding to the BR2 receptor in transfected COS-7 cells, even at 10 μM concentration (Fig. 2A). By contrast, binding in rat
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**Fig. 2.** Displacement of $[^{125}I]$-Tyr$^1$SRIF-14-specific binding to BR2 receptor expressed in transfected COS-7 cell membranes (A) and rat cerebral cortex cell membranes (B) by SRIF-14 and its analog MK678. $[^{125}I]$-Tyr$^1$SRIF-14 (55,000 cpm, 2200 Ci/mmol, Amersham Corp.) was incubated for 60 min at 22°C with transfected COS-7 cell membranes (15 µg of protein) or rat brain cortex cell membranes (40 µg of protein) in the presence of increasing concentration of competitors. Results are expressed as percent displacement of $[^{125}I]$-Tyr$^1$SRIF-14 binding. Values are means of three different experiments, each done in duplicate for each competitor. The total counts bound in the absence of SRIF-14 are 6000 cpm for transfected COS-7 cell membranes and 7000 cpm for rat brain cortex cell membranes. Nonspecific binding in the presence of 1 µM SRIF-14 was less than 10% of total counts bound.

Cerebral cortex is displaced by MK678 in a biphasic pattern. The high-affinity component is inhibited 50% by 0.6 nM MK678. Inhibition by MK678 plateaus at 10 nM with about 40% of total $[^{125}I]$-Tyr$^1$SRIF-14 binding unaffected by 1 µM MK678. In confirmation of the findings of Raynor and Reisine (17), these findings suggest multiple SRIF receptors in rat brain with different affinities for MK678. BR2 protein appears to reflect a MK678-resistant SRIF receptor.

**Northern Analysis and in Situ Hybridization—**Total RNA was extracted from rat brain regions, including cerebellum, cerebral cortex, hippocampus, striatum, as well as pituitary, heart, small intestine, lung, adrenal gland, and stomach. Northern blot reveals a hybridizing band (4.0 kb) in all brain regions with particularly intense hybridization in cerebral cortex and hippocampus (Fig. 3A). There is also a lower level of expression of BR2 receptor in cerebellum, striatum, pituitary, and lung. Most peripheral organs, however, do not show detectable hybridization to BR2 cDNA probe, indicating that the cloned SRIF receptor is likely to be a neuronal SRIF receptor. These findings contrast with the tissue distribution of human homolog SSTR1 that is highly expressed in stomach and jejunum but is undetectable in human cerebral cortex (21). We also examined the hybridization of the BR2 cDNA probe to RNA prepared from human cerebral cortex and cerebellum as well as mouse whole brain and forebrain under the same hybridization condition. We detect a hybridizing band in mouse total brain and forebrain but not in human brain tissue (Fig. 3B). The hybridizing band in mouse brain is the same size as that in rat brain.

We assessed the location of the cloned SRIF receptor in rat brain by in situ hybridization. Substantial hybridization is evident in cerebral cortex and hippocampus (Fig. 4A). Hybridization in hippocampus encompasses pyramidal cells of CA1 through CA4, whereas the label in the dentate gyrus is...
moderately higher than the hippocampus. Substantial mRNA is also evident in granule cells of cerebellum, in the olfactory bulb, and in the olfactory tubercle. Under the same hybridization conditions, control experiments with sense RNA probe reveal only weak nonspecific hybridization to granule cells in cerebellum with no labeling in cerebral cortex and hippocampus (Fig. 4B). Thus, a portion of the hybridization signal in granule cells of cerebellum using antisense RNA probes may be nonspecific. However, the prominent hybridization in cerebral cortex and hippocampus and most of the hybridization in cerebellum reflects specific labeling and is consistent with the results of Northern analysis. Other brain regions, such as corpus striatum and brain stem, show low levels of hybridization signals.

**DISCUSSION**

The diverse functions of SRIF in the mammalian brain are mediated by multiple receptors. In this study we have cloned the cDNA encoding a rat brain SRIF receptor and examined its expression in brain and peripheral tissues. The rat SRIF receptor shares sequence and structural properties with the family of seven transmembrane domain receptors. Unlike other peptide receptors, BR2 receptor also carries an aspartic acid residue (Asp) in the third transmembrane domain. This Asp residue has been found in all G protein-coupled receptors for biogenic amines, including receptors for catecholamines, acetylcholine, dopamine, and histamine (30, 31), but not in receptors for peptides or amino acids. This Asp residue has been thought to be critical for binding of protonated amines, since its replacement with glutamate in &beta;-adrenergic receptors greatly reduces affinity of receptor for ligands and agonist potency (32). Whether this charged residue is also involved in the binding to SRIF peptides remains to be determined.

Very recently, two human SRIF receptors have been cloned from human pancreatic islets (21). The amino acid sequence of rat BR2 protein shows 97.5% identity to one (SSTR1) of the human SRIF receptors. Therefore, human SSTR1 and the cloned rat BR2 receptor reflect the same subtype of SRIF receptor in different species. High affinity SRIF receptors have been demonstrated previously both by homogenate binding assays and autoradiography in rat brain (13, 36). At least two SRIF receptors in rat brain have been identified with SRIF-14 and its analogs (13, 17, 33). SRIF-14 and its biological precursor SRIF-28 display a reverse rank order of potencies in displacing the binding of radiolabeled SRIF agonists to rat SRIF receptors in brain and pituitary (13). By contrast, the cloned human analog SSTR1 does not show dramatically different affinities for SRIF-14 and SRIF-28 (21). In this study we compared the binding of cloned rat receptor to radiolabeled SRIF-14 and its analog MK678. MK678 is a stable cyclohexapeptide analog that distinctly labels a subpopulation of brain SRIF receptors, being almost 4000-fold more potent at brain SRIF receptors labeled by 125I-MK678 than those labeled by 125I-CGP23996 (17). Expression of the cloned BR2 receptor in COS-7 cells confirms that the cloned rat receptor has the same high affinity for SRIF as receptors in rat brain cortex. Our study also shows that MK678 has high and low affinities, respectively, for two populations of SRIF receptors labeled by 125I-Tyr1-SRF-14 in rat brain. MK678 does not displace the binding of 125I-Tyr1-SRF-14 to BR2 receptors in transfected cells, indicating that the BR2 receptor represents a subtype of brain SRIF receptors that is insensitive to MK678.

The regional distribution of BR2 mRNA is similar whether assessed by Northern analysis or in situ hybridization. The in situ hybridization pattern, with substantial densities in the cerebral cortex, the granule cell layer, and the dentate gyrus and pyramidal cell layer of the hippocampus, resembles autoradiographic maps of SRIF receptors (10, 34, 35). Limited differences in the pattern of label in the hippocampus may reflect labeling of different subtypes by autoradiography and in situ hybridization as well as variations in the localization of receptor mRNA versus protein.

One of the striking findings of the present study is the contrasting tissue expression of BR2 receptor and its human homolog SSTR1 (Table 1). These differences are especially notable, considering that the two receptors have 97.5% amino acid sequence identity, and both are derived from a single 4.48-kb mRNA. SSTR1 expression is restricted to peripheral tissues with highest levels in jejunum and stomach, low levels in colon and kidney, and undetectable levels in human cerebral cortex (Ref. 21; Table 1). By contrast, the rat SRIF receptor is brain-selective with high levels in hippocampus and cerebral cortex and undetectable levels in stomach, small intestine, kidney, and many other peripheral tissues. We have confirmed the negligible mRNA level for SSTR1 or BR2-like receptors in human brain by the cross-hybridization of BR2 cDNA probe to RNA from human brain tissues. BR2 cDNA probe detects mRNA from mouse but not human brain. The remarkable differences in tissue localization between BR2 and SSTR1 despite their extremely similar amino acid sequences might have at least two explanations. One is that BR2 and SSTR1 are generally homologous, reflecting species variation of the same gene. In this case the human and rat promoters and enhancers would provide markedly different tissue-specific expression. Another is that BR2 and SSTR1 represent distinct members of a closely related family of genes.

### Acknowledgments

We thank Drs. Michael Chinkers and John Adelman (Vollum Institute, Portland, OR) for providing rat brain cDNA library and suggestion for PCR, respectively; Dr. Terry Reisine (University of Pennsylvania School of Medicine) for providing MK 678; Yan-Na Wu (Vollum Institute) for her technical assistance in studies of expression of BR2 receptor in Xenopus oocytes; and Audrey Bentley for her technical assistance in in situ studies.

### REFERENCES


### TABLE 1

**Comparison of tissue-specific expression of messengers for rat SRIF receptor (BR2) and human SRIF receptor (SSTR1)**

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Identity of amino acids</th>
<th>mRNA (size)</th>
<th>Central nervous system</th>
<th>Peripheral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cortex</td>
<td>Hippocampus</td>
</tr>
<tr>
<td>Ret BR2</td>
<td>97.5%</td>
<td>4</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Human SSTR1</td>
<td></td>
<td>4.8</td>
<td>- ND</td>
<td>+++</td>
</tr>
</tbody>
</table>

* ND, not determined.

See Ref. 21.
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