Differential Expression of Somatostatin Receptor Subtypes in Brain

Christopher D. Breder,¹ Yuichiro Yamada,^{3,a} Kazuki Yasuda,³ Susumu Seino,^{4,5,b} Clifford B. Saper,^{1,2,c} and Graeme I. Bell^{3,4,5}

¹Committee on Neurobiology and the Brain Research Institute, Departments of ²Pharmacological and Physiological Sciences and Neurology, ³Department of Biochemistry, ⁴Department of Medicine, and ⁵Howard Hughes Medical Institute, University of Chicago, Chicago, Illinois 60637

The tetradecapeptide somatostatin has been implicated as an important regulator of neuronal and neuroendocrine function in the CNS. The cellular actions of somatostatin are mediated by specific receptors. The genes encoding two different somatostatin receptors (SSTRs) have been isolated and characterized, and RNA blotting studies have shown that both SSTR1 and SSTR2 are expressed in the brain. In order to gain a better understanding of the functions of somatostatin in the CNS, the distribution of SSTR1 and SSTR2 mRNAs was determined using the technique of *in situ* hybridization. SSTR1 mRNA was present throughout the mouse brain, particularly in the supra- and infragranular layers of the cortex, the amygdala, hippocampus, bed nucleus of the stria terminalis, substantia innominata, hypothalamus, pre-tectum, substantia nigra, parabrachial nucleus, and nucleus of the solitary tract. SSTR2 mRNA was primarily observed in the infragranular layers of the cortex, the amygdala, claustrum, endopiriform nucleus, arcuate and paraventricular nuclei of the hypothalamus, and medial habenular nucleus. Several regions of the brain reported to contain dense somatostatin-like immunoreactive terminal fields and receptor binding sites were devoid of both SSTR1 and SSTR2 mRNA, suggesting the existence of additional SSTR subtypes.

Somatostatin (SST), the somatotropin-release inhibitory factor, is a tetradecapeptide that was first identified in extracts of ovine hypothalamus by the inhibition of secretion of growth hormone (GH) from pituitary cell cultures (Brazeau et al., 1973). Subsequent studies have revealed multiple forms of this peptide in brain (Benoit et al., 1982) and have demonstrated the presence of SST-like immunoreactivity in neurons, nerve fibers, and presumptive terminal fields throughout the brain (Johansson et al., 1984; Vincent et al., 1985; Shimada and Ishikawa, 1989). Phys-

iological studies (e.g., Wang et al., 1989) and the broad distribution of SST in the CNS suggest that SST may regulate many aspects of CNS function including neuroendocrine control (Lumpkin et al., 1981; Murakami et al., 1987; Horvath et al., 1989), autonomic function (Cunningham and Sawchenko, 1989; Nabekura et al., 1989; Rettig et al., 1989), nociception (Shimada and Ishikawa, 1989), appetitive behavior (Lin et al., 1987), and arousal (Danguir, 1986). SST has also been implicated in neuronal development (Kimura, 1989; Villar et al., 1989; Kentroti and Vernadakis, 1990), and altered levels and distribution of SST-like immunoreactivity have been noted in neuronal degenerative states such as Alzheimer's disease (Dawbarn et al., 1986; Unger et al., 1988) and Huntington's disease (Marshall and Landis, 1985).

The cellular actions of SST are mediated by specific high-affinity receptors (Srikant and Patel, 1981). The distribution of these receptors in the rat brain has been mapped by receptor-binding autoradiography using radiolabeled SST-14 and -28 as well as SST analogs such as CGP-23996 and MK-678 (Leroux et al., 1985; Uhl et al., 1985; Martin et al., 1991). These studies have shown differences in the pattern and levels of binding of these peptides in the brain, implying that the actions of SST may be mediated by a family of receptors, an idea supported by binding and physiological studies using SST-14 and its analogs (Wang et al., 1989). In addition, biochemical studies have identified a number of SST-binding proteins of various sizes from about 27 to 90 kDa that can be labeled by cross-linking with SST and its analogs (Thermos et al., 1989; Patel et al., 1990). However, until recently it was not clear whether these proteins represented distinct receptors.

We have recently cloned and characterized the genes for two new members of the family of receptors with a seven-transmembrane segment that bind [¹²⁵I-Tyr¹¹]-SST-14 specifically and with high affinity (Yamada et al., 1992). We termed these two SST receptor (SSTR) isoforms SSTR1 and SSTR2. Although SSTR1 and SSTR2 bind SST-14 with a similar affinity, their sequences are quite divergent and there is only 49% amino acid identity and 64% similarity between them. RNA blotting studies using human SSTR1 and SSTR2 probes revealed high levels of SSTR1 mRNA in the human gastrointestinal tract including small intestine and stomach and high levels of SSTR2 mRNA in the brain and kidney. RNA blotting studies with the mouse probes have showed that both SSTR1 and SSTR2 mRNAs were present in the adult mouse brain. Using *in situ* hybridization, we have determined the anatomical pattern of expression of transcripts of these two receptors in the adult mouse brain. These studies revealed distinct but overlapping patterns of expression

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Correspondence should be addressed to Dr. Graeme Bell, Howard Hughes Medical Institute, University of Chicago, 5841 South Maryland Avenue, MC1028, Chicago, IL 60637.

^aPresent address: Second Department of Medicine, Kyoto University School of Medicine, Kyoto 606, Japan.

^bPresent address: Division of Molecular Medicine, Center for Neurobiology and Molecular Immunology Chiba School of Medicine, Chiba 280, Japan.

^cPresent address: Department of Neurology, Beth Israel Hospital, Boston, MA.

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of each isoform in this tissue. In addition, the absence of noticeable hybridization in certain regions of the brain where previous receptor-binding autoradiography studies showed SST binding suggests that one or more additional SST isoform may be expressed in the mouse CNS.

Materials and Methods

General methods. Standard methods were carried out as previously described (Sambrook et al., 1989; Yamada et al., 1992). For Northern blotting, 20 μ g of total adult mouse brain RNA was denatured with glyoxal, electrophoresed in a 1% agarose gel and blotted using capillary action to a Hybond-N nylon membrane (Amersham, Arlington Heights, IL). The membrane was hybridized with nick-translated 32 P-labeled inserts from the mouse SST1 and SST2 probes described below.

Mouse SST1 and SST2 cDNA probes. The mouse SST1 probe was a 413 base pair (bp) *Ban*II-*Acl*I fragment of the gene that encodes amino acids 214–352, and the mouse SST2 probe was a 458 bp *Bst*EII-*Xba*I fragment of the gene that encodes amino acids 254–369, the stop codon, and 107 nucleotides of the 3'-flanking/untranslated region; the sequences of the mouse SST1 and SST2 genes are in the GenBank database with accession numbers M81831 and M81832, respectively. There is 54% overall nucleotide sequence identity between the mouse SST1 and SST2 sequences in the region of homology between these probes; the longest stretch of identity is 15 nucleotides. We examined the Northern blot for evidence of cross-hybridization with these probes using moderately stringent hybridization and washing conditions [hybridization: 5 $\times$ saline-sodium citrate (SSC), 50% formamide, 20 mM sodium phosphate, pH 6.5, 100 μ g/ml of sonicated and denatured salmon testes DNA, 2 $\times$ Denhardt's solution, and 10% dextran sulfate at 42°C for 16–20 hr; and washing in 0.1 $\times$ SSC and 0.1% sodium dodecyl sulfate at room temperature and 50°C for 1 hr each]. The DNA fragments described above were cloned into pGEM-3Z. To obtain antisense probes, the plasmids were linearized with *Eco*RI and transcribed with the Gemini II system (Promega, Madison, WI) using SP6 RNA polymerase and 35 S-uridine 5'-[α -thio]triphosphate (DuPont-New England Nuclear, Boston, MA). Sense probes were prepared from *Hind*III-linearized plasmid DNA using T7 RNA polymerase. The probes were diluted to 10⁷ cpm/ml in 10 ml aliquots of hybridization mixture that contained 50% formamide, 10 mM Tris HCl, pH 8 (GIBCO-Bethesda Research Labs, Gaithersburg, MD), 5 mg tRNA (Boehringer-Mannheim, Indianapolis, IN), 10 mM dithiothreitol (DTT), 10% dextran sulfate, 0.3 M NaCl, 1 mM EDTA, pH 8.0, and 1 $\times$ Denhardt's solution (Sigma, St. Louis, MO). Aliquots were stored at –20°C. Prior to use for *in situ* hybridization, mixtures were heated to 65°C for 10 min and centrifuged at 4000 rpm.

In situ hybridization. *In situ* hybridization was performed using methods similar to those described by Simmons et al. (1989). Briefly, five 6–8-week-old BalbC mice (Harlan Sprague-Dawley) were deeply anesthetized and perfused transcardially with saline followed by 4% paraformaldehyde solution at pH 6.5 and then at 9.5. Brains were postfixed overnight in the pH 9.5 fixative containing 20% sucrose. Serial 25 μ m coronal sections were cut on a sliding microtome and stored in a cryoprotectant solution (Simmons et al., 1989) containing 50% neutral buffered formalin (Richard-Allan, Richland, MI) at –20°C until they were mounted on gelatin-polylysine-subbed slides. Slides were dried in a vacuum desiccator overnight and immersed in 10% neutral buffered formalin for 2 hr at 4°C immediately prior to hybridization, and then incubated in 0.001% proteinase K (Boehringer-Mannheim, Indianapolis, IN) for 30 min, incubated in 0.025% acetic anhydride (Sigma, St. Louis MO) for 10 min, and dehydrated in ascending concentrations of ethanol. One hundred and twenty microliters of hybridization mixture were applied to each slide, and a glass coverslip was applied (Clay Adams, Lincoln Park, NJ) and sealed with DPX (Gallard and Schlesinger, Calle Place, NY). Slides were incubated overnight at 56°C. The following day, the slides were cooled to room temperature and the coverslips removed and washed four times in 4 $\times$ SSC (Sigma). Sections were incubated in a solution of 0.002% RNase A (Boehringer Mannheim), 0.5 M NaCl, 10 mM Tris HCl, pH 8, and 1 mM EDTA for 30 min at 37°C and rinsed in 2 $\times$, 1 $\times$, 0.5 $\times$, and then 0.1 $\times$ SSC containing 0.25% DTT. Sections were incubated in 0.1 $\times$ SSC with 0.25% DTT for 30 min at 60°C, and dehydrated in 50% and 75% ethanol that contained 0.1 $\times$ SSC and 0.25% DTT, followed by 95% and three changes of 100% ethanol. Slides were air dried and stored overnight in a vacuum desiccator. Sections were dipped in NTB2 photographic emulsion (Inter-

national Biotechnologies Incorporated, New Haven, CT), dried, and stored with desiccant in foil-wrapped slide boxes at 4°C for 2–4 weeks. Slides were developed with D-19 developer (Kodak, Rochester, NY), counterstained for 3 min in thionin, dehydrated in graded ethanols, cleared in xylene, and coverslipped with DPX (Gallard and Schlesinger).

Control studies. The specificity of *in situ* hybridization was determined by comparing the results of hybridization with antisense and sense probes to adjacent tissue sections. Northern blot analysis was also performed on mouse brain RNA using the inserts from the same DNA constructs used for *in situ* hybridization.

Results

In situ hybridization to sections of the adult mouse brain using SST1- and SST2-specific probes showed distinctive patterns of expression of these genes with respect to both anatomical distribution and relative level of expression. In contrast, hybridization with the sense-strand probe controls showed only sparse, diffusely scattered silver grains (Fig. 1). RNA blotting showed the presence of both SST1 and SST2 mRNAs in adult mouse brain (Fig. 2). The mouse SST1 and SST2 probes used for RNA blotting, which were the same as those used for the *in situ* hybridization studies described below, hybridized with distinct transcripts of 3.8 and 2.3 kilobases, respectively, and no cross-hybridization was observed using normal hybridization and washing conditions. The *in situ* hybridization results obtained with antisense cRNA probes were essentially identical in each of the five brains used in this study. SST1 expression was observed throughout the mouse brain, particularly in forebrain regions and brainstem structures, whereas SST2 expression was limited to the forebrain. In most regions that we judged to contain specific hybridization, clusters of autoradiographic silver grains surrounded lightly thionin-stained nuclei. In some regions, especially regions with high densities of labeled cells, it was not possible to identify separate clusters. This was especially true in the piriform cortex, the stratum pyramidale of the hippocampus (Fig. 5A,B), and the granular layer of the dentate gyrus for both probes; in the periamygdaloid cortex, the bed nucleus of the anterior commissure, and the medial accessory olivary nucleus for SST1; and in the medial habenular nucleus for SST2 (Fig. 3C). In the absence of clustering, such hybridization was identified as specific by comparison to adjacent, unlabeled regions in the same section, and by the absence of such labeling in the sections hybridized with the sense probe. An overview of the pattern of hybridization is presented in Table 1. In the description that follows, the term *density* will be used to describe the number of grain clusters or compactness of non-aggregated grains in a nucleus or region; the *intensity* of hybridization will refer to the number of silver grains within an individual cluster.

Anatomical distribution

Telencephalon. Both receptor transcripts were expressed in the *neocortex*. SST1 was primarily observed in the infragranular and, to a lesser extent, in the supragranular layers (Fig. 4A), and SST2 hybridization was observed only in the infragranular layer. Both forms were observed in cortical fields of the *allocortex* with distinct characteristics of laminar distribution and intensity (Fig. 4A,B). We have chosen representative regions of the mouse *allocortex* to present in Table 1. Light-to-medium levels of both forms were observed in the dorsal part of the *hippocampus* (Fig. 5A,B). The density and intensity of SST1 hybridization increased dramatically in a septotemporal and dorsal-to-ventral gradient, particularly in the stratum pyrami-

Figure 1. Dark-field photomicrographs of SSTR and SSTR2 expression in the neocortex of the mouse brain. *A*, SSTR1 expression in the second somatosensory cortex. Expression was densest in the infragranular layers 1 and 2. A moderate level of expression was observed in the supragranular layers 2 and 3. *B*, A dark-field photomicrograph of the adjacent mouse brain section hybridized with the sense-strand SSTR1 probe. *C*, SSTR2 expression in the second somatosensory cortex. Expression was primarily observed in the infragranular layers, although a few scattered clusters were observed in layers 2 and 3. *D*, A dark-field photomicrograph of the adjacent mouse brain section hybridized with the sense-strand SSTR2 probe. Scale bar, 200 μ m.

dale of CA3, dentate granular layer, and subiculum (Fig. 4*B*). SSTR1 was observed throughout the nuclei of the *amygdala* (Fig. 4*A,B*), while SSTR2 expression was primarily observed in the lateral, basal magnocellular (Fig. 3*B*), and posterior cortical nuclei and in the amygdalohippocampal area. In the *subcortical telencephalon*, both forms were observed in the claustrum, endopiriform, and diagonal band nuclei. The claustrum contained the densest and most intense clusters of SSTR2 hybridization

Figure 2. Northern blot analysis of SSTR1 and SSTR2 mRNA in the mouse brain. Twenty micrograms of mouse brain RNA were denatured with glyoxal, electrophoresed in a 1% agarose gel, blotted to a nylon membrane, and hybridized with nick-translated 32 P-labeled inserts of the mouse SSTR1 or SSTR2 probes. Note the absence of cross-hybridization.

in the mouse brain (Fig. 3*A*). SSTR1 hybridization was also observed in the bed nucleus of the stria terminalis, ventrolateral part of the lateral septal nucleus, magnocellular preoptic nucleus (Fig. 6*A*), plexiform layer of the olfactory tubercle, ventral pallidum, globus pallidus, and substantia innominata. The density and intensity of clusters of SSTR1 hybridization in the substantia innominata increased in a rostral-to-caudal gradient. No hybridization with either SSTR1 or SSTR2 was observed in the caudate-putamen.

Diencephalon. There was little hybridization in the *thalamus*, although both SSTR forms were present in a limited distribution. A dense patch of high-intensity SSTR1 hybridization capped the dorsal part of the paraventricular nucleus of the thalamus, while the body of this nucleus was devoid of clusters. Sparse levels of light-intensity clusters were observed in the ventromedial part of the reticular nucleus of the thalamus, adjacent to the zona incerta. SSTR1 was also observed in the internal and dorsal, "relatively cell-free" zones of the ventral lateral geniculate nucleus (Swanson et al., 1974) and in the lateral habenular, posterior intralaminar, posterior limitans, and supragenicular nuclei (LeDoux et al., 1985) and the zona incerta. The densest SSTR2 hybridization was observed in the medial habenular nucleus (Fig. 3*C*). Observation of this nucleus with high-power, bright-field magnification revealed a few very high-intensity clusters within the very dense patch of apparently non-aggregated grains, suggesting that the pattern resulted from dense packing of moderately to intensely labeled clusters over individual cells.

In the *hypothalamus*, both SSTR forms were observed in the medial parvicellular subnucleus of the paraventricular nucleus and in the arcuate and posterior periventricular nuclei (Fig.

Figure 3. *In situ* hybridization showing expression of SSTR2 in the forebrain. *A*, Dark-field photomicrograph demonstrating dense labeling in the claustrum (*Cl*) and endopiriform nucleus. Labeling in the agranular insular cortex (*AIC*; above the rhinal fissure, *rf*) was restricted to layers 5 and 6. *B*, In the amygdala, hybridization was primarily observed in the lateral (*LA*) and basal (*BA*) magnocellular nuclei. Labeling in the adjacent perirhinal cortex (*PRH*) was limited to the deeper layers, while in the piriform cortex (*PIR*), clusters were primarily observed over layer 2 (*arrow*) and to a lesser extent in layer 3 (*arrowhead*). *C*, In the epithalamus, dense SSTR2 hybridization was observed in the medial habenular nucleus (*MH*). 1–6, layers of the insular cortex; *cp*, cerebral peduncle; *ot*, optic tract; *P*, putamen. Scale bar: 250 μ m for *A*; 950 μ m for *B*; 35 μ m for *C*.

4A,B). In the arcuate nucleus, the densest and most intense clusters of hybridization of both SSTR1 and SSTR2 were seen in the dorsal and ventrolateral part of the nucleus, while moderate levels of medium-intensity clusters were observed in the ventromedial part. Dense SSTR1 hybridization was also present in the ventral part of the suprachiasmatic nucleus, and more moderate hybridization was observed throughout the preoptic, anterior, tuberal, and posterior hypothalamus (Fig. 4A,B). In the tuberal part of the lateral hypothalamic area, a group of clusters extended with a gradient of decreasing density from the ventrolateral edge of the fornix to the ventromedial edge of the optic tract (Fig. 4A). SSTR2 hybridization was also observed in

the internal lamina of the median eminence and the retrochiasmatic and lateral hypothalamic areas. In the posterior part of the lateral hypothalamic area, clusters of silver grains extended from the ventrolateral edge of the fornix to the ventromedial edge of the internal capsule.

Midbrain and pretectal area. SSTR1 hybridization was observed in several nuclei in the midbrain including the superior and inferior colliculi, periaqueductal gray matter, substantia nigra pars reticulata, the nucleus of the optic tract, and the anterior pretectal, anteromedian, Edinger–Westphal, and dorsal and median raphe nuclei (Fig. 7A). No SSTR2 expression was observed in these levels of the mouse brain. The density of SSTR1 clusters

Table 1. Pattern of expression of SSTR1 and SSTR2 mRNAs in the brain of the adult mouse

Region	SSTR1 mRNA	SSTR2 mRNA
Telencephalon		
Isocortex		
I	+	—
II–III	++	—
IV	+	—
V–VI	+++	+++
Allocortex (cortical fields)		
Piriform cortex		
I	—	—
II	<u>++</u>	<u>++</u>
III	++	+
Anterior cingulate cortex		
I	—	—
II–IV	++	—
V	+++	++
VI	++	++
Dysgranular insular cortex¹		
I	—	—
II–III	+++	++
IV	+	+
V	+++	++
VI	++	++
Agranular insular cortex²		
I	—	—
II–III	++	+++
V	++	+++
VI	+	++
Granular retrosplenial cortex³		
I	—	—
II–III	<u>++</u>	+
IV	—	—
V	++	+++
VI	++	++
Agranular retrosplenial cortex³		
I	—	—
II–III	—	—
V	++	+++
VI	++	++
Hippocampus		
Dorsal part		
Subiculum	+	+
CA1		
Stratum pyramidale	<u>++</u>	<u>++</u>
Suprapyramidal	+	—
Infrapyramidal	+	—
CA3		
Stratum pyramidale	<u>+</u>	<u>+</u>
Suprapyramidal	+	—
Infrapyramidal	+	—
Dentate gyrus		
Granular layer	<u>++</u>	<u>+</u>
Molecular layer	<u>+</u>	<u>—</u>
Hilus	++	+
Ventral part		
Subiculum	++++	++
CA1		
Stratum pyramidale	++	<u>++</u>
Suprapyramidal	++	+
Infrapyramidal	+	—

Table 1. Continued

Region	SSTR1 mRNA	SSTR2 mRNA
CA3		
Stratum pyramidale	++++	<u>++</u>
Suprpyramidal	+	—
Infrapyramidal	++	+
Dentate gyrus, granular layer	<u>++++</u>	<u>++</u>
Amygdala ⁴		
N. of the lateral olfactory tract, layers 2–3	++	—
Medial n.		
Superficial layer	+++	+
Deep part	++	—
Anterior cortical n.		
Layer 1	+	—
Layer 2	+++	—
Layer 3	++	—
Periamygdaloid cortex		
Layer 1	—	+
Layer 2	<u>++</u>	—
Layer 3	++	—
Lateral n.	+++	+++
Basal n.		
Magnocellular division	++	+++
Parvocellular division	+++	++
Central n.		
Medial part	+	—
Lateral part	++	—
Accessory basal n.	++	—
Posterior cortical n.	+++	++
Amygdalohippocampal area	+++	+++
Subcortical telencephalon		
Clastrum	+++	++++
Endopiriform n.	++	+++
Olfactory tubercle, Plexiform layer	+	—
Bed n. of the stria terminalis		
Medial zone	+	—
Central zone	++	—
Lateral zone	+	—
Preoptic portion	+	—
Bed n. of the anterior commissure	<u>+++</u>	—
Lateral septal n. ⁵ Ventrolateral part	+	—
Nucleus of the diagonal band		
Vertical part	++	+
Horizontal part	++	—
Magnocellular preoptic n. ⁶	+++	—
Substantia innominata	+++	—
Striatum		
Globus pallidus	+	—
Ventral pallidum	+	—
Diencephalon		
Thalamus		
Medial habenular n.	—	<u>++++</u>
Lateral habenular n.	+	—
Paraventricular n. (rostral, dorsal part)	+++	—
Reticular n.	+	—
Ventral lateral geniculate n. ⁷	++	—
Posterior intralaminar n. ⁸	++	—
Supragenulate n. ⁸	+	—
Posterior limitans n. ⁸	+	—
Hypothalamus ⁹		
Vascular organ of the lamina terminalis	+	—

Table 1. Continued

Region	SSTR1 mRNA	SSTR2 mRNA
Median preoptic n.	++	—
Anteroventral periventricular n. ¹⁰	++	—
Periventricular preoptic n.	++	—
Medial preoptic n.	++++	—
Median preoptic area	++	—
Lateral preoptic area	++	—
Anterior hypothalamic area	++	—
Periventricular n.	+	—
Suprachiasmatic n., ventral portion	+++	—
Paraventricular n.		
Anterior parvicellular subn.	++	—
Medial parvicellular subn.	++	++
Dorsal subn.	+	—
Lateral parvicellular subn.	+	—
Posterior magnocellular subn.	++	—
Retrochiasmatic area	+	++
Arcuate and posterior periventricular n.	++++	+++
Median eminence, internal lamina	—	+
Dorsomedial n.	+	—
Ventromedial n. ¹¹		
Dorsomedial part	++	—
Ventrolateral part	+	—
Perifornical area	++	—
Lateral hypothalamic area	++	++
Supramammillary n.	++	—
Posterior hypothalamic area	+++	—
Pretectum ¹² and midbrain		
N. of the optic tract	++	—
Anterior pretectal n.	++	—
Ventral tegmental area	+++	—
Substantia nigra, pars reticulata	+++	—
Peripeduncular n.	+	—
Superior colliculus		
Superficial	+	—
Intermediate	+++	—
Deep	++	—
Inferior colliculus ¹³		
Internal division	+	—
External division	+++	—
Interpeduncular n. ¹⁴		
Rostral dorsal, caudal dorsal and caudal lateral subn.	++	—
Anteromedian n.	++	—
Edinger–Westphal n.	++	—
Periaqueductal gray matter		
Dorsolateral	++	—
Ventrolateral	+++	—
Medial	+	—
Raphe n. ¹⁵		
Dorsal n.	++	—
Median n.	++	—
Cuneiform n.	++	—
Pedunculopontine tegmental n.	++	—
Central tegmental n.	++	—
Pons		
Parabrachial n. ¹⁶		
Central lateral subn.	++	—
Dorsal lateral subn.	++	—
External lateral subn.	+	—

Table 1. Continued

Region	SSTR1 mRNA	SSTR2 mRNA
Internal lateral subn.	++	—
Superior lateral subn.	++	—
Ventral lateral subn.	++	—
Medial n.	+++	—
External medial subn.	+++	—
Kölliker-Fuse n.	++	—
Locus coeruleus	+++	—
Mesencephalic n. of V	++	—
Principle n. of V	++	—
Medulla		
N. of the solitary tract ¹⁷		
Rostral part	++	—
Medial subn.	+++	—
Dorsomedial subn.	++	—
Parvicellular subn.	+++	—
Ventrolateral subn.	++	—
Commissural subn.	++	—
Area postrema		
Lateral part	++++	—
Core	+	—
Subpostrema	++	—
Dorsal motor n. of X	+	—
N. ambiguus	+	—
Hypoglossal n.	+	—
Reticular formation, parvicellular part ¹⁷	++	—
Spinal n. of V ¹⁸		
Subn. zonalis	+++	—
Subn. gelatinosa	+	—
Gigantocellular field, pars alpha	+	—
Medial accessory olive	<u>++</u>	—

Relative distribution of SSTR1 and SSTR2 in the mouse brain is expressed in the following density range for clusters: —, not observed; +, rare clusters; ++, moderate numbers of lightly labeled clusters; +++, moderate numbers of intensely labeled clusters; +++++, frequent, moderately or intensely labeled clusters. Double underline (e.g., ++) denotes the relative density of *nonaggregated grains*. I–IV, cortical laminae; n., N., nucleus; n. of V, nucleus of the trigeminal nerve; n. of X, nucleus of the vagal nerve. Nomenclature derived from specific literature citations is noted: 1. Cechedo and Saper, 1987; 2. Saper, 1982; 3. Zilles, 1985; 4. Price et al., 1987; 5. Swanson and Cowan, 1979; 6. Saper, 1984; 7. Swanson et al., 1974; 8. LeDoux et al., 1985; 9. Swanson, 1987; 10. Saper et al., 1983; 11. Saper et al., 1976; 12. Scalia, 1972; 13. Berman, 1968; 14. Hemmendinger and Moore, 1984; 15. Taber et al., 1960; 16. Fulwiler and Saper, 1984; 17. Herbert et al., 1990; 18. Olszewski, 1960.

in the superior colliculus increased in a rostral-to-caudal gradient and posteriorly covered the intermediate and deep layers with a continuous layer of clusters.

Pons. SSTR1 hybridization was also observed in several nuclei of the pons, particularly the locus coeruleus, mesencephalic and principle sensory nuclei of the trigeminal nerve, and the parabrachial nucleus, while no pontine SSTR2 expression was observed. SSTR1 clusters were especially prominent in the ventral part of the locus coeruleus.

Medulla. SSTR1 hybridization was also observed in several regions of the medulla, and was especially dense in the dorsal vagal complex (Fig. 7*B*). In the spinal trigeminal nucleus, dense clusters of silver grains were grouped inside the medial (deep) border of the gelatinous subnucleus, adjacent to the magnocellular subnucleus (Olszewski, 1960). SSTR2 hybridization was not observed in this level of the brain.

Cerebellum. Neither SSTR1 or SSTR2 hybridization was observed in this part of the adult mouse brain.

Discussion

We have used cRNA probes to investigate the distribution of two SSTR forms, termed SSTR1 and SSTR2, in the mouse brain. SSTR1 expression was observed throughout all levels of the brain, particularly in the cortex, hippocampus, amygdala, and several brainstem structures, while SSTR2 was only observed in forebrain structures (Fig. 8). Sense transcript hybridization resulted in only background levels of silver grains. The data suggest that SSTR1 and SSTR2 have distinct patterns of expression both with regard to location and level of expression in the adult mouse brain.

Cellular localization

In this study, we describe the characteristic regional distribution of SSTR1 and SSTR2 mRNAs and propose that these patterns represent neuronal hybridization. The hybridization was restricted to specific nuclei and areas, and in most areas the ag-

Figure 4. Dark-field photomicrographs of SSTR1 hybridization. *A*, Anterior tuberal hypothalamus, amygdala, and adjacent cortex. At this level, hypothalamic labeling was primarily observed in the arcuate nucleus (ARH), extending in an arc (curved arrow) from the fornix to the ventromedial edge of the optic tract in the lateral hypothalamic area, and in a crescent-shaped cap (straight arrow) corresponding to the dorsomedial part of the ventromedial nucleus of the hypothalamus. Hybridization was observed throughout the amygdala, particularly in the anterior cortical (CoA), medial (MeA), basal magnocellular, lateral, and central nuclei, as well as in the periamygdaloid cortex. Pronounced labeling was observed in the supra- and infragranular layers of the cerebral cortex. *B*, At the posterior tuberal hypothalamic level, expression was observed in the arcuate nucleus and lateral hypothalamic area (LHA). In the telencephalon, very dense labeling was observed in the posterior cortical nucleus of the amygdala (PCo), subiculum (S), dentate granule cell layer (DG), CA3 cell field of the ventral hippocampal formation (CA3), and entorhinal cortex. The arrowhead indicates the rhinal fissure. 1–6, cortical laminae; 3v, third ventricle; Hipp, dorsal part of the hippocampus. Scale bar, 800 μ m.

gregated silver grains constituted the clusters that surrounded thionin-stained neuronal nuclei. We saw only diffuse hybridization over cell groups with a high packing density of labeled cells, and even in these cases, some clusters of silver grains could still be identified over cell bodies. Neither receptor form was observed in regions such as the cerebellum, caudate-putamen (Fig. 3*A*), or ventrobasal nuclei of the thalamus (Fig. 4*B*), nor was labeling observed over fiber tracts. These results suggest that the hybridization was both specific and neuronal, as SSTR

Figure 5. *In situ* hybridization showing expression of SSTR1 in the rostral dorsal part of the hippocampus. *A*, A dark-field photomicrograph illustrating the density of diffuse hybridization over the pyramidal (sp; see labels in *B*) and granule cell layers. The arrowhead delineates the border of between the CA1 and CA2 cell fields of the hippocampus. Sparse clusters of silver grains were observed in the stratum oriens (so) and infrapyramidal cell layers, particularly in the stratum radiatum (sr) of the CA fields. Moderate numbers of grain clusters were observed in the hilus of the dentate gyrus (DG) at this level. *B*, High-power photomicrograph of the boxed area in the CA1 field in *A* depicting the restricted distribution of grains over the pyramidal cell body layer. Note the tendency of silver grains to cluster (arrows) over the densely packed cell layer. CTX, cortex. Scale bar: 400 μ m for *A*; 40 μ m for *B*.

transcripts in astrocytes, microglia, or oligodendroglia would be expected to give rise to a more widespread pattern.

Anatomical considerations: comparison to receptor binding studies

The patterns of SSTR1 and SSTR2 hybridization in the mouse brain were similar to portions of the previously described anatomical distributions of binding sites of SST-14, SST-28, and their analogs in the mammalian brain. Several studies have mapped the distribution of SST-14 binding sites in the rat brain (Leroux et al., 1985; Whitford et al., 1987). Scatchard analysis of the binding of this ligand with rat brain membranes suggested the presence of two high-affinity binding sites (Weightman et al., 1985). The distribution of the regions with the densest binding in these studies (cf. Fig. 1*C* of Whitford et al., 1987) is remarkably similar to the pattern of SSTR2 hybridization, par-

Figure 6. Dark-field photomicrograph of *in situ* hybridization showing expression of SSTR1 at the level of the basal forebrain. *A*, Preoptic level of the diencephalon. Dense hybridization was observed in the medial (MPN) and magnocellular (MgPO) preoptic nuclei. *B*, In the paraventricular nucleus of the hypothalamus (PVH), clusters of silver grains (arrows) were observed in the parvicellular divisions. 3V, third ventricle; LPO, lateral preoptic area; oc, optic chiasm. Scale bar: 300 μ m for *A*; 120 μ m for *B*.

ticularly in the cortex, claustrum, and amygdala (Fig. 3*A,B*). Several SST-14 binding regions such as the lateral septum, nucleus of the lateral olfactory tract, inferior colliculus, and locus coeruleus exhibited only SSTR1 hybridization.

SST-28 (Leroux et al., 1985) and the analog LTT-S28 (Uhl et al., 1985; Patel et al., 1986; Leroux et al., 1991) have been used to investigate the distribution of putative SST-28 binding

Figure 7. Dark-field photomicrographs of SSTR1 mRNA expression in the brainstem. *A*, In the midbrain, heavy clusters were observed in the substantia nigra pars reticulata (SNr). SSTR1 hybridization extended through the ventral tegmental area (VTA), into the periaqueductal gray matter (PAG), and over the Edinger–Westphal nucleus. Labeling also circumscribed the medial geniculate body (MG) and extended into the pretectum and tectum. In anterior tectum, hybridization was primarily observed in the intermediate layer of the superior colliculus (SC). *B*, Dense SSTR1 expression was also observed in the dorsal vagal complex. Dense levels of very high-intensity clusters were observed in the area postrema (AP) and in the medial (m) and parvicellular (pc) subnuclei of the nucleus of the solitary tract. Clusters were also observed in the dorsomedial (dm) and ventrolateral (vl) solitary subnuclei. Few clusters were observed over the dorsal motor vagal nucleus (DMV). AM, anteromedian nucleus; c, commissural subnucleus of the solitary tract; cc, central canal; CTF, central tegmental field; ts, solitary tract. Scale bar: 800 μ m for *A*; 400 μ m for *B*.

Figure 8. Schematic diagram of a sagittal section of the mouse brain illustrating the distribution of SSTR1 and SSTR2. *Shading*, Regions that expressed both SSTR1 and SSTR2; *circles*, SSTR1 only; *triangles*, SSTR2 only. 1–6, cortical laminae; 3v, third ventricle; 4v, fourth ventricle; AMY, amygdala; AP, area postrema; ARH, arcuate nucleus of the hypothalamus; BST, bed nucleus of the stria terminalis; Cb, cerebellum; cc, corpus callosum; Cl, claustrum (and endopiriform nucleus); DR, dorsal raphe nucleus; fx, fornix; HIPP, hippocampus; IC, inferior colliculus; LC, locus coeruleus; LHA, lateral hypothalamic area; LS, lateral septal nucleus; LV, lateral ventricle; ME, median eminence; MH, medial habenular nucleus; MPO, medial preoptic nucleus (and the anteroventral region around the third ventricle); NTS, nucleus of the solitary tract; PB, parabrachial nucleus; PHA, posterior hypothalamic area; PVH, paraventricular nucleus of the hypothalamus; PVT, paraventricular nucleus of the thalamus; RCA, retrochiasmatic area; SC, superior colliculus; scp, superior cerebellar peduncle; SN, substantia nigra, pars reticulata; SNV, spinal nucleus of the trigeminal nerve; VDB, vertical limb of the nucleus of the diagonal band; VMH, ventromedial nucleus of the hypothalamus; VTA, ventral tegmental area.

sites in the rat brain. Both ligands bind to numerous structures in the rat brain, most of which are consistent with the pattern of expression of SSTR1 mRNA. However, the medial habenular nucleus (Leroux et al., 1985; Uhl et al., 1985) and the median eminence (Reubi et al., 1987), where only SSTR2 hybridization was observed, bound moderate-to-high amounts of SST-28 in these studies. Furthermore, SSTR2 hybridization was observed in many of the regions with the highest ligand binding, such as the infragranular layers of the cortex (Fig. 3A), basal and lateral nuclei of the amygdala (Fig. 3B), and the arcuate and paraventricular nuclei of the hypothalamus (Uhl et al., 1985). These results suggest that these ligands may be less selective in labeling specific SSTR forms, or that they may recognize additional subtypes.

Rens-Domiano et al. (1992) recently showed selective binding of the SST analog CGP-23996 to Chinese hamster ovary cells expressing SSTR1 and of another analog, MK-678, to cells expressing SSTR2. In binding studies (Epelbaum et al., 1985; Martin et al., 1991), MK-678 labeled the claustrum more heavily than CGP-23996, which is consistent with the relatively denser level of hybridization of SSTR2 than SSTR1 observed in our study. The relative binding of these ligands in the basal and lateral nuclei of the amygdala (Fig. 4A) and the CA1 field of the hippocampus (Fig. 5A,B) were also congruent with our findings. In addition, higher levels of MK-678 binding were observed in the medial habenular nucleus (Martin et al., 1991), which showed dense SSTR2 hybridization (Fig. 3C). Numerous regions where

MK-678 binding was observed did not display SSTR2 hybridization, particularly the medial and cortical nuclei of the amygdala, paraventricular nucleus of the thalamus, superior colliculus, periaqueductal gray matter, and the interpeduncular and peripeduncular nuclei, yet we observed moderate-to-dense levels of SSTR1 hybridization in these areas (Figs. 4A,B; 6A). Martin et al. (1991) noted a lower level of CGP-23996 binding between the supra- and infragranular layers of the prefrontal cortex and labeling in the substantia nigra restricted to the pars reticulata. Both of these observations were strikingly similar to the pattern of hybridization we observed with the SSTR1 probe (Figs. 4A,B; 6A). Epelbaum et al. (1985) observed CGP-23996 binding in the lateral habenular nucleus and anterior pretectal, trigeminal, and parabrachial nuclei; our studies showed that each of these regions expressed SSTR1 mRNA (Table 1). By contrast, low levels of CGP-23996 binding in the hypothalamus and elevated MK-678 binding in the striatum and substantia nigra pars compacta were inconsistent with the specificity of these ligands for SSTR1 and SSTR2.

Certain discrepancies exist between the pattern of SSTR1 and SSTR2 expression in the adult mouse brain and the distribution of binding sites for SST and its analogs in the rat brain. While this may be a species difference, in part it may reflect the fact that protein levels may be regulated by posttranscriptional events beyond cellular mRNA levels. In addition, there may be other SSTR isoforms expressed in brain in addition to SSTR1 and SSTR2. Finally, considerable ultrastructural (Alonso et al., 1985;

Obata-Tsuto, 1987) and pharmacological (Ipp et al., 1979; Peterfreund and Vale, 1984) data support the existence of SST autoreceptors. In this instance, the presynaptic receptor located on the axon terminal and its mRNA (located in the cell body) would not have the same regional distribution. Despite these caveats, the distribution of SSTR1 and SSTR2 hybridization displays some striking similarities and interesting differences with respect to ligand binding distributions.

Heterogeneity of SSTRs

Functional considerations. SST has been implicated in the control of neuroendocrine (Brazeau et al., 1973), autonomic (Brown, 1988), and cognitive function (Beal et al., 1986). We observed extensive hybridization in regions of the CNS thought to regulate these functions, as well as in nuclei involved in sensory afferent processing. In most of the latter regions, SSTR1 and SSTR2 were observed in divisions or subnuclei that connective and physiological data suggest are involved in the processing of polymodal rather than primary sensory afferent information. These areas are discussed in conjunction with those traditionally thought to be involved in cognitive function.

Neuroendocrine regulation. SST was first isolated from the hypothalamus because of its ability to inhibit pituitary secretion of GH. SST is also active in the hypothalamo-hypophyseal axis in the regulation of thyrotropin- and luteinizing hormone-releasing hormone (-RH) secretion (Lumpkin et al., 1981), as well as in the regulation of the magnocellular secretory system (Brown, 1988; Rettig et al., 1989), particularly vasopressin secretion.

The relationship of central SST systems to hypophyseal GH secretions is currently a topic of intense interest and increasing complexity. Immunohistochemical and pharmacological data provide evidence for at least two functionally distinct populations of SST-immunoreactive neurons in the periventricular and mediobasal hypothalamus. Double-labeling experiments combining subpial injections of HRP into the median eminence with SST immunohistochemistry suggest that neurons in the anterior periventricular hypothalamus and parvicellular portions of the paraventricular nucleus of the hypothalamus give rise to the neurosecretory projection to the neurohemal zone of the median eminence (Merchenthaler et al., 1989). Ultrastructural studies of the periventricular zone reveal SST-immunoreactive innervation of SST-immunoreactive structures (Alonso et al., 1985). This observation led Alonso to suggest that SST may regulate its own secretion in the tuberoinfundibular system. This concept is supported by studies that demonstrated a decrease in SST secretion from hypothalamic explants and cultured neurons after exposure to SST (Peterfreund and Vale, 1984; Epelbaum et al., 1986) and a paradoxical increase of plasma GH after intraventricular injection of SST (Lumpkin et al., 1981; Murakami et al., 1987). We observed both SSTR1 and SSTR2 in the medial parvicellular division of the paraventricular nucleus of the hypothalamus. Only SSTR1 was expressed in the anteroventral preoptic and anterior periventricular nuclei of the hypothalamus and in the anterior parvicellular division of the paraventricular nucleus of the hypothalamus (Fig. 6B). Future experiments injecting ligands specific for these SSTR forms into the periventricular hypothalamus, directed at blocking the secretion of SST, would be of considerable interest.

The primary source of GH-RH in the tuberoinfundibular system is the cells in the ventral and lateral portion of the arcuate nucleus of the hypothalamus (Swanson, 1987). Immunohistochemical studies at the light and ultrastructural level (Horvath

et al., 1989) suggested that these neurons share bidirectional connections with SST-immunoreactive neurons that are also located in the arcuate nucleus. Interestingly, dense hybridization of both SSTR1- and SSTR2-specific probes were observed in the ventrolateral arcuate nucleus, where GH-RH-immunoreactive neurons are observed in the rat brain (Fig. 3A,B), as well as in the dorsomedial portion of the nucleus. Immunohistochemical and *in situ* hybridization double-labeling experiments would be of considerable interest in determining whether SSTR1 and SSTR2 are expressed in GH-RH-immunoreactive neurons. Dense levels of high-intensity SSTR1 expression was also observed in the regions of the brain that project to the arcuate nucleus in the rat brain, such as the medial preoptic nucleus (Fig. 6A; see Simerly and Swanson, 1988), ventral subiculum (Fig. 4B; see Kohler, 1990), and to a lesser extent the bed nucleus of the stria terminalis (Swanson and Cowan, 1979). Our data suggest that SSTR1 and SSTR2 are present in multiple levels of the tuberoinfundibular axis as well as in the forebrain nuclei that project to this zone, implying that these receptors may be involved in the central integration of autonomic, behavioral, and neuroendocrine control.

Central autonomic regulation. Intraventricular infusions of SST-14 and -28 in the rat brain result in a pressor and bradycardic response that is thought to be primarily mediated by an elevation of peripheral levels of vasopressin (Brown, 1988; Rettig et al., 1989). Brown injected SST-28 into several forebrain regions involved in autonomic regulation as well as into the dorsal vagal complex. He concluded that the paraventricular nucleus was the locus of this pressor response, although the volume of the injections (500 nl) employed in this study would preclude precise localization of this effect. We observed SSTR1 in several regions involved in regulation of the magnocellular secretory system, including the division of the paraventricular nucleus containing vasopressin-immunoreactive neurons in the mouse brain (C. D. Breder and C. B. Saper, unpublished observation) as well as forebrain nuclei that project to this region in the rat brain, such as the medial preoptic (Simerly and Swanson, 1988) and anteroventral periventricular (Standaert and Saper, 1988) nuclei and bed nucleus of the stria terminalis (Sawchenko and Swanson, 1983). We also observed SSTR2 expression in the internal lamina of the median eminence, the region of the infundibulum through which the axons of the magnocellular secretory system project to the posterior pituitary gland (Swanson, 1987). Interestingly, Brown (1988) observed a depressor effect after injection of SST-28 into the anterior hypothalamic area, which most likely would have included the medial preoptic nucleus, and also the central nucleus of the amygdala, where we observed SSTR1 hybridization. Injections of smaller volumes of ligands specific for SSTR1 into these nuclei would be of considerable interest in examining the role of SST in central cardiovascular regulation.

Several cardiovascular and pulmonary responses have been described after intraparenchymal or intracisternal infusion of SST, to examine its effects in the dorsal vagal complex and ventral lateral medulla. Five hundred nanoliter injections of SST-28 into the nucleus of the solitary tract resulted in a depressor and bradycardic response (Koda et al., 1985; Brown, 1988). Expiratory apnea was induced after intracisternal SST-14 infusion in the rat (Harfstrand et al., 1984). Jacquin et al. (1988) recorded intracellularly in neurons of the nucleus of the solitary tract of the rat and observed that application of SST-14 and -28 hyperpolarized cells through the augmentation of

I_M , a voltage-dependent potassium current. Striking SSTR1 hybridization was observed in the medial and commissural subnuclei of the nucleus of the solitary tract, which receives visceral sensory input from the cardiovascular and pulmonary systems (Loewy, 1990) and in the ventrolateral subnucleus, also known as the dorsal respiratory group because it contains neurons with inspiratory-related discharges (Smith et al., 1989). Yamamoto et al. (1986) applied SST to the ventral medullary surface of the cat brain and observed strong apnea with a complete cessation of phrenic nerve activity. Injections into the ventrolateral medulla resulted in an apneic and depressor response. Furthermore, Cunningham and Sawchenko (1989) described a dense SST-immunoreactive projection from the nucleus of the solitary tract to the nucleus ambiguus. Sparse SSTR1 and no SSTR2 hybridization was observed throughout these latter medullary structures. The possibility of an additional, yet uncharacterized SSTR in these latter regions involved in crucial roles in central autonomic function is certainly intriguing.

Complex, integrative function: cognition and polymodal sensory processing. SSTR1 and SSTR2 were observed in structures such as the cortex, hippocampus, and basal and lateral nuclei of the amygdala, which are involved in complex, integrative activities (Figs. 2A,B; 3A,B; 5A,B). Previous studies have demonstrated SST-immunoreactive nerve fibers (Shimada and Ishikawa, 1989) and electrophysiological effects of SST (Watson and Pittman, 1988; Wang et al., 1989) in these regions. Both SSTR forms were observed in the claustrum (Fig. 3A), the vertical limb of the nucleus of the diagonal band, and the basal and lateral nuclei of the amygdala (Figs. 3A, 4A), all of which share bidirectional projections with the cerebral cortex (Saper, 1987). SSTR1 expression was also observed in regions that project diffusely to the cerebral cortex such as the magnocellular preoptic nucleus (Fig. 6A), the lateral hypothalamic (Fig. 4A,B) and ventral tegmental areas (Fig. 7A), and the dorsal raphe, pedunculo-pontine, and medial parabrachial nuclei (Saper, 1987).

The distribution of SSTR1 in the hippocampus is particularly intriguing (Fig. 5A,B). The septotemporal gradient of SSTR1 in the hippocampus was similar in topography to the distribution of afferent projections from the medial septal and magnocellular preoptic nuclei and the nucleus of the diagonal band (Wyss et al., 1979; Gage et al., 1984; Saper, 1984), as well as the ventral and lateral part of the nucleus reuniens of the thalamus (Riley and Moore, 1981) and the dorsal raphe nucleus (Wyss et al., 1979) in the rat brain. The basal nuclei of the amygdala (Figs. 3B, 4A), which expressed both SSTR forms, are reciprocally connected with several polymodal association areas, including the medial, orbital, and insular cortices, and portions of the hippocampal formation in the rat brain. It is therefore likely that SST may be active at multiple levels of the pathways influencing complex integrative function. Interestingly, each of the regions implicated in complex, integrative functions, and in which we observed SSTR1 and SSTR2, demonstrates pathological changes in Alzheimer's disease (Saper, 1988).

Different SSTR types

Previous ligand binding (Patel et al., 1986; Reubi et al., 1987; Whitford et al., 1987; Matsumoto et al., 1991), analog cross-linking (Thermos et al., 1989; Patel et al., 1990), and physiological studies (Wang et al., 1989; Dichter et al., 1990) suggested that multiple SSTRs are present in the brain. Our data not only provide evidence for the expression of two distinct forms of SSTRs in the mouse brain but also suggest the existence of other

forms. The neuronal localization of SSTR1 and SSTR2 was indicated by their anatomical distribution and hybridization over thionin-stained cell bodies. Marin (1991) recently reported that SST potentiated α_1 -adrenergic activation of phospholipase C in astrocyte cultures. We did not observe diffuse hybridization, which would be expected if this cell type expressed SSTR1 or SSTR2 (cf. McGrail et al., 1991; Watts et al., 1991). It is possible that astrocytes express another form of SSTR or are induced to express SSTR1 or SSTR2 in culture.

One of our most intriguing findings was the remarkably sparse hybridization in the striatum with both SSTR1 and SSTR2 probes, particularly in the caudate-putamen. This was surprising given previous reports on the high density of SST-immunoreactive fibers and terminals in the striatum (Chesselet and Graybiel, 1986), the profound effects of SST on striatal second messenger systems (Markstein et al., 1989; Marin et al., 1991), and the high level of [125 I-Tyr¹¹]-SST binding to striatal membranes (Srikant and Patel, 1981; Tran et al., 1985). Morphological analysis of striatal SST-immunoreactive neurons suggests that SSTRs would be localized in the extrastriosomal matrix (Chesselet and Graybiel, 1986), perhaps even functioning as autoreceptors on SST-immunoreactive cells. These data strongly suggest the existence of other SSTR subtypes in the mammalian brain.

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