

Characterization of a human gene related to genes encoding somatostatin receptors

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Received 16 September 1996; revised version received 2 October 1996

Abstract We report the identification of a gene, named *SLC-1*¹, encoding a novel G protein-coupled receptor (GPCR). A customized search procedure of a database of expressed sequence tags (dbEST) retrieved a human cDNA sequence that partially encoded a GPCR. A genomic DNA fragment identical to the cDNA was obtained and used to screen a library to isolate the full-length coding region of the gene. This gene was intronless in its open reading frame, and encoded a receptor of 402 amino acids, and shared ~40% amino acid identity in the transmembrane (TM) regions to the five known human somatostatin receptors. Northern blot analysis revealed that *SLC-1* is expressed in human brain regions, including the forebrain and hypothalamus. Expression in the rat was highest in brain, followed by heart, kidney, and ovary. Expression of *SLC-1* in COS-7 cells failed to show specific binding to radiolabelled Tyr¹-somatostatin-14, naloxone, bremazocine, 1,3-di(2-tolyl)-guanidine (DTG), or haloperidol. A repeat polymorphism of the form (CA)_n was discovered in the 5'-untranslated region (UTR) of the gene and *SLC-1* was mapped to chromosome 22, q13.3.

Key words: Polymerase chain reaction; Chromosome; Northern blot; Intronless

1. Introduction

Somatostatin peptides are widely distributed in central and peripheral tissues, participating in numerous and diverse physiological processes, including the regulation of GH and TSH secretion from the pituitary and the inhibition of secretion of gastrointestinal and pancreatic hormones and enzymes [1]. The effects of somatostatin are mediated by G protein-coupled receptors (GPCR) that have affinity for both major peptide products of somatostatin gene expression, SS-14 and SS-28. Five different somatostatin receptors (SSTR) have been identified [2–4], each having a distinct pharmacological profile and distribution. These receptors are structurally similar and studies have revealed that each subtype has a characteristic pattern of tissue expression (reviewed in [5]). It has been suggested that the SSTR family may not be complete, and that

other subtypes may yet be identified. In searching a database of EST cDNAs for sequences encoding GPCRs, we identified a fragment with significant homology to the SSTRs. We isolated this novel gene encoding a receptor related to the somatostatin receptor family, with highest expression in the human forebrain and hypothalamus.

2. Material and methods

2.1. Searching the database of expressed sequence tags (dbEST)

We queried the dbEST maintained by the National Center for Biotechnology Information (NCBI), with the complete amino acid sequence of GPCRs, such as the α -adrenoceptor, using the TBLASTN algorithm [6]. EST sequences that were returned having statistically significant scores were examined further. The conceptualized amino acid sequences of the EST sequences were used to query [7,8] our GPCR database using the FastA algorithm to determine whether the EST cDNAs represented known GPCRs [9]. The amino acid sequences thus filtered were used to query the SwissProt (release 31) database using the FastA algorithm (BLOSUM 50 matrix, ktup=1) [7,8]. The sequence of one EST (cloneID: c-1zf10; GenBank Acc. No.: F07228) that met these criteria was used for further investigation.

2.2. PCR amplification and genomic library screening

As the EST fragment identified from the computerized database searches was unavailable from the IMAGE consortium, human genomic DNA was amplified using the polymerase chain reaction (PCR) using a set of specific oligonucleotides designed based on the EST cDNA sequence (P1: 5'-CGGAATTCCTGGGCATCATCGGGAAGTC CACG; P2: 5'-CGTCTAGACAGGAGGCAGATCAC-CAGGGTGGC). Each primer contained a self-inserted restriction enzyme recognition sequence (*Eco*RI for P1 and *Xba*I for P2) to facilitate subcloning. The PCR conditions were as follows: denaturation at 94°C for 1 min, annealing at 55°C for 2 min and extension at 72°C for 2 min for 30 cycles, followed by a 7 min extension at 72°C [10]. The resultant PCR products were phenol/chloroform extracted, precipitated with ethanol, phosphorylated with T4 polynucleotide kinase, and blunt-ended with Klenow enzyme [11]. Subsequently, they were electrophoresed on a 0.5% low-melting point agarose and a fragment of the expected size was subcloned into the *Eco*RV site of pBluescript SK(–) (pB/S; Stratagene, La Jolla, CA). Colonies were selected, plasmid DNA was purified, and the inserts sequenced. An insert identical in overlapping sequence with the EST cDNA was purified, radiolabeled with [α -³²P]dCTP (NEN, Boston, MA) by nick translation (Amersham, Arlington Heights, IL) and used to screen a EMBL3 SP6/T7 human genomic library (Clontech, Palo Alto, CA). Positive phage clones were plaque purified and DNA was prepared. Following restriction digestion, the DNA was electrophoresed on an agarose gel, transferred to nylon membrane, and hybridized with the same probe used to screen the library, as described by Marchese et al. [12].

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¹The sequences of *SLC-1* also called GPR24 are available under accession numbers U71092 (human) and U77953 (rat).

2.3. PCR amplification of rat orthologue

Rat genomic DNA was PCR-amplified using degenerate oligonucleotides designed based on the sequence encoding putative TM3 (P3: 5'-CTGACCGCATGRSCATTGAC SGCTAC; Y=C or T, R=A or G, S=C or G) and TM7 (P4: 5'-GGGGTTGRSGCAGCTGTTGGCCTA) of the receptor encoded by *SLC-1* and somatostatin receptors. The PCR conditions were as follows: denaturation at 95°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2.5 min for 30 cycles, followed by a 7 min extension at 72°C. The resultant PCR products were subcloned and sequenced as described earlier.

2.4. Northern blot analysis

RNA from several human tissues was extracted as previously described [12]. Briefly, total RNA was extracted by the method of Chomczynski and Sacchi [13], and poly(A)⁺ RNA was isolated using oligo-dT cellulose spin columns (Pharmacia, Sweden). RNA was denatured, size fractionated on a 1% formaldehyde agarose gel, and transferred onto nylon membrane and immobilized by UV irradiation. The blots were hybridized with a 855 bp ³²P-labeled fragment of *SLC-1* obtained from a *Pst*I digestion, washed with 2× SSPE and 0.1% SDS at 50°C for 20 min and with 0.1× SSPE and 0.1% SDS at 50°C for 2 h and exposed to X-ray film at -70°C in the presence of an intensifying screen for 1 week. Northern analysis of rat mRNA was done similarly using a radiolabelled rat orthologue of *SLC-1*.

2.5. In situ hybridization analysis

The PCR-derived rat orthologue of *SLC-1* was used as a probe for in situ hybridization. Preparation of rat brain sections and in situ hybridization procedures were done as described [14].

2.6. Receptor expression and function

A 1.6 kb *Sac*I fragment containing *SLC-1* was subcloned into the expression vector pcDNA3 (Invitrogen, San Diego, CA). This construct, named Phage 8-CA, contained -400 bp 5'-UTR. However, 121 bp upstream of the start codon, a (CA)_n tandem repeat sequence was present. A second construct, named Phage 8/*Sma*I, produced from a *Sma*I digest of the construct in pB/S, eliminated the CA repeat sequence and reduced the 5'-UTR to 67 bp. This was also subcloned into pcDNA3 as described above. Transient expression of both constructs was performed in COS-7 cells using a calcium phosphate transfection system (Gibco BRL, Gaithersburg, MD). Radioligand binding studies were carried out as described in Zastawny et al. [15]. Ligands

used were [¹²⁵I]Tyr¹-somatostatin-14, [³H]naloxone, [³H]bremazocine, [³H]DTG, and [³H]haloperidol (NEN, Boston, MA).

2.7. Chromosomal localization

To determine which chromosome contained *SLC-1*, a monochromosomal somatic cell hybrid panel (Bios Laboratories; code #SCB-5500, blot #2931, enzyme *Eco*RI) consisting of human/hamster somatic cell hybrids and human/mouse hybrids was hybridized overnight at 42°C with the same PCR fragment used to screen the genomic library. The panel was then washed first in 2×SSC, 1% EDTA for 30 min at 23°C followed by a final wash in 0.1×SSC, 1% EDTA for 20 min at 55°C. In a separate experiment, to confirm the chromosomal assignment and to determine more specifically the locus of *SLC-1*, metaphase spread chromosomes derived from human lymphocytes were subjected to FISH and DAPI banding analyses. Slides were prepared and FISH was performed as previously described [16,17].

2.8. Dinucleotide repeat analysis

Upon discovery of a dinucleotide repeat sequence of the form (CA)_n, genomic DNA from 10 different human individuals was amplified using oligonucleotides flanking the repeat sequence (P5: 5'-ACACTCAGGGCTACACATAGG; P6: 5'-TTCCTGTTGCTAATCTTGTC). The resultant PCR products were subcloned and sequenced as described earlier.

2.9. Searching for subtypes

Human genomic DNA was PCR-amplified using the degenerate oligonucleotides used to isolate the rat orthologue. The PCR conditions were as follows: denaturation at 95°C for 1 min, annealing at either 55, 45 or 38°C for 1 min and extension at 72°C for 2.5 min for 30 cycles, followed by a 7 min extension at 72°C. The resultant PCR products were subcloned and sequenced.

3. Results and discussion

3.1. Cloning of *SLC-1*

Using an approach we used to clone novel GPCR genes *GPR19* [14], *GPR21* and *GPR22* [18], we searched a dbEST for other GPCR-encoding sequences. The sequence of one EST cDNA fragment partially encoded a GPCR from TM1 to TM3 which demonstrated significant identity to the soma-

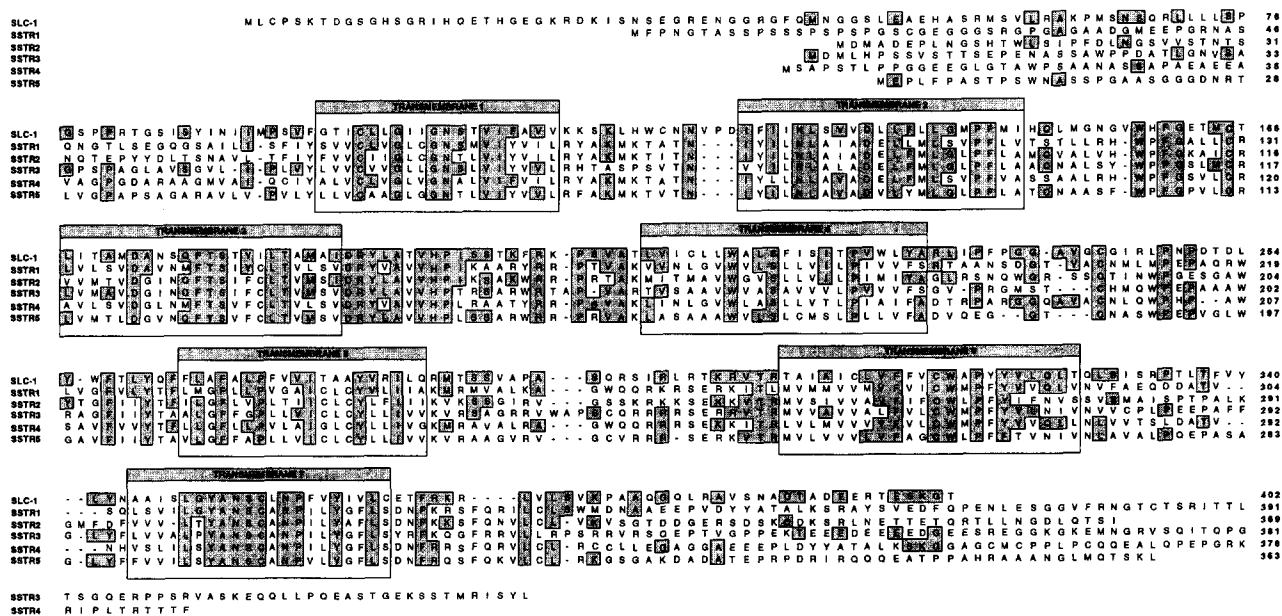


Fig. 1. Amino acid comparison of the receptor encoded by *SLC-1* with related somatostatin receptors, SSTR1 to SSTR5. Amino acids identical with the protein encoded by *SLC-1* are boxed and shaded. The predicted seven-transmembrane domains are indicated. Gaps (-) have been introduced to maximize the alignments among the sequences.

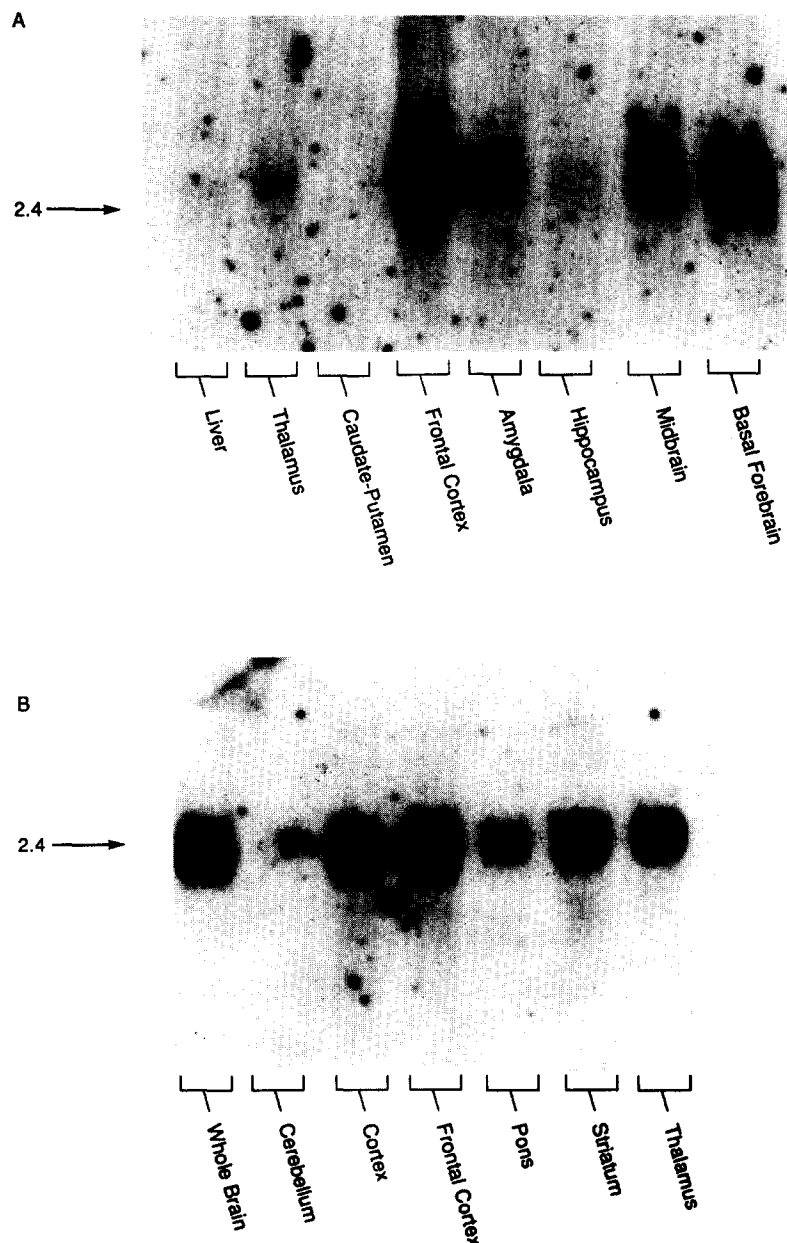


Fig. 2. Northern blot analyses of the brain distribution of *SLC-1* mRNA in: (A) human and (B) rat. The human blot was probed with a fragment isolated from the coding region of *SLC-1* and the rat blot probed with the rat orthologue of *SLC-1*. Each lane contained 5 μ g of poly-(A)⁺ RNA.

tostatin receptors. We cloned a 350 bp genomic DNA fragment amplified from PCR using specific oligonucleotides encompassing the published EST cDNA sequence. This fragment was used to screen a human genomic library to obtain the full-length open reading frame. Eight positive phage clones were isolated, purified and DNA prepared. Following restriction enzyme digestion and Southern blot analysis, a 1.6-kb *SacI* fragment from one phage (phage #8) was isolated, sequenced, and found to contain identical overlapping sequence to the EST cDNA. This genomic clone, named *SLC-1* (aka GPR24), contained a full-length and intronless open reading frame of 1206 bp encoding for a protein of 402 amino acids. Using BLAST search [6], highest identity was observed with the somatostatin receptor gene family. The receptor encoded by *SLC-1* shares ~40% identity with

the SSTRs in the TM domains (Fig. 1). Significantly, an aspartic acid residue (Asp¹⁷²; see Fig. 1) is present in TM3 that aligns with the somatostatin receptors. It has been demonstrated in other GPCRs that this residue in the analogous position is important for receptor-ligand interaction [19–21]. The receptor encoded by *SLC-1* has other residues considered to form the ligand binding pocket of SSTRs, namely Tyr²³⁰, Phe²⁶⁶, Trp³¹⁸, Tyr³²², and Gln³²⁵ [5]. Compared with other SSTRs, the receptor encoded by *SLC-1* has a large amino terminus and a short cytoplasmic tail with no putative N-linked glycosylation sites identified.

Using PCR with oligonucleotides based on the sequence encoding TM3 and TM7 of the receptor encoded by *SLC-1*, we isolated the rat orthologue sequence. The protein sequence

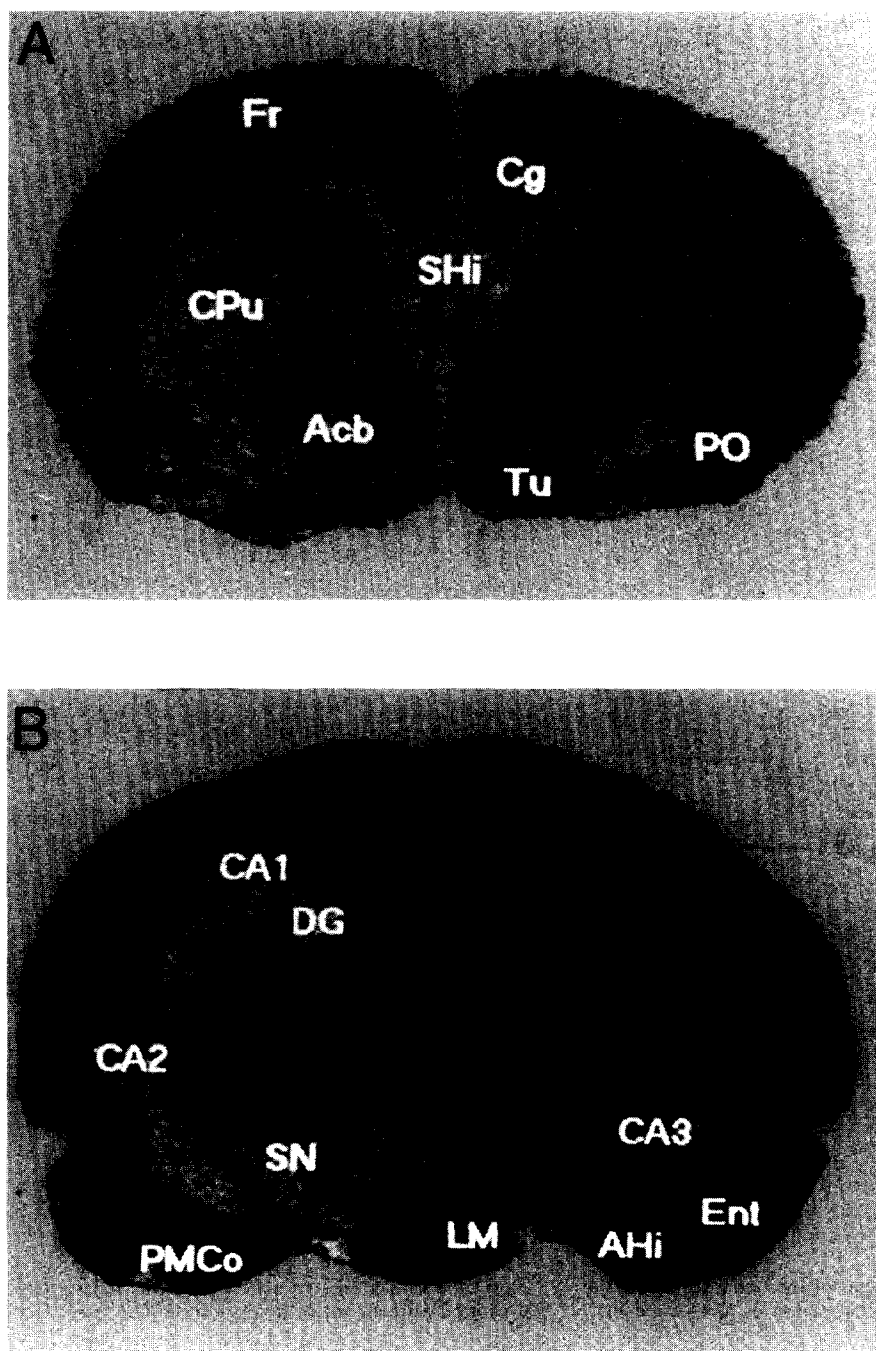


Fig. 3. Darkfield autoradiograms of coronal sections of rat brain showing the localization of *SLC-1* mRNA. Representative sections are shown at levels relative to bregma at (A) +0.7 mm and (B) -4.8 mm, according to the stereotactic coordinates [29]. Fr = frontoparietal cortex; Cg = anterior cingulate cortex; SHi = septohippocampal nucleus; CPu = caudate putamen; Acb = nucleus accumbens; Tu = olfactory tubercle; PO = primary olfactory cortex; CA1, CA2, CA3 = fields of Ammon's horn; DG = dentate gyrus; SN = substantia nigra pars compacta; PMCo = posteromedial cortical amygdaloid nucleus; LM = lateral mammillary nucleus; AHi = amygdalohippocampal area; Ent = entorhinal cortex.

is highly conserved with only three amino acid differences (from a total of 157) between the human and rat.

3.2. Dinucleotide repeat analysis

Eighty base pairs upstream of the start codon is a repeat sequence of the form (CA)_n. To determine the variability in the length of this sequence, DNA samples from 10 individuals were amplified by PCR using oligonucleotides flanking the repeat sequence. Five individuals had homozygous repeat se-

quences of $n = 12$, three individuals $n = 10$, one $n = 11$ and one individual was heterozygous with $n = 13$ and $n = 11$.

3.3. Northern and in situ analyses

Northern blot analysis detected a single 2.4 kb mRNA species for *SLC-1* (in order of decreasing abundance) in human frontal cortex, hypothalamus, basal forebrain, midbrain, amygdala, hippocampus, subthalamus, substantia nigra, thalamus, corpus callosum, liver and heart, while no signals were

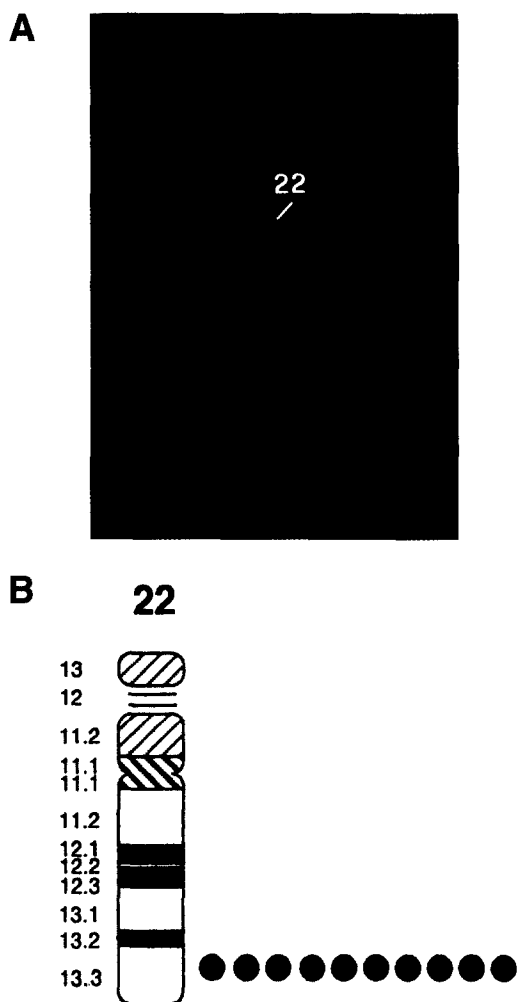


Fig. 4. FISH analysis of *SLC-1*. (A) Demonstrates the results of metaphase spread chromosomes probed with a phage clone encoding *SLC-1*. Arrows point to the FISH signals on a pair of chromosomes. (B) A summary of the FISH analysis, each dot represents the location of a fluorescent signal on the chromosome using phage *SLC-1* as a probe.

detected in the caudate-putamen, pancreas, kidney, muscle, lung, or placenta (selected regions are shown in Fig. 2A). Northern blot analysis of rat brain regions showed transcripts expressed (in order of decreasing abundance) in frontal cortex, striatum, cortex, thalamus, and pons (Fig. 2B), while no hybridizing signals were detected in the cerebellum. Analysis of rat peripheral tissues revealed that *SLC-1* is expressed in (in order of decreasing abundance) kidney, ovary, fetus, and heart (data not shown).

Using in situ hybridization of rat brain sections, the distribution of *SLC-1* mRNA was found to be discretely localized to many areas (Fig. 3A,B). In cerebral cortex, signal was present in the anterior cingulate, frontal, piriform, and somatosensory areas. Dense labeling was present in the septohippocampal nucleus, which may be a component of the primordial hippocampus. Very dense labeling was detected in the olfactory tubercle, islands of Calleja, medial nucleus accumbens, the dentate gyrus and the hippocampal areas CA1, CA2, and CA3. mRNA was also detected in moderate abundance in the lateral mammillary and tuberomammillary nuclei, amygdaloid nuclei and the entorhinal area. Lesser amounts of

SLC-1 mRNA were visualized in claustrum, substantia nigra pars compacta, central grey, lateral geniculate nucleus, subparafascicular nucleus of thalamus, caudate putamen and nucleus accumbens.

3.4. Pharmacological characterization of the receptor encoded by *SLC-1*

A fragment encoding *SLC-1* (having –400 bp of 5'-UTR) was inserted into pcDNA3 and transiently expressed in COS-7 cells. A second construct was also used in which the CA tandem repeat element had been removed (67 bp of 5'-UTR). No specific binding was observed with [125 I]Tyr¹-somatostatin-14 with either construct. Opioid and sigma ligands were also assayed for binding, however, [3 H]naloxone, [3 H]bremazocine, [3 H]DTG, and [3 H]haloperidol failed to show specific binding.

3.5. Chromosomal assignment

A monochromosomal somatic cell hybrid panel was probed with the same PCR fragment used to screen a human genomic library. Only the lane corresponding to chromosome 22 displayed two hybridizing bands (data not shown), indicating *SLC-1* was located on chromosome 22. To localize *SLC-1* specifically on this chromosome, FISH analysis of human metaphase spread chromosomes in combination with DAPI banding patterns was used (Fig. 4A,B) [18]. *SLC-1* is located at human chromosome 22, region q13.3. It is noteworthy that *SSTR3* is located nearby at q13.1 [22].

3.6. Subtype search

To search for other related genes, we used PCR to amplify genomic DNA with oligonucleotides based on *SLC-1* and other somatostatin receptor genes. PCR fragments obtained using a 55°C annealing temperature yielded fragments identical to *SLC-1* (13 samples of 60), *GPR7* (13) [23], *SSTR2* (5), *SSTR5* (4), *SSTR3* (1), *GPR14* (1) [24], bradykinin B2 receptor (1) [25], and non-GPCR encoding fragments (4). Two novel genes encoding for partial novel GPCR were identified, clone 37, showing high identity to *GPR15* [26] and clone 42, to the galanin receptor [27]. An annealing temperature of 45°C produced fragments identical to *SLC-1* (36 of 94), *GPR7* (22), *SSTR5* (15), *SSTR3* (6), clone 37 (4), clone 42 (2), *GPR14* (1), and 5 non-GPCR encoding fragments. Annealing at 38°C gave fragments identical to *SSTR3* (6 of 30), *SSTR5* (5), *GPR7* (5), *SLC-1* (1), *SSTR4* (1), *GPR14* (1), and 10 non-GPCR encoding fragments. One fragment encoded for a GPCR closest to the rat olfactory receptor-like protein I7 [28] sharing 56% amino acid identity.

In summary, we have isolated a novel human gene encoding a GPCR with significant homology to the somatostatin receptors that does not bind somatostatin. The abundance of *SLC-1* mRNA expression in brain with regional localization to discrete areas involved in functions such as emotion, memory and sensory perception make the isolation of the endogenous ligand of this receptor an important priority. This heralds the exciting potential of identifying a novel peptidergic neurotransmitter signalling system in brain.

Acknowledgements: This research was supported by grants from the National Institutes of Drug Abuse, the Medical Research Council of Canada, and the Smokeless Tobacco Research Council to B.F.O. and S.R.G. and was supported in part by a grant from UTHSCSA Research Resources Program for Medical Schools from the Howard

Hughes Medical Institute to L.F.K. We would like to thank Victor Saldivia, Yang Shen, and Jenny Casares for their excellent technical assistance.

References

- [1] Schusdziarra, V. (1992) in: Basic and Clinical Aspects of Neuroscience (Weil, C., Müller, E.E., and Thorner, M.O. eds.), Somatostatin, Vol. 4, pp. 43–54, Springer, Heidelberg.
- [2] Yamada, Y., Post, S.R., Wang, K., Tager, H.S., Bell, G.I. and Seino, S. (1992) *Proc. Natl. Acad. Sci. USA* 89, 251–255.
- [3] Corness, J.D., Demchyshyn, L.L., Seeman, P., Van Tol, H.H.M., Srikant, C.B., Kent, G., Patel, Y.C. and Niznik, H.B. (1993) *FEBS Lett.* 321, 279–284.
- [4] Yamada, Y., Kagimoto, S., Kubota, A., Yasuda, K., Masuda, K., Someya, Y., Ihara, Y., Li, Q., Imura, H., Seino, S. and Seino, Y. (1993) *Biochem. Biophys. Res. Commun.* 195, 844–852.
- [5] Patel, Y.C., Greenwood, M.T., Panetta, R., Demchyshyn, L., Niznik, H. and Srikant, C.B. (1995) *Life Sci.* 57, 1249–1265.
- [6] Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) *J. Mol. Biol.* 215, 403–410.
- [7] Pearson, W.R. and Lipman, D.J. (1988) *Proc. Natl. Acad. Sci. USA* 85, 2444–2448.
- [8] Pearson, W.R. (1995) *Prot. Sci.* 4, 1145–1160.
- [9] Kolakowski, L.F. (1994) *Int. J. Receptors, Channels Transporters* 2, 1–7.
- [10] Nguyen, T., Erb, L., Weisman, G.A., Marchese, A., Heng, H.H.Q., Garrad, R.C., George, S.R., Turner, J.T., and O'Dowd, B.F. (1995) *J. Biol. Chem.* 270, 30845–30848.
- [11] Jin, H., Oksenberg, D., Ashkenazi, A., Peroutka, S.J., Duncan, A.M.V., Rozmahel, R., Yang, Y., Mengod, G., Palacios, J.M., and O'Dowd, B.F. (1992) *J. Biol. Chem.* 267, 5735–5738.
- [12] Marchese, A., Docherty, J.M., Nguyen, T., Heiber, M., Cheng, R., Heng, H.H.Q., Tsui, L.-C., Shi, X., George, S.R. and O'Dowd, B.F. (1994) *Genomics* 23, 609–618.
- [13] Chomczynski, P. and Sacchi, N. (1987) *Anal. Biochem.* 162, 156–159.
- [14] O'Dowd, B.F., Nguyen, T., Lynch, K.R., Kolakowski, F.L., Thompson, M., Cheng, R., Marchese, A., Ng, G., Heng, H.H.Q. and George, S.R. (1996) *FEBS Lett.* 394, 325–329.
- [15] Zastawny, R.L., George, S.R., Nguyen, T., Cheng, R., Tsatsos, J., Briones-Urbina, R. and O'Dowd, B.F. (1994) *J. Neurochem.* 62, 2099–2105.
- [16] Heng, H.H.Q., Squire, J. and Tsui, L.-C. (1992) *Proc. Natl. Acad. Sci. USA* 89, 9509–9513.
- [17] Heng, H.H.Q. and Tsui, L.-C. (1993) *Chromosoma* 102, 325–332.
- [18] O'Dowd, B.F., Nguyen, T., Jung, B.P., Marchese, A., Cheng, R., Heng, H.H.Q., Kolakowski, L.F., Lynch, K.R. and George, S.R. (1997) *Gene*, in press.
- [19] Strader, C.D., Sigal, I.S., Register, R.B., Candelore, M.R., Rands, E. and Dixon, R.A.F. (1987) *Proc. Natl. Acad. Sci. USA* 84, 4384–4388.
- [20] Fraser, C.D., Wang, C.D., Robinson, D.A., Gocayne, J.D. and Venter, J.C. (1989) *Mol. Pharmacol.* 36, 840–847.
- [21] Surrat, C.K., Johnson, P.S., Moriwaki, A., Sidleck, B.K., Blaschak, C.J., Wang, J.B. and Uhl, G.R. (1994) *J. Biol. Chem.* 269, 20548–20553.
- [22] Yamada, Y., Stoffel, M., Espinosa III, R., Xiang, K.-S., Seino, M., Seino, S., Le Beau, M.M. and Bell, G.I. (1993) *Genomics* 15, 449–452.
- [23] O'Dowd, B.F., Scheideler, M.A., Nguyen, T., Cheng, R., Rasmussen, J.S., Marchese, A., Zastawny, R., Heng, H.H.Q., Tsui, L.-C., Shi, Xiaomei, S., Puy, L. and George, S.R. (1995) *Genomics* 28, 84–91.
- [24] Marchese, A., Heiber, M., Nguyen, T., Heng, H.H.Q., Saldivia, V.R., Cheng, R., Murphy, P.M., Tsui, L.-C., Shi, X., Gregor, P., George, S.R., O'Dowd, B.F. and Docherty, J.M. (1994) *Genomics* 29, 335–344.
- [25] Powell, S.J., Slynn, G., Thomas, C., Hopkins, B., Briggs, I. and Graham, A. (1993) *Genomics* 15, 435–438.
- [26] Heiber, M., Marchese, A., Nguyen, T., Heng, H.H.Q., George, S.R. and O'Dowd, B.F. (1996) *Genomics* 32, 462–465.
- [27] Habert-Ortoli, E., Amiranoff, B., Loquet, I., Laburthe, M. and Mayaux, J.-F. (1994) *Proc. Natl. Acad. Sci.* 91, 9780–9783.
- [28] Buck, L. and Axel, R. (1991) *Cell* 65, 175–187.
- [29] Paxinos, G. and Watson, C. (1982) *The Rat Brain*, Academic Press, New York.