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MOLECULAR CLONING AND SEQUENCING OF PORCINE SOMATOSTATIN RECEPTOR 2+

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SUMMARY: The porcine somatostatin receptor gene was isolated from a porcine genomic library. Based on the deduced amino acid sequence, this gene encodes a 369 amino acid protein with seven hydrophobic segments, a characteristic of G-protein coupled receptors, and shows only 13 amino acid difference (identity 96.5%, similarity 99.2%) in amino acid sequence from human somatostatin receptor 2. The data indicate that the amino acid sequence is highly conserved in pig, human, rat and mouse somatostatin receptor 2.

Somatostatin (SST) was originally isolated from an ovine hypothalamic extract based on its ability to inhibit growth hormone release(1). Subsequent studies have shown that SST with the same structure is widely distributed in animals including the rat, pig, and man(2,3). This peptide has diverse biological functions such as as a neurotransmitter or neuromodulator in the central nervous system, and as an inhibitor of secretions from various endocrine and exocrine glands including the pituitary, pancreas, and gastro-intestinal tract through its effects on adenylyl cyclase and ion channel activities(4-7). The fact that the phenotype of somatostatin 14 is so well conserved (as, to a lesser degree,

+The nucleotide sequence reported in this paper has been deposited in the GSDB/DDBJ/EMBL/NCBI DNA databases (accession number D21338).

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is that of somatostatin 28) suggests that the specific configuration of somatostatin 14 has endowed animals with a selective advantage during evolution(2). The biological effects of somatostatin are mediated by specific receptors. Therefore, cloning of the somatostatin receptor (SSTR) is important for determining the mechanism of action of somatostatin. Recently, human, and murine SSTR genes have been identified(8-12), but the porcine SSTR gene has not yet been cloned. To investigate the segment that is important for signal transduction and to evaluate the structural evolution of SSTR in mammals, we cloned and sequenced the porcine SSTR 2 gene from a porcine genomic library.

MATERIALS AND METHODS

General methods

Standard methods of molecular cloning were carried out as described by Sambrook et al. (13). DNA sequencing was done by the dideoxynucleotide chain termination procedure (14), using Sequenase Version 2.0 (USB, Cleveland, OH) after subcloning an appropriate DNA fragment into PUC19. Sequence analyses were performed using a GENETYX software system from Software Development Co. (Tokyo, Japan).

Synthesis of probe for plaque hybridization

The oligonucleotide primers used for the polymerase chain reaction (PCR) were designed based on the nucleotide sequence of cloned human SSTR 2 (8). The primer sequences were:

(1) Upstream primer 5'-AAAGCAGCCATGGACATGGCG-3'

(2) Downstream primer 5'-GATACTGGTTTGGAGGTCTCC-3'

Human genomic DNA was subjected to PCR using these primers. The PCR was performed in a final volume of 50 μl containing 1 μg of human genomic DNA, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 200 μM of each deoxynucleotide, 1 μM of each primer, and 1 unit of Taq DNA polymerase (Perkin Elmer Cetus, USA). The PCR thermal program used was denaturation at 94℃ for 7 min and annealing at 60℃ for 5 min, followed by denaturation at 94℃ for 1 min, annealing at 60℃ for 1 min, and extension at 72℃ for 1 min for a total of 45 cycles followed by a final 10 min incubation at 72℃. The PCR products were separated in 1% agarose gel, and a 1.1 kilobase-pair(kbp) fragment was isolated. One product was observed, and subcloned into the PCR vector (TA cloning kit, Invitrogen). The resulting plasmid containing the 1.1 kbp DNA insert, termed T1, was sequenced by the chain termination procedure (14).

Isolation of a genomic clone

Approximately $5x10^5$ clones of a porcine liver DNA genomic library (EMBL3, Clontech Laboratories) were screened with

[^{32}P] -labelled T1 probe (using a Mutiprime DNA labeling system, Amersham). Hybridization was carried out in 5 × standard saline citrate (1 × SSC = 0.15 M NaCl, 0.015 M Na citrate, pH 7.0), 50% formamide, 2 × Denhardt's solution, 0.1%sodium dodecyl sulfate, 20 mM sodium phosphate buffer (pH 6.5), 100 μ g / ml sonicated and denatured salmon testicular DNA, 10% dextran sulfate, and 1 × 10^6 cpm of ^{32}P-labeled probe per ml at 42℃ for 16h. The final washing was performed in 0.2 × SSC and 0.1% sodium dodecyl sulfate at 65℃ for 1 h before exposure to X-ray film. Positive plaques were picked up, and after repeating the screening twice more a positive plaque, designated as P1, was isolated. P1, containing a 10 kbp insert, was analyzed by restriction mapping and Southern blot hybridizations. A 4.4 kbp BamHI fragment from the genomic clone containing porcine SSTR was separated in 0.7% agarose gel, and subcloned into the PUC19 and the entire coding region was sequenced by the chain termination procedure (14).

RESULTS

As many G-protein coupled receptors are known to be intronless (15), PCR was performed on human genomic DNA. A single product (1116 bp), designated as T1, that contained a 1107 nucleotide long sequence of the human SSTR 2 gene was obtained. This DNA fragment was used to screen a porcine genomic library, and a clone designated as P1 was isolated. From this clone a 4.4 kbp BamHI restriction fragment was subcloned into PUC19 and sequenced. The cloned DNA contained an open reading frame of 1107 bp and encoded 369 amino acids (Fig. 1). This 1107 bp DNA fragment has the highest homology with human SSTR 2 of the 5 human SSTR subtypes and so was designated as porcine SSTR 2. Computer analysis of the hydrophobic profile of the amino acid sequence of porcine SSTR 2 (Fig. 2) showed seven hydrophobic domains separated by stretches of hydrophilic amino acids, a characteristic of G-protein coupled receptors (16). This SSTR lacked introns like other genes of the somatostatin receptor family. The amino acid residues conserved in other G-protein coupled receptors are also conserved in the sequence of porcine SSTR 2, such as the tripeptide sequence (Asp-139, Arg-140 and Tyr-141) that is considered important in coupling to G proteins (17). Of the three cysteine residues, Cys-115 and Cys-193 are likely to form a disulfide bridge between extracellular loops 1 and 2 (18), and Cys-328, analogous to that in other G-protein

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     ctagcctgggactgtcaggcagccatggatatggcgtatgagctactcaac   51
1                              M  D  M  A  Y  E  L  L  N
     gggagccagccgtggctttcctctccattcgacctcaatggctccgtggca   102
10   G  S  Q  P  W  L  S  S  P  F  D  L  N  G  S  V  A
     acagccaacagttcaaaccagacggagccatactatgacctgaccagcaat   153
27   T  A  N  S  S  N  Q  T  E  P  Y  Y  D  L  T  S  N
     gcagtcctcacgttcatatattttgtggtctgcatcattggcctgtgcggc   204
44   A  V  L  T  F  I  Y  F  V  V  C  I  I  G  L  C  G
     aacacgcttgtcatttacgtcatcctccgctacgccaagatgaagacaatc   255
61   N  T  L  V  I  Y  V  I  L  R  Y  A  K  M  K  T  I
     accaacatctacatcctcaacctggccattgccgatgagctcttcatgctg   306
78   T  N  I  Y  I  L  N  L  A  I  A  D  E  L  F  M  L
     ggcctgcccttcctggccatgcaggtggctctggtccactggccctttggc   357
95   G  L  P  F  L  A  M  Q  V  A  L  V  H  W  P  F  G
     aaggccatctgccgggtggtcatgactgtggatggcatcaatcagttcacc   408
112  K  A  I  C  R  V  V  M  T  V  D  G  I  N  Q  F  T
     agcattttctgcttgaccgtcatgagcattgaccggtacctggctgtggtc   459
129  S  I  F  C  L  T  V  M  S  I  D  R  Y  L  A  V  V
     caccccatcaagtcggccaagtggaggagaccccggacagccaagatgatc   510
146  H  P  I  K  S  A  K  W  R  R  P  R  T  A  K  M  I
     aatgtggccgtgtggggcgtctctctgctggtcatcttgcccatcatgata   561
163  N  V  A  V  W  G  V  S  L  L  V  I  L  P  I  M  I
     tatgccgggcttcgaagcaaccagtgggggagaagcagctgcaccatcaac   612
180  Y  A  G  L  R  S  N  Q  W  G  R  S  S  C  T  I  N
     tggccaggcgagtcgggggcatggtacacggggttcattatctacgccttc   663
197  W  P  G  E  S  G  A  W  Y  T  G  F  I  I  Y  A  F
     atcctggggttcctggtgcccctcaccatcatctgtctttgctacctgttc   714
214  I  L  G  F  L  V  P  L  T  I  I  C  L  C  Y  L  F
     attatcatcaaggtgaagtcctccggaatccgagtgggttcctccaagagg   765
231  I  I  I  K  V  K  S  S  G  I  R  V  G  S  S  K  R
     aaaaagtctgagaagaaggtcacccggatggtgtccattgtggtggccgtc   816
248  K  K  S  E  K  K  V  T  R  M  V  S  I  V  V  A  V
     ttcatttttctgctggctccccttctacatcttcaatgtctcttcggtctct   867
265  F  I  F  C  W  L  P  F  Y  I  F  N  V  S  S  V  S
     gtggccatcagtcccaccccagcccttaaaggcatgtttgactttgtggtg   918
282  V  A  I  S  P  T  P  A  L  K  G  M  F  D  F  V  V
     gtcctcacctatgctaacagctgtgccaaccctatcctctatgccttcttg   969
299  V  L  T  Y  A  N  S  C  A  N  P  I  L  Y  A  F  L
     tccgacaacttcaagaagagcttccagaatgtcctctgcttggtcaaggtg  1020
316  S  D  N  F  K  K  S  F  Q  N  V  L  C  L  V  K  V
     agcggcacagatgatggggaacggagtgacagtaagcaggacaaatcgcgg  1071
333  S  G  T  D  D  G  E  R  S  D  S  K  Q  D  K  S  R
     ctgaatgagaccacggagacccagaggaccctcctcaatggagacctccag  1122
350  L  N  E  T  T  E  T  Q  R  T  L  L  N  G  D  L  Q
     accagtatctgaactgcctgaacattcaatccagaaaatgcccc
367  T  S  I
```

Fig. 1. Nucleotide sequence and deduced amino acid sequence of SSTR 2 of the porcine genomic clone. The single-letter amino acid code is used.

coupled receptors, is likely to attach the C-terminal region to the membrane via a palmitoyl anchor (19). In addition, porcine SSTR 2 has four putative N-glycosylation sites (Asn-9, Asn-22, Asn-29 and Asn-32) (17) in the extracellular NH_2-terminal segment. One putative phosphorylation site (Ser-250) could be phosphorylated by cAMP-dependent protein kinase (20),

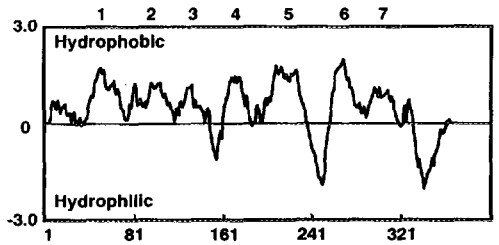

Fig. 2. Hydrophobicity analysis according to the GENETYX software system revealing seven hydrophobic regions that suggest the presence of seven membrane-spanning domains.

and two others (Ser-244, and Ser-343) could be phosphorylated by multifunctional calmodulin- dependent protein kinase II (21).

DISCUSSION

In this study, we cloned and sequenced porcine SSTR 2 (pSSTR2). Its amino acid sequence is shown in comparison with those of human, mouse and rat SSTR 2 (8,10) in Fig. 3. The overall homology of these SSTR 2 is very high. Sequence analysis revealed differences in only 13 amino acids (identity 96.5%, similarity 99.2%) between porcine and human SSTR2 (8), in 17 amino acids (identity 95.4%, similarity 98.9%) between porcine and mouse SSTR 2 (8), and in 20 amino acids (identity 94.6%, similarity 99.2%) between porcine and rat SSTR 2 (12). Comparison of the amino acid sequences of human and porcine SSTR 2 shows that the transmembrane-spanning regions differ in only 4 amino acids, and that the intracellular C-terminal region is completely conserved. Therefore, the transmembrane-spanning regions and intracellular C-terminal region are probably important to maintain signal transduction. All four receptors have a small cytoplasmic loop connecting transmembrane segments V and VI, a characteristic of G- protein coupled receptors that bind peptide hormones and neuropeptides(22). This small loop is completely conserved in all four receptors. Recently, conversion of Asp-89 to Asn-89 in human SSTR 2 was reported to result in a mutant receptor whose affinity for agonists was not altered by Na^+, indicating that Asp-89 is involved in mediating the effects of Na^+ on agonist binding to human SSTR

```
          1                                                    I
pSSTR2 MDMAYELLNGSQPWLSSPFDLNGSVATANSSNQTEPYYDLTSNAVLTFIY
hSSTR2 MDMADEPLNGSHTWLSIPFDLNGSVVSTNTSNQTEPYYDLTSNAVLTFIY
mSSTR2 MEMSSEQLNGSQVWVSSPFDLNGSLGPSNGSNQTEPYYDMTSNAVLTFIY
rSSTR2 MELTSEQFNGSQVWIPSPFDLNGSLGPSNGSNQTEPYYDMTSNAVLTFIY
         51                                  II
pSSTR2 FVVCIIGLCGNTLVIYVILRYAKMKTITNIYILNLAIADELFMLGLPFLA
hSSTR2 FVVCIIGLCGNTLVIYVILRYAKMKTITNIYILNLAIADELFMLGLPFLA
mSSTR2 FVVCVVGLCGNTLVIYVILRYAKMKTITNIYILNLAIADELFMLGLPFLA
rSSTR2 FVVCVVGLCGNTLVIYVILRYAKMKTITNIYILNLAIADELFMLGLPFLA
         101                  III
pSSTR2 MQVALVHWPFGKAICRVVMTVDGINQFTSIFCLTVMSIDRYLAVVHPIKS
hSSTR2 MQVALVHWPFGKAICRVVMTVDGINQFTSIFCLTVMSIDRYLAVVHPIKS
mSSTR2 MQVALVHWPFGKAICRVVMTVDGINQFTSIFCLTVMSIDRYLAVVHPIKS
rSSTR2 MQVALVHWPFGKAICRVVMTVDGINQFTSIFCLTVMSIDRYLAVVHPIKS
         151          IV
pSSTR2 AKWRRPRTAKMINVAVWGVSLLVILPIMIYAGLRSNQWGRSSCTINWPGE
hSSTR2 AKWRRPRTAKMITMAVWGVSLLVILPIMIYAGLRSNQWGRSSCTINWPGE
mSSRE2 AKWRRPRTAKMINVAVWCVSLLVILPIMIYAGLRSNQWGRSSCTINWPGE
rSSTR2 AKWRRPRTAKMINVAVWGVSLLVILPIMIYAGLRSNQWGRSSCTINWPGE
         201                  V
pSSTR2 SGAWYTGFIIYAFILGFLVPLTIICLCYLFIIIKVKSSGIRVGSSKRKKS
hSSTR2 SGAWYTGFIIYTFILGFLVPLTIICLCYLFIIIKVKSSGIRVGSSKRKKS
mSSTR2 SGAWYTGFIIYAFILGFLVPLTIICLCYLFIIIKVKSSGIRVGSSKRKKS
rSSTR2 SGAWYTGFIIYAFILGFLVPLTIICLCYLFIIIKVKSSGIRVGSSKRKKS
         251                  VI                      VII
pSSTR2 EKKVTRMVSIVVAVFIFCWLPFYIFNVSSVSVAISPTPALKGMFDFVVVL
hSSTR2 EKKVTRMVSIVVAVFIFCWLPFYIFNVSSVSMAISPTPALKGMFDFVVVL
mSSTR2 EKKVTRMVSIVVAVFIFCWLPFYIFNVSSVSVAISPTPALKGMFDFVVIL
rSSTR2 EKKVTRMVSIVVAVFIFCWLPFYIFNVSSVSVAISPTPALKGMFDFVVIL
         301
pSSTR2 TYANSCANPILYAFLSDNFKKSFQNVLCLVKVSGTDDGERSDSKQDKSRL
hSSTR2 TYANSCANPILYAFLSDNFKKSFQNVLCLVKVSGTDDGERSDSKQDKSRL
mSSTR2 TYANSCANPILYAFLSDNFKKSFQNVLCLVKVSGTEDGERSDSKQDKSRL
rSSTR2 TYANSCANPILYAFLSDNFKKSFQNVLCLVKVSGAEDGERSDSKQDKSRL
         351              369
pSSTR2 NETTETQRTLLNGDLQTSI
hSSTR2 NETTETQRTLLNGDLQTSI
mSSTR2 NETTETQRTLLNGDLQTSI
rSSTR2 NETTETQRTLLNGDLQTSI
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Fig. 3. Comparison of the deduced amino acid sequences of porcine(p), human (h), mouse (m), and rat (r) SSTR 2. The sequences of human, mouse and rat SSTR 2 are cited from published papers(8,10). The differences in amino acids from those in human SSTR 2 are indicated by boldface type letters. Putative membrane-spanning domains are showed by I to VII with solid bars.

2 (23). An aspartate (Asp-89) in the second transmembrane-spanning region is also conserved in all four SSTRs. On the other hand, the amino acid sequence of the extracellular N-terminal region varies in these four SSTR 2. Computer analysis showed that as in the other three SSTR 2, this extracellular N-terminal region of porcine SSTR 2 has a hydrophobic segment. This hydrophobic segment may be the signal peptide (24).

Somatostatins of similar structure are widely distributed in both lower and higher mammals(2), and SSTRs of similar structure are found in various mammals, indicating strong evolutionary conservation of somatostatin and its receptor.

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REFERENCES

1. Brazeau,P., Vale,W., Burgus,R., Ling,N., Butcher,M., Rivier,J. and Guillemin,R. (1973) Science 179, 77-79.
2. Reichlin,S. (1983) N. Engl. J. Med. 309, 1495-1501, 1556-1563.
3. Schally,A.V., Dupont, A., Arimura, A., Redding, T.W., Nishi,N., Linthicum, G. L. and Schlesinger,D.H. (1976) Biochemistry. 15, 509-514.
4. Jakobs,K., Aktories,K. and Schultz, G. (1983) Nature 303,177-178.
5. Mahy,N., Woolkalis,M., Thermos,K., Carlson,K., Manning,D. and Reisine,T.(1988) J. Pharmacol. Exp. Ther. 246, 779-785.
6. Wang,H., Wang,L., Bogen,C., Reisine,T. and Dichter,M. (1989) Proc. Natl. Acad. Sci. USA 86, 9616-9620.
7. Wang,H., Reisine,T. and Dichter,M. (1990) Neuroscience. 38,335-342.
8. Yamada,Y., Post,S.R., Wang, K., Tager,H.S., Bell,G.I. and Seino,S. Proc. Natl. Acad. Sci. USA 89 251-255.
9. Yamada,Y., Reisine,T., Law, S.F., Ihara,Y., Kubota,A., Kagimoto,S., Seino, M.,Seino,Y., Bell,G.I. and Seino,S.(1992) Mol. Endocrinol. 6, 2136-2142.
10. Rohrer,L., Raulf,F., Bruns,C., Buettner,R., Hofstaedter,F. and Schüle,R. (1993) Proc. Natl. Acad. Sci. USA 90,4196-4200.
11. 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. Biophy. Res. Commun. 195, 844-852.
12. Kluxen,F.W., Bruns,C. and Lübbert,H. (1992) Proc. Natl. Acad. Sci. USA 89, 4618-4622.
13. Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) Molecular Cloning, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
14. Sanger,F., Coulson,A.R., Barrell,B.G., Smith, A.J.H. and Roe,B.A. (1980) J. Mol. Biol. 143, 161-178.

15. Partanen,J.,Mäkelä,T.P., Eerola,E., Korhonen,J., Hirvonen,H., Claesson,W.L. and Alitalo,K. (1991) EMBO J. 10, 1347-1354.
16. Dohlman,H.G., Thorner,J., Caron,M.G. and Lefkowitz,R.J.(1991) Annu. Rev. Biochem. 60, 653-688.
17. Franke,R.R., König,B., Sakmar,T.P., Khorana,H.G. and Hofmann,K.P.(1990) Science 250, 123-125.
18. Strosberg,A.D.(1991) Eur. J. Biochem. 196, 1-10.
19. Probst,W.C., Snyder,L.A., Schuster,D.I., Brosius,J. and Seaflon,S.C. (1992) DNA Cell Biol. 11, 1-20.
20. Kornfeld,R. and Kornfeld,B.E. (1985) Annu. Rev. Biochem. 54, 631-664.
21. Kemp,B.E. and Pearson,R.B. (1990) Trends Biochem. Sci. 15, 342-346.
22. Ross,E.M. (1990) Nature (London) 344, 707-708.
23. Kong,H., Raynor,K., Yasuda,K., Bell,G.I. and Reisine,T. (1993) Mol. Pharmacol. 44, 380-384.
24. Nagayama,Y., Kaufman,K.D., Seto,P., and Rapoport,B. (1989) Biochem. Biophy. Res. Commun. 165, 1184-1190.