Expression of somatostatin receptor subtypes on guinea pig gastric and colonic smooth muscle cells

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Corletto, Vito D., H. Christian Weber, and Robert T. Jensen. Expression of somatostatin receptor subtypes on guinea pig gastric and colonic smooth muscle cells. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G235–G244, 1999.—In vivo and in vitro studies have demonstrated that somatostatin can influence motility and smooth muscle contractility of the stomach and colon. Recent studies have proposed that some of these effects may be mediated by somatostatin receptors (sst) directly on the smooth muscle cells. If this is correct, the sst receptor subtypes that are present are unknown. This study aimed to resolve these points. Because nucleotide sequences of guinea pig sst genes are unknown, we used sst subtype-specific primers based on comparisons of human and rat sst subtypes and performed RT-PCR of DNase I-treated total RNA from guinea pig total brain. PCR products were cloned in pCR II and sequenced and showed 87% (sst1), 90% (sst2), 90% (sst3), 99% (sst4), and 80% (sst5), respectively, nucleotide homology to the same region (transmembrane 4–6) of the human sst genes. Homology to rat sequences were lower. PCR products were obtained from first-strand cDNA derived from DNase I-treated RNA from dispersed guinea pig gastric and colonic smooth muscle cells. In gastric and colonic smooth muscle cells, we detected sst1–sst3 and sst5, and all were confirmed by sequencing. The presence of sst4 was shown by Southern blot analysis and hybridization with a guinea pig sst-specific primer. RT-PCR from cultured colonic and gastric smooth muscle cells devoid of any neural elements gave identical results. These results demonstrate that in the guinea pig all five sst subtypes are present directly on gastric and colonic smooth muscle cells. Previous studies have suggested that a predominant sst3 subtype on gastric and a sst5 subtype on colonic muscle cells mediated somatostatin's contractile effects, but the finding here that all five sst subtypes exist on both of these cells suggests that other sst subtypes have only a small or no contractile effect, sst subtypes in guinea pig have a different pharmacological profile from rat or human sst, or these other sst subtypes have some yet undescribed physiological function in muscle cells.

colon; colonic motility; octreotide; cloning

SOMATOSTATIN IS WIDELY distributed in the gastrointestinal tract, including in specific mucosal cells, in ganglia, and on neurons, some of which project into smooth muscle cells (14, 29). Somatostatin has been shown to influence colonic and gastric motility and contractility both in vivo and in vitro (2, 28, 30). Furthermore, previous studies have demonstrated that somatostatin can cause changes in contractility in isolated and dispersed guinea pig smooth muscle cells, suggesting somatostatin receptors exist on these cells. Specifically, the presence of somatostatin receptors on isolated and dispersed guinea pig gastric smooth muscle cells has been suggested by both binding studies and contraction-relaxation studies (15, 16, 27). Recently, the presence of functionally active somatostatin receptors on guinea pig descending colon smooth muscle cells was supported by contraction studies (9). In that same study, various synthetic somatostatin analogs selective for one sst subtype over the other were used, and it was concluded that sst3 on guinea pig gastric smooth muscle cells and sst5 on guinea pig colonic smooth muscle cells mediate the contractile effect of somatostatin on these cells. However, conclusions from these data are limited by the fact that these synthetic somatostatin analogs have only moderate selectivity for one somatostatin receptor subtype over the other and species differences can occur in affinity for different subtypes (10). At present, there is no direct evidence of somatostatin receptor expression on guinea pig smooth muscle cells, and their detection is complicated by the fact that the structure of the guinea pig somatostatin receptors are unknown, even though guinea pig smooth muscle cells are widely used to study the cellular basis of hormones and/or neurotransmitters (26). Therefore, in the present study, we investigated whether sst receptor expression occurs in guinea pig colonic and gastric isolated smooth muscle cells and investigated which sst subtype was present after first determining specific sequences of the structure of guinea pig sst receptor genes.

MATERIALS AND METHODS

Male guinea pigs (200–300 g) were obtained from the Small Animal Section, Veterinary Resources Branch, National Institutes of Health. HEPES was from Boehringer Mannheim Biochemicals (Indianapolis, IN). Collagenase type II was from Worthington Biochemical (Freehold, NJ). Soybean trypsin inhibitor was from Sigma Chemical (St. Louis, MO). DMEM, PBS, first-strand cDNA synthesis kit, EcoR I, and DNase I were from Gibco BRL (Gaithersburg, MD). RNeasy total RNA kit was from Qiagen (Chatsworth, CA). Taq polymerase was from Perkin-Elmer (Norwalk, CT). The fmol DNA sequencing system was from Promega (Madison, WI), and β-actin primers were from Clontech (Palo Alto, CA). TA Cloning kit was from Invitrogen (San Diego, CA), X-Omat films were from Kodak (Rochester, NY). Synaptophysin antibody was from Zymed (San Francisco, CA). S-100 protein antibody and smooth muscle actin antibody were from Biogenex (San Ramon, CA). [γ-32P]ATP (3,000 Ci/mmol) and [γ-32P]dCTP (3,000 Ci/mmol) were purchased from DuPont NEN (Boston, MA).

Preparation of dispersed colonic and gastric smooth muscle cells. Dispersed smooth muscle cells from guinea pig stomach were prepared as previously described by Bitar and Makhlouf...
(4). The same method, with the modifications described recently (9), was used to obtain dispersed smooth muscle cells from guinea pig descending colon. Briefly, the guinea pig descending colon was removed and cut longitudinally, the mucosa was removed by gentle scraping, and the muscle layer was cut transversely into 5- to 10-mm strips. The muscle strips were incubated at 31°C for two successive 45-min periods in 15 ml of standard incubation solution (4) containing 0.1% (wt/vol) collagenase. The partially digested strips were successively washed with 50 ml of collagenase-free standard incubation solution, and smooth muscle cells were allowed to disperse spontaneously for 30 min and then were harvested by filtration through a 500-µm mesh. Dispersed smooth muscle cells were pelleted at 350 g for 10 min and washed two times with PBS solution. Greater than 95% of all intact cells excluded trypan blue. The isolated cells were lysed in guanidine isothiocyanate solution (11) and stored at −70°C until RNA extraction was performed.

Culture of colonic and gastric smooth muscle cells. A modification of the method described by Chijiiwa et al. (8) was used. After standard dispersion, smooth muscle cells were harvested by filtration through a 500-µm mesh. Dispersed smooth muscle cells were pelleted at 350 g for 10 min and resuspended in DMEM. After a second centrifugation, the cells were resuspended in the same solution with 10% (wt/vol) fetal bovine serum, penicillin (50 U/ml), and streptomycin (50 µg/ml) and finally resuspended in the same medium at a concentration of 2 × 10^5 cells/ml. Two milliliters of the cell suspension were plated in 35-mm-diameter well plates (Falcon, Oxnard, CA) and placed in a 5% CO2 incubator at 37°C. Medium was replaced every 3 days. After 10–14 days, the cells became confluent and were then used for RNA extraction and immunohistochemical studies.

RNA isolation. Total RNA extraction from guinea pig total brain, cerebellum, and isolated dispersed colonic or gastric smooth muscle cells was performed by the guanidine isothiocyanate-cesium chloride method (11). Total RNA from cultured colonic and gastric smooth muscle cells was extracted using the RNeasy total RNA kit (Qiagen).

RT-PCR. All RNA samples were first treated with DNase I to remove genomic DNA contamination from the total RNA preparations according to the manufacturer’s protocol (GIBCO BRL). First-strand cDNA for PCR was synthesized from 1 µg of total RNA by reverse transcription either using random hexamer or oligo(dT) primers according to the standard protocol of the manufacturer (GIBCO BRL). Control experiments for completeness of genomic DNA digestion were performed by PCR without RT and by PCR for β-actin for which the primers were designed on either side of an intron so that genomic DNA contamination could be determined. PCR was carried out in a buffer at pH 8.3, containing 10 mM Tris-HCl, 50 mM KCl, 1.0 mM MgCl2, 0.2 mM of each dNTP, 0.3 mM of each primer, and 2.5 units Taq DNA polymerase in a 50-µl reaction volume. PCR products were obtained either directly (one PCR) or by nested PCR. PCR primers were designed, with some degeneracy, by comparing human and rat somatostatin subtype-specific sequences and identifying areas of high homology and specificity for each subtype. For all sst receptors, a sequence coding for transmembrane regions four to six (TM4–TM6) was amplified. For three receptors, sst1, sst3, and sst5, an additional sequence encoding from the first to the third or fourth transmembrane region was amplified (Fig. 1). Nested PCR was used for five of the sequences amplified: sst1 (TM4–TM6), sst3 (TM4–TM6), sst4 (TM1–TM4), sst4 (TM4–TM6), and sst5 (TM4–TM6). Nested PCR was not required for three sequences: sst1 (TM1–TM4), sst2 (TM3–TM7) and sst3 (TM1–TM4). The specific PCR primers used were as follows: for sst1 (sst1 TM1–TM3), 5'-CACCTGGATGTTCCCCAAATG-3' and 5'-AGCAGTCAAGACAGTAG-3'. For sst2 (TM4–TM6), the first PCR primers were 5'-GACCCTATGTGGCAGAGACC-3' and 5'-GGGTGGGCACTGTTGGCAC-3'. The specific PCR primers used were as follows: for sst3 (sst3 TM1–TM3), 5'-TGTTGAGAGCACCC-3' and 5'-GGGTGGAGACCTGGCATGAG-3'. For sst4 (TM4–TM6), the first PCR primers were 5'-GACCGCTACCTGGGTCAG-3' and 5'-GGGTGGGCAACAGCTGGGATG-3'. Nested amplification was performed with 5'-GGGTGGGCAAGCTGGCATGAG-3'. For sst5 (TM4–TM6), the first PCR primers were 5'-GCCACACGCCCAGCTGCGGCGAG-3' and 5'-GGGTGGGCAAGCTGGCATGAG-3'. Nested amplification was performed with 5'-GGGTGGGCAAGCTGGCATGAG-3'. For sst6 (TM4–TM6), the first PCR primers were 5'-GACCGCTACCTGGGTCAG-3' and 5'-GGGTGGGCAAGCTGGCATGAG-3'. Nested amplification was performed with 5'-GGGTGGGCAAGCTGGCATGAG-3'. For sst7 (TM4–TM6), the first PCR primers were 5'-GACCGCTACCTGGGTCAG-3' and 5'-GGGTGGGCAAGCTGGCATGAG-3'. Nested amplification was performed with 5'-GGGTGGGCAAGCTGGCATGAG-3'.

Fig. 1. Receptor regions of partial guinea pig somatostatin receptor (sst) subtype-specific sequences. The 8 sets of primers were from areas of close homology between human and rat sst sequences for a given sst subtype, which also had high specificity for this subtype. Transmembrane (TM4–TM6) of each subtype was amplified as well as the indicated additional sequences of sst1, sst3, and sst5. After RT-PCR, each PCR product was cloned and sequenced.

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(Clontech) were used under identical conditions in PCR reactions to verify the integrity of the cDNA template.

Cloning and sequencing. PCR products were initially sequenced directly and then cloned into the plasmid vector pCR II using the TA Cloning kit (Invitrogen), when corresponding to a sst sequence. Recombinant clones were identified by restriction enzyme digestion. DNA sequence of partial cDNA guinea pig sst subtypes were also cloned (Fig. 1). Specifically, we obtained a cDNA clone from a different region of sst4 (TM1–TM4–TM6 (Fig. 2). An independently obtained partial sequence of guinea pig sst1 (TM1–TM3) and guinea pig sst2, a 321-bp (sst3 TM1–TM4) fragment for sst4, and a 303-bp (sst5 TM4–TM6) fragment for sst5 had 19 amino acid differences (82% homology) along with an eight amino acid motif that was absent in the human TM4–TM6 region of each somatostatin receptor subtype. A higher degree of homology to human than to rat sequences is shown in 4 of 5 sst subtypes.

**RESULTS**

Cloning of partial gene sequences of guinea pig somatostatin receptor subtypes. After PCR with each of the eight sets of primers corresponding to the sst receptor areas for each sst subtype shown in Fig. 1 and after the PCR products were cloned, eight different cDNA clones were obtained from the guinea pig brain total RNA (Fig. 1). For each sst subtype, one guinea pig cDNA clone containing the receptor TM4–TM6 region was obtained. Additional 5′ regions of guinea pig sst1, sst3, and sst5 were also cloned (Fig. 1). Specifically, we obtained a 459-bp (sst1 TM1–TM3) and a 243-bp (sst4 TM4–TM6) fragment for guinea pig sst1, a 513-bp (sst5 TM3–TM7) fragment for guinea pig sst2, a 321-bp (sst6 TM1–TM4) and a 312-bp (sst7 TM4–TM6) fragment for sst3, a 382-bp (sst8 TM1–TM4) and a 333-bp (sst9 TM4–TM6) fragment for sst4, and a 303-bp (sst10 TM4–TM6) fragment for sst5 (Fig. 1). Comparison of the nucleotide sequences of the guinea pig TM4–TM6 regions to the human sequences demonstrated a higher degree of homology with the human than with the rat sequences in four of the five sst subtypes (Fig. 2). The highest homology (99%) existed for the nucleotide sequence of the sst4 subtype TM4–TM6 (Fig. 2). An independently obtained partial cDNA clone from a different region of sst4 (TM1–TM4; data not shown) also showed 99% homology to the human sequence. In addition, the nucleotide sequence of guinea pig sst1 (TM1–TM3) and guinea pig sst3 (TM1–TM4) clones, compared with the respective human sst subtype, showed the same degree of homology that was found between the TM4–TM6 region of the two species (data not shown). The amino acid sequence of the guinea pig TM4–TM6 nucleotide sequences showed a complete identity with the human homologous area in the case of sst1 (Fig. 3), four amino acid changes for TM4–TM6 of sst2 (96% homology), and six changes for TM4–TM6 of sst3 (94% homology) along with an eight amino acid motif between TM5–TM6 that was absent in the human sequence (Fig. 3). The guinea pig and human TM4–TM6 sst4 sequences differed in only one amino acid (99% homology), whereas the TM4–TM6 sequence of sst5 had 19 amino acid differences (82% homology) (Fig. 3).

Expression of sst subtypes on freshly isolated gastric and colonic smooth muscle cells. Using first-strand cDNAs generated from total RNA preparations from isolated gastric or colonic smooth muscle cells, we performed PCR with the same primer pairs and with the same conditions used to obtain guinea pig sst subtype-specific sequences from guinea pig brain, as specified in MATERIALS AND METHODS. Specific PCR products for sst1, sst2, sst3, and sst5 were obtained from both smooth muscle cell preparations, and the products were identical in molecular weight to those obtained from guinea pig brain on ethidium bromide staining (Fig. 4). Sequence analysis of the PCR products demonstrated that products were identical to the respective subtypes.
guinea pig sst subtypes, previously cloned and sequenced from guinea pig brain. No PCR product was detected using ethidium bromide staining after PCR when using specific guinea pig primer pairs for sst4 (Fig. 4). No PCR products were obtained when water or RT-negative samples were used as a template in the same amplification reaction for any sst subtype (Fig. 4). Furthermore, PCR using β-actin primers demonstrated only a 838-bp product. This is a product expected when only cDNA is present. Because this primer pair spans an intron, if a genomic DNA was present, a larger PCR product would have been obtained.

Immunohistochemical detection of neural elements in cultured smooth muscle cells. Because of the possibility that sst subtypes in the freshly dispersed smooth muscle preparations seen with RT-PCR in the above experiments might exist on contaminating neural elements, we cultured the smooth muscle cells for 10–14 days. Both cultured gastric and cultured colonic smooth muscle cell preparations stained positive using a monoclonal antibody to smooth muscle actin. The same cultured smooth muscle cell preparations did not show any evidence of immunohistochemical staining using antibodies to S-100 protein and synaptophysin, two widely used proteins that identify neural tissue (Fig. 5). These combined immunohistochemical results support the lack of neural elements in our cultured gastric and colonic smooth muscle cells.

Expression of sst subtypes on cultured gastric and colonic smooth muscle cells. When RT-PCR was performed on total RNA from cultured gastric and colonic smooth muscle cells using guinea pig sst subtype-specific primer pairs, amplified products for sst2, sst3, and sst5 subtypes were seen (Fig. 6). The amplified products had the same molecular weight on ethidium bromide staining as that seen when brain RNA was used with these primers. Similar to noncultured dispersed muscle cells, no evidence for sst4 was found. The lack of sst1 after PCR and ethidium bromide staining in cultured cells raised the possibility that the sst1 seen in noncultured cells was from contaminating neural elements or that the ethidium bromide staining was not sufficiently sensitive enough to detect low amounts of the sst1 PCR product. To distinguish these two possibilities, Southern blot analysis was performed (Fig. 7). Furthermore, to determine whether the latter possibility (i.e., low sst1 product) was a possible explanation for not seeing evidence of the sst1 subtype on ethidium
bromide staining after PCR on both cultured and noncultured cells, Southern blot analysis was also performed for this subtype (Fig. 8).

Southern blot analysis of PCR products. With the use of a γ-32P-labeled oligonucleotide under conditions that only identified the sst₁ subtype RT-PCR product, the sst₁ subtype was shown to be present in both cultured colonic and gastric smooth muscle cells (Fig. 7). With the use of a guinea pig sst₄-specific, γ-32P-labeled oligonucleotide, under conditions that identified only the sst₄ subtype (Fig. 8), both dispersed and cultured gastric and colonic smooth muscle cell RT-PCR products were found to possess the sst₄ subtype (Fig. 8).

DISCUSSION

The purposes of the present study were to determine whether somatostatin receptors are expressed on gastric and colonic smooth muscle cells and to define the somatostatin receptor subtypes present. The expression of somatostatin receptor subtypes on smooth muscle cells was investigated using an RT-PCR-based strategy. This was chosen because the amount of RNA in smooth muscle cells is low. Furthermore, the smooth muscle cells that are prepared using methods to limit contaminating elements from which the mRNA is extracted are limited. PCR amplification, because of its high sensitivity, can identify the presence of somatostatin receptor subtypes even when they are present in very low abundance. This approach was complicated by the fact that, although guinea pigs are one of the main laboratory animals used to study the effects of somatostatin and other hormones and/or neuropeptides on gastrointestinal smooth muscle motility (26), no guinea pig somatostatin receptor gene structure sequence was known. To overcome this limitation, we first determined the guinea pig cDNA structure sequences for all five guinea pig sst subtypes. To accomplish this, we assumed, as in other species (rat, human) (5–7, 33, 35, 36, 44, 45), that all five sst subtypes would be expressed in the guinea pig brain; therefore, we first performed RT-PCR using total RNA from guinea pig brain. After the human and rat sst sequences were analyzed, a number of sst subtype-specific primer pairs were designed from conserved areas that were specific for each sst subtype, and each was tested. If a single PCR product was seen on the ethidium bromide-stained gel and it was of the expected size, it was sequenced and the sequence was compared with that of the same sst subtype for the human and rat sequence. Generally, we performed further analysis (cloning into the plasmid vector pCR II) only when the nucleotide homology between the supposed guinea pig sst fragment and the human or rat sequence was higher than 75%. For this...
study, the complete coding sequence of each of the five guinea pig sst subtype mRNAs was not necessary; however, we obtained two-thirds or more of the structure of the coding region of sst\textsubscript{1–4} and one-third of that for the guinea pig sst\textsubscript{5}. The guinea pig TM4–TM6 region, obtained for all five guinea pig sst subtypes, compared with the homologous area of human and rat nucleotide sst subtype structures, demonstrated the close homology between the guinea pig and the human and rat sequences (80–99%). These data confirm in the guinea pig what is known in other species (human, rat), that is, that the membrane-spanning helical domains and intracellular connection loops are highly conserved in different species (7, 36). Higher homology existed between guinea pig and human than between guinea pig and rat for the TM4–TM6 oligonucleotide structure for four of the five sst receptor subtypes, with only the guinea pig sst\textsubscript{5} having a slightly higher homology for rat (84%) than for the human (80%) sequence. Recent studies demonstrated that the guinea pig is closer to human than to rat in the evolutionary scale, and this finding is consistent with the higher homology between guinea pig and human sst subtype sequences than with rat sequences (13). The homology at the amino acid level between guinea pig and human TM4–TM6 region is higher than that seen when the nucleotide sequences

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**Fig. 5. Presence of smooth muscle actin but absence of neural elements in cultured colonic (left) and gastric (middle) smooth muscle cell preparations.** Dispersed smooth muscle cells isolated from guinea pig colon or stomach were cultured for 10–14 days as described in MATERIALS AND METHODS. Immunohistochemical staining for smooth muscle actin and the neural markers S-100 protein and synaptophysin, as well as a positive control (right), was performed, as suggested by the manufacturer. There was positive staining for smooth muscle actin in both muscle cell cultures and lack of staining for neural elements (S-100 and synaptophysin) in both cultured smooth muscle cell types.
are compared from the same area. In fact, 100% identity existed for sst1; 96%, 94%, 99%, and 82% homologies were found for sst 2, sst 3, sst 4, and sst 5, respectively. This result, from comparison with the human and rat protein structure of sst subtypes (7, 36), is consistent with the conclusion that the sst1 subtype is the most highly conserved and the sst 5 subtype is the most divergent sst subtype in the guinea pig.

A number of results in the present study support the conclusion that somatostatin receptors are expressed directly on the gastric and colonic smooth muscle cells. First, the correct nucleotide sequence for sst receptors was generated by RT-PCR from dispersed isolated colonic and gastric smooth muscle cell preparations.

Consistent with this result is the finding that somatostatin alters the contractility of isolated gastric and colonic smooth muscle cells in other studies using an identical preparation (9, 15). Second, because dispersed smooth muscle preparations may contain occasional contaminating neural elements (8), the smooth muscle cells were maintained in culture. After 10–14 days in culture, no neural elements were detected; however, somatostatin receptor expression on the cultured smooth muscle cells was demonstrated. The ability to culture these cells and obtain preparations free of contaminating neural elements will allow in the future a number of important studies to be performed, such as the determination of which promoter for the various receptor subtypes might be present in these smooth muscles, as was done recently for sst1 in a number of cell lines (21).

The second purpose of the present study was to identify the somatostatin receptor subtypes present on the gastric and colonic smooth muscle cells. In a study using receptor-selective somatostatin analogs to assess changes in contractility (9), it was concluded that sst3 and sst5 on guinea pig gastric and colonic smooth muscle cells, respectively, mediated the contractile responses of native somatostatin, which can interact with all five sst subtypes with high affinity (3, 23).
However, in the present study, all five somatostatin receptor subtypes were detected in both isolated gastric and colonic smooth muscle cells. This discrepancy between results of the biological activity study (9) and from the PCR results in the present study could have a number of possible explanations. First, it is possible that some of the sst subtypes could be detected at the mRNA level but are expressed in such low numbers that they cannot be detected by studies of contractility. The present results do not allow reliable conclusions to be made about the relative amounts of expression of the mRNAs for the different somatostatin receptors in the gastric and colonic smooth muscle cells. The amount of PCR product may be proportional not only to the amount of each subtype mRNA but also to the efficiency of different primers and the condition of the mRNA of the different subtypes, which could be differentially affected by the isolated conditions used. Attempts to perform Northern blot analysis, which would allow a direct comparison of the amount of mRNA of each somatostatin receptor subtype, did not give reproducible results, probably because of the low abundance of the sst mRNAs in these cells and the limited number of dispersed cells that could be obtained (data not shown). Second, some sst subtypes, although present, might not be coupled to transduction pathways, causing changes in smooth muscle cell contractility, and therefore would not be detected during contraction-relaxation studies. Third, the conclusions from the study of biological activity (9) were obtained by comparing the relative affinities of different synthetic somatostatin analogs in altering contractility in guinea pig gastric and colonic smooth muscle cells with those for the known relative affinities of these various analogs for the sst subtypes in rat and human. This comparison assumes that the somatostatin analogs have similar relative selectivity for the sst subtypes in guinea pig, rat, and human. These conclusions may not be valid if the pharmacology of guinea pig sst subtypes for these different somatostatin analogs differs from that of the rat and human sst subtypes. Fourth, the possibility exists that the somatostatin receptor subtypes seen with PCR might not be on the isolated smooth muscle cells but instead on a contaminating cell. This possibility cannot be completely excluded. However, the fact that each somatostatin receptor subtype could be detected on cultured gastric and colonic smooth muscle cells that possessed no neural elements supports the conclusion that these somatostatin receptor subtypes are on the smooth muscle cells. Fifth, the possibility could be raised that the sst subtypes found by PCR were actually due to contaminating genomic DNA and not to the cDNA from the mRNA. This possibility is excluded by two important controls. First, when no RT was included after the DNase digestion and PCR was performed, no product was seen. Second, when PCR for β-actin was performed after DNase digestion and synthesis of the cDNA, because primers spanning an intron were used, it could be assessed whether a genomic DNA was present. None was detected in any of the preparations, therefore excluding contamination by genomic DNA as a possibility.

In previous studies, a number of different techniques (i.e., binding studies, Northern blot analysis, in situ hybridization, autoradiography, and RT-PCR) have been used to examine the distribution of the five sst subtypes in different human and rat tissues (6, 22, 25, 35, 41). The sst subtypes were reported to have tissue-specific localization with one subtype expressed on a given tissue and in other cases to have an overlapping pattern of distribution with different subtypes expressed on the same tissue (1, 6, 12, 35, 36). Specifically, in the gastrointestinal tract, somatostatin binding studies report binding sites in a number of different tissues and specific cell types (38, 39), whereas other studies using molecular methods have described the widespread presence of sst subtype mRNAs in rat intestine and pancreas (22, 24, 40). Only a few specific cellular localization studies in the gastrointestinal tract have been reported. Recently, the expression of sst subtypes, evaluated by RT-PCR, has been studied in the rat (34) and human enterochromaffin-like cells as well as in the colonic crypt epithelium (43). In rat enterochromaffin-like cells (34) and colonic crypt epithelium (43), the predominant expression of sst2 was found and sst2 activation accounted for the biological changes caused by somatostatin. Other sst subtypes, although present, were functionally not active (43). Recently, a study using in situ hybridization described results similar to the present study by showing the presence of all five sst subtypes on muscle layers from the whole rat gastrointestinal tract, including stomach and colon (22). Furthermore, in various pathological conditions such as on pancreatic endocrine tumors or carcinoid tumors, frequently all five sst subtypes are found to be present on these cells (19). However, the therapeutic actions of octreotide, such as inhibition of hormonal secretion, are due only to the presence of the sst2 and possibly the sst5 subtypes (17, 18, 20, 23, 32). These results, coupled with the findings in the present study, demonstrate that additional sst subtypes can exist in tissues that are not involved in the main biological actions assessed and therefore, at present, have an unknown function.

Recent studies have demonstrated that different sst receptor subtypes can be coupled to different signaling cascades such as the activation of protein tyrosine phosphates, inhibition of cGMP formation, activation of phospholipase C-β3, and inhibition of adenylate cyclase (31, 37). Now that it is established that all five sst subtypes are present on both gastric and colonic smooth muscle cells, with the increased development of agonists highly selective for each sst subtype, in the future it should be possible to explore the effect of selective activation of each sst subtype on these different cellular transduction pathways.

In conclusion, our study demonstrates that somatostatin receptors are expressed in both guinea pig gastric and colonic smooth muscle cells. Furthermore, this study demonstrates that all five somatostatin subtypes are present in smooth muscle cells from both tissues. These results differ from studies of contractile...
activity, which demonstrated evidence for only a sst3 subtype in gastric and a sst5 subtype in dispersed colonic smooth muscle cells, and suggest that the other four subtypes in each tissue likely have important functions in smooth muscle physiology, which may not be directly related to contractile responses.

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