Somatostatin receptor subtype-5 mediates inhibition of peptide YY secretion from rat intestinal cultures

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Received 13 March 2000; accepted in final form 20 May 2000

Chisholm, Connie, and Gordon R. Greenberg. Somatostatin receptor subtype-5 mediates inhibition of peptide YY secretion from rat intestinal cultures. Am J Physiol Gastrointest Liver Physiol 279: G983–G989, 2000.—Somatostatin-14 (S-14) and somatostatin-28 (S-28) bind to five distinct membrane receptors (SSTRs), but S-28 has higher affinity for SSTR-5. Whether S-28 acting through SSTR-5 regulates inhibition of peptide YY (PYY) secretion was tested in fetal rat intestinal cell cultures. S-28 and S-14 caused dose-dependent inhibition of PYY secretion stimulated by gastrin-releasing peptide, but S-28 was more potent than S-14 (EC\textsubscript{50} 0.04 vs. 13.2 nM). PYY was inhibited by two analogs with affinity for SSTR-5, BIM-23268 and BIM-23052, more potently than S-14 and as effectively as S-28. The SSTR-5 analog L-362855 suppressed PYY equivalent only to S-14, but the structurally related peptide L-372585 (Phe to Tyr at position 2) was equipotent to S-28, whereas L-372587 (Phe to Tyr at position 7) caused no inhibition. An SSTR-2 analog decreased PYY secretion similar to S-14, and an SSTR-3 analog was ineffective. PYY secretion stimulated by phorbol 12-myristate 13-acetate (PMA) also potently stimulated PYY release; forskolin and phorbol 12-myristate 13-acetate and by forskolin was also more potently suppressed by S-28 and the octapeptide SSTR-5 analogs. The results indicate that S-28 mediates inhibition of gastrin-releasing peptide-stimulated PYY secretion through activation of SSTR-5 and includes suppression of cAMP- and protein kinase C-dependent pathways. Substitution of a single hydroxyl group confers differences in SSTR-5 agonist properties, suggesting region specificity for the intrinsic activity of this receptor subtype.

MAMMALIAN SOMATOSTATIN is comprised of two principal bioactive molecular forms, somatostatin-14 (S-14) and somatostatin-28 (S-28), derived through differential processing of a common prosomatostatin precursor (31). The gastrointestinal tract provides the largest source of somatostatin and shows relatively selective distribution of the two molecular forms. S-14 is preferentially localized to mucosal D cells in the stomach, duodenum, and proximal jejunum, whereas S-28 is found predominantly in the mucosa of the ileum and colon (18, 25). Recent observations indicating differential regulation of intestinal S-28 and S-14 secretion (11, 12) and selective inhibitory effects on digestive functions (6, 31, 36) suggest independent physiological roles for the two somatostatin molecular forms. The biological effects of somatostatin are mediated by five distinct G protein-coupled membrane receptors (SSTRs) that are widely expressed in the central nervous system and the periphery, including the gastrointestinal tract (3, 14). Whereas SSTR-1 through -4 show similar affinities for S-14 and S-28, SSTR-5 is distinguished by a higher affinity for S-28 than for S-14 (19, 20, 41, 42). The development of selective somatostatin analogs with relatively high binding affinities for specific subtypes has facilitated examination of the actions of SSTRs. In the gastrointestinal tract, SSTR-2 mediates inhibition of gastrin (9), histamine (26), and gastric acid secretion (9, 16) and SSTR-3 modulates gastric smooth muscle contraction (13). Although SSTR-5 has been shown to regulate inhibition of growth hormone secretion (32), pancreatic enzyme (29) and insulin secretion (34), and colonic smooth muscle contraction (4), a specific role for the SSTR-5 subtype in the regulation of intestinal endocrine secretion has not been identified.

Peptide YY (PYY), a regulator of the ileal break mechanism (33) and an enterogranule (8, 22), is also preferentially found in the ileum and colon, where it is localized to L cells (1). Previous studies from our laboratory have shown that somatostatin is a potent inhibitor of PYY secretion and plays a role in the counterregulation of fat-mediated inhibition of acid secretion by PYY (10). To examine further the mechanisms regulating PYY release from intestinal L cells, a rat intestinal cell culture system that synthesizes and secretes PYY was established (2). Initial investigations with this preparation demonstrated that gastrin-releasing peptide (GRP) is one of the most effective stimulators of PYY release; forskolin and phorbol 12-myristate 13-acetate (PMA) also potently stimulated PYY release, indicating involvement of cAMP- and protein kinase C (PKC)-dependent pathways (2). In the present study, this cell culture model was used to investigate whether PYY release stimulated by GRP is preferentially regulated by S-28 or by S-14 and to determine which of the somatostatin receptor subtypes mediate inhibition by employing somatostatin analogs with different binding affinities to the SSTRs. Postreceptor activation of PYY was also investigated by com-
paring the effects of the somatostatin analogs on forskolin- and PMA-stimulated PYY release.

**MATERIALS AND METHODS**

**Cell isolation and culture.** Fetal rat intestinal cells were placed into monolayer cultures as described in detail previously (2). In brief, small and large intestines from 19- to 21-day-old fetal Wistar rats were dissected free of gastric and pancreatic tissues, and the cells were dispersed by incubation with collagenase (Type H; Sigma, St. Louis, MO), hyaluronidase (type II), and deoxyribonuclease-1 (Sigma) and placed into monolayer culture for 24 h at a density of 0.625 fetal rat intestines/60-mm dish. Cells were then washed with Hanks’ balanced salt solution and incubated with test agents for 2 h in DMEM containing 0.5% (vol/vol) FBS, 1 g/l glucose, 20 μg/ml insulin, 50 IU/ml penicillin, and 50 μg/ml streptomycin both without and, in separate experiments, with 10 μM amastatin and 1 μM phosphoramidon. Groups of two dishes were used for all experiments, and the experiments were repeated 4–6 times. After the incubation period, medium samples were centrifuged to remove any floating cells and made to 0.1% (vol/vol) trifluoroacetic acid. Cells were homogenized in 1 N HCl containing 5% (vol/vol) HCOOH, 1% (vol/vol) trifluoroacetic acid, and 1% (wt/vol) NaCl. PYY contained in the media and cell samples were then collected separately by passage through a cartridge of C18 silica (Sep-Pak; Waters Associates, Milford, MA), as described previously (2). This methodology affords a >95% recovery of exogenously added PYY. Aliquots of each extract were dried in vacuo, and the samples were stored at −20°C for subsequent RIA determinations. Animal protocols were approved by the University of Toronto animal care committee according to Canadian Council on Animal Care standards.

**Somatostatin analogs and test agents.** The analogs D-Phe-Cys-Tyr-d-Trp-Lys-Au-Cys-d-NaL-NH₃ [where NaL is B-(2-naphthyl)alanine and Abu is α-amino-β-butyric acid] (NC8–12), c[Cys-Phe-Phe-d-Trp-Lys-Thr-Phe-Cys]-NH₃ (BIM-23268), d-Phe-Phe-Phe-d-Trp-Lys-Val-Phe-Thr-NH₃ (BIM-23058), d-Phe-Phe-Phe-d-Trp-Lys-Thr-Phe-Thr-NH₃ (BIM-23052), and d-Phe-Phe-Phe-d-Trp-Lys-Val-Phe-d-NaL-NH₃ (BIM-23056) were gifts of Dr. J. E. Taylor (Biomeasure, Milford, MA). The analogs c[Aha-Phe-Phe-d-Trp-Lys-Thr-Phe] (where Aha is 7-aminoheptanoic acid) (L-362855), c[Aha-Tyr-Trp-d-Trp-Lys-Thr-Phe]-NH₃ (L-372587), and c[Aha-Phe-Trp-d-Trp-Lys-Thr-Tyr] (L-372588) were gifts of Dr. R. M. Freidinger (Merck Research Laboratories, West Point, PA). The analogs were dissolved in distilled water at 1 mM, with the exception of BIM-23056, which was dissolved in absolute ethanol, lyophilized, and stored at −20°C until used. GRP, PYY, S-28, and S-14 were obtained from Bachem (Torrance, CA). Forskolin, IBMX, PMA, nitrendipine, orthovanadate, amastatin, and phosphoramidon were obtained from Sigma.

**RIA.** Immunoreactive PYY was quantified by RIA techniques using methods described in detail previously (2). The detection limit of the assay is 0.8 fmol/tube, and the sensitivity (IC₅₀) is 10.0 fmol/tube.

**Data and statistical analysis.** Secretion of PYY was determined as a percentage of the total cell content of PYY (100 × medium PYY/medium plus cellular PYY) at the end of the incubation period. The total cell content of PYY was determined to be constant under all test conditions used in the present study (164 ± 13 fmol/dish; n = 48), consistent with previous findings (2). PYY suppression by each analog was calculated as a percentage of the control PYY secretion (GRP, PMA, or forskolin) above basal in the same experiment. Statistical comparisons of means within a group were evaluated by Student’s t-test, and differences between groups were tested by ANOVA followed by a multiple-comparisons test using Sigma-Stat (Jandel Scientific, San Rafael, CA). Results are expressed as means ± SE; P < 0.05 was considered significant.

**RESULTS**

**Effects of S-14 and S-28.** In the basal state, medium contained 10.6 ± 0.9 fmol PYY/dish or 5.3 ± 0.2% of cell content; the total cell content of PYY remained constant. After 2 h treatment, GRP (100 nM) increased PYY to 12.6 ± 0.4 or by 239 ± 10% of paired basal controls (P < 0.001). Both S-28 and S-14 caused concentration-dependent inhibition of GRP-stimulated PYY secretion (Fig. 1). However, S-28 was more potent compared with S-14 (P < 0.001). Maximal inhibition of basal values occurred at 1 nM S-28 and 1 μM S-14, with EC₅₀ for S-28 and S-14 of 0.04 and 13.2 nM, respectively. The addition of amastatin (10 μM) and phosphoramidon (1 μM) did not alter the potency of either S-28 (EC₅₀ 0.04 nM) or S-14 (EC₅₀ 13.8 nM).

**Effects of somatostatin analogs.** The relatively specific SSTR-5-preferring analogs BIM-23268 and BIM-23052 decreased GRP-stimulated PYY secretion in a concentration-dependent manner, with maximal inhibition to basal values occurring at 1 nM and 10 nM, respectively (Fig. 2). BIM-23268 (EC₅₀ 0.09 nM) was as effective as S-28, whereas BIM-23052 (EC₅₀ 0.17 nM) was marginally less potent than S-28 (P < 0.05). Both BIM-23268 and BIM-23052 were more effective compared with S-14 (both P < 0.001).

The SSTR-2 analog NC8–12 also showed concentration-dependent inhibition of GRP-stimulated PYY secretion, but the profile of inhibition was not different from that obtained with S-14 (Fig. 2). NC8–12 was less potent compared with S-28 (P < 0.001), BIM-23268
The SSTR-3 analog BIM-23058 was without effect at concentrations up to 10 nM (Fig. 2).

**Structure-activity relationships.** The SSTR-5-prefering analog L-362855 has two Phe residues in its structure, and conversion of one of the Phe to a Tyr at position 7 or position 2 generates the peptides L-372588 and L-372587, respectively. As shown in Fig. 3, L-372588 caused concentration-dependent inhibition of PYY secretion (EC\(_{50}\) 0.06 nM) with a potency similar to S-28. However, L-362855 was less effective compared with L-372588 or S-28 (both \(P < 0.001\)), with a profile of inhibition that was not different from S-14 (Fig. 3). L-372587 did not affect PYY secretion at concentrations up to 10 nM (Fig. 3).

Previous studies undertaken in a pituitary cell line expressing SSTR-5 indicated that the hexapeptide L-362855 at a concentration of 1 \(\mu\)M behaved as a partial agonist but at lower concentrations (<100 nM) acted as an SSTR-5 antagonist by blocking the inhibitory effect of BIM-23052 (35). However, coadministration of L-362855 (10 nM) with S-28 (1 nM) did alter the inhibition of GRP-stimulated PYY release caused by S-28 alone, and when coadministered with BIM-23052 (1 nM) further suppressed PYY release to levels that were not different from basal values (Fig. 4). The linear peptide BIM-23056, which shows relative selectivity for SSTR-3 (27), is also reported to act as an SSTR-5 antagonist in Chinese hamster ovary (CHO) cells expressing SSTR-5 (40). However, BIM-23056 (10 nM) caused only a weak agonist effect, with maximal inhibition by 23 ± 4%, and did not alter the PYY inhibition caused by S-28 (1 nM; Fig. 4) or the SSTR-5 analogs (data not shown).

**Effects of somatostatin analogs on signal transduction.** To investigate the cellular basis of action of SSTR-5, S-28 and the analogs were studied in the presence of activators of PKC (PMA) or protein kinase A (PKA; forskolin plus IBMX). Consistent with previous observations (2), PMA (1 \(\mu\)M) stimulated PYY secretion to 12.4 ± 0.6% total cell content at 2 h or by 235 ± 12% of paired basal values (\(P < 0.001\); Fig. 5), a value similar to that observed after stimulation with GRP. Both S-28 (10 nM) and S-14 (10 nM) inhibited

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**Fig. 2.** PYY response to treatment with 100 nM GRP alone and with different concentrations of S-14, S-28, and the analogs BIM-23268, BIM-23052, NC8–12, and BIM-23058. PYY inhibition to each analog is expressed as a percentage of the PYY response to GRP alone above basal. Values are means ± SE of 4–6 experiments.

**Fig. 3.** PYY response to treatment with 100 nM GRP alone and with different concentrations of S-14, S-28, and the analogs L-372588, L-362855, and L-372587. PYY inhibition to each analog is expressed as a percentage of the PYY response to GRP alone above basal. Values are means ± SE of 4–6 experiments.

**Fig. 4.** Secretion of PYY in the basal state and in response to treatment with 100 nM GRP alone, with S-28 alone, with S-28 plus L-362855 (L-855), with BIM-23052 (B-52) plus L-362855 (L-855), or with S-28 plus BIM-23056 (B-56). Secretion is expressed as %TCC. Values are means ± SE of 4 experiments.
PMA-stimulated PYY by 90 ± 5% and 41 ± 8%, respectively, but S-28 was more effective when compared with S-14 (P < 0.01; Fig. 5). BIM-23052 (10 nM) inhibited PMA-stimulated PYY release by 67 ± 5%, whereas L-372588 (10 nM, a concentration that abolished GRP-stimulated PYY secretion) had no effect on PMA-stimulated PYY secretion. L-362855 (10 nM) also did not alter PMA-stimulated PYY secretion (Fig. 5). NC8–12 (10 nM) inhibited PMA-stimulated PYY by 39 ± 6% and was only as effective as S-14 (Fig. 5).

Activation of PKA with forskolin plus IBMX (both 10 μM) increased PYY secretion to 45.2 ± 2.7% total cell content at 2 h (P < 0.001 compared with paired basal values) or about fourfold above PYY release stimulated with GRP or PMA (Fig. 6). Both S-28 (10 nM) and S-14 (10 nM) inhibited forskolin-stimulated PYY release by 58 ± 7% and 18 ± 4%, respectively, but S-28 was more effective compared with S-14 (P, 0.001; Fig. 6). BIM-23052 (10 nM) decreased forskolin-stimulated PYY release by 46 ± 8%, but L-372588 (10 nM) was without effect (Fig. 6). NC8–12 (10 nM) inhibited forskolin-stimulated PYY by 33 ± 7% and was more effective compared with S-14 (P < 0.05).

In contrast to the findings with PMA, L-362855 (10 nM) also reduced forskolin-stimulated PYY secretion by 39 ± 4% but was less potent compared with S-28 (P < 0.05; Fig. 6). L-362855 is also reported to antagonize the inhibition of forskolin-stimulated cAMP accumulation by somatostatin in CHO cells expressing SSTR-5 (35). However, after pretreatment with L-362855 (10 nM), inhibition of forskolin-stimulated PYY by 1 nM S-28 (61 ± 7%) or 1 nM BIM-23052 (49 ± 7%) was not different from the values observed after S-28 (1 nM) or BIM-23052 (10 nM) alone (Fig. 6).

Coadministration of L-362855 (10 nM) with S-14 (1 nM) caused further suppression of forskolin-stimulated PYY release by 41 ± 6% (P < 0.05 compared with S-14 alone), suggesting that L-362855 acted as partial agonist.

Pretreatment of cells with nitrendipine (10 μM) did not alter GRP-stimulated PYY release (12.8 ± 0.5 vs. 12.6 ± 0.4%; n = 4). Similarly, the inhibitory responses with nitrendipine to 1 nM S-28 (103 ± 8%), 10 nM S-14 (48 ± 4%), 1 nM BIM-23052 (67 ± 5%), and 1 nM L-372588 (84 ± 8%) were not different from the values observed with the same analogs alone (Figs. 2 and 3).

Pretreatment of the cells with and without orthovanadate (10 μM) also did not alter GRP-stimulated PYY inhibition by 1 nM S-28 (106 ± 7 vs. 104 ± 6%; n = 4), 10 nM S-14, or the SSTR analogs (data not shown).

DISCUSSION

Somatostatin is known to have diverse inhibitory effects on a number of endocrine cell types, including those found in the gastrointestinal tract. S-14 has generally been regarded as the only bioactive molecular form of somatostatin regulating inhibition of endocrine secretion from the gut, whereas the role of S-28 in counterregulatory endocrine suppression has been thought to be limited to modulation of the pituitary hormones (32) and insulin (6). The present study shows that S-28 is the principal bioactive molecular form of somatostatin mediating inhibition of PYY secretion from rat intestinal cell cultures. Both somatostatin molecular forms caused dose-dependent inhibition of PYY secretion when stimulated by GRP, the principal neurotransmitter regulating PYY release (2). However, S-28 was ~300-fold more potent than S-14. This difference was not attributable to endopeptidase-mediated
degradation of S-14 (7, 38) because the potency of both peptides was not modified by the presence of the peptidase inhibitors amastatin and phosphoramidon. The preferential inhibitory effect by S-28 occurred at an IC_{50} that was well within the range for receptor binding (28) and thus was likely physiologically relevant.

Although five subtypes of structurally related somatostatin receptors have been cloned, SSTR-5 is the only subtype with preferential affinity for S-28 (19, 20). Distribution of SSTR-5 mRNA expression has been observed in the small intestine of rats (14) and humans (20), in accord with the intestinal localization of S-28. In the present study, results obtained with agonists formulated to provide relatively selective affinities for different somatostatin receptors indicated that activation of SSTR-5 mediated the PYY suppression by S-28. Two compounds that show relatively high selectivity for SSTR-5, BIM-23052 (24, 28) and BIM-23268 (5), caused dose-dependent inhibition of PYY more effectively than S-14 and at a magnitude that was similar to S-28. The more potent of the two analogs, BIM-23268, is a unique cyclic octapeptide that begins and ends at the NH_{2} and COOH terminals of the octapeptide and has the highest selectivity so far reported for SSTR-5, with an affinity equivalent to S-28 (5). The selectivity of SSTR-5 activation was further supported by the response to NC8–12, which binds to the two isoforms of the SSTR-2 receptor with high affinity (37) yet caused relatively weaker inhibition of PYY secretion and was no more potent than S-14. Significant involvement of SSTR-3 seems unlikely given the lack of effect by the SSTR-3 analogs. The finding of preferential counter-regulatory suppression of PYY secretion by S-28 mediated through activation of SSTR-5 therefore delineates a novel role for this receptor subtype in the gastrointestinal tract.

Among the signal transduction pathways involved in SSTR-5 activation are suppression of adenyl cyclase, inhibition of L-type Ca^{2+} channels, stimulation of phospholipase C activity, and activation of phosphotyrosine phosphatase (23). In the present study, forskolin was used as a secretagogue to increase cAMP, resulting in potent stimulation of PYY release. S-28 and the SSTR-5 analogs suppressed forskolin-stimulated PYY with a rank order of effectiveness (S-28 > BIM-23052 > NC8–12 > S-14) that accords with the preferential affinity of S-28 over S-14 for SSTR-5, consistent with the suppression of adenyl cyclase described in SSTR-5-transfected CHO cells (20). However, our results implicate the involvement of cAMP-independent pathways mediating S-28 activation of SSTR-5 responses to GRP-stimulated PYY release from intestinal L cells. GRP exerts its biological effects through elevations in intracellular Ca^{2+} and by activation of PKC. (15). Although Ca^{2+} ionophores do not influence PYY release in our model (2), PMA, a direct activator of diacylglycerol-sensitive PKC isozymes, stimulated PYY secretion similar to GRP. Consistent with the notion that S-28 activation of SSTR-5 modulates inhibition of PKC was the finding that PMA-stimulated PYY release was preferentially suppressed by S-28 compared with S-14 and by the SSTR-5 analogs compared with the SSTR-2 analog. Similar observations were made in studies using isolated colonic smooth cells, in which S-28 inhibited contraction mediated by bombesin and the phorbol ester tetradecanoyl phorbol 13-acetate, although the effect of SSTR-5 analogs was not examined (4). Together, these findings suggest that S-28 activation of SSTR-5 in normal intestinal cells modulates suppression of PKC pathways, in contrast to results obtained in transfected cells in which SSTR-5 appears to stimulate phospholipase C-dependent inositol triphosphate production (40).

Certain lines of evidence indicate an important role for single hydroxyl groups in ligand binding to SSTR-5. Mutagenesis studies on cloned rat SSTR-5 receptors indicate that replacement of Phe with Tyr^{265} in SSTR-5 increased the affinity of the receptor for S-14 to a level comparable to that observed for S-28. (21). Recent structure-activity studies in the AtT-20 pituitary cell line also point to the involvement of a single hydroxyl group in SSTR-5 activation by showing that a group of structurally-related hexapeptide SSTR-5 analogs cause differential inhibitory responses on L-type Ca^{2+} channel current conductance (35). The present study lends further support to the notion that single hydroxyl groups modulate SSTR-5 activity. L-362855, a hexapeptide with relatively high affinity for both rat and human SSTR-5 (28, 35), was only as effective as S-14 in causing PYY inhibition. However, the addition of a hydroxyl group (Phe to Tyr) at position 7 of L-362855 yields L-372588, and this analog was ~300-fold more potent than L-362855, causing suppression of PYY secretion equivalent to S-28. In contrast, the addition of a hydroxyl group at position 2 of L-362855 yields L-372587, and this analog had no effect on PYY secretion. Similar differences in the agonist properties of these analogs were shown in AtT-20 cells (35). These findings suggest that the Phe at position 2 may facilitate the interaction of this group of hexapeptides with SSTR-5. The observations also imply that, in addition to the role of the transmembrane domain 6 Phe (21), ligand binding may be modulated by hydroxyl group interactions with an additional element of the SSTR-5 receptor. Notably, a d-Tyr^{1} version of the octapeptide BIM-23052 shows improved affinity for SSTR-5 and with similar selectivity (5). Whether conversion of the Phe between the two Thr residues to a Tyr would further improve selectivity and potency of the SSTR-5 octapeptide analogs requires evaluation.

The SSTR-5 hexapeptide analogs were also remarkable for their differential effects on signal transduction compared with the octapeptide analogs. L-362855 reduced forskolin-stimulated PYY secretion, similar to effects on cAMP described for SSTR-5-transfected cells (35). However, unlike BIM-23052, L-362855 did not influence PYY stimulated by GRP or PMA, suggesting that inhibition of cAMP-dependent pathways may be a relatively selective effect of this analog. Furthermore, notwithstanding the potent inhibition of GRP-stimulated PYY secretion by L-372588, interestingly, this analog had no effect on PYY secretion stimulated by
PMA or forskolin, and the suppressive effects were not altered by pretreatment with orthovanadate, an inhibitor of protein tyrosine phosphatase activity. Responses to L-372558 also were not influenced by nitrendipine, an L-type Ca\(^{2+}\) channel blocker, indicating that SSTR-5 activation was not mediated by inhibition of Ca\(^{2+}\) influx. This latter finding contrasts with the inhibitory mechanism described for L-372558 in AtT-20 cells (35). The signal transduction pathway associated with L-372558 inhibition of PYY, therefore, is uncertain and requires further study. However, the observation that single hydroxyl group substitutions can selectively influence SSTR-5 inhibition of different signal transduction pathways in the same cell type could provide an additional approach to the assessment of cellular events mediating SSTR-5 effects.

The linear octapeptide BIM-23056 has been reported to act as competitive antagonist to S-14 in CHO cells, expressing SSTR-5 by selectively attenuating the changes in intracellular Ca\(^{2+}\) concentrations produced by somatostatin (39). We found that BIM-23056 acted only as a weak agonist on PYY secretion and did not identify any antagonist properties on the inhibitory responses to S-28 or any of the other analogs studied. Similarly, we were unable to confirm an antagonist effect for L-362855 on the inhibition of GRP- or forskolin-stimulated PYY by S-28 and BIM-23052, as reported in investigations of AtT-20 and CHO cells (35). This divergent finding may, in part, reflect variability in the expression of SSTR receptors in cell lines compared with normal intestinal cells, because antagonism by BIM-23056 also was not found in studies of ion transport on colonic epithelial cells (17).

In conclusion, the present studies indicate a novel role for S-28 acting through SSTR-5 in the counter-regulation of PYY secretion from the gastrointestinal tract. Activation of SSTR-5 inhibits regulation of PYY secretion through both PKA- and PKC-dependent pathways. That alterations of a single hydroxyl group can modulate SSTR-5 agonist properties and cause selective effects on signal transduction pathways suggests an important region for intrinsic activity of this receptor subtype and may facilitate development of more potent agonists.

This work was supported in part by a grant from the Medical Research Council of Canada (MA-6763).

REFERENCES


