Intracisternal Injection of Somatostatin Receptor 5-Preferring Agonists Induces a Vagal Cholinergic Stimulation of Gastric Emptying in Rats¹

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ABSTRACT

We previously showed that the somatostatin receptor 5 (sst₅) -preferring agonist BIM-23052 injected intracisternally (i.c.; 0.8 nmol/rat) stimulated gastric emptying of a non-nutrient meal in conscious rats. In this study, we investigated the neural pathways and specificity of BIM-23052 action. BIM-23052 (0.4, 0.8, and 1.2 nmol/rat i.c.) stimulated gastric transit; values of gastric emptying were 65.5 ± 6.5, 77.4 ± 5.3, and 77.7 ± 1.9%, respectively, compared with 43.2 ±3.2% in i.c. saline group. Intravenous injection of BIM-23052 (0.8 nmol/rat) had no effect. BIM-23052 (0.8 nmol/rat i.c.) action was prevented by subdiaphragmatic vagotomy or atropine. Medullary thyrotropin-releasing hormone (TRH) is known to play a physiological role in the vagal stimulation of gastric motor function. TRH receptor antisense oligodeoxynucleotides injected i.c. with a regimen that prevented TRH (0.3 nmol/rat i.c.)-induced enhanced gastric emptying did not influence BIM-23052 stimulatory action. Somatostatin-28 (0.2–1.2 nmol/rat i.c.), which possesses a higher affinity than somatostatin-14 for sst₅, and the cyclic octapeptide SMS 201-995, also known as octreotide (des-AA¹,2,4,5,12,13[O-Trp⁸]somatostatin) and sub-class 2, including sst₁ and sst₃ receptors that show extremely low affinity for SMS 201-995 (Hoyer et al., 1995). Ligands with preferential affinities for the five cloned receptors have been used to assess the role of different receptor subtypes in mediating the pleiotropic peripheral and central actions of somatostatin to regulate gastrointestinal function (Martínez et al., 1995, 1996; Wyatt et al., 1996, 1998a). Pharmacological, morphological, and functional studies clearly established that sst₅ receptor is the main subtype whereby peripheral endogenous somatostatin inhibits gastric acid secretion in rats, mice, and dogs (Rossowski and Coy, 1993; Wyatt et al., 1996; Lloyd et al., 1997; Martínez et al., 1998a; Kawakubo et al., 1999). In the central nervous system, the pattern of somatostatin actions to influence gastric acid secretion varies with the somatostatin agonists used and the brain sites of injection because both inhibitory and stimulatory responses are observed in rats (Martínez et al., 1995, 1996; Yoneda and

Somatostatin is a peptide widely distributed throughout the mammalian gastrointestinal tract and central nervous system, including the hypothalamus and brainstem (McIntosh, 1985; Fitzpatrick-McElligott et al., 1988). Somatostatin interacts with five G-protein-coupled, 7-transmembrane-spanning receptors that have been identified by molecular cloning techniques in mammals and characterized pharmacologically (Hoyer et al., 1995; Rohrer et al., 1998; Patel, 1999). These receptors have been named somatostatin receptor subtypes 1 to 5 (sst₁ to sst₅; Hoyer et al., 1995). They are grouped into somatostatin class 1, including sst₂, sst₃, and sst₅ receptors that display high relative affinity for the cyclic octapeptide SMS 201-995, also known as octreotide (des-AA¹,2,4,5,12,13[O-Trp⁸]somatostatin) and sub-class 2, including sst₁ and sst₃ receptors that show extremely low affinity for SMS 201-995 (Hoyer et al., 1995). Ligands with preferential affinities for the five cloned receptors have been used to assess the role of different receptor subtypes in mediating the pleiotropic peripheral and central actions of somatostatin to regulate gastrointestinal function (Martínez et al., 1995, 1996; Wyatt et al., 1996, 1998a). Pharmacological, morphological, and functional studies clearly established that sst₅ receptor is the main subtype whereby peripheral endogenous somatostatin inhibits gastric acid secretion in rats, mice, and dogs (Rossowski and Coy, 1993; Wyatt et al., 1996; Lloyd et al., 1997; Martínez et al., 1998a; Kawakubo et al., 1999). In the central nervous system, the pattern of somatostatin actions to influence gastric acid secretion varies with the somatostatin agonists used and the brain sites of injection because both inhibitory and stimulatory responses are observed in rats (Martínez et al., 1995, 1996; Yoneda and

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ABBREVIATIONS: sst, somatostatin receptor subtype; ODT8-SST, des-AA¹,2,4,5,12,13[O-Trp⁸]somatostatin; i.c., intracisternal; DMN, dorsal motor nucleus of the vagus; TRH, thyrotropin-releasing hormone; CSF, cerebrospinal fluid.
A role for a somatostatin receptor belonging to the class 1 is suggested by the demonstration that SMS 201-995 (octreotide) or somatostatin-14 injected into the lateral brain ventricle or into specific hypothalamic nuclei inhibits gastric acid secretion in rats (Yoneda and Taché, 1995; Martínez et al., 1996). In contrast, the somatostatin agonists des-4,8,9-somatostatin (ODT8-SST) and SMS 201-995 (Reubi et al., 1981; Raynor et al., 1993a,b; Patel and Srikant, 1994) injected intracerebrally (i.c.) or microinjected directly into the dorsal motor nucleus of the vagus (DMN) stimulated gastric acid output through vagal-dependent pathways in rats (Taché et al., 1981; Yoneda et al., 1991; Yoneda and Taché, 1995).

A number of peptides shown to act in the brain to influence gastric acid secretion also modulate gastric emptying (Taché et al., 1990; Martínez et al., 1998b). Among them, thyrotropin-releasing hormone (TRH), which activates directly neurons in the DMN and gastric vagal outflow (Travaglì et al., 1992; O’Lee et al. 1997), plays a physiological role in specific stimuli such as cold exposure-induced stimulation of gastric emptying (Martínez et al., 1998b). An earlier study indicates that microinjection of somatostatin-14 into the DMN stimulates gastric motility in urethane-anesthetized rats (Hermann and Rogers, 1989). Preliminary evidence also shows that BIM-23052, a linear somatostatin agonist, which displays high binding affinity for the cloned rat sst2 receptor (O’Carroll et al., 1994), injected i.c. at 0.8 nmol/rat stimulated gastric emptying in rats independently from the TRH receptor (Martínez et al., 1998b). This study aims to assess whether BIM-23052 injected i.c. stimulates gastric emptying through central vagal cholinergic pathways. We also investigated the selectivity of BIM-23052 for activation of the sst2. We used somatostatin-28 because it has a higher affinity than somatostatin-14 for the cloned rat and human sst5 (O’Carroll et al., 1994; Patel and Srikant, 1994; Patel, 1999), and ODT8-SST, which after injection into the cerebrospinal fluid (CSF) exhibits a similar potency and pattern of biological actions as somatostatin-28, unlike somatostatin-14 (Brown et al., 1981, 1984; Taché et al., 1981; Vecsei and Widerlov, 1990). Based on recent in vitro studies suggesting that BIM-23056 may function as an sst5 receptor antagonist (Wilkinson et al., 1996; Siezies and Hoyer, 1999), we investigated whether this analog exhibits antagonist action when injected i.c. before somatostatin-28. We also tested somatostatin agonists with preferential affinities for somatostatin receptor subtypes established in vitro on human cloned somatostatin receptors 1 to 4, namely, CH-275 (sst1) (Liapakis et al., 1996; Leroux et al., 1997; Patel, 1999), NC-8-12 (sst2), and BIM-23056 (sst3) (Raynor et al., 1993a,b; Patel, 1999) as well as the recently developed nonpeptide-selective agonists L-803,087 and L-796,778 (Reubi et al., 1981; Raynor et al., 1993a,b; Patel and Srikant, 1994) injected intracisternally (i.c.) or microinjected directly into the dorsal motor nucleus of the vagus (DMN) stimulated gastric acid output through vagal-dependent pathways in rats (Taché et al., 1981; Yoneda et al., 1991; Yoneda and Taché, 1995).

**Chemicals**

The following peptides, somatostatin-14 and TRH (Peninsula Laboratories, Belmont, CA), somatostatin-28 (Bachem Inc., Torrance, CA), and CH-275 (Peptide Biology Laboratory, Salk Institute, La Jolla, CA) were dissolved immediately before use in 0.9% sterile saline (Sigma, St. Louis, MO) to an initial concentration of 1 µg/µl; NC-8-12 (also known as DC-32-87), BIM-23052, and BIM-23056 (Peptide Research Laboratories, Tulane University, New Orleans, LA) were dissolved in 0.01% acetic acid to a concentration of 1 µg/µl immediately before use. The chemical structures of the peptides are listed in Table 1. The nonpeptide agonists L-803,087 and L-796,778 (Merck Research Laboratories, Rahway, NJ) were dissolved in 100% dimethyl sulfoxide (Sigma) to a concentration of 1 µg/µl before use. In all cases, further dilutions were made in 0.9% sterile saline to reach appropriate concentrations for i.c. injection of 10 µl. Saline alone, 0.01% acetic acid, and 100% dimethyl sulfoxide (both diluted 1:10 in saline) were used as vehicle controls.

**Treatments**

Drug administrations were performed in rats under short enflurane anesthesia (2–3 min; 5% vapor concentration in O2; Etrane, Anquest, Madison, WI). For i.c. injection, rats were placed on ear bars of a stereotaxic frame and the occipital membrane was punctured with the needle of a Hamilton syringe. Correct positioning of the needle into the cisterna magna was determined by the reflux of CSF into the syringe. Drugs were administered in 10 µl manually over 20 s. For i.v. administrations, a small incision was made in the ventral right side of the neck, the jugular vein was exposed, and drugs or vehicle were injected manually in 0.1 ml over 15 s.

**Measurement of Gastric Emptying**

Gastric emptying was determined by the phenol red method, as previously described (Martínez et al., 1998b). The liquid meal consisted of methyl cellulose (Sigma) dispersed in hot water at a final concentration of 1.5% under continuous stirring in which phenol red (50 mg/100 ml; Sigma) was added as a nonabsorbable marker. The meal was given intragastrically (1.5 ml of methyl cellulose-phenol red solution at room temperature) by oral intubation of conscious rats at appropriate times.

**TABLE 1**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Structures</th>
<th>Relative Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somatostatin-14</td>
<td>Ala-Gly-c[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys]-OH</td>
<td>sst2−sst3−sst4−sst5−</td>
</tr>
<tr>
<td>Somatostatin-28</td>
<td>Ser-Ala-Ase-Asn-Pro-Ala-Met-Ala-Pro-Arg-Glu-Arg-Lys-Ala-Gly-c[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys]-OH</td>
<td>sst2−sst3−sst4−sst5−</td>
</tr>
<tr>
<td>ODT8-SST</td>
<td>c[Cys-Phe-Phe-d-Trp-Lys-Thr-Phe-Cys]-OH</td>
<td>Unknown</td>
</tr>
<tr>
<td>CH-275</td>
<td>c[Cys-Lys-Phe-Phe-d-Trp-Lamp-Thr-Phe-Thr-Ser-Cys]-OH</td>
<td>sst1−sst4−</td>
</tr>
<tr>
<td>NC-8-12</td>
<td>d-Phe-c[Cys-Tyr-d-Trp-Lys-Abu-Cys]-Nal-NH2</td>
<td>sst2−sst3−</td>
</tr>
<tr>
<td>BIM-23056</td>
<td>d-Phe-Phe-Tyr-d-Trp-Lys-Val-Phe-d-Nal-NH2</td>
<td>sst2−sst5−</td>
</tr>
<tr>
<td>BIM-23052</td>
<td>d-Phe-Phe-Phe-d-Trp-Lys-Thr-Phe-Thr-NH2</td>
<td>sst2−sst5−</td>
</tr>
</tbody>
</table>

*Based on binding affinity for cloned human receptors (from Patel, 1999).

**Materials and Methods**

**Animals**

Adult male Sprague-Dawley rats (Harlan, San Diego, CA) weighing 250 to 280 g were housed in group cages under controlled conditions of 12-h light/dark cycle and temperature (21–23°C). Animals had free access to food (Purina Rat Chow) and tap water up to 18 to 20 h before experiments when food, but not water, was removed. Studies were conducted under the Veterans Administration Animal Component of Research Protocol no. 98-080-08.
rats with a stainless steel cannula. After a 20-min period, rats were euthanized by CO₂ inhalation. The abdominal cavity was opened, the gastroesophageal junction and the pylorus were clamped, and the stomach was excised and rinsed in 0.9% saline. After removing the clamps, the stomach was placed in 100 ml of 0.1 N NaOH and homogenized (Polytron; Brinkmann Instruments Inc., Westbury, NY). The suspension was allowed to settle for 1 h at room temperature and then 5 ml of the supernatant was added to 0.5 ml of 20% trichloroacetic acid (w/v; Sigma) and centrifuged at 3000 rpm at 4°C for 20 min. The supernatant was mixed with 4 ml of 0.5 N NaOH, and the absorbance of the sample read at 560 nm (Shimazu 260 spectrophotometer). Phenol red recovered from animals euthanized immediately after the administration of the methylcellulose solution was used as a standard (0% emptying). Percentage of emptying in the 20-min period was calculated according to the following equation: 

\[
\text{Percentage of emptying} = \left(1 - \frac{\text{absorbance of test sample}}{\text{absorbance of standard}}\right) \times 100.
\]

**Experimental Procedures**

**Effects of BIM-23052 Injected i.c. or i.v. on Gastric Emptying.** Under short anesthesia, fasted rats were injected i.c. with BIM-23052 (0.2, 0.4, 0.8, or 1.2 nmol/rat) or vehicle (1 µl of 0.01% acetic acid + 9 µl of saline) and returned to their home cages. Ten minutes later, the phenol red-methylcellulose viscous solution was administered to awake rats and gastric emptying was determined 20 min later. A similar protocol was used for i.v. injection of vehicle or BIM-23052 with the dose that induces maximal gastric response when injected i.c.

**Effects of Vagotomy and Cholinergic Blockade.** Subdiaphragmatic vagotomy was performed 48 h before the experiments in fasted rats under ketamine hydrochloride (75 mg/kg i.p.; Ketaset, Fort Dodge Laboratories, Fort Dodge, IA) and xylazine (5 mg/kg i.p.; Rompun, Mobay, Shawnee, KS) anesthesia. Vagotomy was achieved by a circular seromuscular myotomy of the esophagus, at a level ~2 cm from the gastroesophageal junction. Sham-operated animals (laparotomy and manipulation of the stomach) were used as controls. Vagotomized and sham-operated animals were injected i.e. either with BIM-23052 (0.8 nmol/rat) or vehicle. Other groups of animals were injected i.p. with either atropine sulfate (0.1 mg/kg; Sigma) or vehicle (saline, 0.5 ml) and 30 min later, BIM-23052 (0.8 nmol/rat) or vehicle was injected i.c. In all experiments, 10 min after i.c. injection, conscious rats received the phenol red-methylcellulose solution and gastric emptying was determined after 20 min.

**Effect of TRH Receptor Antisense Oligodeoxynucleotide Pretreatment.** Antisense oligodeoxynucleotides complementary to the first 18 bases downstream from the initiation codon of the rat TRHr mRNA were synthesized with phosphorothionate derivatives of each nucleotide (5'-GAGGTTTCATTCCTCAAT-3'; UCLA Molecular Biology Core, Los Angeles, CA). Mismatch antisense oligonucleotides (5'-GATGGTTCATTCCTCAT-3') mutated at four different positions (underlined bases), but kept identical in composition to the antisense, also were synthesized (UCLA Molecular Biology Core) and used as control treatment. The mismatch sequence has neither significant complementarity to any part of the TRHr mRNA nor significant complementarity to any other gene sequence in the Gene Bank database. The oligodeoxynucleotides were purified by polyacrylamide gel electrophoresis, diluted in sterile saline to a final concentration of 10 µg/µl, and aliquots (20 µl) were maintained at −70°C until use. Animals were injected i.c. with a total dose of 200 µg of TRHr antisense or mismatch oligodeoxynucleotides, in two injections (100 µg each), 48 and 24 h before the measurement of gastric emptying. This protocol for TRHr antisense treatment was previously shown to be effective in blocking the stimulation of gastric emptying induced by i.c. TRH and endogenous medullary TRH released by cold (Martínez et al., 1998b). Pretreated rats were injected i.c. with either vehicle, BIM-23052 (0.8 nmol/rat), or TRH (0.3 nmol/rat). After 10 min, the phenol red-methylcellulose solution was administered intragastrically and gastric emptying was determined. 

**Effects of Various Somatostatin Agonists Injected i.c. on Gastric Emptying.** Saline, somatostatin-28 (0.2, 0.4, 0.8, or 1.2 nmol/rat), or OT8-SST (0.2, 0.4, 0.8, or 1.2 nmol/rat) was injected i.c. and gastric emptying was monitored with the same experiment protocol as for BIM-23052. In other studies, somatostatin-14, CH-275, NC-8-12, L-796,778, L-803,087 (0.8 nmol/rat), or BIM-230256 (0.8 or 1.2 nmol/rat) and appropriate vehicles were injected i.c. and gastric emptying was monitored with similar experimental conditions as for BIM-23052. The dose 0.8 nmol/rat injected i.c. was selected based on maximal changes in gastric acid secretion induced by BIM-23056 and NC-8-12 (Martínez et al., 1995, 1996) and gastric emptying elicited by BIM-23052 (present study) after i.c. injection in conscious rats.

**Effects of Coinjection of BIM-23056 and Somatostatin-28.** BIM-23056 was injected i.c. at 4 or 8 nmol/rat (5 µl), immediately before the i.c. injection of somatostatin-28 (0.8 nmol/rat; 5 µl). The 20-min gastric emptying was determined during the 10- to 30-min period after administration of peptides.

**Statistical Analysis**

Results are expressed as mean ± S.E. Comparisons between groups were performed with one-way ANOVA followed by a Student-Newman-Keuls multiple comparison test. When the effects of two treatments and their reciprocal interactions were studied, data were analyzed by a two-factor ANOVA with replication. When the two-way ANOVA revealed significant effects of treatments, data were reanalyzed with one-way ANOVA and Student-Newman-Keuls multiple comparison test. P values <.05 were considered statistically significant.

**Results**

**Effect of BIM-23052 Injected i.c. or i.v. on Gastric Emptying.** In rats injected i.c. with vehicle (0.01% acetic acid/saline), gastric emptying of a non-nutrient viscous solution was 43.2 ± 3.2% (n = 7) as assessed during the 10- to 30-min period after the i.c. injection. BIM-23052 injected i.c. (0.2, 0.4, and 0.8 nmol/rat) induced a dose-related stimulation of gastric emptying with values reaching 49.3 ± 1.5% (n = 4), 65.5 ± 6.5% (n = 4; P < .05 versus vehicle), and 77.4 ± 5.3% (n = 8; P < .05 versus vehicle), respectively (Fig. 1); this corresponds to a 14.1, 51.6, and 79.2% increase, respectively, above values of the vehicle-treated group. At 1.2 nmol/rat, BIM-23052 did not further elevate gastric emptying values (77.7 ± 1.9%; n = 5; P < .05 versus vehicle; Fig. 1). By contrast, BIM-23052 injected i.v. at 0.8 nmol/rat did not significantly influence gastric emptying (i.v. BIM-23052: 47.0 ± 5.7%; i.v. vehicle: 50.5 ± 8.7%; n = 6 in each group; P > .05).

**Effect of Vagotomy, Atropine, or TRH Receptor Antisense Pretreatment on i.c. BIM-23052-Induced Stimulation of Gastric Emptying.** In sham-vagotomized rats, i.c. injection of BIM-23052 (0.8 nmol/rat) increased gastric emptying (F1,20 = 10.559; P = .004). Subdiaphragmatic vagotomy, which did not alter the gastric emptying compared with sham operation in i.c. saline-injected rats (F1,20 = .78; P = .388), prevented the stimulation of gastric emptying elicited by the i.c. injection of BIM-23052 (sham operation + BIM-23052: 69.9 ± 4.8%; vagotomy + BIM-23052: 48.1 ± 5.2%; F1,20 = 7.833; P = .011; Fig. 2). The muscarinic receptor antagonist atropine significantly inhibited gastric emptying compared with vehicle-treated animals (F1,20 = 46.001, P = .001; Fig. 2). The stimulation of gastric emptying induced by i.c. BIM-23052 (0.8 nmol/rat; F1,20 = 5.367, P = .031) was
Pretreatment with the antisense or mismatch oligodeoxynucleotides against the TRH receptor did not modify basal gastric emptying in vehicle-treated animals (antisense: 49.1 ± 1.4%; F1,119 = 15.68; P = .02; mismatch: 50.6 ± 1.3%).

The stimulatory effect of i.c. BIM-23052 (0.8 nmol/rat) on gastric emptying (F1,118 = 144.367; P < .001) was not modified by pretreatment with i.c. injection of TRH receptor antisense (F1,118 = 0.26; P = .616) and values (74.0 ± 2.5%) were similar to TRH receptor mismatch plus BIM-23052 (73.9 ± 1.4%, Fig. 3). Intracisternal TRH (0.3 nmol/rat) increased gastric emptying to 77.8 ± 3.3% compared with 50.6 ± 3.1% in i.c. vehicle-treated animals (F1,12 = 8.92; P = .011). Intracisternal injection of the TRH receptor mismatch oligodeoxynucleotides did not modify the stimulatory effect of TRH. In contrast, the TRH receptor antisense oligodeoxynucleotides completely prevented the stimulatory effect of i.c. TRH on gastric emptying (47.1 ± 2.7%; interaction between TRH receptor antisense and TRH: F1,12 = 14.459; P = .003; Fig. 3).

**Effects of Various Somatostatin Agonists on Gastric Emptying.** Somatostatin-28 and ODT8-SST injected i.c. induced a dose-related stimulation of gastric emptying similar to that observed after BIM-23052 injected i.c. (Fig. 1). In animals injected i.c. with vehicle (0.9% saline), the gastric emptying of methylcellulose in 20 min was 51.2 ± 3.3% (n = 9). After i.c. injection of somatostatin-28 (0.2, 0.4, 0.8, or 1.2 nmol/rat), gastric emptying values reached 59.9 ± 3.4 (n = 5), 65.1 ± 3.4 (n = 5; P < .05 versus vehicle), 71.3 ± 4.6 (n = 5; P < .05 versus vehicle), and 73.4 ± 2.7% (n = 5; P < .05 versus vehicle), respectively (Fig. 1). After i.c. injection of ODT8-SST (0.2, 0.4, 0.8, or 1.2 nmol/rat), the percentages of gastric emptying were 52.8 ± 4.0 (n = 5), 63.4 ± 2.2 (n = 5; P < .05 versus vehicle), 71.8 ± 2.0 (n = 5; P < .05 versus vehicle), and 73.6 ± 3.2 (n = 5; P < .05 versus vehicle), respectively. Gastric emptying was not different after i.c. injection of somatostatin-14 (0.8 nmol/rat) or saline (Fig. 1).

CH-275, NC-8-12, L-796,778, and L-803,087 injected i.c. at 0.8 nmol/rat did not modify gastric emptying (Table 2). BIM-

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**Fig. 1.** The sst₅-prefering agonists BIM-23052, somatostatin-28, and ODT8-SST, injected i.c., induced a dose-related stimulation of gastric emptying of a non-nutrient solution in rats. Animals, under short enflurane anesthesia, were injected i.c. with agonists or vehicle (0.0), which was saline or 0.01% acetic acid in saline (for BIM-23052). Gastric emptying was determined during the 10- to 30-min period after peptide administration. Data are mean ± S.E. of four to eight animals per group. *P < .05 versus control vehicle (peptide dose of 0.0 nmol/rat; one-way ANOVA).

**Fig. 2.** Effect of subdiaphragmatic vagotomy and cholinergic blockade on i.c. BIM-23052-induced stimulation of gastric emptying in conscious rats. Subdiaphragmatic vagotomy was performed 48 h before the i.c. injection of BIM-23052. Gastric emptying was determined during the 10- to 30-min period after i.c. injection. Data are expressed as mean ± S.E. of the number of animals shown in parentheses. *P < .05 versus sham vagotomy or vehicle-treated animals. #P < .05 versus vehicle + BIM-23052 or sham vagotomy + BIM-23052. □, vehicle (10 μl, i.c.); ■, BIM-23052 (0.8 nmol/rat, i.c.).

blocked by atropine (vehicle + BIM-23052: 74.5 ± 5.9%; atropine + BIM-23052: 32.3 ± 9.2%; F1,20 = 5.061; P = .037; Fig. 2).

**Fig. 3.** Effect of pretreatment with TRH receptor antisense or mismatch oligodeoxynucleotides on i.c. TRH- and BIM-23052-induced stimulation of gastric emptying in conscious rats. Animals were injected i.c. with TRH receptor mismatch or TRH receptor antisense oligodeoxynucleotides (100 μg; 48 and 24 h before the experiments). Gastric emptying was determined during the 10- to 30-min period after the i.c. injection of either vehicle (saline or 0.01% acetic acid in saline), TRH, or the sst₅-prefering agonist BIM-23052. Data are expressed as mean ± S.E. of the number of animals shown in parentheses. *P < .05 versus respective vehicle-treated group. **P < .05 versus no treatment + TRH or TRH receptor mismatch + TRH. □, vehicle (10 μl, i.c.); ■, TRH (0.3 nmol/rat, i.c.); ♦, BIM-23052 (0.8 nmol/rat, i.c.).
23056 (0.8 nmol/rat i.c.) showed a slight, although not significant, tendency to stimulate gastric emptying that was not maintained at 1.2 nmol/rat (Table 2).

**Effect of i.c. BIM-23056 on i.c. Somatostatin-28-Induced Stimulation of Gastric Emptying.** Preliminary experiments showed that BIM-23056 (8 nmol/rat i.c.; n = 3) induced barrel rotation-like behavior and apnea. Animals injected i.c. with BIM-23056 at 4 nmol/rat still exhibited signs of behavioral changes but with less intensity. In these animals (n = 2), somatostatin-28 increased gastric emptying to 68.4 and 77.7%, respectively (gastric emptying in vehicle-injected rats was 52.2% with n = 1). Due to the behavioral effects of BIM-23056 and its apparent lack of antagonistic activity on somatostatin-28 effects, these experiments were not pursued.

**Discussion**

We previously reported that the preferential sst₂ receptor agonist BIM-23052 injected i.c. at 0.8 nmol/rat (1 μg/rat) stimulated gastric emptying in rats (Martínez et al., 1998b). In this study, we showed that BIM-23052 injected i.c. at 0.2, 0.4, or 0.8 nmol/rat dose-dependently increases gastric emptying. The lack of change in gastric emptying when the peptide was administered i.v. at the maximal effective dose injected i.c. indicates that the stimulatory effect of i.c. BIM-23052 is initiated in the brain and not related to its leakage into the peripheral circulation (Tannenbaum and Patel, 1986). The action of BIM-23052 was prevented by subdiaphragmatic vagotomy and atropine, suggesting mediation through vagal cholinergic pathways. The DMN, which contains >90% of the preganglionic cell bodies of vagal efferent fibers projecting to the stomach (Okumura and Namiki, 1990), is the responsive site for SMS 201-995-induced vagal cholinergic gastric acid secretion (Yoneda et al., 1991). In addition, the similar pattern of central actions of ODT8-SST and somatostatin-28 when injected into the CSP (Brown et al., 1981, 1984; Taché et al., 1981). Likewise, we showed that somatostatin-28 and ODT8-SST injected i.c. mimic the dose-related increase in gastric emptying induced by BIM-23052, whereas somatostatin-14 had no effect. Preferential activation of sst₂ may underlie the gastric motor response induced by BIM-23052, somatostatin-28, and ODT8-SST. BIM-23052 displays high binding affinity for the cloned rat sst₂ (O’Carroll et al., 1994) while having less affinity for the other somatostatin receptor subtypes (Patel and Srikant, 1994). In vitro reports have established that somatostatin-28 exhibits preferential affinity for rat and human sst₂ compound to somatostatin-14 (O’Carroll et al., 1994; Patel and Srikant, 1994; Siehler and Hoyer, 1999; Patel, 1999). Although in vitro binding affinity for ODT8-SST to somatostatin receptor subtypes is not known, functional studies indicate that ODT8-SST injected i.v. does not display sst₂-like agonist effects as does somatostatin-14 (Taché et al., 1981; Yoneda et al., 1991). In addition, the similar pattern of central actions of ODT8-SST and somatostatin-28 when injected into the CSP (Brown et al., 1981, 1984; Taché et al., 1981; Vecsei and Widerlov, 1990) suggests a possible sst₂ compound to somatostatin-14 (O’Carroll et al., 1994; Patel and Srikant, 1994; Siehler and Hoyer, 1999; Patel, 1999). Although in vitro binding affinity for ODT8-SST to somatostatin receptor subtypes is not known, functional studies indicate that ODT8-SST injected i.v. does not display sst₂-like agonist effects as does somatostatin-14 (Taché et al., 1981; Yoneda et al., 1991). In addition, the similar pattern of central actions of ODT8-SST and somatostatin-28 when injected into the CSP (Brown et al., 1981, 1984; Taché et al., 1981; Vecsei and Widerlov, 1990) suggests a possible sst₂ compound to somatostatin-14 (O’Carroll et al., 1994; Patel and Srikant, 1994; Siehler and Hoyer, 1999; Patel, 1999).

Earlier reports indicated that i.c.v. or i.c. injection of somatostatin-28 and ODT8-SST exerts similar potent actions in the brain to influence glucoregulation, thermoregulation, gastric acid secretion, and stress-related pituitary-adrenal responses, whereas somatostatin-14 had little or no effect (Brown et al., 1981, 1984; Taché et al. 1981). Likewise, we showed that somatostatin-28 and ODT8-SST injected i.c. mimic the dose-related increase in gastric emptying induced by BIM-23052, whereas somatostatin-14 had no effect. Preferential activation of sst₂ may underlie the gastric motor response induced by BIM-23052, somatostatin-28, and ODT8-SST. BIM-23052 displays high binding affinity for the cloned rat sst₂ (O’Carroll et al., 1994) while having less affinity for the other somatostatin receptor subtypes (Patel and Srikant, 1994). In vitro reports have established that somatostatin-28 exhibits preferential affinity for rat and human sst₂ compound to somatostatin-14 (O’Carroll et al., 1994; Patel and Srikant, 1994; Siehler and Hoyer, 1999; Patel, 1999). Although in vitro binding affinity for ODT8-SST to somatostatin receptor subtypes is not known, functional studies indicate that ODT8-SST injected i.v. does not display sst₂-like agonist effects as does somatostatin-14 (Taché et al., 1981; Yoneda et al., 1991). In addition, the similar pattern of central actions of ODT8-SST and somatostatin-28 when injected into the CSP (Brown et al., 1981, 1984; Taché et al., 1981; Vecsei and Widerlov, 1990) suggests a possible sst₂ compound to somatostatin-14 (O’Carroll et al., 1994; Patel and Srikant, 1994; Siehler and Hoyer, 1999; Patel, 1999).

So far, the only neuropeptide established to play a physiological role in the vagal stimulation of gastric motor function through direct stimulation of DMN neurons is TRH (Travagli et al., 1992; O-Lee et al., 1997; Martínez et al., 1998b). TRH injected i.c. or into the DMN enhanced gastric vagal efferent discharge (O-Lee et al., 1997), leading to a vagal cholinergic-dependent stimulation of gastric emptying and contractility in both conscious and anesthetized rats (Rogers and Hermann, 1987; Raybould et al., 1989). In addition, cold exposure-induced vagal cholinergic-mediated stimulation of gastric emptying is mediated by activation of medullary TRH receptors (Martínez et al., 1998b). In this study, TRH receptor antisense oligodeoxynucleotide pretreatment abolished i.c. TRH-induced gastric emptying but did not alter i.c. BIM-23052 action. These observations are consistent with our previous report in which BIM-23052 was used to assess receptor specificity of the TRH receptor antisense pretreatment (Martínez et al., 1998b). Collectively, these results demonstrate that BIM-23052-induced central vagal cholinergic stimulation of gastric emptying is independent from medullary TRH.

**Table 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose n mol/rat</th>
<th>Gastric Emptying %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>9</td>
<td>51.2 ± 3.3</td>
</tr>
<tr>
<td>SST-14</td>
<td>0.8</td>
<td>53.0 ± 1.1</td>
</tr>
<tr>
<td>CH-275</td>
<td>0.8</td>
<td>51.0 ± 1.5</td>
</tr>
<tr>
<td>Acetic acid/saline</td>
<td>7</td>
<td>43.2 ± 3.2</td>
</tr>
<tr>
<td>NC 8-12</td>
<td>0.8</td>
<td>52.3 ± 9.3</td>
</tr>
<tr>
<td>BIM-23056</td>
<td>0.8</td>
<td>62.4 ± 8.1</td>
</tr>
<tr>
<td>BIM-23056</td>
<td>1.2</td>
<td>57.1 ± 7.0</td>
</tr>
<tr>
<td>DMSO/saline</td>
<td>5</td>
<td>43.2 ± 4.5</td>
</tr>
<tr>
<td>L-796,778</td>
<td>0.8</td>
<td>51.4 ± 3.3</td>
</tr>
<tr>
<td>L-803,087</td>
<td>0.8</td>
<td>46.8 ± 3.3</td>
</tr>
</tbody>
</table>

Fasted rats were injected i.c. with the compounds listed or the appropriate vehicle (10 μl). Ten minutes later, the phenol red-methyl cellulose solution was administered and the gastric emptying determined 20 min later. Data represent mean ± S.E. of the number of animals (n) shown for each treatment.
Inhibition for NC-8-12) in conscious rats (Martinez et al., 1996; Raynor 1993a,b); however, a dose-related effect could not be demonstrated with 1.2 nmol/rat. In contrast, we previously reported that NC-8-12 or BIM 23056 injected i.c. at a similar dose (0.8 nmol/rat) displays maximal biological action to inhibit gastric acid secretion (stimulation for BIM 23056 and inhibition for NC-8-12) in conscious rats (Martinez et al., 1995, 1996). Although the results of this study are consistent with the sst3 being preferentially involved in the vagal stimulation of gastric motor function, the possibility of other somatostatin receptor subtypes in the medulla regulating gastric motor function through neural pathways cannot be ruled out.

Morphological observations with in situ hybridization techniques established a very restricted distribution of sst5 gene expression in the medulla, with the strongest expression in the DMN (Thoss et al., 1995). Whether sst5-prefering agonists could directly modulate the excitability of preganglionic vagal neurons in the DMN, as established for TRH (Travaglini et al., 1992), requires further investigation with an electrophysiological approach. The distribution of somatosta-28 cell bodies and fibers in the rat dorsal vagal complex, including cell groups that receive sensory input from the vagus (Higgins and Schwaber, 1983; Sawchenko et al., 1990), along with the present pharmacological results suggest a role for somatostatin-28 acting on sst5 located in the DMN in the vagal regulation of gastric motor function. However, the presence, although in low density, of other receptor subtypes, including sst1, sst3, and sst5 in the rat dorsal vagal complex and area postrema (Dournaud et al., 1996; Hervieu and Emerson, 1998; Handel et al., 1999; Schindler et al., 1999) may suggest a more complex interaction between receptor subtypes.

In summary, the present findings show that the sst5-prefering ligand BIM-23052 injected i.c. (0.2–0.8 nmol/rat) induced a dose-related and vagal cholinergic-dependent stimulation of gastric emptying in conscious rats. The lack of action of BIM-23052 injected into the peripheral circulation indicates that responsive sites are most likely located in the medulla, including the DMN, where high levels of sst5 gene expression have been reported. Although medullary TRH is a physiological stimulant of vagal outflow regulating gastric motor function, the action of BIM-23052 is not secondary to the activation of medullary TRH receptors. A role for sst5 to induce vagal stimulation of gastric emptying is further suggested by the similar increase in gastric transit elicited by i.c. injection of the sst5-prefering native ligand somatostatin-28 and an oligosomatostatin analog ODT8-SS. Somatostatin-14 and sst1, sst3, and sst5-prefering peptide agonists and non-peptide-selective sst3 and sst4 agonists under the conditions tested did not alter gastric emptying. These findings suggest a possible involvement of medullary sst5 in the vagal stimulation of gastric motor function in rats.

Acknowledgments

We thank Dr. Vincent Wu (Digestive Diseases Research Center, Department of Medicine, UCLA, Los Angeles) for the help in the preparation of the TRH receptor oligodeoxynucleotides. Paul Kirsch is acknowledged for help in the preparation of the peptide-selective sst3 and sst4 agonists under the conditions tested did not alter gastric emptying. These findings suggest a possible involvement of medullary sst5 in the vagal stimulation of gastric motor function in rats.

References

Skrikant, 1994; Rosowskii and Coy, 1993; Liapakis et al., 1996; Leroux et al., 1997; Rohrer et al., 1998; Patel, 1999) were injected i.c. at 0.8 nmol/rat, no significant changes in gastric emptying were observed. The tendency to increase gastric emptying when BIM-23056 was injected i.c. may be related to its weak affinity for sst3 (Patel and Skrikant, 1994; Raynors 1993a,b); however, a dose-related effect could not be demonstrated with 1.2 nmol/rat. In contrast, we previously reported that NC-8-12 or BIM 23056 injected i.c. at a similar dose (0.8 nmol/rat) displays maximal biological action to influence gastric acid secretion (stimulation for BIM 23056 and inhibition for NC-8-12) in conscious rats (Martinez et al., 1995, 1996). Although the results of this study are consistent with the sst3 being preferentially involved in the vagal stimulation of gastric motor function, the possibility of other somatostatin receptor subtypes in the medulla regulating gastric motor function through neural pathways cannot be ruled out.

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