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Intracerebroventricular injection of somatostatin sst_5 receptor agonist inhibits gastric acid secretion in rats

Vicente Martínez [a,*], David H. Coy [b], K.C. Kent Lloyd [a], Yvette Taché [a]

[a] *CURE: Digestive Diseases Research Center, VA Medical Center, Department of Medicine and Brain Research Institute, UCLA, Los Angeles, CA 90073, USA*

[b] *Peptide Research Laboratories, Department of Medicine, Tulane University, New Orleans, LA 70112, USA*

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Abstract

Somatostatin and its analogs act in the brain to influence gastric acid secretion. Five different somatostatin receptor subtypes have been characterized (sst_1 to sst_5). We studied the influence of somatostatin (0.18–0.6 nmol/rat) and selective sst_2, sst_3 and sst_5 receptor ligands on basal gastric acid secretion in conscious rats equipped with chronic gastric and intracerebroventricular (i.c.v.) cannulae. Somatostatin-14 (0.36 nmol/rat), the sst_2, sst_3 and sst_5 receptor agonist, Des-AA^{1,2,4,5,12,13}-[D-Tryp^8,D-Cys^{14}]somatostatin (SMS 201-995) (0.18–0.36 nmol/rat) and the sst_5 receptor agonist, BIM-23052, (0.8–1.2 nmol/rat) injected i.c.v. inhibited gastric acid secretion. Maximal inhibition reaching 42%, 60% and 42% was induced by somatostatin-14 (0.36 nmol/rat), SMS 201-995 (0.18 nmol/rat) and BIM-23052 (0.8 nmol/rat) respectively. The sst_2 receptor agonist, DC 32-87 (0.2–0.8 nmol/rat) and sst_3 receptor agonist, BIM-23056 (0.2–1.2 nmol/rat), did not modify gastric acid secretion, except the sst_3 receptor agonist at 0.4 nmol/rat which increased acid output at 20 min post-injection. The sst_2 receptor agonists (0.4 nmol/rat) co-injected i.c.v with a subthreshold dose of sst_5 (0.4 nmol/rat) inhibited gastric acid secretion. These results show that i.c.v. injection of somatostatin-14 inhibits basal gastric acid secretion in conscious rats through an action on sst_5 receptor subtype which can be potentiated by sst_2 receptor subtype.

Keywords: Somatostatin sst_2 receptor; Somatostatin sst_3 receptor; Somatostatin sst_5 receptor; SMS 201-995; Somatostatin receptor subtype; Gastric acid secretion; Brain; Somatostatin analog; (Rat)

1. Introduction

Somatostatin is a peptide widely distributed throughout the mammalian central nervous system (Finley et al., 1981; Rossowski and Coy, 1993). Previous studies indicated that somatostatin acts in the brain to influence gastric secretion in rats and dogs (Taché et al., 1981; Pappas et al., 1985). However, the pattern of gastric acid secretory response induced by somatostatin or somatostatin analogs varies depending on the peptides and the brain sites of administration. Intracisternal injection of the oligosomatostatin analogs with deletion of amino acids 1, 2, 4, 5, 12 and 13, Des-AA^{1,2,4,5,12,13}-[D-Tryp^8]somatostatin (ODT8-SS) and Des-AA^{1,2,4,5,12,13}-[D-Tryp^8,D-Cys^{14}]somatostatin (SMS 201-995 or octreotide) stimulated gastric acid output in conscious pylorus-ligated rat, mostly by increasing the volume of gastric secretion through vagal cholinergic mechanisms while intracisternal injection of somatostatin had no effect (Taché et al., 1981; Yoneda et al., 1991; Stephens, 1991). Intracerebroventricular (i.c.v.) injection of ODT8-SS also stimulated gastric acid secretion in conscious rats with pylorus ligation and enhanced the acid response to a meal or pentagastrin in conscious dogs (Pappas et al., 1985; Lenz et al., 1989). Likewise, direct microinjection of somatostatin or SMS 201-995 into the dorsal motor nucleus of the vagus stimulated gastric acid secretion and motility in urethane-anesthetized rats (Hermann and Rogers, 1989; Yoneda et al., 1991; Yoneda and Taché, 1995). By contrast, the injection of somatostatin or SMS 201-995 into the lateral ventricle, or the hypothalamus (lateral and paraventricular nucleus) dose dependently

* Corresponding author. CURE: Digestive Diseases Research Center, VAMC West Los Angeles, Building 115, Room 203, 11301 Wilshire Boulevard, Los Angeles, CA 90073, USA. Tel.: 310-312 92 85; fax: 310-268 49 63.

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inhibited pentagastrin-stimulated acid secretion in urethane-anesthetized rats (Osumi et al., 1979; Yoneda and Taché, 1995).

Five different somatostatin receptor subtypes (sst_1 to sst_5) have been cloned that are structurally, pharmacologically and functionally distinct (Raynor et al., 1993a; Yamada et al., 1993). Recently, the somatostatin receptors cloned by Bruno et al. (1992) and O'Carroll et al. (1992) have been re-named sst_4 and sst_5, respectively (Bell and Reisine, 1993; Reisine and Bell, 1995, Hoyer et al., 1995). Raynor et al. have characterized specific peptide analogs which display a high degree of selectivity for binding to sst_2, sst_3 or sst_5 receptor subtypes while no analogs so far are selective for sst_1 and sst_4 (formerly sst_5) receptor subtypes (Raynor et al., 1993a, b). Current studies using these selective analogs indicate that different receptor subtypes may mediate the multiple peripheral actions of somatostatin. The sst_2 receptor is the subtype that mediates the inhibition of growth hormone release and gastric acid secretion induced by peripheral somatostatin (Raynor et al., 1993a; Rossowski et al., 1994; Lloyd et al., 1995), while the inhibition of glucose-stimulated pancreatic insulin and amylase release involved the sst_5 receptor subtype (Rossowski and Coy, 1993; Rossowski et al., 1994). In vitro studies also showed that the sst_3 receptor subtype mediates the inhibitory action of somatostatin on guinea pig gastric smooth muscle cells (Gu et al., 1995) and that the sst_2 and sst_5 receptor subtypes might serve as the major receptors mediating inhibition of cell growth (Buscail et al., 1995).

In the rat, the brain distribution of messenger RNA encoding each of the receptor subtypes shows a distinct, but overlapping, pattern of expression (Breder et al., 1992; Bruno et al., 1993; Kong et al., 1994; Senaris et al., 1994; Pérez and Hoyer, 1995). Activation of different receptor subtypes may account for the different patterns of acid response upon injection of various somatostatin analogs in different parts of the brain. In the present study, we examined the influence of i.c.v. injection of the somatostatin selective agonists, DC 32-87 (sst_2), BIM-23056 (sst_3), BIM-23052 (sst_5) and SMS 201-995 (sst_5, sst_2 and sst_3) (Raynor et al., 1993a, b; Rossowski and Coy, 1993) on basal gastric acid secretion in conscious rats with chronic gastric fistula.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (270–350 g) (Harlan Laboratories, San Diego, CA) were housed under controlled conditions (22–24°C; 12-h light cycle, starting at 6:30 a.m.). Animals were maintained ad libitum on Purina Chow (Ralson Purina, St. Louis, MO) and tap water. All experiments were performed in rats deprived of food for 18–24 h but allowed free access to water up to the beginning of the studies.

2.2. Chronic gastric fistula and i.c.v. cannulation

Animals were anesthetized with a mixture of ketamine hydrochloride (75 mg/kg, i.p.; Ketaset, Fort Dodge Laboratories, Fort Dodge, IA) and xylazine (5 mg/kg, i.p.; Rompun, Mobay Corporation, Shawnee, KS) and a chronic guide cannula (22 gauge, Plastic Products, Roanoke, VA) was implanted into the right lateral brain ventricle according to coordinates derived from Paxinos and Watson's brain atlas (Paxinos and Watson, 1986) (mm from bregma: antero-posterior: −0.8; lateral: −1.5; dorsoventral: −3.5). 2–3 days later, under the same conditions of anesthesia, the stomach was exteriorized through a median celiotomy and the non-glandular portion of the stomach was incised to insert a modified stainless steel Thomas cannula. The cannula was sutured to the stomach wall, exteriorized through the abdominal wall and capped. Rats were housed in individual cages with indirect bedding. Experiments began at least 1 week after surgery, and during this period, animals were accustomed to Bollman cages and to the handling of the cannulae.

2.3. Measurement of gastric acid secretion

Experiments were performed between 8:00 am and 1:00 pm. The stomach was rinsed until clean with 0.9% saline solution (NaCl 0.9%, pH 7.0, 37°C) through the opened cannula. Then, free secretion was allowed for a 30–45 min period. Thereafter, gastric acid secretion was measured before and after i.c.v. injection by flushing the gastric lumen with a 5-ml bolus of saline

Table 1
List of somatostatin analogs and characterized affinity to the receptor subtypes

Peptide	Structure	Receptor affinity
Somatostatin-14	Ala-Gly-*c*[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys]	$sst_3 \geq sst_1 \geq sst_2 \geq sst_4 \geq sst_5$
SMS 201-995	D-Phe-*c*[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr(ol)	$sst_5 > sst_2 \geq sst_3$
DC 32-87	D-Phe-Cys-Tyr-D-Trp-Lys-Abu-Cys-Nal-NH_2	sst_2
BIM-23056	D-Phe-Phe-Tyr-D-Trp-Lys-Val-Phe-D-Nal-NH_2	sst_3
BIM-23052	D-Phe-Phe-Phe-D-Tryp-Lys-Thr-Phe-Thr-NH_2	sst_5

From: Raynor et al., 1993a, b; Rossowski and Coy, 1993.

solution followed by a 5-ml bolus of air at the end of each 10-min period. Acid output was determined by titration (autotitrator, Radiometer Corp., Copenhagen, Denmark) of the perfusate to pH 7.0 with 0.01 N NaOH. A period of 4–5 days was allowed between experiments on the same animal. A maximum of four to six experiments was performed on each rat, and the same treatment was given only once per animal.

2.4. Drugs

The peptides listed in Table 1 were used: somatostatin-14 (Peninsula Laboratories, Belmont, CA) and SMS 201-995 (Sandostatin, octreotide acetate, Sandoz Laboratories, Basel, Switzerland). Somatostatin-14 and SMS 201-995 were dissolved in 0.9% sterile saline (Sigma Chemical Co., St Louis, MO). The selective somatostatin receptor subtype ligands listed in Table 1, were synthetized, purified and characterized as previously described (Rossowski and Coy, 1993). They were dissolved in 0.01% acetic acid to a concentration of 0.8 nmol/μl. 5 μl aliquots were stored frozen (−70° C) until use. Further dilutions of stock solution were made in 0.9% sterile saline before use.

2.5. Experimental procedures

Basal acid secretion was collected for 40 min before and 120 min after i.c.v. injection of vehicle or peptides in conscious rats. The i.c.v. injection was performed in a volume of 10 μl using a 26-gauge injection cannula connected to a PE-50 catheter. Peptides were: somatostatin-14 (0.18, 0.36, 0.6 or 1.2 nmol/rat; $n = 4$–5 for each dose), SMS 201-995 (0.06, 0.18 and 0.36 nmol/rat, $n = 5$ for each dose except for the 0.36 nmol/rat dose, $n = 3$) and the selective sst_2, sst_3 and sst_5 receptor agonists (0.2, 0.4, 0.8 and 1.2 nmol/rat, $n = 3$–6 for each peptide and dose). The existence of a possible interaction between receptor subtypes was tested by simultaneously injecting i.c.v. the different somatostatin receptor agonists, sst_2 with sst_3, sst_2 with sst_5 or sst_3 with sst_5, each at the dose of 0.4 nmol/rat (total dose administered 0.8 nmol/rat in 10 μl). The vehicle control groups were injected i.c.v. (10 μl) with either 0.01% acetic acid diluted in 0.9% sterile saline (pH 5) or sterile saline alone (pH 8).

At the end of the experiments, rats were killed by decapitation and the correct location of the cannula in the lateral ventricle was verified by injecting 10 μl of dye (0.1% toluidine blue). The visualization of dye on the walls of the lateral ventricle indicates the accuracy of i.c.v. injection.

2.6. Statistics

Data are presented as means ± S.E.M. Acid output is expressed in μmol/time or as net acid output (μmol/40 min) representing the net changes between the acid output during the 40-min period before and after injection of vehicle or peptides. Negative net acid output denotes inhibition of acid secretion while positive values indicate an increase in acid secretion from basal pre-injection values. Differences in acid output within groups over time were assessed by analysis of variance (ANOVA) followed, when necessary, by a Student-Newman-Keuls multiple comparisons test. Data were considered statistically significant when $P \leq 0.05$.

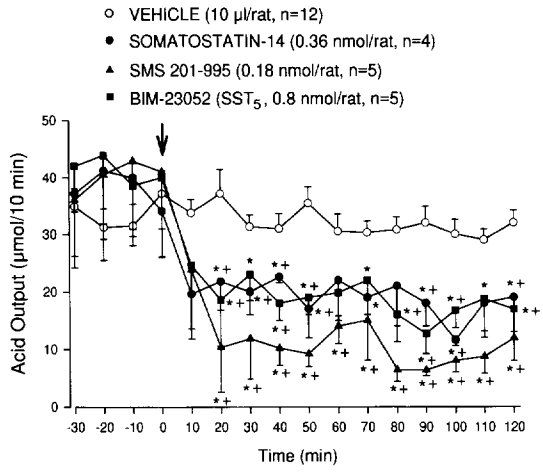

Fig. 1. Time course changes in basal gastric acid secretion after the i.c.v. injection (time 0) of either vehicle, somatostatin-14, the stable somatostatin analog, SMS 201-995, or the selective sst_5 receptor agonist, BIM-23052 in conscious rats with chronic gastric and i.c.v. cannulae. Each point represents the mean ± S.E.M. of number of rats indicated in parentheses. * $P < 0.05$ compared with respective pre-injection levels. + $P < 0.05$ compared with vehicle (pooled values from both vehicles used in the experiments, 0.9% saline solution and 0.01% acetic acid diluted in 0.9% saline).

3. Results

3.1. Effect of i.c.v. injection of somatostatin-14 and SMS 201-995 on basal gastric acid secretion

The i.c.v. injection of 0.9% sterile saline (10 μl, $n = 5$) did not modify basal gastric acid secretion during the 2-h experimental period in conscious rats with chronic gastric fistula and i.c.v. cannula. Somatostatin-14 (0.36 nmol/rat) injected i.c.v. induced a maximal decrease in gastric acid secretion (μmol/10 min) from 34.3 ± 3.8 before to 19.6 ± 6.1 ($P < 0.05$, $n = 4$) 10 min after peptide injection (Fig. 1). Thereafter, values remained significantly decreased by 40–57% compared with pre-injection levels or vehicle-treated group throughout the 2-h experimental period (Fig. 1). The net decrease in acid output was −78.7 ± 25.7 μmol/40 min compared with −1.5 ± 8.7 μmol/40 min in rats injected i.c.v. with vehicle ($P < 0.05$) (Fig. 2). By con-

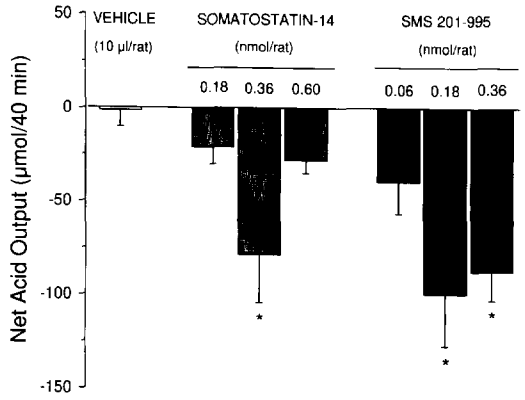

Fig. 2. Net acid output during the first 40 min period after the i.c.v. injection of either vehicle, somatostatin-14 or the somatostatin analog, SMS 201-995 in conscious rats with chronic gastric and i.c.v. cannulae. Each column represents the mean ± S.E.M. of three to five rats. * $P < 0.05$ compared with vehicle (0.9% saline).

trast, somatostatin-14 at lower (0.18 nmol/rat) or higher doses (0.6 or 1.2 nmol/rat) did not induce a significant net decrease in gastric acid secretion compared with vehicle-treated group (Fig. 2 and data not shown).

The compound SMS 201-995 injected i.c.v. (0.06, 0.18 and 0.36 nmol/rat) induced a net decrease in acid output (µmol/40 min) reaching -39.4 ± 17.2 ($P > 0.05$, $n = 5$), -99.4 ± 27.9 ($P < 0.05$, $n = 5$) and -87.3 ± 15.6 ($P < 0.05$, $n = 3$), respectively (Fig. 2). The peak inhibition, for the dose of 0.18 nmol/rat, was observed at 20 min post-injection (Fig. 1); gastric acid output (µmol/10 min) decreased from 40.0 ± 13.4 before the i.c.v. injection to 10.3 ± 7.8 ($P < 0.05$, $n = 5$) 20 min after SMS 201-995 administration. Acid secretion was still inhibited by $71 \pm 11\%$ at 120 min after peptide injection ($P < 0.05$ compared with vehicle) (Fig. 1).

3.2. Effect of i.c.v. injection of somatostatin analogs selective for receptor subtypes on basal gastric acid secretion

The i.c.v. injection of 0.01% acetic acid diluted in 0.9% sterile saline (10 µl, $n = 7$) did not modify basal gastric acid secretion during the 2-h experimental period in conscious rats with chronic gastric fistula and i.c.v. cannula. Net acid output during the first 40 min after i.c.v. injection of vehicle was 5.2 ± 9.0 µmol (Fig. 3). The sst_5 receptor agonist, BIM-23052, (0.4, 0.8 and 1.2 nmol/rat) injected i.c.v. induced a net decrease in gastric acid output within the 40-min period post-injection (µmol/40 min) reaching -19.0 ± 8.0 ($P > 0.05$, $n = 6$), -80.0 ± 17.0 ($P < 0.05$, $n = 5$) and -51.3 ± 6.2 ($P < 0.05$, $n = 5$), respectively (Fig. 3). The lower dose (0.2 nmol/rat, $n = 3$) did not decrease gastric acid secretion during the experimental period (Fig. 3). Time course studies show that i.c.v. injection of sst_5 receptor agonist at 0.8 nmol/rat induced a peak inhibition of acid secretion at 20 min post-injection from pre-injection values of 40.8 ± 9.5 µmol/10 min to 18.6 ± 7.2 µmol/10 min ($P < 0.05$, $n = 5$) (Fig. 1). The 100 min thereafter, acid secretion was inhibited by 32–50% (Fig. 1). The sst_3 receptor agonist, BIM-23056, injected i.c.v. at 0.2, 0.8 or 1.2 nmol/rat, ($n = 3$–7) did not induce significant net change in gastric acid secretion (Fig. 3). By contrast, the dose of 0.4 nmol/rat tends to increase net gastric acid secretion during the first 40-min period after administration (Fig. 3). The peak stimulation of acid secretion occurring at 20 min post-injection reached 47.8 ± 6.7 µmol/10 min ($P < 0.05$ compared with pre-injection values of 27.4 ± 1.5 µmol/10 min, $n = 6$). The specific sst_2 receptor agonist, DC 32-87, tested under the same conditions (0.2, 0.4 and 0.8 nmol/rat, $n = 3$–6) did not induce change in net gastric acid secretion (Fig. 3). The sst_2 receptor agonist injected i.c.v. at the dose of 1.2 nmol/rat caused behavioral changes characterized by rotating behavior, and therefore was not studied further.

3.3. Effect of co-injections of selective somatostatin analogues on basal gastric acid secretion

The somatostatin sst_5 receptor agonist, BIM-23052, or the sst_2 receptor agonist, DC 32-87, injected i.c.v. at 0.4 nmol/rat ($n = 6$) did not significantly modify basal acid secretion (net acid output during the first 40 min post-injection, µmol/40 min: -20.7 ± 9.4 and 8.4 ± 9.7 respectively, $P > 0.05$ compared with vehicle) (Fig. 4). Simultaneous i.c.v. injection of sst_2 and sst_5 agonists DC 32-87 and BIM-23052 (0.4 nmol each, $n = 6$) resulted in a significant reduction in gastric acid output (µmol/10 min) from basal of 33.8 ± 5.1 to 13.0 ± 3.1

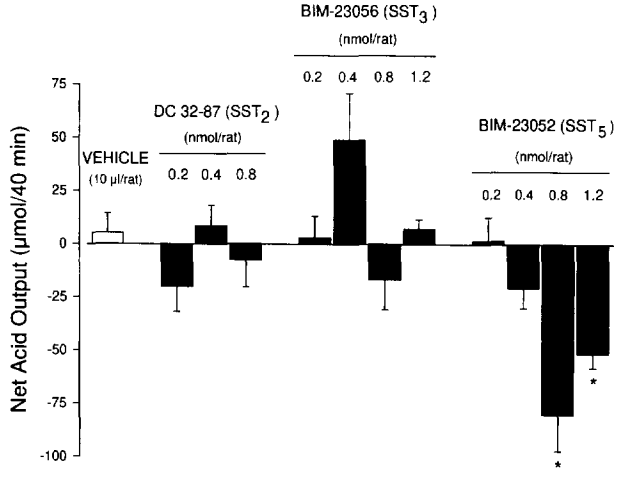

Fig. 3. Net acid output during the first 40-min period after the i.c.v. injection of the selective sst_2 (DC 32-87), sst_3 (BIM-23056) and sst_5 (BIM-23052) receptor agonists in conscious rats with chronic gastric and i.c.v. cannulae. Each column presents the mean ± S.E.M. of three to seven rats. * $P < 0.05$ compared with vehicle (0.01% acetic acid diluted in 0.9% saline).

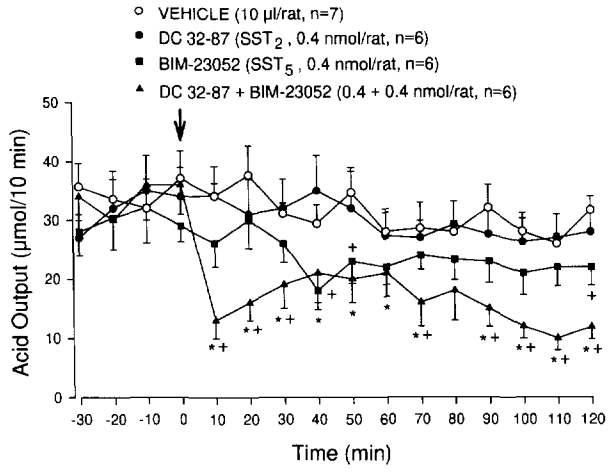

Fig. 4. Time course changes in basal gastric acid secretion after the i.c.v. injection of either vehicle, or the selective sst_2 and sst_5 receptor agonists DC 32-87 and BIM-23052 given alone or in combination in conscious rats with chronic gastric and i.c.v. cannulae. Each point represents the mean ± S.E.M of number of rats indicated in parentheses. * $P < 0.05$ compared with respective basal levels before i.c.v. injection. + $P < 0.05$ compared with vehicle (0.01% acetic acid diluted in 0.9% saline).

within 10 min post-injection (Table 2). The inhibition was maintained throughout the 2-h experimental period (Table 2, Fig. 4). By contrast, the combined i.c.v. injection of $sst_3 + sst_5$ receptor agonists, BIM-23056 and BIM-23052 (0.4 nmol/rat each, $n = 5$), did not modify basal gastric acid secretion compared with pre-injection levels except at a significant decrease at the 20–30 min collection period (17.0 ± 4.2 µmol/10 min; $P < 0.05$, $n = 6$, Table 2); thereafter values were not different from pre-injection values or vehicle-treated group (data not shown). Co-injection of the sst_2 and sst_3 receptor agonists, DC 32-87 and BIM-23056 ($n = 5$; 0.4 nmol/rat each), did not modify basal gastric acid secretion during the 2-h experimental period (Table 2 and data not shown).

Table 2
Time-course changes in basal gastric acid secretion during the first 40 min after combined i.c.v injections of sst_2, sst_3 and sst_5 receptor agonists [a]

Time	Treatment [b]			
(min)	Vehicle	$sst_2 + sst_5$	$sst_3 + sst_5$	$sst_2 + sst_3$
Basal [c]	31.8 ± 5.2	33.8 ± 5.1	26.2 ± 4.6	27.0 ± 4.0
0–10	34.0 ± 5.7	13.0 ± 3.1 [d,e]	22.8 ± 4.0	27.6 ± 7.8
10–20	30.8 ± 6.1	16.1 ± 2.9 [d,e]	19.5 ± 5.8	26.5 ± 5.0
20–30	31.6 ± 6.2	19.3 ± 4.2 [d,e]	17.0 ± 4.2 [d]	26.7 ± 6.1
30–40	34.2 ± 7.6	21.0 ± 5.1 [e]	19.0 ± 4.4	28.1 ± 10.0

[a] Data are means ± S.E.M. (gastric acid secretion, µmol/10 min) of five to seven animals per group. [b] sst_2, sst_3 and sst_5 receptor agonists were administered at a dose of 0.4 nmol/rat each, in 10 µl volume. [c] Values for basal secretion correspond to the mean for the 40-min control period before the i.c.v. injection. [d] $P < 0.05$ vs. vehicle (0.01% acetic acid in 0.9% saline). [e] $P < 0.05$ vs. basal.

4. Discussion

Somatostatin-14 injected into the lateral ventricle at a dose of 0.36 nmol/rat inhibited basal gastric acid secretion by 40–57% throughout the 2-h post-injection period in conscious rats with chronic gastric fistula and i.c.v. cannula. In previous studies, somatostatin-14 injected i.c.v. was also reported to inhibit basal acid secretion in urethane-anesthetized rats (Osumi et al., 1979). Results obtained using selective analogs for somatostatin receptor subtypes injected i.c.v. indicate that sst_5 receptor subtype is the main mediator of i.c.v. somatostatin-induced inhibition of basal gastric acid secretion in conscious rats. The compound BIM-23052, which has the highest selectivity for sst_5 receptor subtypes (Raynor et al., 1993a, b), injected i.c.v. at 0.8 nmol/rat inhibited basal gastric acid secretion for the 2-h experimental period. The inhibitory responses induced by somatostatin-14 and BIM-23052 were of similar magnitude (50% inhibition of basal rate of acid secretion) and duration (over 2 h). The selectivity of the response to sst_5 receptors was demonstrated by the lack of significant inhibition of basal gastric acid secretion in response to i.c.v. injection of similar doses of the selective sst_2 and sst_3 receptor ligands, DC 32-87 and BIM-23056 (Raynor et al., 1993a, b).

There is evidence that somatostatin-14 injected i.c.v. at 3–6 nmol/rat induces massive hypersomatostatinemia resulting from leakage into the peripheral circulation in rats (Tannenbaum and Patel, 1986). It is unlikely that the inhibitory response induced by i.c.v. injection represents leakage of the peptides into the peripheral circulation. First, the doses of BIM-23052 and somatostatin-14 injected i.c.v. were 5–10-fold lower than in the study reporting leakage of somatostatin-14 injected i.c.v. (Tannenbaum and Patel, 1986). Moreover, i.c.v. and peripheral injections of BIM-23052 result in different patterns of acid response. Peripheral administration of selective ligands for the sst_5 receptor subtype, including BIM-23052, did not modify gastric acid secretion at a dose of 10 µg/kg per h (8 nmol/kg per h) while the sst_2 receptor agonist, DC 32-87, at the same dose, inhibited pentagastrin-stimulated acid secretion (Rossowski et al., 1994; Lloyd et al., 1995).

The present study also shows that the stable analog of somatostatin, SMS 201-995, injected i.c.v. inhibited basal acid secretion in conscious rats with chronic gastric fistula. Likewise, in a previous study, we also observed that SMS 201-995 injected i.c.v. inhibited pentagastrin-stimulated gastric acid secretion in urethane-anesthetized rats (Yoneda and Taché, 1995). SMS 201-995 displays a higher selectivity for sst_5 receptors, compared with the sst_2 and sst_3 receptor subtypes and lacks affinity for the sst_1 and sst_4 subtypes (Raynor et al., 1993a, b). These data further support a role for the sst_5 receptor subtype in mediat-

ing the inhibition of gastric acid secretion induced by injection of somatostatin into the forebrain. As reported for other biological actions of SMS 201-995 (Pless et al., 1986), the stable somatostatin analog was found more potent than somatostatin-14 or the sst_5 receptor agonist. The peak acid inhibition reached $84 \pm 8\%$ when SMS 201-995 was injected i.c.v. at 0.18 nmol/rat compared with $50 \pm 11\%$ and $55 \pm 17\%$ peak inhibition when somatostatin-14 and BIM-23052 (sst_5 receptor agonist) were injected i.c.v. at 0.36 nmol/rat and 0.8 nmol/rat respectively. In addition, lower doses of somatostatin or the sst_5 receptor agonist did not significantly modify basal gastric acid secretion.

At 0.6 nmol/rat dose, i.c.v. injection of somatostatin-14 no longer exerts an inhibitory effect. These results may be explained on the basis of differences in the affinity of somatostatin-14 for the various receptor subtypes which were $sst_3 \geq sst_1 \geq sst_2 \geq sst_4 \geq sst_5$ (Raynor et al., 1993a, b) whereas SMS 201-995 has a higher affinity for the $sst_5 > sst_2 > sst_3$. In our studies, i.c.v. injection of the sst_3 receptor agonist at 0.4 nmol/rat slightly increased gastric acid secretion within the first 40-min period after the injection in conscious rats with chronic gastric fistula. These results are in agreement with our previous report showing that the intracisternal injection of this sst_3 receptor agonist also increased gastric acid secretion in conscious rats with chronic gastric fistula (Martínez et al., 1995). The stimulation elicited by the sst_3 receptor agonist was not dose-dependent and higher doses (0.8 and 1.2 nmol/rat) did not stimulate acid secretion. This effect may be due to the action on other receptor subtypes at higher doses since the compound BIM-23056 has a low affinity for the sst_5 receptor subtype (Raynor et al., 1993b). Taken together, these data suggest that brain sst_3 receptors may mediate at least some of the gastric secretory response observed after the central administration of somatostatin. In dogs, i.c.v. somatostatin-14 increased gastric acid secretion stimulated by a submaximal dose of pentagastrin while a higher dose had no effect (Pappas et al., 1985). By contrast, previous results show that somatostatin-14 microinjected into the lateral and paraventricular nucleus of the hypothalamus inhibited pentagastrin-stimulated acid secretion in urethane-anesthetized rats or basal acid secretion in conscious rats upon i.c.v. injection (Yoneda and Taché, 1995; present study). The variation in the pattern of responses may be related to differences among species in the relative distribution of somatostatin receptor subtypes at hypothalamic or medullary sites.

The inhibitory action of the sst_5 receptor agonist appears to be potentiated by i.c.v. injection of the sst_2 receptor agonist. When the sst_5 agonist BIM-23052 was injected i.c.v. at a subthreshold effective dose in combination with the sst_2 receptor ligand, DC 32-87, which did not influence gastric acid secretion, there was a 47% significant reduction in basal gastric acid secretion. This represents a 125% increase in the inhibitory response above that expected from an additive effect. This synergistic effect may be related either to the presence of both receptor subtypes in the same cells and therefore sst_2 facilitates the inhibitory action of sst_5, or in different cell populations that interact in the neural pathways controlling acid secretion. Neuroanatomical studies showed that single rat brain neurons can co-express both sst_3 and sst_4 receptor messenger RNAs supporting the idea that somatostatin can interact with different receptors in the same neuron (Pérez and Hoyer, 1995). Furthermore, similar synergistic interactions at the central level have been reported for other family-related peptides such as thyrotropin-releasing hormone (TRH) and prepro-TRH-(160–169) (Roussell et al., 1991; Yang and Taché, 1994). The localization of the site and molecular mechanisms of this interaction need to be further studied, including the possible co-expression of sst_2 and sst_5 receptor subtypes in the rat brain and the characterization of the pharmacological profile of both receptor subtypes when transfected simultaneously in the same cell. These findings suggest that both the sst_2 and sst_5 receptor subtypes may mediate the inhibitory action of somatostatin in the brain. They can also explain the greater potency of SMS 201-995, which displays a high affinity for both sst_5 and sst_2, compared with somatostatin or the compound BIM-23052 which showed $sst_5 \gg sst_3 > sst_4 > sst_1 > sst_2$ (Raynor et al., 1993a, b).

The potentiating action observed appears to be specific to the sst_2-sst_5 receptor agonists interaction since the sst_3 receptor ligand, BM-23056 (0.4 nmol/rat), injected i.c.v. concomitantly with a similar dose of either the sst_5 or the sst_2 receptor ligand, did not influence basal gastric acid secretion. Although the sst_3 receptor agonist at a dose of 0.4 nmol stimulated gastric acid secretion, this action was no longer observed when this agonist was injected concomitantly with either the sst_2 or the sst_5 receptor agonists. The lack of stimulatory effect of the combinations sst_3/sst_5 and sst_3/sst_2 may result from opposite actions of somatostatin receptor subtypes.

Peptides injected into the lateral ventricle would spread into the cerebrospinal fluid to the third ventricle and diffuse to the hypothalamus (Feldberg, 1982), acting on somatostatin receptors located in this area. Microinjection experiments further established that SMS 201-995 acts specifically in the paraventricular nucleus and lateral nucleus of the hypothalamus to inhibit the gastric acid response to pentagastrin in urethane-anesthetized rats (Yoneda and Taché, 1995). Messenger RNA coding for each one of the somatostatin receptor subtypes is widely distributed in the central nervous system. In particular, in the rat hypothalamus, high concentrations of sst_5 receptor mRNA,

followed by that of sst_2 receptor and, to a lesser extent, sst_3 receptor, expressions have been observed autoradiographically (Breder et al., 1992; Bruno et al., 1993; Pérez and Hoyer, 1995). Somatostatin nerve terminals are located at hypothalamic nuclei regulating autonomic outflow to the stomach (Finley et al., 1981; Hisano and Daikoku, 1991). These neuroanatomical data provide support for a possible physiological relevance of these observations.

In conclusion, results obtained in this study show that i.c.v. injection of somatostatin and SMS 201-995 inhibits basal gastric acid secretion in conscious rats equipped with chronic gastric fistulae through a central action, most likely occurring in the forebrain. This inhibitory action seems to be exerted mainly through interaction with the sst_5 receptor subtype. The sst_2 receptor subtype may potentiate the inhibitory effect of the sst_5 receptor subtype, and therefore central inhibitory actions of somatostatin may be driven mainly by these two receptor subtypes. On the other hand, the sst_3 receptor subtype seems to mediate stimulatory actions at a central level. These findings indicate that the central action of somatostatin modulating basal gastric acid secretion reflects interactions with various receptor subtypes having potentiating or opposite effects. The selective activation of specific brain areas with a predominance of one or several somatostatin receptor subtypes can explain the gastric inhibitory and stimulatory responses, as well as the absence of responses, reported in the literature depending upon the somatostatin analog used and brain sites at which the peptide is injected.

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References

Bell, G.I. and T. Reisine, 1993, Molecular biology of somatostatin receptors, Trends Neurosci. 16, 34.

Breder, C.D., Y. Yamada, K. Yasuda, S. Seino, C.B. Saper and G.I. Bell, 1992, Differential expression of somatostatin receptor subtypes in brain, J. Neurosci. 12, 3920.

Bruno, J.F., Y. Xu, J. Song and M. Berelowitz, 1992, Molecular cloning and functional expression of a brain-specific somatostatin receptor, Proc. Natl. Acad. Sci. USA 89, 11151.

Bruno, J.F., Y. Xu, J. Song and M. Berelowitz, 1993, Tissue distribution of somatostatin receptor subtype messenger ribonucleic acid in the rat, Endocrinology 133, 2561.

Buscail, L., J.-P. Estève, N. Saint-Laurent, V. Bertrand, T. Reisine, A.-M. O'Carroll, G.I. Bell, A.V. Schally, N. Vaysse and C. Susini, 1995, Inhibition of cell proliferation by the somatostatin analogue RC-160 is mediated by somatostatin receptor subtypes SSTR2 and SSTR5 through different mechanisms, Proc. Natl. Acad. Sci. USA, 92, 1580.

Feldberg, W.S., 1982, Fifty years on: Looking Back on Some Developments in Neurohumoral Physiology (Liverpool University Press, Liverpool) p. 31.

Finley, J.C.W., J.L. Maderdrut, L.J. Roger and P. Petrusz, 1981, The immunocytochemical localization of somatostatin-containing neurons in the central nervous system, Neuroscience 6, 2173.

Gu, Z.-F., V.D. Corleto, S.A. Mantey, D.H. Coy, P.N. Naton, and R.T. Jensen, 1995, Somatostatin receptor subtype 3 mediates the inhibitory action of somatostatin on gastric smooth muscle cells, Am. J. Physiol. 268, G739.

Hermann, G.E. and R.C. Rogers, 1989, Extrinsic neural control of brainstem gastric vago-vagal reflex circuits, in: Nerves and the gastrointestinal tract – Falk Symposium No. 50, eds. M.V. Singer and H. Goebell (MTP Press Ltd., Lancaster) p. 345.

Hisano, S. and S. Daikoku, 1991, Existence of mutual synaptic relations between corticotropin-releasing factor-containing and somatostatin-containing neurons in the rat hypothalamus, Brain Res. 545, 265.

Hoyer, D., G.I. Bell, M. Berelowitz, J. Epelbaum, W. Feniuk, P.P.A. Humphrey, A.-M. O'Carroll, Y.C. Patel, A. Schonbrunn, J.E. Taylor, and T. Reisine, 1995, Classification and nomenclature of somatostatin receptors, Trends Pharmacol. Sci. 16, 86.

Kong, H., A.M. DePaoli, C.D. Breder, K. Yasuda, G.I. Bell and T. Reisine, 1994, Differential expression of messenger RNAs for somatostatin receptor subtypes SSTR1, SSTR2 and SSTR3 in adult rat brain: analysis by RNA blotting and in situ hybridization histochemistry, Neuroscience 59, 175.

Lenz, J.H., I. Forquignon, G. Druge and H. Greten, 1989, Effects of neuropeptides on gastric acid and duodenal bicarbonate secretions in freely moving rats, Reg. Pept. 24, 293.

Lloyd, K.C.K., J. Wang, K. Aurang, P. Grönhed, D.H. Coy and J.H. Walsh, 1995, Activation of somatostatin receptor subtype 2 inhibits acid secretion in rats, Am. J. Physiol. 268, G102.

Martínez, V., D.H. Coy and Y. Taché, 1995, Influence of intracisternal injection of somatostatin analog receptor subtypes 2, 3 and 5 on gastric acid secretion in conscious rats, Neurosci. Lett. 186, 79.

O'Carroll, A.M., S.J. Lolait, M. Konig and L.C. Mahan, 1992, Molecular cloning and expression of a pituitary somatostatin receptor with preferential affinity for somatostatin-28, Mol. Pharmacol. 42, 939.

Osumi, Y., T. Ishikawa and M. Fujiwara, 1979, Effect of intraventricularly applied somatostatin on gastric acid secretion and mucosal blood flow in rats, in: Integrative Control Functions of the Brain, Vol. II, eds. M. Ito, K. Kubota, N. Tsukahara and K. Yagi (Elsevier/North Holland Biomedical Press, Kodansha Ltd., Tokyo) p. 261.

Pappas, T., Y. Taché and H. Debas, 1985, Cerebro-ventricular somatostatin stimulates gastric acid secretion in the dog, in: Regulatory Peptides in Digestive, Nervous and Endocrine Systems, INSERM Symposium No. 25, eds. M.J.M. Lewin and S. Bonfils (Elsevier Science Publishers BV, Amsterdam) p. 323.

Paxinos, G. and C. Watson, 1986, The rat brain in stereotaxic coordinates (Academic Press, Orlando) p. 1.

Pérez, J. and D. Hoyer, 1995, Co-expression of somatostatin SSTR-3 and SSTR-4 receptor messenger RNAs in the rat brain, Neuroscience 64, 241.

Pless, J., W. Bauer, U. Briner, W. Doepfner, P. Marbach, R. Maurer, T.J. Petcher, J.C. Reubi and J. Vonderscher, 1986, Chemistry and

pharmacology of SMS 201-995, a long-acting octapeptide analogue of somatostatin, Scand. J. Gastroenterol. 21 (Suppl. 119), 54.

Raynor, K., W.A. Murphy, D.H. Coy, J.E. Taylor, J.P. Moreau, K. Yasuda, G.I.Bell and T. Reisine, 1993a, Cloned somatostatin receptors: identification of subtype-selective peptides and demonstration of high affinity binding of linear peptides, Mol. Pharmacol. 43, 838.

Raynor, K., A.M. O'Carroll, H. Kong, K. Yasuda, L.C. Mahan, G.I. Bell and T. Reisine, 1993b, Characterization of cloned somatostatin receptors SSTR4 and SSTR5, Mol. Pharmacol. 44, 385.

Reisine, T., G.I. Bell, 1995, Molecular biology of somatostatin receptors, Endocrinol. Rev. 16, 427.

Roussell, J.P., F. Hollande, M. Bulant and H. Astier, 1991, A prepro-TRH connecting peptide (prepro-TRH 160-169) potentiates TRH-induced TSH release from rat perifused pituitaries by stimulating dihydropyridine- and ω-conotoxin-sensitive Ca^{2+} channels, Neuroendocrinology 54, 559.

Rossowski, W.J. and D.H. Coy, 1993, Potent inhibitory effects of a type four receptor-selective somatostatin analog on rat insulin release, Biochem. Biophys. Res. Commun. 197, 366.

Rossowski, W.J., Z.-F. Gu, U.S. Akarca, R.T. Jensen and D.H. Coy, 1994, Characterization of somatostatin receptor subtypes controlling rat gastric acid and pancreatic amylase release, Peptides 15, 1421.

Senaris, R.M., P.P.A. Humphrey and P.C. Emson, 1994, Distribution of somatostatin receptors 1, 2 and 3 mRNA in rat brain and pituitary, Eur. J. Neurosci. 6, 1883.

Stephens, R.L., Jr., 1991, Disparate effects of intracisternal RX 77368 and ODT8-SS on gastric acid secretion and serotonin release: role of adrenal catecholamines, Reg. Pept. 36, 21.

Taché, Y., J. Rivier, W. Vale and M. Brown, 1981, Is somatostatin or a somatostatin-like peptide involved in central nervous system control of gastric secretion?, Reg. Pept. 1, 307.

Tannenbaum, G.S. and Y.C. Patel, 1986, On the fate of centrally administered somatostatin in the rat: massive hypersomatostatinemia resulting from leakage into the peripheral circulation has effects on growth hormone secretion and glucoregulation, Endocrinology 118, 2137.

Yamada, Y., S. Kagimoto, A. Kubota, K. Yasuda, K. Masuda, Y. Someya, Y. Ihara, Q. Li, H. Imura, S. Seino and Y. Seino, 1993, Cloning, function expression and pharmacological characterization of a fourth (hSSTR4) and a fifth (hSSTR5) human somatostatin receptor subtype, Biochem. Biophys. Res. Commun. 195, 844.

Yang, H. and Y. Taché, 1994, Prepro-TRH-(160–169) potentiates gastric acid secretion stimulated by TRH microinjected into the dorsal motor nucleus of the vagus, Neurosci. Lett. 174, 43.

Yoneda, M. and Y. Taché, 1995, The somatostatin analog, SMS 201-995 stimulates gastric acid secretion via the dorsal vagal complex and inhibits it via the hypothalamus in anesthetized rats, Br. J. Pharmacol. 116, 2303.

Yoneda, M., H. Raybould and Y. Taché, 1991, Central action of somatostatin analog, SMS 201-995, to stimulate gastric acid secretion in rats, Peptides 12, 401.