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SMS 201-995-induced stimulation of gastric acid secretion via the dorsal vagal complex and inhibition via the hypothalamus in anaesthetized rats

Masashi Yoneda & Yvette Taché

CURE/Gastroenteric Biology Center, West Los Angeles VA Medical Center, Department of Medicine and Brain Research Institute, University of California, Los Angeles, CA, U.S.A.

1 SMS 201-995, a somatostatin analogue which interacts with highest affinities at somatostatin receptor subtypes $5>2\geqslant3$, was microinjected into selective brain sites and its influence on pentagastrin (10 $\mu g\ kg^{-1}\ h^{-1}$, i.v.) -stimulated gastric acid secretion was investigated in rats anaesthetized with urethane. Gastric acid secretion was measured by flushing the stomach with saline through a gastric cannula every 10 min.

2 SMS 201-995 microinjected into the dorsal vagal complex (DVC, 7, 15, 30 and 60 ng) dose-dependently increased pentagastrin-stimulated gastric acid secretion. The peak acid response was reached within 20 min and returned to basal level 50 min post-injection. SMA 201-995 (30 ng) microinjected into the surrounding area or the central amygdala did not modify pentagastrin-stimulated acid secretion.

3 SMS 201-995 injected into the lateral ventricle (i.c.v., 100, 200, or 300 ng), paraventricular nucleus (PVN) or lateral hypothalamus (LH) (7.5, 15, or 30 ng) dose-dependently inhibited pentagastrin-stimulated gastric acid secretion. SMS 201-995 (30 ng) microinjected into the area surrounding the PVN or LH did not modify the acid secretion response to pentagastrin.

4 Vagotomy prevented the effects of SMS 201-995 (30 ng) microinjected into the DVC and LH.

5 Spinal cord transection abolished the inhibitory action of SMS 201-995 (30 ng) microinjected into the PVN but not the LH.

6 These results demonstrate that SMS 201-995 acts in the DVC to enhance and in the LH and PVN to inhibit pentagastrin-stimulated gastric acid secretion. The action is mediated through vagal (DVC, LH) or spinal (PVN) pathways. The site specific pattern of acid responses to SMS 201-995 may be linked to the distribution of receptor subtypes at these sites that convey the different biological actions of somatostatin.

Keywords: Somatostatin analogue, SMS 201-995; vagotomy; spinal cord; vagus; dorsal vagal complex; lateral hypothalamus; paraventricular nucleus of the hypothalamus; central amygdala

Introduction

Somatostatin is a peptide widely distributed throughout the mammalian central nervous systems (Finley *et al.*, 1981; Rossowski & Coy, 1993). Previous reports indicate that somatostatin injected into the cisterna magna 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 response induced by somatostatin-14 or somatostatin analogues varies, depending on the peptides and the brain site of administration. The oligosomatostatin analogues, Des-AA^{1,2,4,5,12,13}- [D-Tryp^{8}]-somatostatin (ODT8-SST), Des-AA^{1,2,4,5,12,13}-[D-Tryp^{8}, D-Cys^{14}]-somatostatin or the clinically used hexapeptide somatostatin analogue, SMS 201-995 (Moreau & DeFeudis, 1987) injected into the cisterna magna stimulated gastric acid output, through vagal- and adrenal-dependent mechanisms in conscious pylorus-ligated rats while somatostatin-14 had no effect (Taché *et al.*, 1981; Yoneda *et al.*, 1991; Stephens, 1991). Intracerebroventricular injection of ODT8-SST also stimulated gastric acid secretion in conscious rats and enhanced the acid response to a meal or pentagastrin in dogs (Pappas *et al.*, 1985; Lenz *et al.*, 1989). By contrast, injection of somatostatin-14 or SMS 201-995 into the lateral ventricle inhibited pentagastrin-stimulated acid secretion in rats anaesthetized with urethane or basal acid secretion in conscious rats with a chronic gastric fistula and an intracerebroventricular cannula (Osumi *et al.*, 1979; Martinez *et al.*, 1994).

Five different somatostatin receptor subtypes ($SSTR_1$: to $SSTR_5$) 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'Carrol *et al.* (1992) have been renamed $SSTR_4$ and $SSTR_5$, respectively (Reisine & Bell, 1995). The brain distribution of messenger RNA encoding for each of the receptor subtypes shows a distinct but overlapping pattern of expression in the rat brain (Breder *et al.*, 1992; Bruno *et al.*, 1993; Kong *et al.*, 1994). For example, in the hypothalamus high concentrations of $SSTR_5$ followed by those of $SSTR_2$ and to a lesser extent, $SSTR_3$ have been observed by autoradiographic densitometry (Breder *et al.*, 1992; Bruno *et al.*, 1993) while in the brainstem $SSTR_1$ and $SSTR_3$ expression are mainly represented (Breder *et al.*, 1992). In Chinese hamster ovary or COS-1 cells transfected with genes encoding these receptor subtypes, SMS 201-995 has highest affinities for $SSTR_5>SSTR_2\geqslant SSTR_3$ and does not bind to $SSTR_1$ and $SSTR_4$ receptors. Under the same conditions, somatostatin-14 has almost equal affinity for the 5 subtypes of receptors (Raynor *et al.*, 1993a, b; Rossowski & Coy, 1993). The selective activation of somatostatin receptor subtypes in brain areas with a predominance of different receptor subtypes might therefore explain the opposite inhibitory and excitatory responses observed when somatostatin or analogues are injected into the cisterna magna vs the cerebral ventricle. However, little is known about the brain sites where somatostatin or analogues act to influence gastric acid secretion.

In the present study, we investigated the influence of SMS 201-995 microinjected into selective hypothalamic and medullary nuclei on pentagastrin-stimulated gastric acid secretion in rats anaesthetized with urethane. The analogue was micro-

[1] Author for correspondence at: CURE, Bldg, 115, Room 115, West Los Angeles VA Medical Center, 11301 Wilshire Blvd, Los Angeles, CA 90073, U.S.A.

injected into the lateral hypothalamus (LH), paraventricular nucleus of the hypothalamus (PVN), central amygdala (CA) as well as the dorsal vagal complex (DVC) known to contain somatostatin receptors and somatostatin immunoreactive terminals (Millhorn *et al.*, 1987; Breder *et al.*, 1992; Bruno *et al.*, 1993; Shiraishi *et al.*, 1993; Kong *et al.*, 1994). These brain nuclei are also involved in the autonomic regulation of gastric function (Taché & Yang, 1990).

Methods

Animals

Male Sprague-Dawley, albino rats weighing 280–310 g (Harlan Laboratory, San Diego, CA, U.S.A.) were housed in group cages under conditions of controlled temperature (22–24°C) and illumination (12 h light cycle starting at 06 h 00 min) for at least 7 days before the experiments. Animals were maintained on Purina Laboratory Chow (Ralson Purina, St. Louise, MO) *ad libitum* and tap water. Experiments were performed in rats deprived of food for 24 h but given free access to water up to the beginning of the study.

Measurement of gastric acid secretion

Rats were anaesthetized with urethane (1.5 g kg^{-1}, ip), the oesophagus was ligated at the cervical level and a laparotomy was performed. The pylorus was ligated and a double-lumen cannula was inserted through a small incision into the forestomach. Gastric secretion was collected by flushing the gastric lumen twice with 5 ml boluses of saline at room temperature followed by one 5 ml bolus of air at 10 min intervals. Acid output was measured by titration (autotitrator; Radiometer Corp., Copenhagen, Denmark) of the flushed perfusate with 0.01 N NaOH to pH 7.0. Basal secretion was collected for 30 min before pentagastrin infusion.

Brain microinjections

For microinjection into the DVC, rats under urethane-anaesthesia and with a gastric cannula were placed in a stereotaxic instrument (Kopf model 900, David Kopf Instruments, Tujunga, CA) in a mouth down position (−15 mm) for the duration of microinjection. The obex region of the dorsal medulla was exposed by resecting the dorsal cervical musculature and removing the occipital skull plate and small pieces of the cerebellum. A single-barrel glass micropipette 50–70 μm diameter) was positioned unilaterally (right side). For microinjection into the CA, PVN, LH, or lateral brain ventricle, urethane-anaesthetized rats were placed in a stereotaxic instrument in the mouth down position (−5 mm). The bregma region of the parietal skull was exposed. A glass micropipette was positioned unilaterally (right side) through a small hole in the skull made by a drill. Saline (0.9%) or SMS 201-995 was microinjected in a 50 nl volume delivered by pressure ejection over 1 min utilizing a 1 μl Hamilton syringe (Hamilton Co, Reno, NV, U.S.A. The micropipette was left in place for an additional 3 min, then withdrawn. For injection into the lateral brain ventricle, the volume of injection was 10 μl delivered with a 50 μl Hamilton syringe. Coordinates (DV: dorsoventral, AP: anteroposterior, and L: lateral in mm) used for microinjections were as follows: DVC (DV: 0.6; AP from obex: 0.5; L from obex: 0.5); CA (DV: 8.0; AP from bregma: −2.3; L from bregma: 4.0); PVN (DV: 8.0; AP from bregma: −1.8; L from bregma: 0.3); LH (DV: 8.2; AP from bregma: −1.8; L from bregma: 2.0); lateral ventricle (DV: 4.5; AP from bregma: −0.8; L from bregma: 1.5).

At the end of experiments, rats were killed by decapitation. Brains were removed and fixed in 10% formalin and 20% sucrose solution at least for 2 days. Frozen sections were sliced at 30 μm, mounted and stained with 0.1% toluidine blue. Histological sections were examined microscopically. The location of microinjection sites was identified as the point of termination of cannula track and marked on plates reproduced from the atlas of Paxinos & Watson (1986).

Experimental protocols

After 30 min of basal measurement of gastric acid secretion in rats under urethane anaesthesia, pentagastrin (10 μg kg^{-1} h^{-1}) was infused through the jugular vein to obtain submaximal stimulation of gastric acid secretion throughout the experiment. Sixty minutes after the start of pentagastrin infusion, SMS 201-995 was injected into the lateral ventricle (100, 200, 300 or 500 ng), CA (30 ng), DVC, PVN or LH (7.5, 15, 30 or 60 ng). Control groups received saline under the same conditions. Gastric acid response was monitored for 120 min after injection of peptide or vehicle.

Drugs and treatments

The following substances were used; somatostatin analogue, D-Phe-c[Cys-Phe-D-Trp-Lys-Thr-Cys]-Thr-ol (SMS 201-995, Sandoz Pharmaceuticals, East Hanover, NJ, U.S.A.) and pentagastrin (Ayerst Laboratories, New York, NY, U.S.A.). SMS 201-995, in powder form, was freshly dissolved in saline before microinjection. Pentagastrin solution was diluted in saline and infused through the jugular vein at a rate of 1.1 ml h^{-1}. Bilateral cervical vagotomy and spinal cord transection at the level of C6 or sham operation were performed 120 min before the basal gastric acid measurement as previously described (Taché *et al.*, 1986).

Statistics

Net acid secretion during the 60 min after injection of somatostatin analogue or vehicle into various brain sites was calculated for each rat by subtracting from each value after the injection, the average of the two collection periods before the injection. Results are expressed as mean ± s.e.mean. Student's *t* test was used to compare differences between two groups. One way ANOVA followed by Duncan's or Dunnet's test was used to compare differences between groups. $P<0.05$ was considered statistically significant.

Results

Effect of SMS 201-995 microinjected into the DVC on pentagastrin-stimulated gastric acid secretion

In urethane-anaesthetized rats, basal gastric acid secretion was low (2.8±0.2 μmol 10 min^{-1}, $n=26$). Intravenous infusion of pentagastrin (10 μg kg^{-1} h^{-1}) stimulated gastric acid secretion that reached a plateau of 17.7±0.8 μmol 10 min^{-1} within 40-50 min (Figure 1). The plateau was not altered by microinjection of vehicle into the DVC (net acid change: 7.6±3.3 μmol 60 min^{-1}). Microinjection of SMS 201-995 into the DVC at 7.5, 15, 30 and 60 ng increased the acid response to pentagastrin (μmol 60 min^{-1}) by 12.0±1.7 ($P>0.05$), 21.1±4.3 ($P<0.05$), 50.9±6.1 ($P<0.01$) and 62.2±14.5 ($P<0.01$) respectively (Figure 2). After microinjection of SMS 201-995 into the DVC at 30 ng, the peak response reached 33.6±2.2 μmol 10 min^{-1} within 20 min, then values returned to preinjection levels 50 min later (Figure 1). Bilateral cervical vagotomy abolished the stimulatory effect of SMS 201-995 (30 ng) microinjected into the DVC (net change: 9.7±1.2 μmol 60 min^{-1}). Vagotomy by itself did not modify the acid response to pentagastrin (acid output: sham group: 17.8±0.3 μmol 10 min^{-1}, $n=6$; vagotomized group: 18.4±0.7 μmol 10 min^{-1}, $n=5$). SMS 201-995 (30 ng) microinjected outside of the DVC into sites illustrated in Figure 3 did not significantly modify the gastric acid secretion stimulated by pentagastrin (net increase 2.4±4.1 μmol 60 min^{-1}, $n=5$).

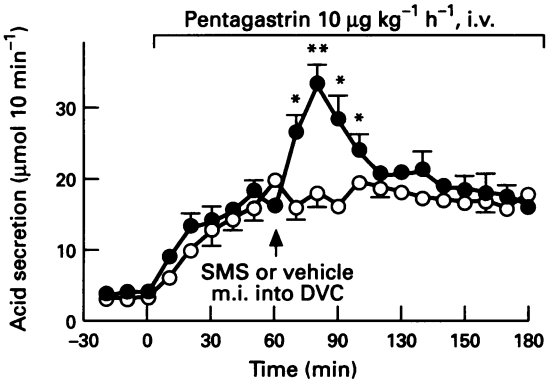

Figure 1 Time course of the stimulatory effect of the somatostatin analogue, SMS 201-995, microinjected (m.i.) into the dorsal vagal complex (DVC) on pentagastrin-stimulated gastric acid secretion in urethane-anaesthetized rats. After monitoring basal gastric acid secretion, pentagastrin was infused through the jugular vein. After 60 min, vehicle (○, $n=5$) or SMS 201-995 (●, n-6) was microinjected unilaterally into the DVC and gastric acid secretion was monitored for 120 min. Each point represents the mean ± s.e.mean. Points without bar have s.e.mean < 0.4 μmol 10 min^{-1}. $*P<0.05$; $**P<0.01$ compared with saline microinjected group.

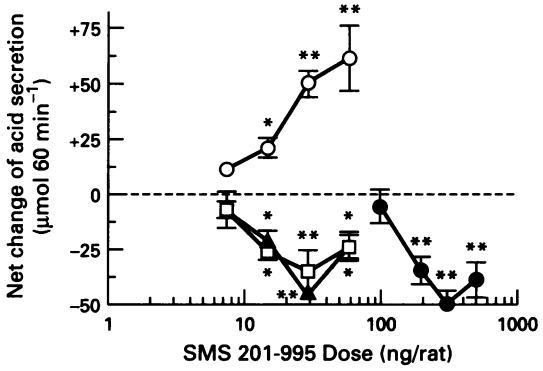

Figure 2 Dose-related effect of net gastric acid changes induced by unilateral injection of SMS 201-995 into the dorsal vagal complex (○, $n=5-6$), lateral ventricle (●, $n=5$) paraventricular nucleus of the hypothalamus (▲, $n=5$) and lateral hypothalamus (□, $n=5$) 60 min after the start of pentagastrin infusion (10 μg kg^{-1}) in urethane-anaesthetized rats. Each point represents the mean ± s.e.mean of net acid changes for 60 min after microinjection. $*P<0.05$; $**P<0.05$ compared with respective average pre-injection values.

Effect of SMS 201-995 microinjected into the lateral brain ventricle, hypothalamic or limbic brain sites on pentagastrin-stimulated gastric acid secretion

SMS 201-995, injected i.c.v. at 100, 200 or 300 ng, dose-dependently inhibited the pentagastrin-stimulated acid secretion for the 60 min period after the injection (net decrease in μmol 60 min^{-1}: −4.9 ± 7.9, −33.7 ± 6.2, −48.9 ± 5.4 and −38.1 ± 7.6 respectively). (Figure 2). There was no change in acid secretion after i.c.v. injection of saline (−1.2 ± 3.8 μmol 60 min^{-1}). Peak inhibition of acid secretion was observed at 30 min after i.c.v. injection of SMS 201-995 (300 ng) and lasted up to 70 min after peptide injection (Figure 4).

SMS 201-995 microinjected into the PVN at 7.5, 15, 30 and 60 ng, dose-dependently reduced the pentagastrin-stimulated

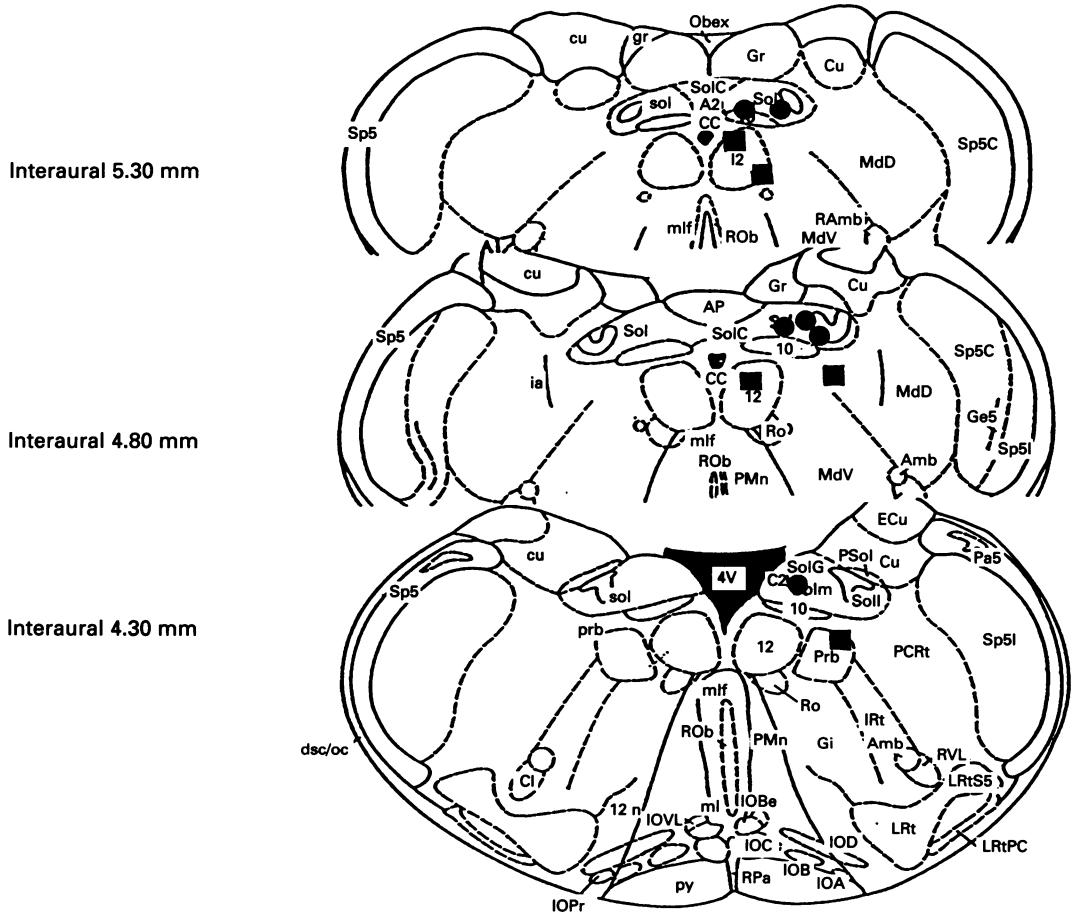

Figure 3 Diagram of coronal sections of rat medulla: circles and squares represent sites microinjected with SMS 201-995 (30 ng) which increased pentagastrin-stimulated acid secretion (●) or had no effects (■). Plates are adapted from Paxinos & Watson (1986).

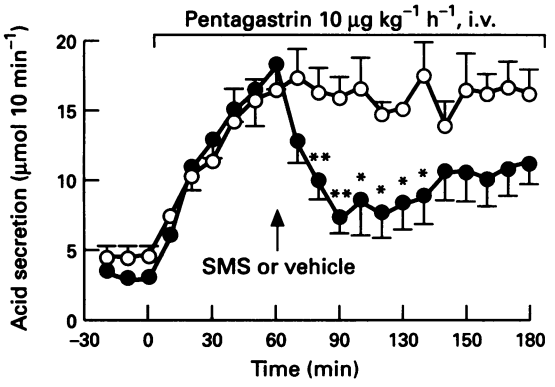

Figure 4 Time course of the inhibition of pentagastrin-stimulated gastric acid secretion by somatostatin analogue, SMS 201-995, injected intracerebroventricularly (i.c.v.) in urethane-anaesthetized rats. After monitoring basal gastric acid secretion, pentagastrin was infused through the jugular vein. After 60 min, vehicle (○, $n=5$) or SMS 201-995 (300 ng 10 μl^{-1}, ●, $n=5$) was injected unilaterally i.c.v. and gastric acid secretion was monitored for 120 min. Each point represents the mean ± s.e.mean of number of rats indicated in parentheses. Points without bar have s.e.mean less than 0.4 μmol 10-min^{-1}. $*P<0.05$, $**P<0.01$ compared with saline microinjected group.

acid secretion (net decreases in μmol 60 min^{-1}: −6.8±8.4, −21.0±3.9, −43.9±8.4 and −23.5±4.6 respectively). SMS 201-995 microinjected into the LH at 7.5, 15, 30 and 60 ng also induced a net decrease of acid secretion (μmol 60 min^{-1}) of −6.2±3.3, −25.7±3.2, −34.5±9.3 and −23.3±5.9 respectively (Figure 2). The maximum peak inhibition occurred after 30–40 min and reached 46.2% and 42.1% compared with the pre-injection values when SMS 201-995 (30 ng) was microinjected into the PVN and LH respectively. The localization of hypothalamic sites responsive and unresponsive to microinjection of SMS 201-995 (30 ng) is illustrated in Figure 5. Microinjection of SMS 201-995 (30 ng) into the reuniens thalamic nucleus ($n=3$), entopeduncular nucleus ($n=1$), anterior hypothalamic area ($n=1$) and zona incerta ($n=3$) did not alter the acid response to pentagastrin. Microinjection of SMS 201-995 (30 ng) into the CA also did not significantly modify pentagastrin-stimulated gastric acid secretion (net changes: 10.7±4.7 μmol 60 min^{-1}, $n=5$).

Spinal cord transection at the level of C6 completely prevented the inhibitory effect of SMS 201-995 (30 ng) microinjected into the PVN, but did not influence the inhibitory response induced by SMS 201-995 microinjected into the LH (Figure 6). Spinal cord transection by itself modified neither basal acid secretion (2.9±0.16 μmol 10 min^{-1}, $n=5$ vs 2.0±0.2 μmol 10 min^{-1} in sham-operated group, $n=5$) nor the acid response to pentagastrin (23.1±1.6 μmol 10 min^{-1} vs 20.8±1.5 μmol 10 min^{-1} vs 20.8±1.5 μmol 10 min^{-1} in sham-operated group, $n=5$). Bilateral cervical vagotomy completely prevented the inhibition of gastric acid response to pentagastrin induced by SMS 201-995 injected into the LH. (Figure 7).

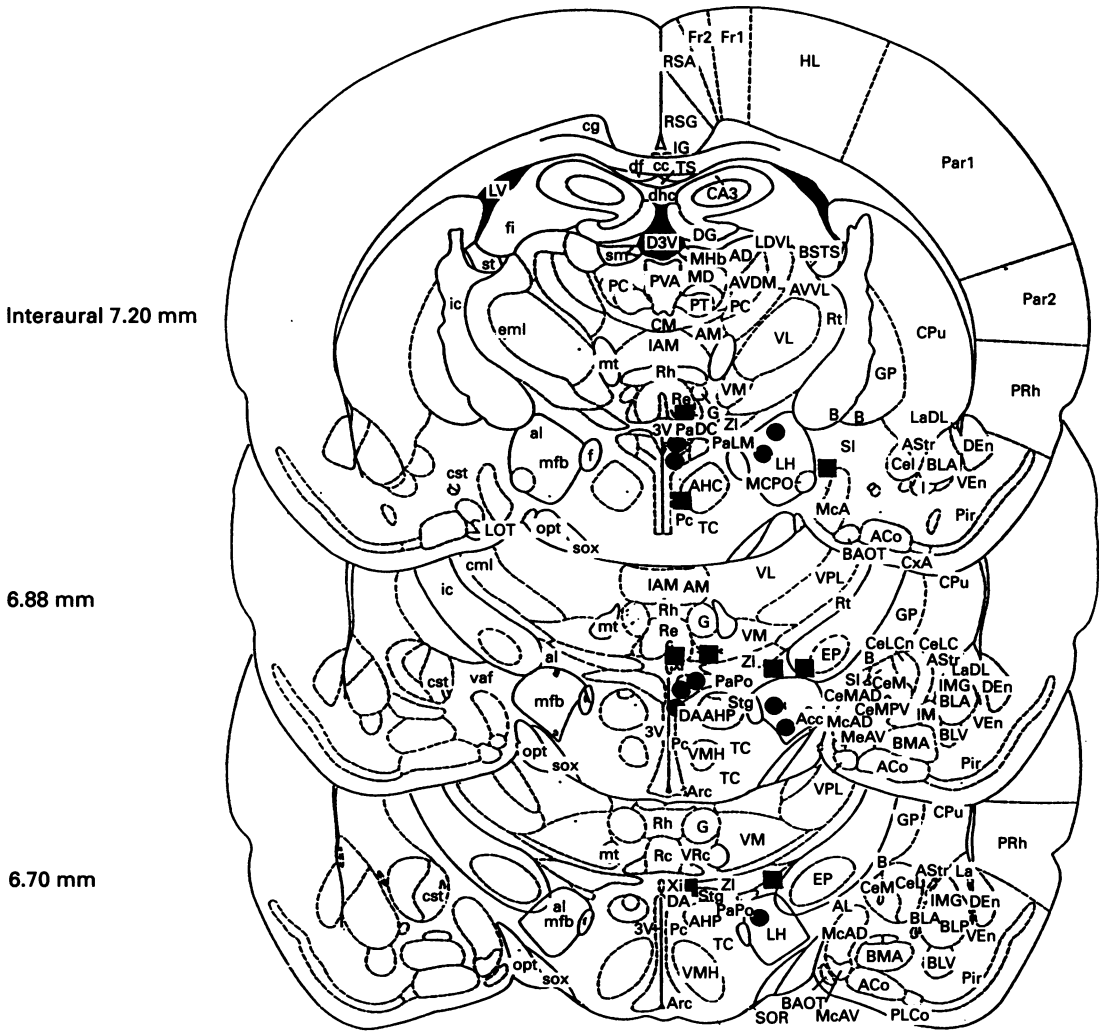

Figure 5 Diagram of coronal sections of rat hypothalamic region. Circles and squares represent sites microinjected with SMS 201-995 (30 ng) which decreased acid secretion (●) or had no effects (■). Plates are adapted from Paxinos & Watson (1986).

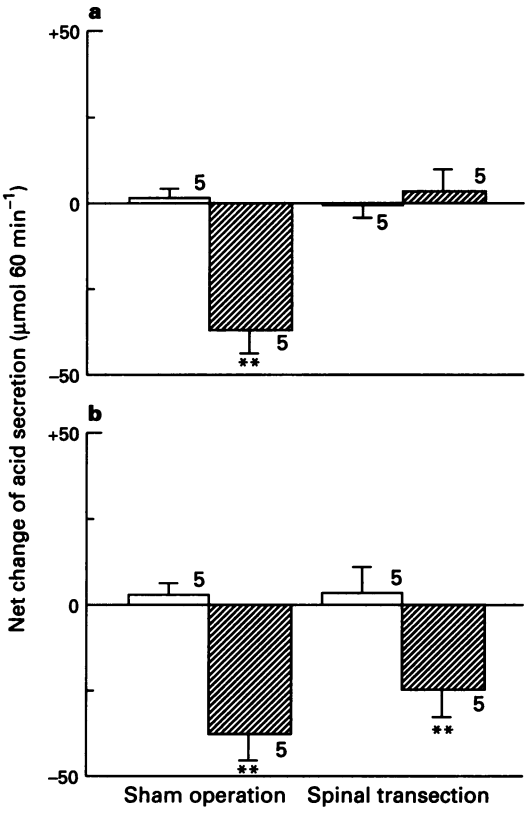

Figure 6 Effect of spinal cord transection on the inhibition of pentagastrin-stimulated gastric acid secretion induced by unilateral microinjection of SMS 201-995 into the paraventricular nucleus of hypothalamus (PVN, a) and lateral hypothalamus (LH, b) in urethane-anaesthetized and pentagastrin-stimulated rats. Spinal cord transection or sham operation was performed 120 min before the basal gastric acid measurement. Each column represents the mean ± s.e.mean of net changes in gastric acid secretion for the 60 min period after the microinjection of vehicle (open columns) or SMS 201-995, 30 ng (hatched columns) compared with individual preinjection values. Number of rats is indicated at the top of each column. $^{**}P<0.01$ compared with respective control groups microinjected with vehicle.

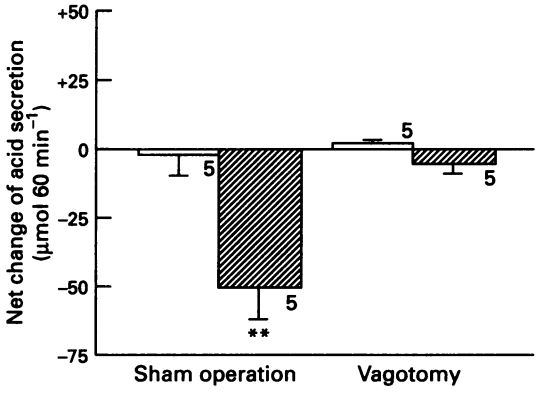

Figure 7 Effect of bilateral cervical vagotomy on the inhibition of pentagastrin-stimulated gastric acid secretion induced by unilateral microinjection of SMS 201-995 into the lateral hypothalamus in urethane-anaesthetized rats. Bilateral vagotomy or sham operation was performed 120 min before the basal gastric acid measurement. Each column represents the mean ± s.e.mean of net changes in gastric acid secretion for the 60 min period after the microinjection of vehicle (open columns) or SMS 201-995, 30 ng (hatched columns) compared with individual preinjection values. Number of rats is indicated at the top of each column. $^{**}P<0.01$ compared with respective vehicle-injected group.

Discussion

The oligosomatostatin analogues, ODT-8SST (0.1 – 1 μg) and SMS 201-995 (0.2 – 1 μg) injected intracisternally were previously reported to stimulate gastric acid output in conscious rats with pylorus ligation (Taché *et al.*, 1981; Stephens, 1991; Yoneda *et al.*, 1991). The present results demonstrate that SMS 201-995 microinjected unilaterally into the DVC at 30 ng increases pentagastrin-induced acid secretion with a peak response observed within 20 min in rats anaesthetized with urethane. The net increase in pentagastrin-stimulated gastric acid secretion for the 60 min period after microinjection of SMS 201-995 was dose-related over the range 15 to 60 ng. The site-specificity of SMS 201-995 microinjected into the DVC was shown by the absence of changes in the acid response to pentagastrin when the peptide was microinjected into the hypoglossal nucleus, nucleus of Probst's bundle and medullary reticular field. These data indicate that SMS 201-995 acts in the DVC to increase gastric acid secretion during the pentagastrin-stimulated state as previously observed under basal conditions (Yoneda *et al.*, 1991) in urethane-anaesthetized rats. Somatostatin-14 microinjected into the DVC was also reported to increase basal gastric motility in rats anaesthetized with urethane (Hermann & Rogers, 1989).

The neuronal circuitry and cellular mechanisms through which SMS 201-995 acts in the DVC to stimulate gastric acid secretion requires further investigation using an electrophysiological approach. Dense labelling of [Try^3] SMS 201-995 in the nucleus tractus solitarius was visualized by autoradiography (Reubi & Maurer, 1985). SMS 201-995 has highest affinity for $SSTR_5 > SSTR_2 \geq SSTR_3$ and very low affinity for $SSTR_1$ and $SSTR_4$ receptors while somatostatin 14 has similar affinities for all 5 subtypes of receptor (Raynor *et al.* 1993a, b). These data suggest that $SSTR_5$, $SSTR_2$ and/or $SSTR_3$, unlike $SSTR_1$ or $SSTR_4$, are involved in mediating the gastric secretory responses to SMS 201-995. Preliminary studies using somatostatin analogues selective at the $SSTR_2$, $SSTR_3$ or $SSTR_5$ receptor subtype, indicate that intracisternal injection of the $SSTR_3$ and $SSTR_5$ analogue stimulated basal gastric acid secretion in conscious rats (Martinez *et al.*, 1995). Although detailed knowledge of the localization of all somatostatin receptor subtypes present in the DVC are still unknown, *in situ* hybridization studies have shown the presence of $SSTR_3$ mRNA in the DVC (Perez *et al.*, 1994).

The enhanced acid response to pentagastrin induced by SMS 201-995 microinjected into the DVC was abolished by vagotomy. Mediation through vagal pathways is consistent with the fact that the dorsal motor nucleus contained over 90% of the preganglionic cell bodies of vagal efferent fibres projecting to the stomach (Shapiro & Miselis, 1985; Berthoud *et al.*, 1990). Likewise, in previous studies, the secretory response induced by ODT8-SST and SMS 201-995 injected into the cisterna magna also involved vagal cholinergic-dependent mechanisms (Taché *et al.*, 1981; Yoneda *et al.*, 1991). We previously reported that the acid response to central vagal stimulation in urethane-anaesthetized rats is peripherally mediated by the dual action of histamine and acetylcholine and that gastrin does not play a role (Yanagisawa *et al.*, 1990). It is likely that the enhanced acid response to pentagastrin induced by SMS 201-995 microinjected into the DVC results from these additional peripheral mechanisms recruited by vagal activation. This is supported by the magnitude of the peak acid response (33.6 ± 2.2 μmol 10 min^{-1}) induced 20 min after SMS 201-995 microinjection into the DVC (30 ng) in pentagastrin-infused rats which is similar to that obtained by adding responses observed 20 min after microinjection into the DVC of saline during pentagastrin infusion (17.9 ± 1.6 μmol 10 min^{-1}) and of SMS 201-995 (30 ng) alone (12.8 ± 1.7 μmol 10 min^{-1}) in urethane-anaesthetized rats (Yoneda *et al.* 1991; present observations).

By contrast to the intracisternal or DVC route of administration which enhanced the gastric acid secretory response to pentagastrin, intracerebroventricular injection of SMS 201-995

at 300 ng inhibited pentagastrin-stimulated acid secretion. In previous studies, somatostatin-14 injected into the lateral ventricle was also reported to induce a long lasting inhibition of basal acid secretion in urethane-anaesthetized or conscious rats (Osumi *et al.*, 1979; Martinez *et al.*, 1994). The inhibitory response can be mimicked by unilateral microinjection of SMS 201-995 into the PVN or LH at 10 fold lower doses than injected intracerebroventricularly. Previous radioautoradiographic studies using [^{125}I]-SMS 204-090 as a ligand showed labelling in the PVN and LH which support an interaction of the peptide with receptors in these nuclei (Krantic *et al.*, 1989). Site-specificity of the action of SMS 201-995 in the PVN and LH was ascertained by the lack of an inhibitory response when the somatostatin analogue was microinjected outside the boundary of these nuclei including the reuniens thalamic nucleus, entopeduncular nucleus, anterior hypothalamic area and zona incerta or into the CA. Taken together, the present results indicate that the PVN and LH are responsive sites in the hypothalamus for SMS 201-995-induced inhibition of pentagastrin-stimulated gastric acid secretion in urethane-anaesthetized rats. Several other peptides including bombesin, corticotropin-releasing factor, interleukin-1, calcitonin, calcitonin gene-related peptide and neuropeptide Y also act in the PVN and/or the LH to inhibit gastric acid secretion in rats (Taché & Yang, 1990; Saperas *et al.*, 1992). These findings further support the plurality of transmitters in the LH and PVN that inhibit gastric acid secretion in rats.

The receptor subtype involved in mediating such inhibitory responses has still to be assessed with somatostatin analogues selective for the various receptor subtypes. Preliminary studies indicate that intracerebroventricular injection of a $SSTR_5$ -selective ligand inhibited basal gastric acid secretion in conscious rats with chronic gastric fistulae while the $SSTR_2$ ligand had no effect and the $SSTR_3$ agonist tended to increase basal acid secretion (Martinez *et al.*, 1994). The decrease in the inhibitory response when the higher dose (60 ng) of SMS 201-995 was microinjected into the LH or PVN may reflect interaction of the somatostatin analogue with the $SSTR_3$ receptors since this analogue has higher affinities for the $5 > 2 \geqslant 3$ subtypes (Raynor *et al.*, 1993a, b). Messenger RNA (mRNA) encoding for each one of the somatostatin receptor subtypes in the hypothalamus was observed by autoradiography with high concentrations of $SSTR_5$ expression followed by that of $SSTR_2$ and to a lesser extent $SSTR_3$ (Breder *et al.*, 1992; Bruno *et al.*, 1993).

The inhibitory effect of SMS 201-995 microinjected into the PVN was completely blocked by spinal cord transection whereas the inhibitory action induced by microinjection of the somatostatin analogue into the LH was not modified. Previous studies also showed that electrical stimulation of the PVN inhibits vagally stimulated acid secretion through splanchnic pathways whereas LH stimulation did not (Okuma & Osumi, 1989). There are direct monosynaptic neuronal projections between the PVN and sympathetic preganglionic neurones in the spinal cord (Swanson & Kuypers, 1980; Sawchenko & Swanson, 1982; Luiten *et al.*, 1985) suggesting a possible mediation through modulation of the sympathetic nervous system. The oligosomatostatin analogue, ODT8-SST and somatostatin-14 injected i.c.v. influence plasma adrenaline secretion and efferent activity in the gastric and adrenal branches of the splanchnic nerve (Fisher & Brown, 1980; Somiya & Tonoue, 1984). Spinal cord transection did not modify the acid response to pentagastrin in urethane-anaesthetized rats as previously reported for the gastric acid secretion in conscious, pylorus-ligated rats (Taché *et al.*, 1985; 1986).

The inhibition of pentagastrin secretion by microinjection of SMS 201-995 into the LH was prevented by vagotomy suggesting a mediation through vagal-dependent pathways. Identified gastric motor neurones in the dorsal motor nucleus of the vagus can be modulated by descending input from the LH (Shiraishi, 1980). These data provide electrophysiological support for a mediation of LH action through vagal pathways. Moreover, intracerebroventricular injection of somatostatin was shown to decrease efferent activity in the gastric branch of the vagus (Somiya & Tonoue, 1984). Neuroanatomical studies showing that the distribution of somatostatin nerve terminals at hypothalamic nuclei regulating autonomic outflow to the stomach (Finley *et al.*, 1981; Hisano & Daikoku, 1991) added to these functional observations suggest a possible physiological relevance of somatostatin in these hypothalamic neurones for regulation of gastric secretion.

In summary, the present findings showed that SMS 201-995, a somatostatin analogue with high affinity for the $SSTR_5$, $SSTR_2$ and $SSTR_3$ receptors acts in the DVC to stimulate, and in the LH and PVN to inhibit pentagastrin-stimulated gastric acid secretion in urethane-anaesthetized rats. The acid response to somatostatin analogue microinjected into the DVC is mediated through vagal pathways. The inhibition of gastric secretion by SMS 201-995 microinjected into the PVN and LH involves spinal sympathetic and parasympathetic pathways respectively. The pattern of gastric acid stimulatory or inhibitory acid response at specific brain sites may be linked to the relative distribution of somatostatin receptor subtypes that convey the different biological actions of the SMS 201-995 through autonomic pathways.

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