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Selective agonists of somatostatin receptor subtype 1 or 2 injected peripherally induce antihyperalgesic effect in two models of visceral hypersensitivity in mice

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Abstract

Somatostatin interacts with 5 G-protein-coupled receptor (sst₁₋₅). Octreotide, a stable sst_{2/3/5} agonist, octreotide, exerts a visceral anti-hyperalgesic effect in experimental and clinical studies. Little is known on the receptor subtypes involved. We investigated the influence of the stable sst₁₋₅ agonist, ODT8-SST and selective receptor subtype peptide agonists (3 or 10 µg/mouse) injected intraperitoneally (ip) on visceral hypersensitivity in mice induced by repeated noxious colorectal distensions (4 sets of 3 CRD, each at 55 mmHg) or corticotropin-releasing factor receptor 1 agonist, cortagine given between 2 sets of graded CRD (15, 30, 45, and 60 mmHg, 3 times each pressure). The mean visceromotor response (VMR) was assessed using a non-invasive manometry method and values were expressed as percentage of the VMR to the 1st set of CRD baseline or to the 60 mmHg CRD, respectively. ODT8-SST (10 µg) and the sst₂ agonist, S-346-011 (3 and 10 µg) prevented mechanically-induced visceral hypersensitivity in the 3 sets of CRD, the sst₁ agonist (10 µg) blocked only the 2nd set and showed a trend at 3 µg while the sst₄

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Disclosures

AM, ML, MB, MM, and YT have nothing to declare. JR is the Dr. Frederik Paulsen Chair in Neurosciences Professor. No conflicts of interest exist.

Author contributions

The contributions of each author to the paper were as follows: AM designed the experiments, carried out the research, analyzed the data, discussed and wrote the manuscript; ML designed the experiments, carried out the research, analyzed the data, discussed and reviewed the manuscript; MB carried out the research, analyzed the data; MM analyzed the data and reviewed the manuscript; JR provided the somatostatin receptors agonists and cortagine, discussed and reviewed the manuscript; YT designed the experiments, evaluated the data, discussed and wrote the manuscript.

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agonist had no effect. The selective sst₂ antagonist, S-406-028 blocked the sst₂ agonist but not the sst₁ agonist effect. The sst₁ agonist (3 and 10 µg) prevented cortagine-induced hypersensitivity to CRD at each pressure while the sst₂ agonist at 10 µg reduced it. These data indicate that in addition to sst₂, the sst₁ agonist may provide a novel promising target to alleviate visceral hypersensitivity induced by mechanoreceptor sensitization and more prominently, stress-related visceral nociceptive sensitization.

Keywords

Colorectal distension; cortagine; somatostatin agonist; visceral hypersensitivity

1. Introduction

Somatostatin is a regulatory neuropeptide widely distributed throughout endocrine, neuronal and immune cells of the gastrointestinal tract [60] that exerts a broad spectrum of biological actions under physiological and pathophysiological conditions [8,23]. In particular, convergent clinical reports established that the stable somatostatin agonist, octreotide (also named SMS 201–995 or sandostatin) [9] administered peripherally decreased the perception of colonic or rectal distension in healthy human subjects [24,40] and normalized the heightened visceral perception in patients with irritable bowel syndrome (IBS) without affecting gut wall compliance [11,25,49]. By contrast, there is a paucity of experimental studies on the influence of somatostatin on visceral pain induced by colorectal distension (CRD). One previous report indicates that octreotide attenuated the visceromotor response (VMR) to CRD only upon intrathecal administration while having no effect upon systemic injection in rats [55]. This contrasts with clinical studies that support the inhibitory effect of peripherally administered octreotide at extrinsic afferent neurons with receptive field within the intestinal mucosa [11,24,40,49]. Furthermore the inhibitory effect of somatostatin or stable agonists injected peripherally on somatic pain in rodents is well established [39].

Somatostatin actions are mediated by the peptide high affinity to five distinct G-protein-coupled membrane receptors referred to as sst₁, sst₂, sst₃, sst₄, and sst₅ that are linked with various transduction-signaling pathways [36]. Among the broad spectrum of somatostatin effects, several responses have been identified to involve distinct receptor subtypes. For instance the inhibition of gastric acid secretion and gastrin release occurs through sst₂ [34] whereas the inhibition of insulin release involves sst₂ and sst₅ [52]. However, there is limited knowledge on the somatostatin receptor subtype(s) involved in modulating visceral pain. The previously used stable somatostatin agonist, octreotide binds with high subnanomolar affinity to sst₂ and only with moderate affinity to sst₃ and sst₅ while displaying no affinity to sst₁ and sst₄ [22] suggesting a primary involvement of sst₂ in the modulation of visceral pain perception in clinical studies. However, somatic pain studies provide experimental evidence that additional receptor subtypes may also mediate somatostatin anti-nociceptive action. For instance, a recent report indicates that subcutaneous injection of octreotide-induced attenuation of mechanical and thermal hyperalgesia in a monoarthritic mice model is no longer observed in sst₂ knockout mice [26]. By contrast, the anti-nociceptive effect of the stable pan-somatostatin agonist,

pasireotide is retained in those animals [26]. Moreover, the expression of sst₁₋₅ in the gut [28] and sst₁, sst₂, sst₄, unlike sst₃ or sst₅ in mouse dorsal root ganglia (DRG) [5] provide anatomical support that additional somatostatin receptor subtypes may also modulate visceral pain.

In the present study, we first investigated the influence of a stable somatostatin mimetic ODT8-SST that binds to sst₁₋₅ [7,20] injected intraperitoneally (ip) on visceral hypersensitivity induced by repeated isobaric noxious CRD in mice, an established protocol to test anti-hyperalgesic substances in rodents [12]. Second, we assessed whether somatostatin agonists selective for sst₁, S-406-062 [19], sst₂ S-346-011 [21], or sst₄, S-315-297 [41] mimic the effect of the pan-somatostatin agonist on repeated CRD-induced visceral hypersensitivity. Lastly, based on clinical and experimental evidence that stress is an important modulator of visceral pain [32], we also tested whether the selective sst₁ and sst₂ agonists, found to be effective in the repeated CRD model, would also influence visceral hypersensitivity induced by the activation of peripheral corticotropin releasing factor (CRF) receptor subtype 1 using the selective peptide CRF₁ agonist, cortagine [59]. We previously established that cortagine induces IBS-like manifestations including visceral hypersensitivity in rodents [31].

2. Materials and methods

2.1. Animals

Adult male C57Bl/6 mice (body weight 25–33 g, Harlan Laboratory, Indianapolis, IN, USA) were maintained group-housed (2–4/cage) under standard housing conditions with controlled illumination (12:12 h light/dark cycle, lights on at 6:00 am) and temperature (22 ± 2 °C). Animals were fed with a standard rodent diet (Prolab RMH 2500; LabDiet, PMI Nutrition, Brentwood, MO, USA) and tap water *ad libitum*. They were acclimated to the animal facility for 1 week before the study. Experimental protocols followed NIH guidelines and were approved by the IACUC Committee of the VA Greater Los Angeles Healthcare System (#11084-03), which is under the auspices of an OLAW Assurance of Compliance (A3002-01).

2.2. Peptides

The stable pan-somatostatin agonist, ODT8-SST (des-AA^{1,2,4,5,12,13}-(DTrp⁸)-SRIF, MW 1078.5, compound #1 in [20]), the sst₁ agonist, S-406-062 (des-AA^{1,4-6,10,12,13}-[DTyr², D-Agl(NMe,2naphthoyl)⁸,IAmp⁹]-SRIFThr-NH₂, MW 1238.5, compound #25 in [19]), the sst₂ agonist, S-346-011 (des-AA^{1,4-6,11-13}-[DPhe²,Aph⁷ (Cam),DTrp⁸]-Cbm-SRIF-Thr-NH₂, MW 1132.5, compound #2 in [21]), the sst₄ agonist, S-315-297 (des-AA^{1,2,4,5,12,13}-[Aph⁷]-Cbm-SRIF, MW 1137.4, compound #15 in [41]), the selective sst₂ antagonist, S-406-028, des-AA(1,4–6,11–13)-[pNO(2)-Phe(2),DCys(3),Tyr(7),DAph(Cbm)8]-SST-2Nal-NH(2), compound 4 in [14] and cortagine, a CRF₁ agonist ([Glu²¹,Ala⁴⁰][sauvagine₁₋₁₂][rat CRF₁₄₋₃₀][sauvagine₃₀₋₄₀]) [42,59] (all from The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla CA, USA) were synthesized by the solid phase approach and purity was characterized by high pressure liquid chromatography, capillary zone electrophoresis and mass spectrometry as previously described [19–21,41,42]. The

chemical structure and binding affinities of somatostatin analogs on human somatostatin receptor-transfected cells were detailed previously [53]. The peptides were stored in powder form at -80°C , weighed and dissolved immediately before administration in vehicle as specified in the experimental protocols. The volume of ip injection was 0.1 or 0.2 ml/mouse as specified.

2.3. Measure of visceral pain

2.3.1. Assessment of visceral pain response to CRD—Visceral sensitivity to CRD was assessed using the non-invasive manometric method that we have recently developed and validated for use in mice and rats [30,31,33]. Briefly, a PE50 catheter was taped 2 cm below the pressure sensor of a miniaturized pressure transducer catheter (SPR-524 Mikro-Tip catheter; Millar Instruments, Houston, TX). A custom-made balloon (1 cm wide $\times$ 2 cm long), prepared from an infinitely compliant polyethylene plastic bag was tied over the catheter at 1 cm below the pressure sensor with silk 4.0 (Henry Schein Inc., Melville, NY). At the beginning of each experiment, the “balloon-pressure sensor” was calibrated at constant pressures of 0, 20, 40 and 60 mmHg using a barostat (Distender Series II, G&J Electronics Inc, Toronto, Canada), and voltage output was converted to pressure using CED digital analog converter (Micro1401, Cambridge Electronic Design, Cambridge, UK) and Spike 2 software (CED, Ltd., Cambridge). On the day of the experiment, mice were briefly anesthetized with isoflurane (3% in O_2) and the lubricated “balloon-pressure sensor” catheter was introduced into the colorectum such that the distal end of the balloon was at 0.5 cm from the anus. The catheter was secured to the tail with tape, and each mouse was placed in an adjustable mouse restrainer (3.3 cm diameter $\times$ 9 cm length, #51325, Stoelting Co, Wood Dale, IL, USA), covered with a light tissue blanket and left to rest for 30 min, before the CRD procedure, to habituate to the conditions. Each balloon was connected to the barostat and the miniaturized pressure transducer to a preamplifier (model 600; Millar Instruments, Houston, TX). The intracolonic pressure (ICP) signal was acquired using CED Micro1401/SPIKE2 program.

2.3.2. Induction of visceral hypersensitivity using repeated noxious isobaric phasic CRD—The protocol was based on previous studies [12]. Mice were subjected to 4 sets of isobaric phasic distensions (each set: 3 CRDs at 55 mmHg, 10-s duration, and 5-min intervals). Baseline VMR was recorded during the 1st CRD set, followed by a rest period of 30 min, after which 3 consecutive CRD sets were performed without the 30 min rest interval period (Fig. 1A).

2.3.3. Induction of visceral hypersensitivity to CRD with cortagine—The CRD protocol was based on our previous studies in models of acute stress-related assessment of visceral hyperalgesia in mice [30,31]. Mice were subjected to 2 sets of graded phasic distension at 15, 30, 45, and 60 mmHg (each pressure three times, 10-s duration, 4-min inter-stimulus interval) before and after treatment as illustrated in Fig. 1B. The interval between the 1st set of graded distension (basal CRD) and the 2nd set was 75 min (Fig. 1B).

2.3.4. Data analysis—The phasic component of the intracolonic pressure (pICP) was extracted from the ICP signal recorded by applying the “DC Remove” process in Spike 2

(CED, Cambridge Electronic Design, U.K.) with a time constant of 1 s, to exclude the slower, tonic changes in ICP resulting from colonic smooth muscle activity, and by applying the “root mean square amplitude” process with a time constant of 1 s to the resulting trace. The VMR was defined as the increase in the area under the curve (AUC) of pICP during CRD over the mean value of pre- and post-distension 10 s periods and was quantified using the “modulus” process in Spike 2 as we previously described [30,31]. The pre- and post-distension periods consist of 10 s immediately preceding and following the end of each 10 s CRD. As CRD was repeated 3 times at the same pressure, the 10 s pre-during and post-distension were averaged.

2.3.5. Adjustment for inter-individual variations of the signal—To examine the pressure-response relationship and adjust for inter-individual variations of the signal, pICP amplitudes were normalized for each mouse. With the repeated constant pressure (55 mmHg) protocol, the mean values of the VMR obtained for each set of distension (set 1 to 4, 3 distensions at 55 mmHg each) were averaged. The VMR recorded during the baseline CRD (1st set of CRD) was expressed as 100% response. The VMR of the sets 2, 3 and 4 were then normalized to the baseline response and expressed in %. For the graded phasic CRD protocol, as each pressure was repeated 3 times, the pre-post CRD and during CRD values were averaged for each pressure then the ICP amplitudes at each pressure were normalized to the highest pressure (60 mmHg) in the 1st set of CRD. This value served as 100% response in the baseline period of data collection before treatment and represented the baseline VMR as in previous studies [30].

2.4. Experimental protocols

Experiments started between 7:00–8:00 am each day to avoid variations due to the circadian rhythm.

2.4.1. Effect of ODT8-SST and selective somatostatin receptor subtype agonists injected ip on visceral hypersensitivity induced by repeated noxious isobaric CRD—Naïve male mice were subjected to the 1st set of noxious CRD (55 mmHg, 10-s duration each, 5-min inter-stimulus interval, 3 times) during which the baseline VMR was measured. Then, after a 30-min rest period, one group was exposed to 3 consecutive sets of noxious CRD with no injection. The other groups, immediately after recording the baseline VMR, were injected ip with vehicle (0.1 ml saline, pH~7), ODT8-SST, sst₁ (S-406-062), sst₂ (S-346-011), sst₄ (S-315-297) receptor agonist – each peptide at 3 and 10 µg/mouse (1 µg ~ 0.7 nmol each corrected for 20% ions and water content) and 30 min later, the 3 consecutive sets of noxious CRD were performed (Fig. 1A). In a separate set of experiments, the sst₂ antagonist, S-406-028 (20 µg/mouse) or vehicle (saline, 0.1 ml, ip) was injected 5 min before the sst₁ (S-406-062) or the sst₂ (S-346-011) receptor agonist. The peptide doses were selected based on our preliminary studies (data not shown) and previous studies [54].

2.4.2. Effect of selective somatostatin sst₁ or sst₂ receptor agonist injected ip on visceral hypersensitivity induced by the CRF₁ agonist, cortagine—Naïve mice were subjected to the 1st set of graded phasic distensions (15, 30, 45, and 60 mmHg, 3

times at each pressure, 10-s duration, 4-min inter-stimulus interval). The VMR to the 1st CRD served as a baseline. Cortagine (30 $\mu\text{g kg}^{-1}$, $\sim 1 \mu\text{g}/\text{mouse}$) dissolved in sterile double distilled (dd)H₂O (0.2 ml) was injected ip 15 min before starting the 2nd set of graded phasic CRD performed 75 min after the 1st CRD set (Fig. 1B). This regimen of administration has been previously used in the mouse model of ip cortagine-induced visceral hypersensitivity [31]. The sst₁ receptor agonist, S-406-062 (3 or 10 $\mu\text{g}/\text{mouse}$) or vehicle (0.1 ml saline) was administered directly before cortagine due to its shorter action time observed in the first part of the experiment described above. The selective sst₂ agonist, S-346-011 (10 $\mu\text{g}/\text{mouse}$) or vehicle (0.1 ml saline) was injected 15 min before cortagine (Fig. 1B).

2.5. Statistical analysis

Statistical analyses were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). For the repeated noxious CRD protocol, repeated-measure one-way ANOVA followed by Dunnett's *post hoc* test was used when comparing sets of repeated noxious CRD within one group of mice, or one-way ANOVA followed by Dunnett's *post hoc* test when comparing between groups of mice for the same pressure of CRD. For the cortagine-induced visceral hypersensitivity protocol, repeated one-way ANOVA followed by Newman Keuls *post hoc* test was used when performing comparisons within groups and 2-way ANOVA followed by Bonferroni *post hoc* test when performing between groups comparison. Results are expressed as means $\pm$ SEM, and *p* values < 0.05 were considered statistically significant.

3. Results

3.1. The stable pan-somatostatin agonist, ODT8-SST prevents repeated noxious isobaric CRD- induced visceral hypersensitivity in mice

In non-injected mice (n=9), a significant increase in VMR values was observed at the 2nd, 3rd, and 4th CRD sets reaching values of $159 \pm 9.5\%$, $152.7 \pm 14.6\%$ and $143.2 \pm 10.5\%$, respectively ($p < 0.05$) compared to the 1st set of CRD taken as 100% baseline (Fig. 2). There was a trend toward a lowering of VMR in vehicle-injected group (n=12) compared to non-injected group, however the rise was significant at each subsequent set of CRD compared to the 1st CRD and values were not significantly different from those in vehicle-treated group (Fig. 2).

The ip injection of ODT8-SST (10 $\mu\text{g}/\text{mouse}$, n=10) completely prevented the visceral hypersensitivity induced by repeated noxious CRD compared to ip saline injection (Fig. 3). The VMR values at the 2nd, 3rd, and 4th set were $91.7 \pm 3.4\%$, $79.6 \pm 4.0\%$ and $90.1 \pm 6.9\%$, respectively in ODT8-SST-treated mice and significantly different compared to ip saline-injected mice ($141.3 \pm 8.5\%$, $p < 0.01$, $125.5 \pm 11.5\%$, $p < 0.05$, and $137.6 \pm 9.8\%$, $p < 0.01$, respectively) (Fig. 3). VMR values in ODT8-SST injected mice were not significantly different from their baseline at the 2nd and 4th set of CRD but significantly lower at the 3rd set (Fig. 3). ODT8-SST injected ip at 3 $\mu\text{g}/\text{mouse}$ had VMR values not significantly different from those of ip saline-treated group, although statistical significance was only reached for the 3rd set of CRD compared to baseline ($p < 0.05$) (Fig. 3).

3.2. Selective agonists to sst₁, S-406-062, or sst₂, S-346-011, but not to sst₄, S-315-297, prevent repeated noxious isobaric CRD-induced visceral hypersensitivity in mice

The ip saline group (n=12) shows a significant increase in VMR compared to baseline at each set of (Fig. 4A). Mice (n=8) injected immediately after the first baseline CRD with the sst₁ agonist, S-406-062 (3 µg/mouse), did not show a significant increase in VMR compared to their baseline (114.5±9.8%, 103.8±9.6%, and 108.1±9.9% at the 2nd, 3rd and 4th set of CRD respectively), however values were not significantly different from the saline group (Fig. 4A). At 10 µg/mouse, the sst₁ agonist completely prevented the visceral hypersensitivity induced by the 2nd set of CRD compared to saline-treated animals (VMR: 93.6±4.8% vs 159±9.5%, p<0.001) but no longer during the 3rd and 4th sets of CRD (n=9) (Fig. 4A). Pre-treatment with the sst₂ antagonist did not significantly modify the inhibitory influence of the sst₁ agonist on the visceral hypersensitivity, which was still observed during the 2nd set of CRD and became statistically significant for the 4th set of CRD (Fig. 4B).

The sst₂ agonist, S-346-011 injected ip under the same conditions completely prevented the noxious repeated CRD-induced visceral hypersensitivity observed during 2nd, 3rd and 4th sets of CRD at both 3 or 10 µg/mouse as shown by values similar to baseline levels (n=7–10; Fig. 5A). Pre-treatment with the sst₂ antagonist reversed the suppressive effect of the sst₂ agonist at all pressures of CRD (n=8; Fig. 5B). By contrast, the selective sst₄ agonist, S-315-297 (3 and 10 µg/mouse) injected ip under otherwise similar conditions, did not significantly influence the VMR observed during the 3 consecutive sets of CRD (n=11–14; p>0.05) (Table 1).

3.3. Selective sst₁, S-406-062 or sst₂, S-346-011 agonists prevents visceral hypersensitivity induced by ip cortagine in mice

Saline injections *per se* had an analgesic effect on the VMR to CRD for the pressures of 30 and 60 mmHg when compared to baseline (66.4±5.0% vs 76.9±4.6% and 66.1±7.9% vs 100±0.0%; p<0.01 and p<0.001, respectively). Cortagine at 30 µg kg⁻¹ induced a significant increase in the VMR to the 2nd set of phasic CRD at graded pressures of 45 and 60 mmHg compared to saline-pretreated mice (110.1±12.4% vs 66.4±5.0% and 132.9 ± 14.3% vs 66.1±7.9%; p<0.01 and p<0.05, respectively, n=8–11/group) (Fig. 6).

The sst₁ agonist S-406-062 injected immediately before cortagine at 3 µg/mouse (n=7) blocked cortagine-induced enhanced VMR at 45 and 60 mmHg (47.9±8.0% and 77.3±15.8%, p<0.001 each, respectively vs ip saline + cortagine), bringing values back to baseline level. At 10 µg/mouse (n=8), the sst₁ agonist blocked the visceral hypersensitivity induced by cortagine at 45 and 60 mmHg and induced visceral analgesia at 30, 45 and 60 mmHg when compared to baseline (25.9±6.6% vs 51.9±11.1%, 45.1±9.1% vs 92.0±6.8% and 49.7±9.9% vs 100.0±0.0%; p<0.05, p<0.001 and p<0.001, respectively) (Fig. 6).

In this model, the sst₂ agonist reduced ip cortagine-induced increased VMR at 45 and 60 mmHg: 72.2±8.1 vs 110.1±12.4% (p<0.05), and 69.4±7.9 vs 132.9 ± 14.3% (p<0.01), respectively and induced visceral analgesia at 60 mmHg when compared to baseline (p<0.01) (Fig. 6).

4. Discussion

In the present study, we used the non-invasive manometric assessment of VMR to CRD in naive mice [1,30] which does not involve prior surgery and single housing unlike other commonly used methods to measure visceral pain [16]. We showed that one set of isobaric phasic CRD at a noxious range (55 mmHg, 10-s duration, 3 times at 5-min intervals,) reliably induced visceral hypersensitivity as shown by the 59% increase above the basal VMR at each of the 3 subsequent sets of similar CRD performed 30 min later. The hyperalgesic response to noxious CRD was also reproducibly evoked in ip saline-injected mice allowing us to test the influence of somatostatin agonists. The stable pan-somatostatin agonist, ODT8-SST binds to ss_{1-5} with the same nanomolar affinity as somatostatin [20]. The ODT8-SST, at the ip dose of 10 μ g/mouse, completely prevented the visceral hypersensitivity occurring in the 3 sets of isobaric phasic CRD at 55 mmHg. Moreover, we found that the selective ss_2 agonist, S-346-011, injected under similar conditions, also inhibited repeated noxious CRD-induced visceral hypersensitivity, in a ss_2 -selective manner, as shown by the blockade by the ss_2 antagonist, S-406-028 [14,54]. Notably, the ss_2 agonist appears to be more potent than ODT8-SST. This is supported by the full preventive action induced by the ss_2 agonist at 3 μ g/mouse (2.65 nmol) while ODT8-SST had no effect when injected at a similar molarity/0.1 ml (2.78 nmol = 3 μ g). ODT8-SST injected peripherally displays a long duration of action in experimental animals and human studies [7]. Therefore, it is unlikely that the lack of ODT8-SST effect when injected ip at 3 μ g/mouse relates to its pharmacokinetics. It may rather reflect the 5.4 times lower receptor binding affinity at the ss_2 displays by ODT8-SST compared to the selective ss_2 agonist (IC_{50} 41 vs 7.5 nmol L^{-1}) [20,21]. Other studies indicate that octreotide injected ip prevented jejunal hypersensitivity to intrajejunal distension at nociceptive range in rats infected with *Cryptosporidium* [3]. Collectively, the present and previous reports, except one [55], indicate that stable pan-somatostatin agonist, and preferential or selective ss_2 agonist administered peripherally exert an antihyperalgesic effect in rodent model of visceral hypersensitivity induced mechanically by CRD (present study) or jejunal distension [3,10].

In addition to the ss_2 agonist, we demonstrated that the selective ss_1 agonist, S-406-062 injected ip at 10 μ g/mouse inhibited the hypersensitivity induced by repeated noxious CRD in mice only during the 2nd set of CRD performed at 30–45 min after injection. At 3 μ g/mouse, the peptide had a slight reducing effect over all the sets of distension as indicated by the VMR being no longer significantly different from baseline; however, values did not reach statistical significance compared to the ip saline-treated group. The ss_1 mediated response was further demonstrated by pretreatment with the ss_2 antagonist, S-406-028, that did not influence ss_1 agonist action when administered under similar conditions reversing the inhibition of visceral hypersensitivity induced by ss_2 agonist. This provides the first report indicating that peripheral activation of ss_1 exerts a visceral antihyperalgesic effect. By contrast, the selective ss_4 agonist administered peripherally under similar conditions than the ss_1 agonist did not influence the VMR to repeated noxious CRD. In the somatic field, there is growing evidence that the $ss_{1,4}$ somatostatin agonist, TT-232 [17,51] and the ss_4 agonist, J-2156 [18], exert peripheral anti-inflammatory and analgesic effects in various acute and chronic inflammatory rodent models including chronic arthritis, or mono- and

polyneuropathy conditions [45,56,57,61]. Further studies using different models of visceral pain, such as chemically-induced colonic inflammation or post-infectious hyperalgesia [32] will be required to elucidate the exact role of the sst₄ in visceral nociception linked with past or present gut inflammatory components.

Next, we evaluated the effects of selective sst₁ and sst₂ agonists found to be effective in the hypersensitivity to repeated CRD in a stress-related model of enhanced VMR induced by peripheral CRF₁ activation [31]. Cortagine, a CRF₁ agonist, injected ip in mice reproduces the main manifestations of diarrhea-predominant IBS including watery diarrhea, increased colonic motility and permeability as well as visceral hyperalgesia to phasic ascending CRD as we observed in the present study [31]. We found that the sst₂ and more prominently the sst₁ agonists at 10 µg/mouse significantly prevented the visceral hypersensitivity observed at 45 and 60 mmHg in ip cortagine mice. In addition, at a lower dose (3 µg/mouse) the sst₁ agonist was still effective in preventing cortagine-induced visceral hypersensitivity to CRD at 45 and 60 mmHg. This contrasts with the repeated isobaric CRD model in which during the same time interval the sst₁ at 10 µg/mouse blocked the hypersensitivity only for the first set of CRD and at 3 µg dose, the VMR was not significantly different compared to ip saline. These findings point toward a differential selectivity of sst₁ and sst₂ to inhibit visceral hyperalgesia in different models. The sst₁ agonist may interfere preferentially with stress-related mechanisms of visceral hypersensitivity while sst₂ is more effective on sensitization induced by mechanosensitive nociceptive afferents. Interestingly, at the highest dose, the sst₁ agonist was able to boost visceral analgesic mechanisms as shown by the strong decrease in VMR values below baseline levels at all pressures of CRD.

The underlying site(s) of action and cellular mechanisms through which sst₁ and sst₂ agonists injected ip exert their inhibitory effects are likely to take place at peripheral site(s). Pharmacokinetic studies in mice indicate that systemically injected oligosomatostatin agonists hardly pass the blood-brain barrier [4]. Moreover, *in vitro* experiments in isolated jejunal segments harvested from wild type mice showed that octreotide reduced afferent nerves activity evoked by intrajejunal distension while this was no longer observed in jejunal preparation from sst₂ knockout mice [43]. Likewise, in rats, sst₂ activation by octreotide or the sst₂ agonist, BIM 23027, decreased selectively spinally-projecting mechanosensitive fibers activated by intestinal distension [10]. Furthermore, neuroanatomical studies established that sst₁ and sst_{2a} are expressed at the gene and protein levels on medium-sized neurons of mice, rat and human dorsal root ganglia [5,26,48,50]. The sst₁ is also expressed on intestinal mucosal nerve fibers [62] and the expression of sst₁ throughout the gastrointestinal tract is 10-fold higher than that of sst₂ [46,47]. These neuroanatomical and functional studies support a possible direct site of action of sst₁ and sst₂ agonists on nerve terminals of mechanosensitive visceral afferents as established in the somatic pain field [13]. In addition, somatostatin inhibits basal or stimulated secretion of intestinal mucosal mast cells [44,58] which are known to express somatostatin receptors including sst₁ [44,62]. The suppression of neuroexcitatory substances release from mast cells might therefore be a potential additional target of sst₁ and sst₂ agonists. This is based on the involvement of mast cells in the development of visceral hypersensitivity [6,38], particularly in the context of stress-related models [29,63]. Whether the more prominent

antihyperalgesic effect of sst₁ agonist in the cortagine model and sst₂ in the repeated CRD model reflect preferential sites of action on mast cells vs terminal afferents related based on their preponderant receptor distributions at these different sites warrant further investigations.

Visceral hypersensitivity is a key pathophysiological factor in IBS and the modulation of visceral perception seems to be one of the most effective therapeutic approaches in this disorder [2,35]. Somatostatin itself is not suitable for drug development due to its short duration of action and a broad-spectrum of side-effects, thus there is a need for stable and receptor-selective agonists. Consistent reports showed the inhibitory effect of octreotide on visceral perception in healthy volunteers and IBS patients [11,24,25,40], although in one clinical study, the long-term treatment of octreotide had no visceral analgesic effect (the thresholds of first sensation increased however) and failed to improve IBS symptoms [27]. Some adverse events associated with octreotide treatment may additionally limit its use in clinical practice (e.g., inhibition of gallbladder emptying and endocrine effects) [37]. The identification of sst₁, in addition to sst₂, to prevent visceral hypersensitivity more prominently in stress-related mechanisms may be promising as a novel pharmacological target and treatment strategy in stress-sensitive IBS symptoms [15]. This will also avoid the widespread inhibitory effects of octreotide or sst₂ agonist on endocrine functions.

In summary, the present results have shed some light on somatostatin receptor subtype able to influence the hyperalgesia to CRD in two mice models of visceral hypersensitivity. We showed that the stable pan-somatostatin agonist ODT8-SST and new selective sst₁ or sst₂ agonist injected ip in nmol range prevented visceral hyperalgesia induced by repeated noxious CRD and evoked antihyperalgesic effects in a model of visceral hypersensitivity induced by activation of peripheral efficiency of sst₁ agonist preferentially in the stress-related CRF₁. The hypersensitivity and sst₂ agonist in mechanical sensitization model underscore the importance of underlying mechanisms of visceral hypersensitivity in the selection of the sst receptor subtype to curtail visceral hypersensitization.

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Glossary

CRD	colorectal distension
CRF	corticotropin-releasing factor
DRG	dorsal root ganglia
IBS	irritable bowel syndrome
ICP	intracolonic pressure
ip	intraperitoneal

sst₁₋₅ somatostatin receptor subtypes 1–5
VMR visceromotor response

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Highlights

- ODT8-SST, a pan-somatostatin agonist, i.p. blocks visceral hyperalgesia in mice.
- The selective sst1 or sst2 agonist prevent hyperalgesia in two visceral pain models.
- The sst4 agonist has no effect on colorectal distension-induced hypersensitivity.
- The sst1 or sst2 agonist may be a promising treatment strategy for visceral hypersensitivity.

Fig. 1.

Experimental protocol design: (A) repeated isobaric noxious colorectal distensions (text in bold represents experiments with sst₂ antagonist pretreatment), and (B) intraperitoneal cortagine-induced visceral hypersensitivity.

Fig. 2.

Visceral hyperalgesia induced in rats without or with an ip injection of saline. For experimental design see Fig. 1A. The VMR value to the baseline CRD was expressed as 100% of response in each group. Data are means $\pm$ SEM of %VMR compared to the baseline response; $n = 9-12$ as indicated in the parenthesis; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs baseline (1st CRD).

Fig. 3. Antihyperalgesic effect of the stable pan-sst₁₋₅ agonist, ODT8-SST injected intraperitoneally. For experimental design see Fig. 1A. Data are means $\pm$ SEM of % VMR compared to the baseline response taken as 100%; n = 10–12 as indicated in the parenthesis; *p<0.05, **p<0.01, ***p<0.001 vs baseline (1st CRD); +p<0.05, ++p<0.01 vs saline in respective set.

Fig. 4.

Antihyperalgesic effect of the selective sst₁ receptor agonist, S-406-062. (A) The selective sst₁ receptor agonist injected ip prevents repeated noxious CRD-induced visceral hypersensitivity in mice in a time-dependent manner. (B) Pre-treatment with the selective sst₂ antagonist, S-406-028 does not affect the inhibition of visceral hypersensitivity induced by the sst₁ agonist. For experimental design see Fig. 1A. Data are means $\pm$ SEM of %VMR compared to the baseline response; n = 5–12 as indicated in the parenthesis; *p<0.05, **p<0.01, ***p<0.001 vs baseline (1st CRD); +p<0.05, ++p<0.01 and +++p<0.001 vs saline in respective set.

Fig. 5.

Antihyperalgesic effect of the selective sst₂ receptor agonist, S-346-011. (A) The selective sst₂ receptor agonist injected ip suppresses the visceral hypersensitivity induced by repeated noxious CRD in mice. (B) Pre-treatment with the selective sst₂ antagonist S-406-028 prevents the inhibition of visceral hypersensitivity by the sst₂ agonist. For experimental design see Fig. 1A. Data are means $\pm$ SEM of %VMR compared to the baseline response; n = 7–12 as indicated in the parenthesis; *p<0.05, **p<0.01, ***p<0.001 vs baseline (1st CRD); +p<0.05, ++p<0.01, +++p<0.001 vs saline in respective set.

Fig. 6.

The selective *sst*₁ and *sst*₂ receptor agonists prevent visceral hypersensitivity induced by receptors in mice. For experimental design see Fig. 1B. Data are activation of peripheral CRF₁ means $\pm$ SEM of VMR expressed as percentage of 60 mmHg response to the 1st CRD; **p*<0.05, ***p*<0.01, ****p*<0.001 vs same CRD pressure for respective baseline CRD; ++ *p*<0.05, +++ *p*<0.01 vs saline + cortagine for same CRD pressure.

Table 1

The selective sst₄ receptor agonist, injected i.p. did not influence visceral hyperalgesia induced by repeated noxious CRD in mice.

	Saline n=12	sst ₄ ago (3 µg/mouse) n=14	sst ₄ ago (10 µg/mouse) n=11
1 st CRD set (baseline)	100 ± 0	100 ± 0	100 ± 0
2 nd CRD set	141.3 ± 8.5 [‡]	120.2 ± 12.6 [†]	124.1 ± 10.9 [†]
3 rd CRD set	125.5 ± 11.5 [†]	123.6 ± 14.95 [†]	124.7 ± 12.2 [†]
4 th CRD set	137.6 ± 9.8 [‡]	126.5 ± 17.2 [†]	125.5 ± 7.2 [†]

Data are means ± SEM of % visceromotor response compared to the 1st CRD;

[†] p < 0.05,

[‡] p < 0.01 vs baseline.