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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 (sst_{1-5}). Octreotide, a stable $sst_{2 \gg 3 \ 5}$ 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 sst_{1-5} 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 sst_2 agonist, S-346-011 (3 and 10 μg) prevented mechanically-induced visceral hypersensitivity in the 3 sets of CRD, the sst_1 agonist (10 μg) blocked only the 2nd set and showed a trend at 3 μg while the sst_4

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agonist had no effect. The selective sst_2 antagonist, S-406-028 blocked the sst_2 agonist but not the sst_1 agonist effect. The sst_1 agonist (3 and 10 μg) prevented cortagine-induced hypersensitivity to CRD at each pressure while the sst_2 agonist at 10 μg reduced it. These data indicate that in addition to sst_2, the sst_1 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 sst_1, sst_2, sst_3, sst_4, and sst_5 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 sst_2 [34] whereas the inhibition of insulin release involves sst_2 and sst_5 [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 sst_2 and only with moderate affinity to sst_3 and sst_5 while displaying no affinity to sst_1 and sst_4 [22] suggesting a primary involvement of sst_2 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 sst_2 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 sst_{1-5} in the gut [28] and sst_1, sst_2, sst_4, unlike sst_3 or sst_5 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 sst_{1-5} [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 sst_1, S-406-062 [19], sst_2 S-346-011[21], or sst_4, 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 sst_1 and sst_2 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 CRF_1 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}-(DTrp^8)-SRIF, MW 1078.5, compound #1 in [20]), the sst_1 agonist, S-406-062 (des-AA$^{1,4-6,10,12,13}$-[DTyr^2,D-Agl(NMe,2naphtoyl)^8,IAmp^9]-SRIFThr-NH$_2$, MW 1238.5, compound #25 in [19]), the sst_2 agonist, S-346-011 (des-AA$^{1,4-6,11-13}$-[DPhe^2,Aph^7 (Cam),DTrp^8]-Cbm-SRIF-Thr-NH$_2$, MW 1132.5, compound #2 in [21]), the sst_4 agonist, S-315-297 (des-AA^{1,2,4,5,12,13}-[Aph^7]-Cbm-SRIF, MW 1137.4, compound #15 in [41]), the selective sst_2 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 CRF_1 agonist ([Glu^{21}, Ala^{40}][sauvagine$_{1-12}$][rat CRF$_{14-30}$]_[sauvagine$_{30-40}$]) [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×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×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 hyperalgesis 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, sst_1 (S-406-062), sst_2 (S-346-011), sst_4 (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 sst_2 antagonist, S-406-028 (20 μg/mouse) or vehicle (saline, 0.1 ml, ip) was injected 5 min before the sst_1 (S-406-062) or the sst_2 (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 sst_1 or sst_2 receptor agonist injected ip on visceral hypersensitivity induced by the CRF_1 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 μg kg^{-1}, ~ 1 μg/mouse) dissolved in sterile double distilled (dd)H$_2$O (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 sst$_1$ receptor agonist, S-406-062 (3 or 10 μg/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 sst$_2$ agonist, S-346-011 (10 μg/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 ± 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±9.5%, 152.7±14.6% and 143.2±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 μg/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±3.4%, 79.6±4.0% and 90.1±6.9%, respectively in ODT8-SST-treated mice and significantly different compared to ip saline-injected mice (141.3±8.5%, p<0.01, 125.5±11.5%, p<0.05, and 137.6±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 μg/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 sst_1, S-406-062, or sst_2, S-346-011, but not to sst_4, 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 sst_1 agonist, S-406-062 (3 μg/mouse), did not show a significant increase in VMR compared to their baseline ($114.5\pm9.8\%$, $103.8\pm9.6\%$, and $108.1\pm9.9\%$ 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 sst_1 agonist completely prevented the visceral hypersensitivity induced by the 2nd set of CRD compared to saline-treated animals (VMR: $93.6\pm4.8\%$ *vs* $159\pm9.5\%$, $p<0.001$) but no longer during the 3rd and 4th sets of CRD (n=9) (Fig. 4A). Pre-treatment with the sst_2 antagonist did not significantly modify the inhibitory influence of the sst_1 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 sst_2 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 sst_2 antagonist reversed the suppressive effect of the sst_2 agonist at all pressures of CRD (n=8; Fig. 5B). By contrast, the selective sst_4 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 sst_1, S-406-062 or sst_2, 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 ($66.4\pm5.0\%$ vs $76.9\pm4.6\%$ and $66.1\pm7.9\%$ vs $100\pm0.0\%$; $p<0.01$ 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 ($110.1\pm12.4\%$ vs $66.4\pm5.0\%$ and $132.9 \pm 14.3\%$ vs $66.1\pm7.9\%$; $p<0.01$ and $p<0.05$, respectively, n=8–11/group) (Fig. 6).

The sst_1 agonist S-406-062 injected immediately before cortagine at 3 μg/mouse (n=7) blocked cortagine-induced enhanced VMR at 45 and 60 mmHg ($47.9\pm8.0\%$ and $77.3\pm15.8\%$, $p<0.001$ each, respectively vs ip saline + cortagine), bringing values back to baseline level. At 10 μg/mouse (n=8), the sst_1 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 ($25.9\pm6.6\%$ vs $51.9\pm11.1\%$, $45.1\pm9.1\%$ vs $92.0\pm6.8\%$ and $49.7\pm9.9\%$ vs $100.0\pm0.0\%$; $p<0.05$, $p<0.001$ and $p<0.001$, respectively) (Fig. 6).

In this model, the sst_2 agonist reduced ip cortagine-induced increased VMR at 45 and 60 mmHg: 72.2 ± 8.1 vs $110.1\pm12.4\%$ ($p<0.05$), and 69.4 ± 7.9 vs $132.9 \pm 14.3\%$ ($p<0.01$), 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 sst_{1-5} with the same nanomolar affinity as somatostatin [20]. The ODT8-SST, at the ip dose of 10 μg/mouse, completely prevented the visceral hypersensitivity occurring in the 3 sets of isobaric phasic CRD at 55 mmHg. Moreover, we found that the selective sst_2 agonist, S-346-011, injected under similar conditions, also inhibited repeated noxious CRD-induced visceral hypersensitivity, in a sst_2-selective manner, as shown by the blockade by the sst_2 antagonist, S-406-028 [14,54]. Notably, the sst_2 agonist appears to be more potent than ODT8-SST. This is supported by the full preventive action induced by the sst_2 agonist at 3 μg/mouse (2.65 nmol) while ODT8-SST had no effect when injected at a similar molarity/0.1 ml (2.78 nmol = 3 μg). 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 μg/mouse relates to its pharmacokinetics. It may rather reflect the 5.4 times lower receptor binding affinity at the sst_2 displays by ODT8-SST compared to the selective sst_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 sst_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 sst_2 agonist, we demonstrated that the selective sst_1 agonist, S-406-062 injected ip at 10 μg/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 3μg/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 sst_1 mediated response was further demonstrated by pretreatment with the sst_2 antagonist, S-406-028, that did not influence sst_1 agonist action when administered under similar conditions reversing the inhibition of visceral hypersensitivity induced by sst_2 agonist. This provides the first report indicating that peripheral activation of sst_1 exerts a visceral antihyperalgesic effect. By contrast, the selective sst_4 agonist administered peripherally under similar conditions than the sst_1 agonist did not influence the VMR to repeated noxious CRD. In the somatic field, there is growing evidence that the $sst_{1,4}$ somatostatin agonist, TT-232 [17,51] and the sst_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 sst_4 in visceral nociception linked with past or present gut inflammatory components.

Next, we evaluated the effects of selective sst_1 and sst_2 agonists found to be effective in the hypersensitivity to repeated CRD in a stress-related model of enhanced VMR induced by peripheral CRF_1 activation [31]. Cortagine, a CRF_1 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 sst_2 and more prominently the sst_1 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 sst_1 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 sst_1 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 sst_1 and sst_2 to inhibit visceral hyperalgesia in different models. The sst_1 agonist may interfere preferentially with stress-related mechanisms of visceral hypersensitivity while sst_2 is more effective on sensitization induced by mechanosensitive nociceptive afferents. Interestingly, at the highest dose, the sst_1 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 sst_1 and sst_2 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 sst_2 knockout mice [43]. Likewise, in rats, sst_2 activation by octreotide or the sst_2 agonist, BIM 23027, decreased selectively spinally-projecting mechanosensitive fibers activated by intestinal distension [10]. Furthermore, neuroanatomical studies established that sst_1 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 sst_1 is also expressed on intestinal mucosal nerve fibers [62] and the expression of sst_1 throughout the gastrointestinal tract is 10-fold higher than that of sst_2 [46,47]. These neuroanatomical and functional studies support a possible direct site of action of sst_1 and sst_2 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 sst_1 [44,62]. The suppression of neuroexcitatory substances release from mast cells might therefore be a potential additional target of sst_1 and sst_2 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 sst_1 agonist in the cortagine model and sst_2 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 sst_1, in addition to sst_2, 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 sst_2 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 sst_1 or sst_2 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 sst_1 agonist preferentially in the stress-related CRF_1. The hypersensitivity and sst_2 agonist in mechanical sensitization model underscore the importance of underlying mechanisms of visceral hypersensivity in the selection of the sst receptor subtype to curtail visceral hypersensitization.

Acknowledgments

Glossary

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

sst**1–5** somatostatin receptor subtypes 1–5

VMR visceromotor response

References

1. Arvidsson S, Larsson M, Larsson H, Lindstrom E, Martinez V. Assessment of visceral pain-related pseudo-affective responses to colorectal distension in mice by intracolonic manometric recordings. J Pain. 2006; 7:108–18. [PubMed: 16459276]

2. Azpiroz F, Bouin M, Camilleri M, Mayer EA, Poitras P, Serra J, et al. Mechanisms of hypersensitivity in IBS and functional disorders. Neurogastroenterol Motil. 2007; 19:62–88. [PubMed: 17280586]

3. Bai J, Khaldi S, Gargala G, Baishanbo A, Francois A, Ballet JJ, et al. Effects of octreotide on jejunal hypersensitivity triggered by Cryptosporidium parvum intestinal infection in an immunocompetent suckling rat model. Neurogastroenterol Motil. 2011; 23:1043–50. e499. [PubMed: 21320238]

4. Banks WA, Schally AV, Barrera CM, Fasold MB, Durham DA, Csernus VJ, et al. Permeability of the murine blood-brain barrier to some octapeptide analogs of somatostatin. Proc Natl Acad Sci U S A. 1990; 87:6762–66. [PubMed: 1975697]

5. Bar KJ, Schurigt U, Scholze A, Segond VB, Stopfel N, Brauer R, et al. The expression and localization of somatostatin receptors in dorsal root ganglion neurons of normal and monoarthritic rats. Neuroscience. 2004; 127:197–206. [PubMed: 15219682]

6. Barbara G, Wang B, Stanghellini V, De Giorgio R, Cremon C, Di Nardo G, et al. Mast cell-dependent excitation of visceral-nociceptive sensory neurons in irritable bowel syndrome. Gastroenterology. 2007; 132:26–37. [PubMed: 17241857]

7. Barnes AJ, Long RG, Adrian TE, Vale W, Brown MR, Rivier JE, et al. Effect of a long-acting octapeptide analogue of somatostatin on growth hormone and pancreatic and gastrointestinal hormones in man. Clin Sci (Lond). 1981; 61:653–6. [PubMed: 6116553]

8. Barnett P. Somatostatin and somatostatin receptor physiology. Endocrine. 2003; 20:255–64. [PubMed: 12721505]

9. Bauer W, Briner U, Doepfner W, Haller R, Huguenin R, Marbach P, et al. SMS 201–995: a very potent and selective octapeptide analogue of somatostatin with prolonged action. Life Sci. 1982; 31:1133–40. [PubMed: 6128648]

10. Booth CE, Kirkup AJ, Hicks GA, Humphrey PP, Grundy D. Somatostatin sst(2) receptor-mediated inhibition of mesenteric afferent nerves of the jejunum in the anesthetized rat. Gastroenterology. 2001; 121:358–69. [PubMed: 11487545]

11. Bradette M, Delvaux M, Staumont G, Fioramonti J, Bueno L, Frexinos J. Octreotide increases thresholds of colonic visceral perception in IBS patients without modifying muscle tone. Dig Dis Sci. 1994; 39:1171–8. [PubMed: 8200249]

12. Brusberg M, Arvidsson S, Kang D, Larsson H, Lindstrom E, Martinez V. CB1 receptors mediate the analgesic effects of cannabinoids on colorectal distension-induced visceral pain in rodents. J Neurosci. 2009; 29:1554–64. [PubMed: 19193902]

13. Carlton SM, Du J, Davidson E, Zhou S, Coggeshall RE. Somatostatin receptors on peripheral primary afferent terminals: inhibition of sensitized nociceptors. Pain. 2001; 90:233–44. [PubMed: 11207395]

14. Cescato R, Erchegyi J, Waser B, Piccand V, Maecke HR, Rivier JE, et al. Design and in vitro characterization of highly sst2-selective somatostatin antagonists suitable for radiotargeting. J Med Chem. 2008; 51:4030–7. [PubMed: 18543899]

15. Chang L. The role of stress on physiologic responses and clinical symptoms in irritable bowel syndrome. Gastroenterology. 2011; 140:761–5. [PubMed: 21256129]

16. Christianson JA, Gebhart GF. Assessment of colon sensitivity by luminal distension in mice. Nat Protoc. 2007; 2:2624–31. [PubMed: 17948005]

17. Elekes K, Helyes Z, Kereskai L, Sandor K, Pinter E, Pozsgai G, et al. Inhibitory effects of synthetic somatostatin receptor subtype 4 agonists on acute and chronic airway inflammation and hyperreactivity in the mouse. Eur J Pharmacol. 2008; 578:313–22. [PubMed: 17961545]

18. Engstrom M, Tomperi J, El Darwish K, Ahman M, Savola JM, Wurster S. Superagonism at the human somatostatin receptor subtype 4. J Pharmacol Exp Ther. 2005; 312:332–8. [PubMed: 15333679]

19. Erchegyi J, Cescato R, Grace CR, Waser B, Piccand V, Hoyer D, et al. Novel, potent, and radio-iodinatable somatostatin receptor 1 (sst1) selective analogues. J Med Chem. 2009; 52:2733–46. [PubMed: 19351180]

20. Erchegyi J, Grace CR, Samant M, Cescato R, Piccand V, Riek R, et al. Ring size of somatostatin analogues (ODT-8) modulates receptor selectivity and binding affinity. J Med Chem. 2008; 51:2668–75. [PubMed: 18410084]

21. Grace CR, Erchegyi J, Koerber SC, Reubi JC, Rivier J, Riek R. Novel sst2-selective somatostatin agonists. Three-dimensional consensus structure by NMR. J Med Chem. 2006; 49:4487–96. [PubMed: 16854054]

22. Grace CR, Erchegyi J, Samant M, Cescato R, Piccand V, Riek R, et al. Ring size in octreotide amide modulates differently agonist versus antagonist binding affinity and selectivity. J Med Chem. 2008; 51:2676–81. [PubMed: 18410083]

23. Guillermet-Guibert J, Lahlou H, Cordelier P, Bousquet C, Pyronnet S, Susini C. Physiology of somatostatin receptors. J Endocrinol Invest. 2005; 28:5–9. [PubMed: 16625838]

24. Hasler WL, Soudah HC, Owyang C. A somatostatin analogue inhibits afferent pathways mediating perception of rectal distention. Gastroenterology. 1993; 104:1390–7. [PubMed: 8097733]

25. Hasler WL, Soudah HC, Owyang C. Somatostatin analog inhibits afferent response to rectal distention in diarrhea-predominant irritable bowel patients. J Pharmacol Exp Ther. 1994; 268:1206–11. [PubMed: 8138933]

26. Imhof AK, Gluck L, Gajda M, Lupp A, Brauer R, Schaible HG, et al. Differential antiinflammatory and antinociceptive effects of the somatostatin analogs octreotide and pasireotide in a mouse model of immune-mediated arthritis. Arthritis Rheum. 2011; 63:2352–62. [PubMed: 21506098]

27. Klooker TK, Kuiken SD, Lei A, Boeckxstaens GE. Effect of long-term treatment with octreotide on rectal sensitivity in patients with non-constipated irritable bowel syndrome. Aliment Pharmacol Ther. 2007; 26:605–15. [PubMed: 17661764]

28. Krempels K, Hunyady B, O'Carroll AM, Mezey E. Distribution of somatostatin receptor messenger RNAs in the rat gastrointestinal tract. Gastroenterology. 1997; 112:1948–60. [PubMed: 9178687]

29. Larauche M. Novel insights in the role of peripheral corticotropin-releasing factor and mast cells in stress-induced visceral hypersensitivity. Neurogastroenterol Motil. 2012; 24:201–5. [PubMed: 22316289]

30. Larauche M, Gourcerol G, Million M, Adelson DW, Tache Y. Repeated psychological stress-induced alterations of visceral sensitivity and colonic motor functions in mice: influence of surgery and postoperative single housing on visceromotor responses. Stress. 2010; 13:343–54. [PubMed: 20536336]

31. Larauche M, Gourcerol G, Wang L, Pambukchian K, Brunnhuber S, Adelson DW, et al. Cortagine, a CRF1 agonist, induces stresslike alterations of colonic function and visceral hypersensitivity in rodents primarily through peripheral pathways. Am J Physiol Gastrointest Liver Physiol. 2009; 297:G215–27. [PubMed: 19407218]

32. Larauche M, Mulak A, Tache Y. Stress and visceral pain: From animal models to clinical therapies. Exp Neurol. 2012; 233:49–67. [PubMed: 21575632]

33. Larauche M, Mulak A, Yuan PQ, Kanauchi O, Tache Y. Stress-induced visceral analgesia assessed non-invasively in rats is enhanced by prebiotic diet. World J Gastroenterol. 2012; 18:225–36. [PubMed: 22294825]

34. Martinez V, Curi AP, Torkian B, Schaeffer JM, Wilkinson HA, Walsh JH, et al. High basal gastric acid secretion in somatostatin receptor subtype 2 knockout mice. Gastroenterology. 1998; 114:1125–32. [PubMed: 9609748]

35. Mertz H, Naliboff B, Munakata J, Niazi N, Mayer EA. Altered rectal perception is a biological marker of patients with irritable bowel syndrome. Gastroenterology. 1995; 109:40–52. [PubMed: 7797041]

36. Olias G, Viollet C, Kusserow H, Epelbaum J, Meyerhof W. Regulation and function of somatostatin receptors. J Neurochem. 2004; 89:1057–91. [PubMed: 15147500]

37. Page MD, Millward ME, Taylor A, Preece M, Hourihan M, Hall R, et al. Long-term treatment of acromegaly with a long-acting analogue of somatostatin, octreotide. Q J Med. 1990; 74:189–201. [PubMed: 2111918]

38. Park JH, Rhee PL, Kim HS, Lee JH, Kim YH, Kim JJ, et al. Mucosal mast cell counts correlate with visceral hypersensitivity in patients with diarrhea predominant irritable bowel syndrome. J Gastroenterol Hepatol. 2006; 21:71–8. [PubMed: 16706815]

39. Pinter E, Helyes Z, Szolcsanyi J. Inhibitory effect of somatostatin on inflammation and nociception. Pharmacol Ther. 2006; 112:440–56. [PubMed: 16764934]

40. Plourde V, Lembo T, Shui Z, Parker J, Mertz H, Tache Y, et al. Effects of the somatostatin analogue octreotide on rectal afferent nerves in humans. Am J Physiol. 1993; 265:G742–51. [PubMed: 8238358]

41. Rivier J, Erchegyi J, Hoeger C, Miller C, Low W, Wenger S, et al. Novel sst(4)-selective somatostatin (SRIF) agonists. 1. Lead identification using a betide scan. J Med Chem. 2003; 46:5579–86. [PubMed: 14667212]

42. Rivier J, Gulyas J, Kunitake K, DiGruccio M, Cantle J, Perrin MH, et al. W Stressin1-A, a potent corticotropin releasing factor receptor 1 (CRF1)-selective peptide agonist. J Med Chem. 2007; 50:1668–74. [PubMed: 17335188]

43. Rong W, Winchester WJ, Grundy D. Spontaneous hypersensitivity in mesenteric afferent nerves of mice deficient in the sst2 subtype of somatostatin receptor. J Physiol. 2007; 581:779–86. [PubMed: 17363388]

44. Saavedra Y, Vergara P. Somatostatin inhibits intestinal mucosal mast cell degranulation in normal conditions and during mast cell hyperplasia. Regul Pept. 2003; 111:67–75. [PubMed: 12609751]

45. Sandor K, Elekes K, Szabo A, Pinter E, Engstrom M, Wurster S, et al. Analgesic effects of the somatostatin sst4 receptor selective agonist J-2156 in acute and chronic pain models. Eur J Pharmacol. 2006; 539:71–5. [PubMed: 16697366]

46. Schafer J, Baumeister H, Lorenz A, Meyerhof W. Localization of somatostatin receptor subtype mRNA in the rat gastrointestinal tract and regulation of SSTR1 gene expression. Z Ernahrungswiss. 1998; 37 (Suppl 1):75–9. [PubMed: 9558732]

47. Schafer J, Meyerhof W. sst1 mRNA is the prominent somatostatin receptor mRNA in the rat gastrointestinal tract: reverse transcription polymerase chain reaction and in situ-hybridization study. Neuropeptides. 1999; 33:457–63. [PubMed: 10657524]

48. Schulz S, Schreff M, Schmidt H, Handel M, Przewlocki R, Hollt V. Immunocytochemical localization of somatostatin receptor sst2A in the rat spinal cord and dorsal root ganglia. Eur J Neurosci. 1998; 10:3700–8. [PubMed: 9875349]

49. Schwetz I, Naliboff B, Munakata J, Lembo T, Chang L, Matin K, et al. Anti-hyperalgesic effect of octreotide in patients with irritable bowel syndrome. Aliment Pharmacol Ther. 2004; 19:123–31. [PubMed: 14687174]

50. Shi TJ, Xiang Q, Zhang MD, Barde S, Kai L, Fried K, et al. Somatostatin and its 2A receptor in dorsal root ganglia and dorsal horn of mouse and human: expression, trafficking and possible role in pain. Mol Pain. 2014; 10:12. [PubMed: 24521084]

51. Simon A, Czajlik A, Perczel A, Keri G, Nyikos L, Emri Z, et al. Binding crevice for TT-232 in a homology model of type 1 somatostatin receptor. Biochem Biophys Res Commun. 2004; 316:1059–64. [PubMed: 15044092]

52. Singh V, Brendel MD, Zacharias S, Mergler S, Jahr H, Wiedenmann B, et al. Characterization of somatostatin receptor subtype-specific regulation of insulin and glucagon secretion: an in vitro study on isolated human pancreatic islets. J Clin Endocrinol Metab. 2007; 92:673–80. [PubMed: 17105845]

53. Stengel A, Goebel-Stengel M, Wang L, Larauche M, Rivier J, Tache Y. Central somatostatin receptor 1 activation reverses acute stress-related alterations of gastric and colonic motor function in mice. Neurogastroenterol Motil. 2011; 23:e223–36. [PubMed: 21564422]

54. Stengel A, Goebel-Stengel M, Wang L, Shaikh A, Lambrecht NW, Rivier J, et al. Abdominal surgery inhibits circulating acyl ghrelin and ghrelin-O-acyltransferase levels in rats: role of the somatostatin receptor subtype 2. Am J Physiol Gastrointest Liver Physiol. 2011; 301:G239–48. [PubMed: 21636529]

55. Su X, Burton MB, Gebhart GF. Effects of octreotide on responses to colorectal distension in the rat. Gut. 2001; 48:676–82. [PubMed: 11302968]

56. Szolcsanyi J, Bolcskei K, Szabo A, Pinter E, Petho G, Elekes K, et al. Analgesic effect of TT-232, a heptapeptide somatostatin analogue, in acute pain models of the rat and the mouse and in streptozotocin-induced diabetic mechanical allodynia. Eur J Pharmacol. 2004; 498:103–9. [PubMed: 15363982]

57. Szolcsanyi J, Pinter E, Helyes Z, Petho G. Inhibition of the function of TRPV1-expressing nociceptive sensory neurons by somatostatin 4 receptor agonism: mechanism and therapeutical implications. Curr Top Med Chem. 2011; 11:2253–63. [PubMed: 21671870]

58. Tang C, Lan C, Wang C, Liu R. Amelioration of the development of multiple organ dysfunction syndrome by somatostatin via suppression of intestinal mucosal mast cells. Shock. 2005; 23:470–5. [PubMed: 15834315]

59. Tezval H, Jahn O, Todorovic C, Sasse A, Eckart K, Spiess J. Cortagine, a specific agonist of corticotropin-releasing factor receptor subtype 1, is anxiogenic and antidepressive in the mouse model. Proc Natl Acad Sci U S A. 2004; 101:9468–73. [PubMed: 15192151]

60. Van, OpdB; Adriaensen, D.; Van Nassauw, L.; Timmermans, JP. The role(s) of somatostatin, structurally related peptides and somatostatin receptors in the gastrointestinal tract: a review. Regul Pept. 2009; 156:1–8. [PubMed: 19362110]

61. Van, OpdB; Torfs, P.; De Winter, BY.; De Man, JG.; Pelckmans, PA.; van Marck, E., et al. Effect of genetic SSTR4 ablation on inflammatory peptide and receptor expression in the non-inflamed and inflamed murine intestine. J Cell Mol Med. 2009; 13:3283–95. [PubMed: 19426160]

62. Van, OpdB; Van Nassauw, L.; Lantermann, K.; van Marck, E.; Timmermans, JP. Effect of intestinal inflammation on the cell-specific expression of somatostatin receptor subtypes in the murine ileum. Neurogastroenterol Motil. 2007; 19:596–606. [PubMed: 17593141]

63. Wallon C, Yang PC, Keita AV, Ericson AC, McKay DM, Sherman PM, et al. Corticotropin-releasing hormone (CRH) regulates macromolecular permeability via mast cells in normal human colonic biopsies in vitro. Gut. 2008; 57:50–8. [PubMed: 17525093]

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.
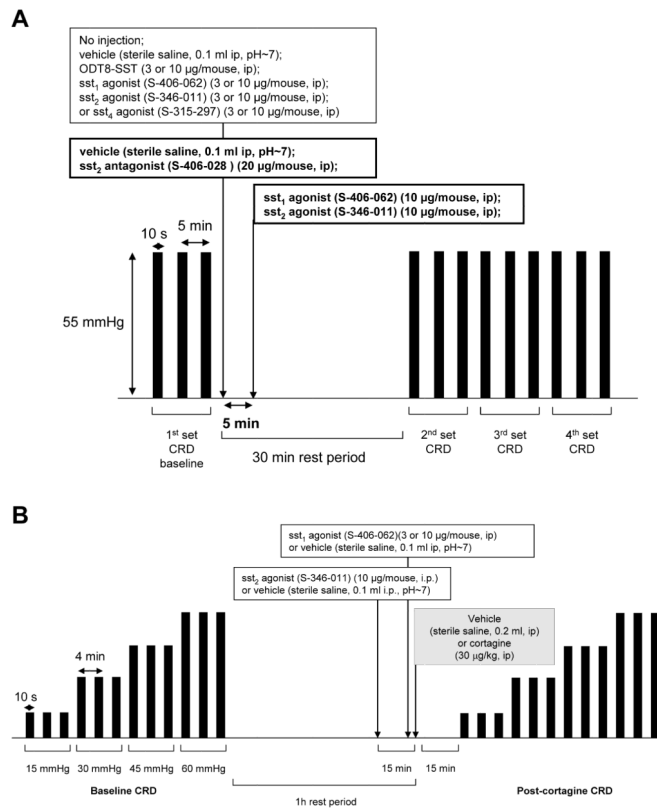
Fig. 1.
Experimental protocol design: (A) repeated isobaric noxious colorectal distensions (text in bold represents experiments with sst$_2$ antagonist pretreatment), and (B) intraperitoneal cortagine-induced visceral hypersensitivity.
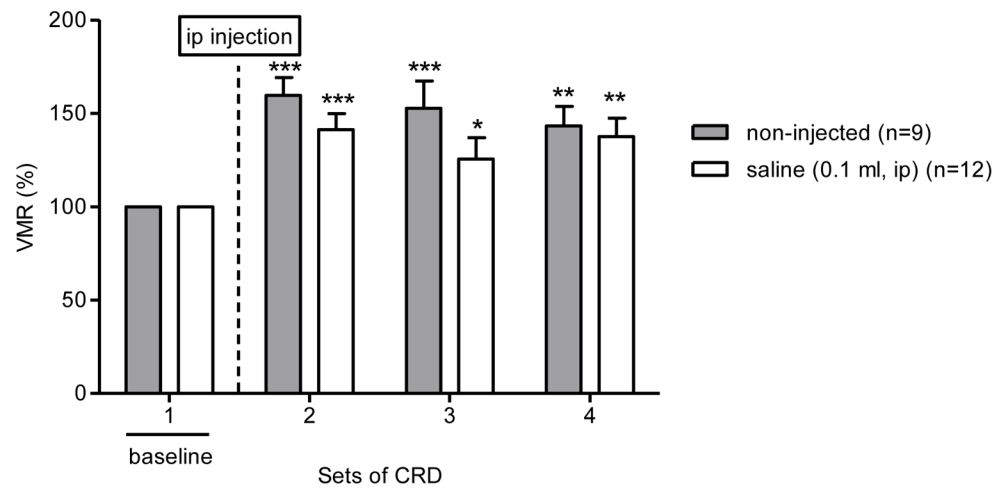
Fig. 2.
Visceral hyperalgesic induced in rats without or with an ip injection of saline. For experimental design see Fig. 1A. The VMR value to the baseline CRD was expressed as 100% of response in each group. Data are means ± SEM of %VMR compared to the baseline response; n = 9–12 as indicated in the parenthesis; *p<0.05, **p<0.01, ***p<0.001 *vs* baseline (1st CRD).
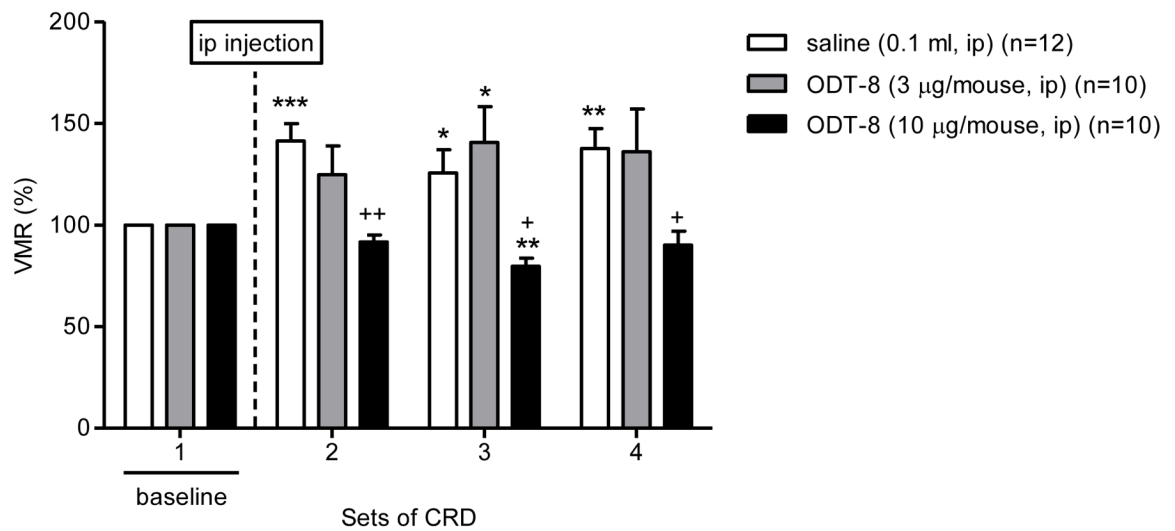
Fig. 3.

Antihyperalgesic effect of the stable pan-sst$_{1-5}$ agonist, ODT8-SST injected intraperitoneally. For experimental design see Fig. 1A. Data are means ± 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.
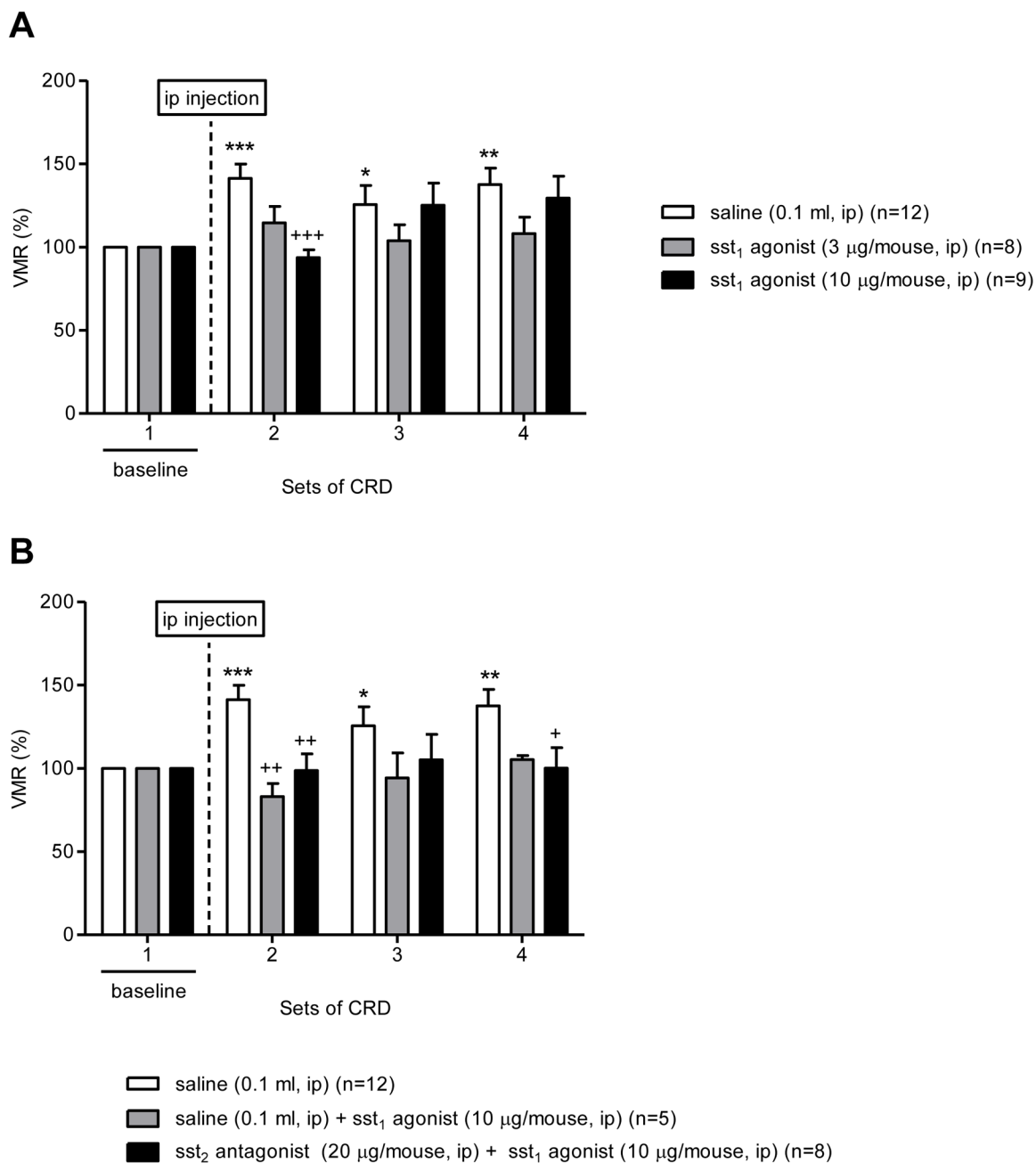
A

B

Fig. 4.

Antihyperalgesic effect of the selective sst_1 receptor agonist, S-406-062. (A) The selective sst_1 receptor agonist injected ip prevents repeated noxious CRD-induced visceral hypersensitivity in mice in a time-dependent manner. (B) Pre-treatment with the selective sst_2 antagonist, S-406-028 does not affect the inhibition of visceral hypersensitivity induced by the sst_1 agonist. For experimental design see Fig. 1A. Data are means ± 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.
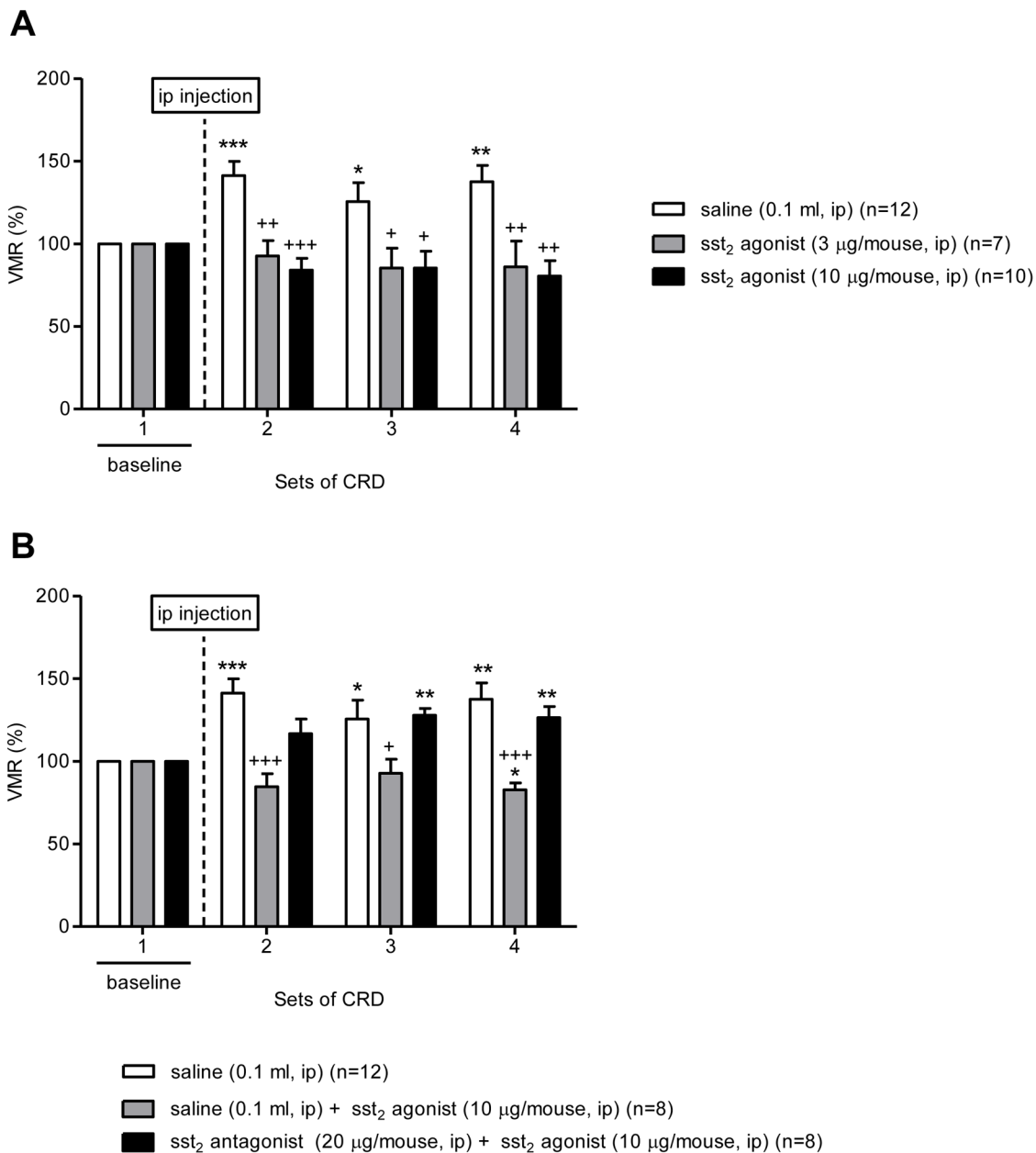
Fig. 5.
Antihyperalgesic effect of the selective sst_2 receptor agonist, S-346-011. (A) The selective sst_2 receptor agonist injected ip suppresses the visceral hypersensitivity induced by repeated noxious CRD in mice. (B) Pre-treatment with the selective sst_2 antagonist S-406-028 prevents the inhibition of visceral hypersensitivity by the sst_2 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.
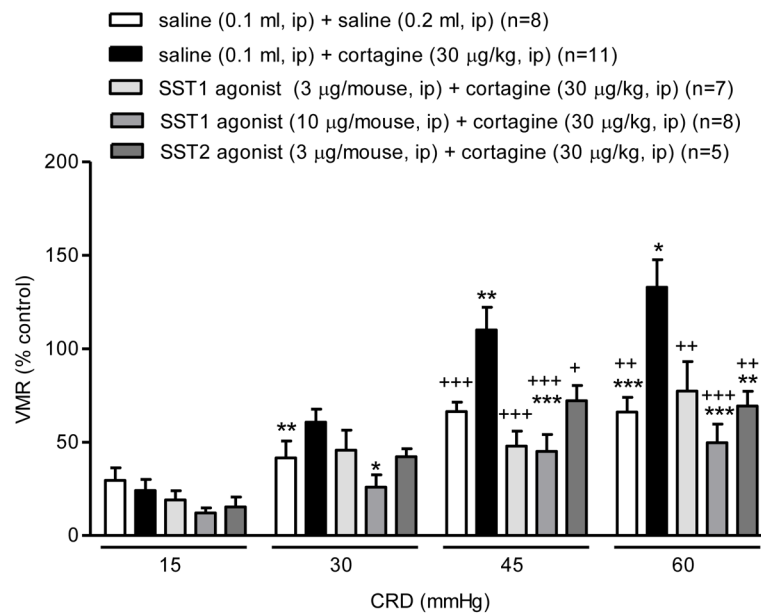
Fig. 6.
The selective sst₁ and sst₂ receptor agonists prevent visceral hypersensitivity induced by receptors in mice. For experimental design see Fig. 1B. Data are activation of peripheral CRF_1 means ± SEM of VMR expressed as percentage of 60 mmHg response to the 1st CRD; *p<0.05, **p<0.01, ***p<0.001 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 sst_4 receptor agonist, injected i.p. did not influence visceral hyperalgesia induced by repeated noxious CRD in mice.

	Saline n=12	sst₄ ago (3 μg/mouse) n=14	sst₄ ago (10 μg/mouse) n=11
1st CRD set (baseline)	100 ± 0	100 ± 0	100 ± 0
2nd CRD set	$141.3 \pm 8.5^{\ddagger}$	$120.2 \pm 12.6^{\dagger}$	$124.1 \pm 10.9^{\dagger}$
3rd CRD set	$125.5 \pm 11.5^{\dagger}$	$123.6 \pm 14.95^{\dagger}$	$124.7 \pm 12.2^{\dagger}$
4th CRD set	$137.6 \pm 9.8^{\ddagger}$	$126.5 \pm 17.2^{\dagger}$	$125.5 \pm 7.2^{\dagger}$

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

† p <0.05,

‡ p<0.01 *vs* baseline.