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Central somatostatin receptor 1 activation reverses acute stress-related alterations of gastric and colonic motor function in mice

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Abstract

Background—Corticotropin-releasing factor (CRF) signaling induced by stress is well established to delay gastric emptying (GE) and stimulate colonic functions. The somatostatin receptor (*sst*₁₋₅) agonist, ODT8-SST acts in the brain to inhibit stress-induced adrenocorticotrophic hormone and epinephrine secretion. We investigated whether ODT8-SST acts in the brain to influence stress-related alterations of gastric and colonic motor function and *sst* receptor subtype(s) involved.

Methods—Peptides were injected intracerebroventricularly (i.c.v.) under short isoflurane anesthesia and GE, fecal pellet output (FPO) and distal colonic motility monitored in conscious mice.

Key results—The stress of anesthesia/vehicle i.c.v. injection reduced GE by 67% and increased defecation by 99% compared to non-injected controls. Both responses were abolished by ODT8-SST (1μg=0.75nmol) or *sst*₁ agonist (3μg=1.95nmol). The *sst*₁ agonist also prevented the abdominal surgery-induced delayed GE. Octreotide (*sst*₂>*sst*₅>*sst*₃) and the *sst*₂ or *sst*₄ agonists (1μg=0.78 or 0.70nmol, respectively) injected i.c.v. did not influence FPO while i.c.v. somatostatin-28 mimicked ODT8-SST's effect. The ODT8-SST-induced increased food intake was inhibited by i.c.v. *sst*₂ antagonist while the reduced FPO was unchanged. ODT8-SST i.c.v. reduced distal colonic motility in semi-restrained mice compared with vehicle and blocked water avoidance- and i.c.v. CRF (0.5μg=0.09 nmol)-induced stimulated FPO while a similar colonic secretomotor response to i.p. 5-hydroxytryptophane (10mg/kg=36.4μmol/kg) was unaltered.

Conclusions & Inferences—ODT8-SST counteracts stress/i.c.v. CRF-related stimulation of colonic motor function and delayed GE which can be reproduced mainly by activation of *sst*₁ receptors. These data opens new insight to brain somatostatinergic signaling pathways interfering with brain circuitries involved in gut motor responses to acute stress.

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DISCLOSURE A.S., M.G.-S., L.W., M.L. and Y.T. have nothing to disclose. J.R. is Founder of Sentia Medical Sciences, Inc. No conflicts of interest exist.

AUTHOR CONTRIBUTIONS A.S. planned and performed the experiments, analyzed the data and wrote the manuscript; M.G.-S., M.L. and L.W. performed components of experiments and reviewed the paper; J.R. provided the *sst* agonists and *sst*₂ antagonist and reviewed the manuscript; Y.T. planned the experiments, gave critical input throughout the study and in the writing of the manuscript.

Keywords

colonic motility; CRF; gastric emptying; ODT8-SST; somatostatin agonists; stress

INTRODUCTION

Somatostatin (SST, somatotropin release-inhibiting factor, SRIF) was identified as a 14 amino acid peptide isolated from ovine hypothalami and involved in the inhibition of growth hormone (GH) secretion.¹ This landmark discovery was followed by the identification of an amino-terminal-extended biologically active form, SRIF-28 from the rat hypothalamus.² Somatostatin in the brain exerts diverse GH-independent biological actions³⁻⁵ that are mediated through interaction with five somatostatin receptor (sst) subtypes, sst₁₋₅ that belong to the G-protein-coupled receptor family inducing multiple transmembrane signaling systems.⁶ In particular, we initially established that ODT8-SST, des-AA^{1,2,4,5,12,13}-[DTrp⁸]-SST, a stable oligosomatostatin agonist⁷ that binds to all sst receptors with high affinity⁸ when injected into the cisterna magna (intracisternally, i.c.) stimulates gastric acid secretion⁹ and accelerates gastric emptying of a liquid non-nutrient solution¹⁰ and injected intracerebroventricularly (i.c.v.) stimulates gastric emptying of a solid meal in rats.¹¹ Studies using various selective SRIF receptor subtype agonists indicated that the acceleration of gastric emptying after i.c. injection of ODT8-SST or SRIF-28 is mimicked mainly by the activation of brain sst₅.¹⁰ Recently, we also reported that ODT8-SST injected i.c.v. robustly increases light phase food intake¹¹ through activation of sst₂ as indicated by the complete blockade of the ODT8-SST effect on food intake using a selective sst₂ antagonist in rats¹¹ and the similar orexigenic effect of i.c.v. injection of a selective peptide sst₂ agonist in rats¹² and mice.¹³

Several studies indicate that various transmitters acting in the brain to regulate food intake such as corticotropin releasing factor (CRF), peptide YY, neuropeptide Y, thyrotropin releasing hormone, ghrelin, opioids, endocannabinoids, oxytocin, neuropeptide S and cholecystokinin¹⁴⁻¹⁷ also alter colonic motor function.¹⁸⁻²⁶ However, the central action of SRIF to influence colonic motility is unknown so far, while there is neuroanatomic evidence for the distribution of sst receptors and ligands in brain nuclei involved in the brain regulation of colonic function²⁷⁻²⁹ such as the paraventricular nucleus (PVN) of the hypothalamus (sst₂, sst₃ and sst₄), locus coeruleus/subcoeruleus (sst₂, sst₃ and sst₄) and the arcuate nucleus (sst₁, sst₂, sst₃, sst₄ and sst₅).³⁰⁻³² Moreover, previous studies showed that brain injection of the oligosomatostatin agonist, ODT8-SST or SRIF-28 inhibits acute stress-induced adrenocorticotrophic hormone (ACTH) and adrenal epinephrine secretion in rats.³³ This effect was exerted by an inhibition of CRF release³³ pointing towards a negative interaction between activation of somatostatin receptors and CRF. Central CRF is well established to be recruited under stress conditions and involved in stress-related delayed gastric emptying and stimulation of colonic propulsive motor activity.³⁴

Therefore, in the present study we investigated the central action of ODT8-SST to influence colonic motor alterations induced by acute stressors or i.c.v. injection of CRF¹⁸ in mice. This was compared with direct peripheral stimulation of the colon by intraperitoneal (i.p.) injection of 5-hydroxytryptophane (5-HTP).³⁵ To get insight into receptor subtype(s) contributing to the i.c.v. ODT8-SST effect, we used the selective sst₁³⁶, sst₂³⁷ and sst₄ peptide agonists³⁸ developed so far along with the recently characterized sst₂ peptide antagonist.³⁹ Based on the relationship between peptides influencing food intake and colonic motility, we simultaneously monitored changes in food intake and defecation upon i.c.v. injection of ODT8-SST and tested whether the sst₂ antagonist, known to block the orexigenic action of i.c.v. ODT8-SST in rats,¹¹ would influence the colonic motor response

to an acute stress. Lastly, we assessed whether i.c.v. ODT8-SST and the selective sst₁ agonist identified to be effective in counteracting the acute stress stimulatory effect on the colon would also prevent the same stressor-induced delayed gastric emptying. We also tested the sst₁ agonist in a well established model of visceral stress, abdominal surgery.⁴⁰

MATERIALS AND METHODS

Animals

Adult male C57Bl/6 mice (23-28g, Harlan Laboratories, San Diego, CA) were housed 4/ cage under controlled illumination (06:00-18:00 h) and temperature (21–23°C). Animals had *ad libitum* access to standard rodent diet (Prolab RMH 2500; LabDiet, PMI Nutrition, Brentwood, MO) and tap water. Protocols were approved by the Veterans Administration Institutional Animal Care and Use Committee (# 10043-09 and # 9906-820).

Compounds

ODT8-SST (MW 1078.5, compound #1 in⁸), the sst₁ agonist (MW: 1238.5, compound #25 in³⁶), sst₁ agonist CH-275 (MW: 1485.5, compound #2 in³⁶), sst₂ agonist (MW: 1132.5, compound #2 in³⁷), sst₄ agonist (MW: 1137.4, compound #15 in³⁸), the sst₂ antagonist (MW: 1208.4, compound #4 in³⁹), somatostatin-14 (MW: 1637.9), somatostatin-28 (MW: 3146.5) and rat/human/mouse CRF (Clayton Foundation Laboratories, Salk Institute, La Jolla, CA) were synthesized as we previously described and purity was characterized by high pressure liquid chromatography, capillary zone electrophoresis and mass spectrometry.^{8, 36-39} No peptide agonists selectively binding to the sst₃ or sst₅ have been developed yet and therefore we used the stable sst_{2/5/3} agonist, octreotide also known as SMS201-995⁴¹ (MW 1019.3, Bachem America, Inc., Torrance, CA). The chemical structure as well as previously established binding affinities of these peptides on sst receptor-transfected human cells are detailed in Table 1. Peptides and the exogenous serotonin (5-HT) precursor, 5-hydroxytryptophane, L-2-amino-3-(5-hydroxyindolyl)propionic acid (Sigma-Aldrich, St. Louis, MO) were kept in powder form at –80 °C and immediately before administration, were dissolved in vehicle as specified in the experimental protocols. As the conversion of µg injected to nmol does not accurately reflect the amount of nmol injection due to the fact that each lyophilized peptide preparation contains counter ions (TFA) and water of lyophilization that together amount 15 to 30% in weight we calculated the peptide amount injected in nmol/mouse based on a 20% correction factor of the formula weight established previously (J.R.).

Procedures

Intracerebroventricular (i.c.v.) injections—Mice were acutely anesthetized by brief (2-3 min) exposure to isoflurane (4.5% vapor concentration in oxygen; VSS, Rockmart, GA), then the intracerebroventricular (i.c.v.) injection (5 µl) was performed using an adaptation of the freehand method⁴² as in our previous studies.¹³ The injection site was localized at the apex of the equal triangle between the eyes and the back of the head and cleaned with Povidone-Iodine 10% (Aplicare Inc., Meriden, CT). The skull was punctured at the point of least resistance with a 30-gauge needle equipped with a polyethylene tube leaving 4-4.5 mm of the needle tip exposed and attached to a Hamilton syringe. On average, mice completely recovered from anesthesia within 4-5 min. Accuracy of injections was assessed in our previous studies by injecting cresyl violet dye i.c.v. under similar conditions in 50 mice.¹⁸

Water avoidance stress—(WAS) lasted for 1 h and was performed as in our previous studies.⁴³ It consisted of placing the mice individually on a rectangular platform (3cm length

× 3 cm width × 6 cm height) positioned inside a container (31 cm length × 31 cm width × 21 cm height) filled with hand warm water up to 1 cm below the top of the platform.

Abdominal surgery in mice was performed as described before.⁴⁰ Briefly, overnight fasted mice anesthetized with isoflurane underwent laparotomy and cecal exteriorization and palpation for 60 s and the abdominal incision was closed in one layer with a running stitch. Sham operation consisted of same duration of anesthesia without surgery.

Measurements

Fecal pellet output—The number of fecal pellets expelled was monitored as in our previous studies¹⁹ during WAS exposure or after injection of peptides. The occurrence of diarrhea was assessed using the recently described diarrhea score¹⁹ with 0 indicating no diarrhea, 1: 1 watery and/or loose pellets, 2: 2 watery and/or loose pellets and 3 indicating 3 watery and/or loose pellets.

Food intake was assessed manually as in our previous studies.¹¹ After injection, pre-weighed rodent chow was made available and food intake corrected for spillage was monitored at 1, 2, 4 and 6 h. Feeding experiments were repeated in a crossover design.

Distal colonic motility—Distal intracolonic pressure monitoring in conscious mice was performed as recently developed.⁴⁴ Briefly, naïve mice anesthetized for 2-3 min with isoflurane (4.5% vapor concentration in oxygen; VSS) were injected i.c.v. with ODT8-SST or vehicle as described above and a miniature pressure transducer catheter (SPR-524 Mikro-Tip catheter; Millar Instruments, Houston, TX) lubricated with chlorhexidine gluconate (Surgilube, E. Fougera & Co., Atlanta Inc., NY) was inserted 2 cm into the distal colon and secured to the tail with tape. Then, mice regained the righting reflex and were placed individually in a mouse semi-restraint tube and distal intracolonic pressure recording started approximately 4 min after the injection. The pressure transducer was connected to a preamplifier (model 600; Millar Instruments). The signal was amplified using a transducer amplifier (TBM4, World Precision Instruments, Boca Raton, FL), acquired using a Micro1401 A/D interface (Cambridge Electronic Design) and recorded using Spike 2 version 5 data acquisition software. Abdominal contractions were excluded by smoothing the original trace with a time constant of 2 s. The phasic component of intra-colonic pressure was extracted from the original trace by removing the DC (direct current) component with a time constant of 10 s from the 2-s smoothed original trace. The distal colonic contractile pressure changes were quantified by measuring the phasic component of the intraluminal pressure trace of the area under the curve (pAUC) using quantifications for every minute as previously described.⁴⁴ Results are expressed as 1) the time course of the phasic contractions, by calculating the mean pAUC for every minute (pAUC_m) from the rolling average of pAUC for the period between 2 min before and 2 min after each minute's pAUC (i. e., pAUC_m = average of pAUC_{±2 min}) or 2) the mean pAUC over a period of 1 h.

Gastric emptying of ingested standard rodent chow was determined as in our previous studies.¹¹ Briefly, mice were fasted overnight for 15 h and re-fed with pre-weighed standard chow starting at 08:00 h for 1 h. Then, food and water were removed and i.c.v. injection was performed under short inhalation anesthesia. Gastric emptying was assessed 2 h later. Mice were euthanized by cervical dislocation, the stomach quickly removed, the stomach content weighed and gastric emptying calculated as (1-gastric content/food intake) × 100.

Gastric emptying of a liquid non-nutrient solution was performed as described before with small modifications.⁴⁰ Briefly, the phenyl-red methyl cellulose solution (0.3 ml) was gavaged orogastrically at 30 min after the end of the surgery or sham procedure in conscious overnight fasted mice and gastric emptying assessed 20 min later. Mice were euthanized by

CO₂ inhalation and the stomachs removed, cleaned, placed in 0.1N NaOH and homogenized. The homogenate was allowed to settle for 1 h, and 5 ml of the supernatant were added to 0.5 ml of 20% trichloroacetic acid. This solution was centrifuged at 4°C for 20 min at 3000 rpm, and 3 ml of the supernatant added to 4 ml of 0.5N NaOH which was read spectrophotometrically. Two non-treated animals were euthanized following gavage and served as control animals (0% emptying). The percentage of gastric emptying was calculated using the following equation: % emptying = (1 - absorbance of test sample/absorbance of standard) × 100.

Experimental protocols

All experiments were performed in freely fed mice with continued access to food and water after treatment except otherwise stated. Animals were single housed for the duration of the experiment starting between 09:00 and 10:00 h. Mice were trained for single housing at least three times before for 1-9 h/d during the week before the experiment.

Effects of ODT8-SST and selective somatostatin receptor agonists injected i.c.v. on stimulated propulsive colonic motor function—Vehicle (ddH₂O alone or containing 0.1% bovine serum albumin, BSA) and the following SRIF peptide agonists were injected i.c.v. in mice: ODT8-SST (0.3 μg = 0.22 nmol or 1 μg = 0.75 nmol/mouse in ddH₂O), SRIF-14 (5.1 μg = 2.5 nmol/mouse in ddH₂O containing 0.1% BSA), SRIF-28 (9.3 μg = 2.4 nmol/mouse in ddH₂O containing 0.1% BSA), octreotide (0.3 μg = 0.24 nmol or 1 μg = 0.78 nmol/mouse in ddH₂O) or the selective sst₁ (0.3 μg = 0.19 nmol, 1 μg = 0.65 nmol or 3 μg = 1.95 nmol/mouse in ddH₂O), sst₁ CH-275 (3 μg = 1.6 nmol or 10 μg = 5.4 nmol/mouse in ddH₂O), sst₂ (0.3 μg = 0.21 nmol or 1 μg = 0.70 nmol/mouse in ddH₂O) and sst₄ agonists (0.3 μg = 0.21 nmol or 1 μg = 0.70 nmol/mouse in ddH₂O) and fecal pellet output was monitored for 1 h afterwards. Doses of ODT8-SST were based on our previous i.c.v. dose-response study on food intake in rats and mice¹¹ and those of SRIF agonists adapted accordingly. Based on the observed effects in these experiments, in all subsequent studies, ODT8-SST was injected at the dose of 1 μg = 0.75 nmol/mouse. To assess basal fecal pellet output, a control group of mice without anesthesia and i.c.v. injection was monitored for 1 h.

To investigate the time course of the ODT8-SST effect on propulsive colonic motor function, mice were injected i.c.v. with ODT8-SST or vehicle and thereafter did or did not have access to food. Fecal pellet output was assessed at 1, 2, 4, 6 and 9 h post injection. Food intake was monitored at the same time points. In other studies, the sst₂ antagonist (1 μg = 0.66 nmol/mouse in 2.5 μl ddH₂O) or vehicle (2.5 μl ddH₂O) was injected i.c.v. followed by i.c.v. ODT8-SST (in 2.5 μl ddH₂O) or vehicle (2.5 μl ddH₂O) and food was provided throughout these experiments. Fecal pellet output and food intake were assessed at 1, 2, 4 and 6 h post injection. The sst₂ antagonist was injected i.c.v. at a dose shown to block the i.c.v. ODT8-SST-induced increased food intake in rats.¹¹

Water avoidance stress: Mice were injected i.c.v. with ODT8-SST, sst₂ agonist (0.70 nmol/mouse in 5 μl ddH₂O) or vehicle and allowed to completely recover (within ~ 4 min). Afterwards, mice were subjected to water avoidance stress without access to food and fecal pellet output assessed at 15, 30, 45 and 60 min. Non i.c.v. injected control mice were placed in single housing cages and 1-h fecal pellet output was monitored in 15-min intervals.

Intracerebroventricular CRF: Mice were injected i.c.v. with ODT8-SST (in 2.5 μl ddH₂O) or vehicle (2.5 μl ddH₂O) followed by CRF (0.5 μg = 0.09 nmol/mouse in 2.5 μl ddH₂O) or vehicle (2.5 μl ddH₂O) and fecal pellet output and diarrhea score were assessed at 15, 30, 45 and 60 min.

Intraperitoneal 5-HTP: Mice were injected i.c.v. with ODT8-SST or vehicle. After complete recovery from anesthesia, mice received an i.p. injection of 5-HTP (10 mg/kg = 36.4 μ mol/kg in 100 μ l saline) or vehicle (100 μ l saline) and fecal pellet output and diarrhea score were monitored at 15, 30, 45 and 60 min post i.p. injection. The dose of i.c.v. CRF and i.p. 5-HTP was based on our previous reports showing a robust stimulation of fecal pellet output and diarrhea score in mice under these conditions.^{18, 19, 35}

Effects of ODT8-SST injected i.c.v. on distal colonic motility in mice—Mice were injected i.c.v. with ODT8-SST or vehicle, then the miniaturized transducer catheter was inserted into the distal colon and secured to the tail with tape and mice were placed in a mouse semi-restraint tube. Recording of distal colonic contractions started at approximately 4 min post injection in mice recovered from anesthesia and was monitored for a 1-h period in animals deprived of food and water.

Effects of ODT8-SST and the selective sst₁ agonist injected i.c.v. on gastric emptying of a solid meal—Overnight fasted mice were re-fed for 1 h and afterwards injected i.c.v. with ODT8-SST, sst₁ agonist (1.95 nmol/mouse in 5 μ l ddH₂O) or vehicle or received no treatment and gastric emptying for the solid meal was assessed 2 h later.

Effects of the selective sst₁ agonist injected i.c.v. on abdominal surgery-induced delayed gastric emptying of a liquid meal—Overnight fasted mice were injected i.c.v. with sst₁ agonist (1.95 nmol/mouse in 5 μ l ddH₂O) or vehicle and directly afterwards underwent abdominal surgery or sham procedure. Animals received an orogastric gavage of a liquid non-nutrient solution 30 min after the end of the procedure and gastric emptying was assessed 30 min later.

Statistical analysis

Data are expressed as mean \pm SEM and analyzed by one way analysis of variance (ANOVA) followed by Tukey *post hoc* test or two-way ANOVA followed by Holm-Sidak method. All analyses were performed using Sigma Stat 3.1 (Systat Software, Inc., Point Richmond, CA). Differences between groups were considered significant when $P < 0.05$.

RESULTS

ODT8-SST, SRIF-28 and the selective sst₁ agonist injected intracerebroventricularly (i.c.v.) prevent defecation induced by the acute stress of i.c.v. injection under short anesthesia

The i.c.v. injection of saline under short anesthesia resulted in an increase in FPO compared to non-anesthetized, non-treated mice (5.8 ± 0.5 vs. 2.9 ± 0.4 fecal pellets/1 h, $P < 0.01$; $n=10$). ODT8-SST (0.22 and 0.75 nmol/mouse) injected i.c.v. dose-dependently reduced the 1-h cumulative fecal pellet output by 41% and 74% respectively in freely fed mice compared to vehicle (5.4 ± 1.4 fecal pellets/1 h), with the highest dose reaching statistical significance ($P < 0.05$; Fig. 1A). Based on these data, the dose of 1 μ g = 0.75 nmol/mouse ODT8-SST was used for all further i.c.v. injections. Likewise, SRIF-28 (2.4 nmol/mouse, i.c.v.) significantly reduced the number of excreted fecal pellets by 60% compared to vehicle (2.3 ± 0.6 vs. 5.8 ± 1.3 fecal pellets/1 h, $P < 0.05$; Fig. 1B), whereas SRIF-14 (2.5 nmol/mouse, i.c.v.) had no effect (Fig. 1B).

Next, we investigated the receptor subtype(s) reproducing the ODT8-SST and SRIF-28 i.c.v. inhibitory effect. The selective sst₁ agonist (0.19, 0.65 or 1.95 nmol/mouse) injected i.c.v. reduced fecal pellet output by 44% and 61% respectively at the highest doses compared with i.c.v. vehicle (3.8 ± 0.7 and 2.6 ± 0.9 vs. 6.8 ± 1.3 fecal pellets/1 h, $P < 0.05$) and had no effect at the lowest dose (Fig. 1D). The sst₁ agonist CH-275 injected i.c.v. (1.6 nmol/mouse)

did not significantly reduce fecal pellet output compared to vehicle (4.0 ± 0.8 vs. 3.6 ± 0.9 fecal pellets/1 h), although the higher dose of 5.4 nmol/mouse tended to reduce 1-h fecal pellet output (2.5 ± 0.8) without reaching statistical significance ($P > 0.05$). The oligosomatostatin ($ss_{2>sst_{5>sst_{3}}$) agonist, octreotide (0.24 or 0.78 nmol/mouse, Fig. 1C) and the selective ss_{2} or ss_{4} agonist (0.21 or 0.70 nmol/mouse) injected i.c.v. did not alter the 1-h cumulative fecal pellet output compared to vehicle, however, both the ss_{2} and ss_{4} agonist showed a 67% and 50% trend towards an increase of the 1-h FPO ($P > 0.05$, Figs. 1E, F).

Time course of i.c.v. ODT8-SST action: the ss_{2} receptor is involved in the orexigenic effect of ODT8-SST but not in the colonic inhibitory response to the acute stress of i.c.v. injection

The time course of i.c.v. ODT8-SST action showed a short lasting 74% inhibition of defecation during the first h (Fig. 2A), whereas the fecal pellet output was increased compared to vehicle at 4 h (20.0 ± 1.5 vs. 11.5 ± 1.5 fecal pellets, $P < 0.05$). Two-way ANOVA indicated a significant influence of treatment ($F_{(1,90)}=15.2$, $P < 0.001$), time ($F_{(4,90)}=34.5$, $P < 0.001$) and treatment \times time ($F_{(4,90)}=5.6$, $P < 0.001$). When expressed as fecal pellet output/period the main increase of fecal pellet output was observed during the 1-2 and 2-4 h periods compared to vehicle ($P < 0.001$; Fig. 2A). Simultaneous monitoring of food intake showed, as previously reported in mice,¹¹ that i.c.v. injected ODT8-SST significantly increases cumulative food intake at 1, 2, 4 and 6 h post injection compared to vehicle in freely fed mice ($P < 0.05$; data not shown).

Then, we assessed the influence of the concomitant orexigenic effect in the dual inhibitory/stimulatory effects of ODT8-SST on propulsive colonic motor function. When mice did not have access to food after the i.c.v. injection of ODT8-SST, a significant 89% inhibitory effect on fecal pellet output was induced during the first hour post injection (5.4 ± 1.4 vs. 0.6 ± 0.2 fecal pellets/1 h, $P < 0.01$; Fig. 2B) which was not significantly different from that observed in mice with access to food ($P > 0.05$). However, the cumulative fecal pellet output was not increased thereafter as observed in freely fed mice (Fig. 2B). Two-way ANOVA showed a significant influence of treatment ($F_{(1,55)}=3.9$, $P < 0.05$) and time ($F_{(4,55)}=7.7$, $P < 0.001$).

To further assess the differential role of ss receptors in the dual feeding and colonic responses to i.c.v. ODT8-SST, the ss_{2} antagonist (0.66 nmol/mouse, i.c.v.) previously established to suppress the feeding response to i.c.v. ODT8-SST in rats¹¹ was used in freely fed mice. The ss_{2} antagonist completely blocked the i.c.v. ODT8-SST-induced increased food intake (e.g. 1 h: 0.11 ± 0.04 vs. 0.27 ± 0.05 g/25g bw, $P < 0.05$; Figs. 2D) along with the significantly increased fecal pellet output during the 1-2 and 2-4 h periods post injection (Fig. 2C), while not influencing the ODT8-SST-induced 71% inhibition of fecal pellet output during the first hour post injection ($P > 0.05$, Fig. 2D). Two-way ANOVA indicated a significant influence of treatment ($F_{(3,112)}=19.7$, $P < 0.001$), time ($F_{(3,112)}=61.6$, $P < 0.001$) and treatment \times time ($F_{(9,112)}=4.3$, $P < 0.001$). The ss_{2} antagonist injected i.c.v. alone had no effect on fecal pellet output or food intake (Figs. 2C-D).

ODT8-SST injected intracerebroventricularly inhibits water avoidance stress and intracerebroventricular CRF-induced stimulation of propulsive colonic motor function

Water avoidance stress increased fecal pellet output compared to single-housed controls (+64%, $P < 0.05$; Fig. 3A). ODT8-SST injected i.c.v. completely blocked the water avoidance stress-induced increase of fecal pellet output (0.8 ± 0.4 vs. 4.8 ± 0.9 fecal pellets/60 min, $P < 0.001$; Fig. 3A). Two-way ANOVA indicated a significant influence of treatment ($F_{(1,88)}=56.9$, $P < 0.001$), time ($F_{(3,88)}=8.9$, $P < 0.001$) and treatment \times time

($F_{(3,88)}=4.3$, $P < 0.01$). In contrast, i.c.v. injection of the ss_{2} agonist (0.70 nmol/mouse) showed a trend to increase defecation response to water avoidance stress which did not reach statistical significance ($P > 0.05$; Fig. 3B).

The i.c.v. injection of CRF (0.09 nmol/mouse) enhanced defecation and the stimulatory effect was completely blocked by the i.c.v. injection of ODT8-SST at all time points (15, 30, 45 and 60 min) compared to CRF alone (60 min: 2.0 ± 0.4 vs. 9.5 ± 1.8 fecal pellets/60 min, $P < 0.01$; Fig. 4A and data not shown). The linear time related increase in the number of excreted fecal pellets from the 15 to 60 min period post i.c.v. injection of vehicle/vehicle was also suppressed by the i.c.v. injection of ODT8-SST at all time points. Two-way ANOVA indicated a significant influence of treatment ($F_{(3,92)}=36.2$, $P < 0.001$) and time ($F_{(3,92)}=7.8$, $P < 0.001$). Likewise, i.c.v. ODT8-SST injected mice had a very low diarrhea score at all time points investigated compared to i.c.v. CRF alone (60 min: 0.1 ± 0.1 vs. 2.8 ± 0.2 , $P < 0.001$) or the vehicle/vehicle group (Fig. 4B).

Peripherally injected 5-HTP (36.4 μ mol/kg, i.p.) significantly increased fecal pellet output ($P < 0.05$; Fig. 5A). Injection of ODT8-SST i.c.v. did not influence the 5-HTP-induced increase in fecal pellet output (Fig. 5A). However, i.c.v. ODT8-SST decreased defecation compared with the i.c.v. vehicle group (Fig. 5A). Two-way ANOVA showed a significant influence of treatment ($F_{(3,112)}=46.7$, $P < 0.001$) and time ($F_{(3,112)}=3.7$, $P < 0.05$). Similarly, 5-HTP i.p. significantly increased the diarrhea score compared to vehicle ($P < 0.05$) and this effect was not influenced by pre-treatment with ODT8-SST i.c.v. (Fig. 5B).

ODT8-SST injected intracerebroventricularly reduces distal colonic contractions in semi-restrained mice

Mice injected i.c.v. with vehicle and placed in the semi-restraint tube displayed high-amplitude (>25 mmHg) contractions in the distal colon throughout the 1-h observation period (Fig. 6A). The overall 60-min pAUC was 153.1 ± 7.0 mmHg \times min in i.c.v. vehicle and decreased to 64.2 ± 4.6 mmHg \times min in mice treated with i.c.v. ODT8-SST ($P < 0.01$; Fig. 6C). The time course response showed a rapid onset in the reduction of pAUC induced by i.c.v. ODT8-SST as indicated by the decrease already occurring within 4 min post injection when recording started and this was maintained throughout the 1-h recording period (Fig. 6B). Two-way ANOVA showed a significant influence of treatment ($F_{(1,720)}=133.6$, $P < 0.001$), whereas time had no effect ($F_{(59,720)}=0.9$, $P > 0.05$).

ODT8-SST and ss_{1} agonist injected intracerebroventricularly prevent the delayed gastric emptying induced by acute stress of i.c.v. injection and anesthesia and abdominal surgery

Non-treated mice fasted overnight and re-fed for 1 h emptied 53.8 ± 9.1 % of their stomach content and this value was reduced to 17.8 ± 4.6 %, under conditions of i.c.v. injection of vehicle under short anesthesia as monitored 2 h after the injection ($P < 0.01$; Fig. 7A). ODT8-SST injected i.c.v. completely prevented the acute i.c.v. injection-induced delayed gastric emptying of a solid meal and values (64.5 ± 4.4 %, $P < 0.001$) were similar to those of non-treated mice ($P > 0.05$; Fig. 7A). Similarly, the selective peptide ss_{1} agonist (1.95 nmol/mouse, i.c.v.) abrogated the inhibition of gastric emptying of a standard rodent meal induced by the acute stress of i.c.v. injection under short anesthesia (60.5 ± 6.5 %, $P < 0.001$; Fig. 7A).

Abdominal surgery reduced gastric emptying of a liquid non-nutrient solution compared to sham-treated animals (9.7 ± 4.4 vs. 33.1 ± 5.7 %, $P < 0.05$; Fig. 7B). Pre-treatment with the ss_{1} agonist (1.95 nmol/mouse, i.c.v.) completely prevented the decrease in gastric emptying (40.0 ± 8.6 %), whereas injected in animals receiving sham treatment, the ss_{1} agonist had no significant effect on gastric emptying (41.2 ± 4.0 %, $P > 0.05$; Fig. 7B).

DISCUSSION

In the present study we show that the oligosomatostatin agonist, ODT8-SST injected i.c.v. at $1 \mu\text{g} = 0.75 \text{ nmol/mouse}$ counteracts both the inhibition of gastric emptying and the stimulation of propulsive colonic motor function induced by exposure to an acute stressor in mice. The peptide action is centrally mediated likely through interaction with brain CRF pathways known to be involved in such a dual response³⁴ and can be mimicked by a sst_1 agonist but not by sst_2 , sst_4 or $\text{sst}_2>\text{sst}_5>\text{sst}_3$ (octreotide) agonists suggesting a primary involvement of sst_1 receptor.

Injection of vehicle i.c.v. under short inhalation anesthesia inhibits gastric emptying of a solid meal by 67% while stimulating distal propulsive colonic motor function as shown by a 99% increase in number of pellets expelled compared to non-injected mice. This reproduces a pattern of gut motor alterations similar to that commonly induced by various acute stressors.^{45, 46} Under these stress conditions, ODT8-SST injected i.c.v. at 0.75 nmol/mouse completely blocked both the delayed gastric emptying and the increased defecation. Convergent evidence supports that ODT8-SST acts in the brain to inhibit brain CRF-related systems involved in these gut motor alterations. First, i.c.v. injection of CRF induces a pattern of stress-like changes in gastro-colonic motor function: inhibition of gastric emptying of a solid meal while stimulating distal colonic transit and defecation in mice and rats.^{18, 34} (present study) In addition, previous studies established that central injection of CRF receptor antagonists alleviates various acute stressor-induced gut motor alterations in rodents indicative of a physiologically relevant role of brain CRF receptor activation.^{18, 34, 46} Second, we showed that ODT8-SST injected i.c.v. inhibits colonic responses in mice exposed to other acute stressors such as the defecation after water avoidance stress, high amplitude phasic contractions in the distal colon of semi-restrained mice and defecation and diarrhea induced by i.c.v. injection of CRF while not influencing defecation and diarrhea elicited by i.p. 5-HTP. We previously established that i.p. 5-HTP injected at the same dose in mice induces defecation and diarrhea through peripheral 5-HT_4 mediated activation of colonic myenteric neurons.³⁵ As the defecation and diarrhea scores elicited by i.c.v. CRF and i.p. 5-HTP were of similar magnitude and had a similar early 15 min onset, this rules out a differential effect of i.c.v. ODT8-SST linked with differential intensity/time course of colonic responses to central CRF and peripheral 5-HTP. Taken together, these data are indicative of a selective central action of i.c.v. ODT8-SST toward brain circuitries involved in the gut motor response to stress, while not interfering with a peripherally initiated stimulation of the colonic secretomotor function. In further support, previous studies showed that ODT8-SST injected i.c.v. prevents the stimulation of adrenal epinephrine secretion induced by ether anesthesia, tail suspension or cold exposure and i.c.v. injection of CRF, while not influencing the epinephrine plasma elevation related to peripheral baroreceptor-dependent mechanisms in rats.⁴⁷ ODT8-SST injected i.c.v. also acts in the brain to inhibit the increase in ACTH plasma levels evoked by either hanging the rats by their tail or placing the animals over an ether-soaked cotton ball for 3 min.³³ Collectively, i.c.v. ODT8-SST inhibits acute stress/CRF coordinated actions to stimulate pituitary ACTH hormone secretion³³, sympathetic adrenal outflow⁴⁸ and to alter gastro-colonic propulsive function (present study).

The somatostatin agonist ODT8-SST binds to the five sst receptor subtypes with nanomolar affinity (Table 1).⁸ To get insight into the contribution of specific receptor subtypes in the central inhibitory effect of ODT8-SST, we used available selective sst peptide agonists. The potent selective sst_1 agonist ($\text{IC}_{50} 0.19 \pm 0.04 \text{ nM}$)³⁶ injected i.c.v. also prevented the increased defecation and delayed gastric emptying resulting from the acute stress of i.c.v. vehicle injection and anesthesia. Similarly, the sst_1 agonist prevented the abdominal-surgery-induced delay of gastric emptying as assessed by liquid gastric emptying. However,

the sst₁ agonist CH-275 injected i.c.v. did not significantly alter fecal pellet output under the present experimental conditions which is likely due to the 150-fold lower affinity of CH-275 to the sst₁ (IC₅₀: 30.9 ± 13 nM)⁴⁹ compared to the sst₁ agonist described above. In addition, peptides lacking affinity to the sst₁ receptor including octreotide (IC₅₀: sst₁ < 1000 nM)⁵⁰, the selective sst₂ (IC₅₀: sst₂ 0.75 ± 0.2; sst₁ > 1000; nM)³⁷ or sst₄ agonist (IC₅₀: sst₄ = 1.4 ± 0.1; sst₁ 650 ± 115 nM)³⁸ tested under similar conditions had no effect on defecation. The role of sst₂ which is the most prominent receptor subtype expressed in the mouse brain⁵¹ was further ruled out by the demonstration that the selective sst₂ antagonist³⁹ did not modify the i.c.v. ODT8-SST inhibitory effect on acute stress-related defecation when injected i.c.v. at a dose preventing the orexigenic effect of ODT8-SST monitored simultaneously. Moreover, the selective sst₂ agonist has an opposite effect by further increasing the number of excreted fecal pellets during water avoidance stress after 30 min of stress exposure. With regard to the endogenous ligands, SRIF-28 injected i.c.v. also reduced defecation while i.c.v. SRIF-14 injected at a similar nanomolar dose as SRIF-28 had no effect. Likewise, other studies showed that several central actions displayed by SRIF-28 and ODT8-SST to influence glucoregulation, thermoregulation, ACTH and epinephrine plasma levels as well as gastric secretory and motor functions are not reproduced by i.c.v. or i.c. injection of SRIF-14 in rats.^{9, 10, 33, 52} *In vitro* studies also showed that SRIF-14 and SRIF-28 induce opposite effects on potassium currents in rat neocortical neurons.⁵³ The differential central actions of SRIF-28 and SRIF-14 are so far little understood. In the present study, it may be speculated that in addition to the primary effect mediated by the activation of the brain sst₁ receptor, the sst₅ receptor also contributes since SRIF-28 displays a 100-fold selectivity on the mouse sst₅ receptors compared to SRIF-14⁵⁴ and in recombinant systems, sst₁ and sst₅ receptors may form heterodimers which confers distinct signaling properties.⁵⁵ In the present study, we observed that ODT8-SST injected i.c.v. at 0.22 or 0.75 nmol/mouse reduces the 1-h fecal pellet output by 41% and 74% respectively and the sst₁ agonist by 0% and 41% respectively *versus* i.c.v. vehicle. Therefore, possible additional contributions of sst₅ receptors in the central effects of SRIF-28 and ODT8-SST will need to be further characterized using selective peptide sst₅ agonists and antagonists which so far have not been developed. Irrespectively of the potential additional involvement of sst₅, the demonstration that the selective sst₁ agonist mimics the ODT8-SST-induced blockade of both gastric and colonic motor alterations induced by acute stressors provides the first pharmacological evidence that central activation of sst₁ receptors prevents acute stress-related gut motor functions in mice.

The brain sites involved in the ODT8-SST and sst₁ agonist actions to prevent the acute stress-induced delay of gastric emptying and the stimulation of propulsive distal colonic motility may involve those regulating CRF release and downstream CRF action since i.c.v. injection of ODT8-SST counteracts both, acute stress- and i.c.v. CRF-induced stimulatory effects on colonic propulsive motor function. Previous studies indicate that the PVN is responsive to exogenous CRF and involved in acute restraint stress-induced inhibition of gastric emptying and stimulation of colonic motor function in rats.⁵⁶ A number of investigations using autoradiography, *in situ* hybridization and immunohistochemistry have established the regional distribution of the sst₁ receptor in the mouse brain including the PVN and in addition the hippocampus, central amygdala, median raphe nucleus, magnocellular preoptic nucleus, arcuate nucleus and cortical areas^{31, 57} indicative of other possible sites of action as part of stress-related circuitries at which the peptides may act.⁵⁸ At the cellular level, sst₁ receptor activation unlike that of other sst receptor subtypes has been reported to reduce hippocampal excitability.⁵⁹ It may be speculated that activation of sst₁ receptors dampens the stress-related increase in neuronal activity well established to occur in specific brain nuclei in response to stress.⁶⁰ In that context, it will be of relevance to establish whether other previously established central effects of i.c.v. injected ODT8-SST or SRIF-28, to block the acute stress-related rise in circulating ACTH and epinephrine

plasma levels in rats, are also mimicked and primarily mediated by the activation of $ss1$ receptors. It was recently shown that central oxytocin signaling counteracts the water avoidance stress stimulation of defecation as well as distal colonic motility in rats.²⁵ Oxytocin injected i.c.v. decreased the number of CRF immunoreactive cells in the PVN and inhibited the stimulated corticosterone release²⁵ giving rise to a negative interaction with the central CRF signaling system. Similarly, oxytocin injected i.c.v. restored the delayed gastric emptying following acute restraint or a chronic heterotypic stress in rats.⁶¹ In addition, the acute and chronic stress-induced increased CRF mRNA levels were reduced by i.c.v. injection of oxytocin.⁶¹ Therefore, one may also speculate that ODT8-SST recruits oxytocin pathways to exert its effects on stress-induced altered gastric and colonic motility. This assumption is further supported by our previous findings in rats showing that ODT8-SST injected i.c.v. activates a large proportion of oxytocin positive neurons in the supraoptic nucleus as well as magnocellular PVN.⁶²

The time course study of the central action of ODT8-SST on the colon showed that the rapid onset inhibition occurring during the first hour is followed by an increase in fecal pellet output. Convergent evidence supports that the additional stimulatory effect on defecation compared to that of i.c.v. vehicle injection is secondary to the $ss2$ mediated concurrent increase in food intake. We showed that ODT8-SST significantly increases food consumption by 3.5- and 1.7-times during the 2nd hour and 2-4 h period post i.c.v. injection respectively consistent with our previous report in rats and mice.¹¹ Moreover, the $ss2$ antagonist injected i.c.v. completely blocked the ODT8-SST-induced stimulation of food intake and fecal pellet output occurring at 2-h post injection while not altering the reduction of defecation during the 1st h. Lastly, when mice did not have access to food after the i.c.v. injection of ODT8-SST, there was no enhanced defecation at 2-4 h while the inhibitory effect during the 1st hour was maintained. These data are consistent with the previously established $ss2$ mediated long-lasting orexigenic effect of i.c.v. ODT8-SST in freely fed rodents.^{11, 13} In particular, the activation of brain $ss2$ receptors was characterized to increase the number of meals, the rate of ingestion and reduce the inter-meal intervals in mice.¹³ The $ss2$ mediated stimulation of defecation is secondary to the orexigenic effect most likely related to increased distal colonic motility associated with a meal as reported in other species.⁶³

In conclusion, ODT8-SST and SRIF-28, which interact with the 5 sst receptor subtypes, when injected i.c.v. counteract the colonic stimulation and gastric motor inhibition in response to acute stressors. The peptide's anti-stress action is mimicked mainly by the activation of the $ss1$ receptor suggesting an important role for this brain sst receptor subtype in the modulation of the gastrointestinal response to activation of brain CRF pathways. As a number of peptides including NPY, cocaine amphetamine-regulated transcript, glucagon-like peptide and interleukin-1 act in the brain to stimulate colonic motor function through activation of CRF pathways,^{20, 64-66} these data suggest that SRIF-28 and activation of $ss1$ receptors may have the potential to interfere with a number of peptides recruiting brain CRF signaling to exert their stimulatory action on the colon. Of importance, the present data point toward a potential role of brain SRIF-28 as an endogenous modulator of stress-related circuitries regulating gastrointestinal motor functions.

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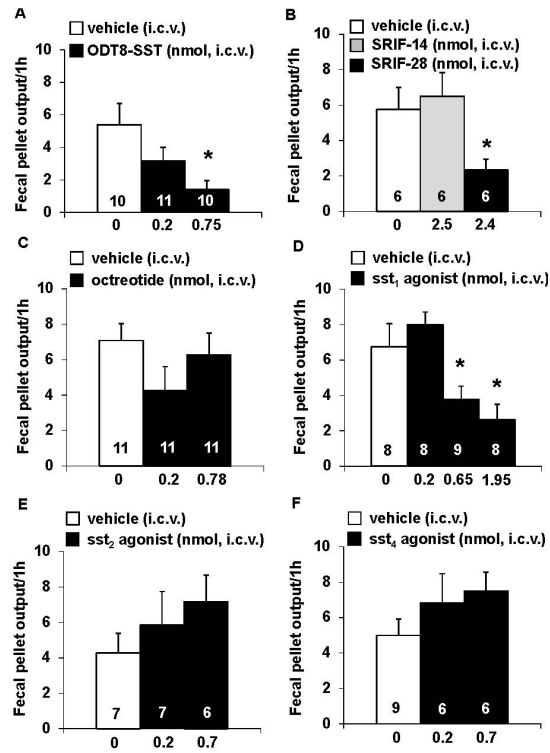
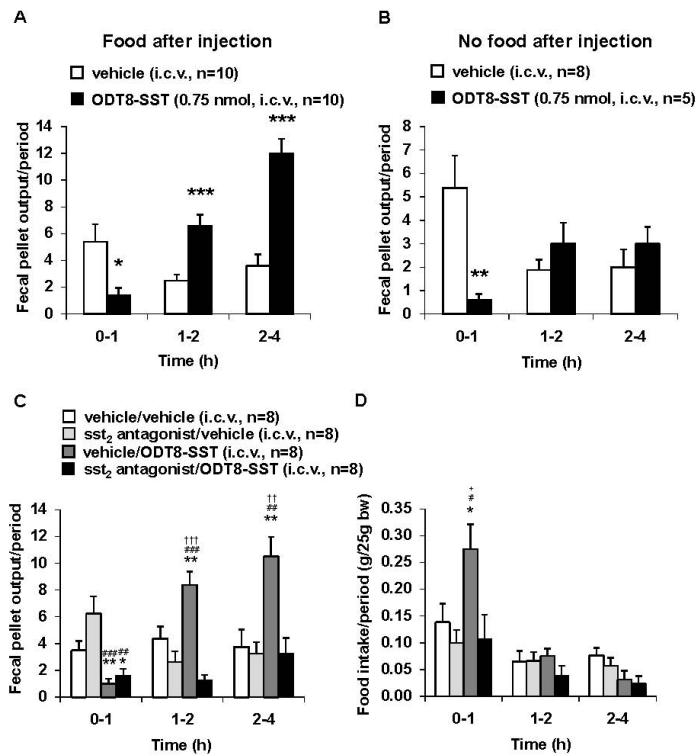


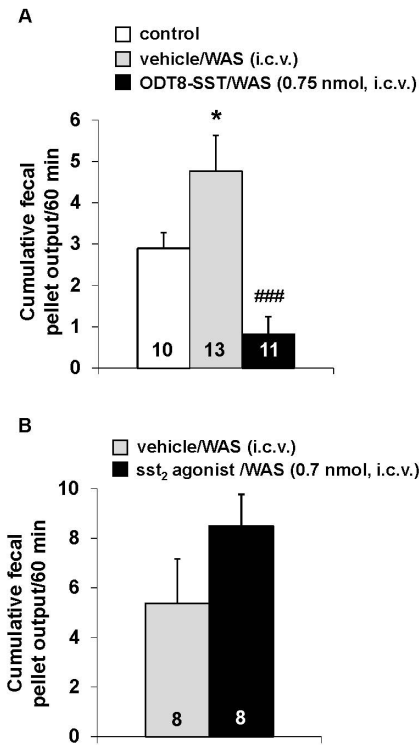
Figure 1.

ODT8-SST, the selective sst₁ agonist and somatostatin-28 injected intracerebroventricularly reduced fecal pellet output whereas somatostatin-14, octreotide and other selective sst₂ and sst₄ agonists did not. *Ad libitum* fed mice were acutely injected i.c.v. under short isoflurane anesthesia with ODT8-SST (0.22 or 0.75 nmol/mouse in 5 μ l ddH₂O, A), somatostatin-14 (2.5 nmol/mouse in 5 μ l ddH₂O containing 0.1% BSA, B), somatostatin-28 (2.4 nmol/mouse in 5 μ l ddH₂O containing 0.1% BSA, B), octreotide (0.24 or 0.78 nmol/mouse, C) the selective sst₁ (0.19, 0.65 or 1.95 nmol/mouse, D), sst₂ (0.21 or 0.70 nmol/mouse, E) and sst₄ agonists (0.21 or 0.70 nmol/mouse, F) and or vehicle (5 μ l ddH₂O or 5 μ l ddH₂O containing 0.1% BSA) and fecal pellet output was assessed for 1 h. Each bar represents the mean \pm sem of number of mice indicated at the bottom of the columns. * $P < 0.05$ vs. vehicle.

**Figure 2.**

(A, B) Time course of dual effects of ODT8-SST injected intracerebroventricularly on fecal pellet output: role of food intake post injection. Mice were injected i.c.v. with ODT8-SST (0.75 nmol/mouse in 5 μ l ddH₂O) or vehicle (5 μ l ddH₂O) and thereafter had access (A,) or no access (B) to food. Fecal pellet output was assessed at 1, 2 and 4 h post injection and expressed as fecal pellet output/period. Each bar represents the mean \pm sem of number of 5-10 mice. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. vehicle.

(C, D) The sst₂ antagonist injected intracerebroventricularly (i.c.v.) blocked the stimulatory effect of i.c.v. ODT8-SST on food intake but not the inhibition of fecal pellet output. Mice were injected i.c.v. with sst₂ antagonist (0.66 nmol/mouse in 2.5 μ l ddH₂O) or vehicle (2.5 μ l ddH₂O) followed by ODT8-SST (0.75 nmol/mouse in 2.5 μ l ddH₂O) or vehicle (2.5 μ l ddH₂O) and fecal pellet output (C) and food intake (D) were assessed at 1, 2, and 4 h post injection and expressed as food intake as well as fecal pellet output/period. Each bar represents the mean \pm sem of number of 5-10 mice. * $P < 0.05$ and ** $P < 0.01$ vs. vehicle/vehicle; # $P < 0.05$, ## $P < 0.01$ and ### $P < 0.001$ vs. sst₂ antagonist/vehicle; + $P < 0.05$, ++ $P < 0.01$ and +++ $P < 0.001$ vs. vehicle/ODT8-SST; †† $P < 0.01$ and ††† $P < 0.001$ vs. sst₂ antagonist/ODT8-SST.

**Figure 3.**

ODT8-SST injected intracerebroventricularly blocked the water avoidance stress-induced increased fecal pellet output whereas the selective *sst*₂ agonist temporarily further increased fecal pellet output. Mice fed *ad libitum* until the start of the experiment were injected i.c.v. with ODT8-SST (0.75 nmol/mouse in 5 μ l ddH₂O, A) *sst*₂ agonist (0.70 nmol/mouse in 5 μ l ddH₂O, B) or vehicle (5 μ l ddH₂O) under short inhalation anesthesia and allowed to completely recover within ~ 4 min. Afterwards, mice were subjected to water avoidance stress (WAS) and fecal pellet output assessed for 60 min. Control mice were placed in single housing cages and 1-h fecal pellet output was monitored in 15-min intervals. Each bar represents the mean \pm sem of number of mice indicated at the bottom of the columns. * $P < 0.05$ vs. control; ### $P < 0.001$ vs. vehicle/stress.

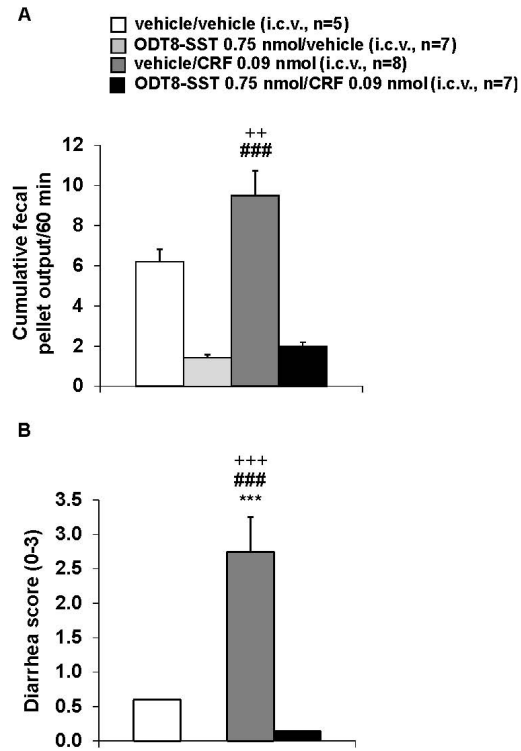


Figure 4.

ODT8-SST injected intracerebroventricularly (i.c.v.) completely blocked the i.c.v. CRF-induced increase in fecal pellet output and diarrhea score in mice. Mice were injected i.c.v. with ODT8-SST (0.75 nmol/mouse in 2.5 μ l ddH₂O) or vehicle (2.5 μ l ddH₂O) followed by i.c.v. CRF (0.09 nmol/mouse in 2.5 μ l ddH₂O) or vehicle (2.5 μ l ddH₂O) under short inhalation anesthesia and after recovery fecal pellet output and diarrhea score (0 indicating no diarrhea, 1: 1 watery and/or loose pellets, 2: 2 watery and/or loose pellets and 3 indicating 3 watery and/or loose pellets) were assessed for 60 min. Each bar represents the mean \pm sem of 5-8 mice/group. *** $P < 0.001$ vs. vehicle/vehicle; ## $P < 0.01$ and ### $P < 0.001$ vs. ODT8-SST/vehicle; +++ $P < 0.001$ vs. ODT8-SST/CRF.

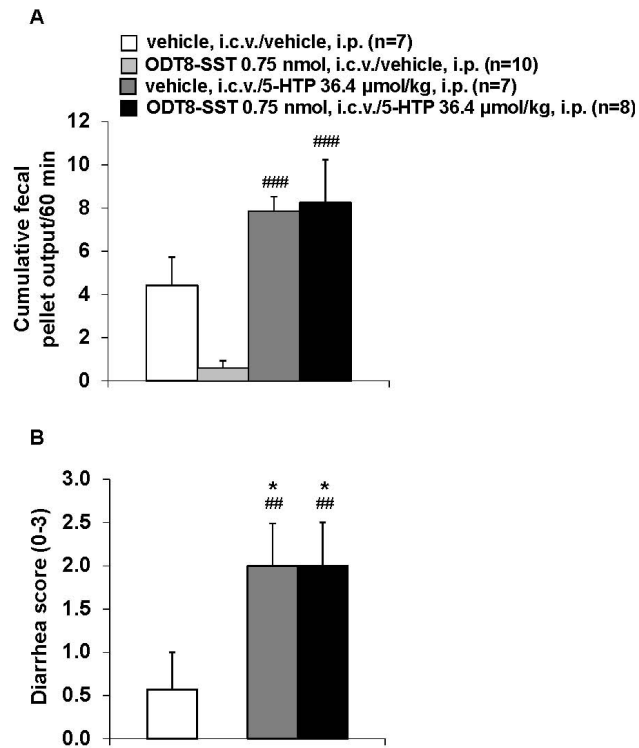


Figure 5.

ODT8-SST injected intracerebroventricularly did not influence the intraperitoneal 5-HTP-induced increased fecal pellet output and diarrhea score. Mice were injected i.c.v. with ODT8-SST (0.75 nmol/mouse in 5 μl ddH₂O) or vehicle (5 μl ddH₂O). After complete recovery from anesthesia, mice received an i.p. injection of 5-HTP (36.4 μmol/kg in 100 μl saline) or vehicle (100 μl saline) and fecal pellet output and diarrhea score (0 indicating no diarrhea, 1: 1 watery and/or loose pellets, 2: 2 watery and/or loose pellets and 3 indicating 3 watery and/or loose pellets) were monitored for 60 min post i.p. injection. Each bar represents the mean ± sem of number 7-10 mice/group. * $P < 0.05$ and vs. vehicle/vehicle; ## $P < 0.01$ and ### $P < 0.001$ vs. ODT8-SST/vehicle.

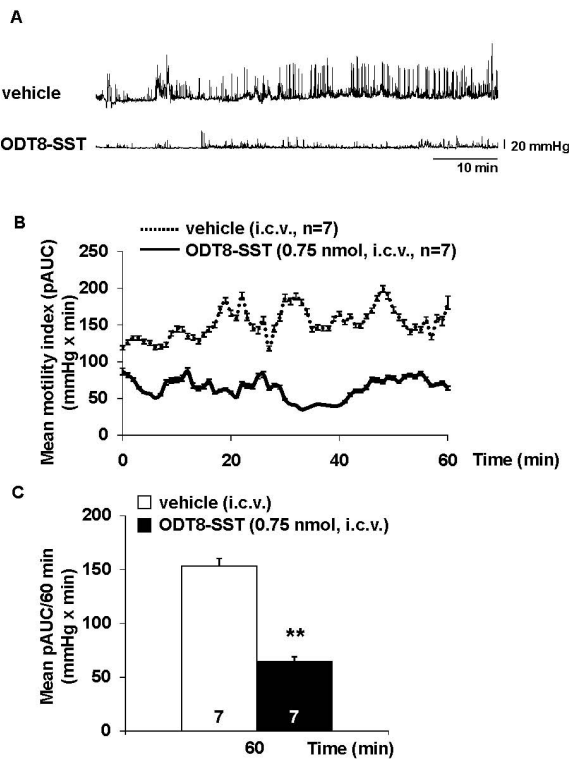


Figure 6.

ODT8-SST injected intracerebroventricularly reduced distal colonic contractions in mice. Mice were injected i.c.v. with ODT8-SST (0.75 nmol/mouse in 5 μ l ddH₂O) or vehicle (5 μ l ddH₂O) under short inhalation anesthesia and immediately thereafter a miniature pressure transducer catheter was inserted into the distal colon (2 cm proximal to the anus). Mice were placed in partial restraint tubes and distal colonic contraction recorded for 1 h starting ~ 4 min post i.c.v. injection. (A) shows a representative raw trace of distal colonic pressure after i.c.v. vehicle and ODT8-SST. (B) Time course (during 60 min) expressed as intraluminal pressure trace of the area under the curve (pAUC) quantified every minute. (C) mean pAUC calculated over a period of 1 h. Data are expressed as mean \pm sem of 7 mice/group. ** $P < 0.01$ vs. vehicle.

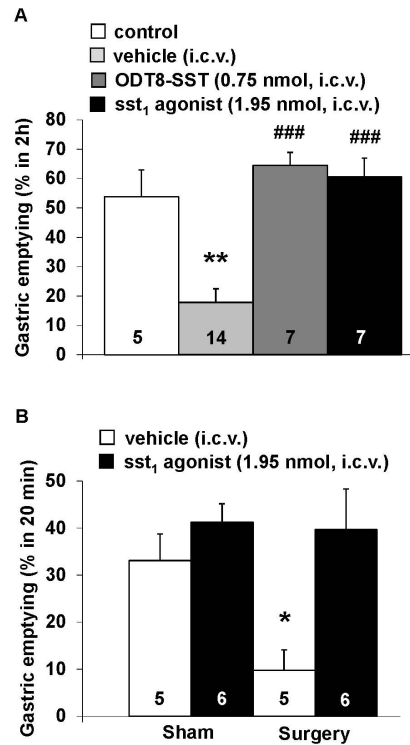


Figure 7.

ODT8-SST and the selective sst₁ agonist injected intracerebroventricularly prevented the stress-related delayed gastric emptying of a solid meal and the sst₁ agonist prevented the abdominal surgery-induced delayed gastric emptying of a liquid meal. (A) Mice were fasted overnight for 15 h and re-fed with pre-weighed standard chow starting at 08:00 h for 1 h. Then, food and water were removed and injected i.c.v. with ODT8-SST (0.75 nmol/mouse in 5 μ l ddH₂O), sst₁ agonist (1.95 nmol/mouse in 5 μ l ddH₂O) or vehicle (5 μ l ddH₂O) and gastric emptying for the solid meal was assessed 2 h later. Control mice were left undisturbed without i.c.v. injection and gastric emptying was assessed at 2 h after re-feeding. Each bar represents the mean \pm sem of number of mice indicated at the bottom of the columns. ** $P < 0.01$ vs. control; ### $P < 0.001$ vs. vehicle i.c.v. (B) Overnight fasted mice were injected i.c.v. with sst₁ agonist (1.95 nmol/mouse in 5 μ l ddH₂O) or vehicle (5 μ l ddH₂O) and afterwards underwent abdominal surgery or sham procedure. Animals received an orogastric gavage of a liquid non-nutrient solution 30 min after the end of the procedure and gastric emptying was assessed 30 min later. Each bar represents the mean \pm sem of number of mice indicated at the bottom of the columns. * $P < 0.0$ vs. all other groups.

Table 1

Structure and receptor binding affinity of somatostatin receptor agonists.

Peptide ^{Reference}	Structure	Receptor binding affinity (IC ₅₀ , nM) ^a				
		sst ₁	sst ₂	sst ₃	sst ₄	sst ₅
ODT8-SST ⁸	des-AA ^{1,2,4,5,12,13} -(DTrp ⁸)-SRIF	27.0 ± 3.4	41.0 ± 8.7	13.0 ± 3.2	1.8 ± 0.7	46.0 ± 27.0
sst ₁ agonist (compound 25) ³⁶	des-AA ^{1,4-6,10,12,13} -[DTyr ² ,D-Agl(NMe,2naphthoyl) ⁸ ,IAmp ⁹]-SRIF-Thr-NH ₂	0.19 ± 0.04	> 1K	158.0 ± 14.0	27.0 ± 7.5	> 1K
sst ₁ agonist CH-275 (compound 2) ³⁶	des-AA ^{1,2,5} -[DTrp ⁸ ,IAmp ⁹]-SRIF	31 ± 13	> 1K	540; 150	>1K	>1K
sst ₂ agonist (compound 2) ³⁷	des-AA ^{1,4-6,11-13} -[DPhe ² ,Aph ⁷ (Cbm),DTrp ⁸]-Cbm-SRIF-Thr-NH ₂	> 1K	7.5 - 20	942 - 1094	872 - 957	109 - 260
sst ₄ agonist (compound 15) ³⁸	des-AA ^{1,2,4,5,12,13} -[Aph ⁷]-Cbm-SRIF	650 ± 115	> 1K	780 ± 62	1.5 ± 0.07	> 1K
somatostatin-14 (SRIF-14) ⁶⁷	Ala-Gly-c[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys]-OH	0.1 - 1.5	1.7	1.7	1.0 - 1.6	0.2 - 2.2
somatostatin-28 (SRIF-28) ⁶⁷	Ser-Ala-Asn-Ser-Asn-Pro-Ala-Met-Ala-Pro-Arg-Glu-Arg-Lys-Ala-Gly-c[Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys]-OH	0.1 - 4.7	0.4 - 5.2	0.2	0.3 - 1.1	0.05 - 0.19
octreotide ⁵⁰	H-(D)-Phe ² -c[Cys ³ -Phe ⁷ -DTrp ⁸ -Lys ⁹ -Thr ¹⁰ -Cys ¹⁴]-Thr ¹⁵ (ol)	> 1K	1.9 ± 0.3	39 ± 14	> 1K	5.1 ± 1.1
sst ₂ antagonist (compound 4) ³⁹	des-AA ^{1,4-6,11-13} -[pNO ₂ -Phe ² ,DCys ³ ,Tyr ⁷ ,DAph(Cbm) ⁸]-SRIF-2Nal-NH ₂	> 1K	2.6 ± 0.7	384.0 ± 97.0	> 1K	> 1K

Derived from competitive radio ligand displacement assays in cells stably expressing the cloned human receptor using ¹²⁵I-[Leu⁸DTrp²²Tyr²⁵]SRIF-28⁸, 36-39, 50 except for SRIF-14 and SRIF-28.⁶⁷