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SELECTIVE CENTRAL ACTIVATION OF SOMATOSTATIN RECEPTOR 2 INCREASES FOOD INTAKE, GROOMING BEHAVIOR AND RECTAL TEMPERATURE IN RATS

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Abstract

The consequences of selective activation of brain somatostatin receptor-2 (sst_2) were assessed using the sst_2 agonist, des-AA$^{1,4–6,11–13}$-[DPhe^2,Aph7(Cbm),DTrp^8]-Cbm-SST-Thr-NH_2. Food intake (FI) was monitored in *ad libitum* fed rats chronically implanted with an intracerebroventricular (i.c.v.) cannula. The sst_2 agonist injected i.c.v. at 0.1 and 1 μg/rat dose-dependently increased light phase FI from 2 to 6 hours post injection (2.3±0.5 and 7.5±1.2 respectively *vs*. vehicle: 0.2±0.2 g/300 g bw, $P<0.001$). Peptide action was reversed by i.c.v. injection of the sst_2 antagonist, des-AA$^{1,4–6,11–13}$–[pNO_2-Phe^2,DCys^3,Tyr^7,DAph(Cbm)^8]-SST-2Nal-NH_2 and not reproduced by intraperitoneal injection (30 μg/rat). The sst_2 antagonist alone i.c.v. significantly decreased the cumulative 14-hours dark phase FI by 29.5%. Other behaviors, namely grooming, drinking and locomotor activity were also increased by the sst_2 agonist (1 μg/rat, i.c.v.) as monitored during the 2nd hour post injection while gastric emptying of solid food was unaltered. Rectal temperature rose 1 hour after the sst_2 agonist (1 μg/rat, i.c.v.) with a maximal response maintained from 1 to 4 hours post injection. These data show that selective activation of the brain sst_2 receptor induces a feeding response in the light phase not associated with changes in gastric emptying. The food intake reduction following sst_2 receptor blockade suggests a role of this receptor in the orexigenic drive during the dark phase.

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Keywords

somatostatin; *somatostatin receptor-2*; *behavior*; *body temperature*; *food intake*; *gastric emptying*; *rat*; *feeding*; *grooming*

INTRODUCTION

Somatostatin-14 was originally isolated from ovine hypothalami and established as physiological inhibitor of pituitary growth hormone (GH) secretion (1). Like a number of other hypothalamic releasing hormones, soon after its characterization, somatostatin was shown to act in the brain to induce a number of pituitary-independent effects (2, 3). In particular, somatostatin was reported to alter food intake upon central administration, although responses diverged with evidence for an increase (4–8), a decrease (5, 9, 10), or a biphasic effect (11) in food intake. These discrepancies may be related to the doses used as somatostatin increased food intake when injected at low picomolar doses (0.4–4.0 pmol/rat) intracerebroventricularly (i.c.v.) or into the anterior piriform cortex, whereas nanomolar doses (2–3 nmol/rat) decreased 1-hour food ingestion (5, 10, 12). High doses of i.c.v. injected somatostatin may also result in peptide leakage into the circulation increasing plasma levels of somatostatin (13) which could inhibit the orexigenic peptide, ghrelin (14, 15) and thereby food intake.

Somatostatin can interact with five distinct receptor subtypes termed $sst_{1–5}$ which belong to the seven transmembrane domain superfamily of G-protein coupled receptors (16). Pharmacological characterization of these receptors showed that somatostatin displays a nanomolar affinity to sst_2 including the variant forms sst_{2A} and sst_{2B} generated by alternative splicing of sst_2 mRNA (17) as well as to $sst_{3–5}$, while exhibiting lower affinity to sst_1 (16, 18). Somatostatin receptors are widely expressed in the brain with specific patterns of distribution, although regional overlap exists between receptor subtypes (19, 20). The expression of the sst_{2A} receptor at the gene and protein levels in the brain, determined by *in situ* hybridization and immunohistochemistry, encompasses, among others, hypothalamic nuclei regulating food intake such as the supraoptic, paraventricular and arcuate nuclei as well as the ventromedial and lateral hypothalamus in rats and mice (21–23). So far, receptor subtypes involved in somatostatin's influence on food intake have been largely unexplored and recruitment of different subtypes may have a bearing with differential actions of somatostatin on food intake depending upon the dose injected. Although recently somatostatin analogues displaying selectivity toward specific somatostatin receptor subtypes have been developed (24, 25), they have been scantly used as tools to delineate receptor subtypes involved in the established central actions of somatostatin to influence food intake (4, 26), behavior (27) or thermoregulation (28). The most commonly used stable oligosomatostatin receptor analog is octreotide (SMS 201–995) that binds to three of the five receptor subtypes, namely sst_2, sst_3 and sst_5 with a higher affinity for sst_2 (16, 24, 29). We previously reported that this oligosomatostatin analog also acts in the brain to promote digestive function by stimulating gastric acid secretion (30). The high affinity of somatostatin and octreotide to sst_2 receptors (16, 24, 29), and the broad distribution at the gene and protein levels in the rat hypothalamus compared with the more restricted

hypothalamic distribution of other somatostatin receptor subtypes (22, 23), provide neuroanatomic support for a role of the sst_2 receptor subtype in mediating the food intake, behavioral and thermogenic responses induced by somatostatin.

In the present study, we examined in *ad libitum* fed rats whether the recently developed selective sst_2 peptide agonist, des-$AA^{1,4–6,11–13}$-[$DPhe_2$,Aph^7(Cbm),$DTrp^8$]-Cbm-SST-Thr-NH_2 (sst_2 IC_{50}: 7.5–20 nM) (24) promotes feeding in the light phase associated with low drive for spontaneous food intake following i.c.v. *versus* intraperitoneal (i.p.) injection of the peptide. We further investigated the receptor specificity of the sst_2 agonist's action and the potential implication of the sst_2 receptor in the modulation of dark phase food intake in *ad libitum* fed rats using the novel sst_2 antagonist, des-$AA^{1,4–6,11–13}$-[pNO_2-Phe^2,$DCys^3$,Tyr^7,$DAph(Cbm)^8$]-SST-2Nal-NH_2. (31) In addition, as somatostatin-28 or specific oligosomatostatin analogs were previously shown to act in the brain to increase rectal temperature and gastric emptying in rats, we explored possible mechanisms involved in the i.c.v. sst_2 agonist's orexigenic action by assessing associated alterations in thermoregulation and gastric transit time. We also assessed whether the sst_2 agonist's action on food intake was selective for feeding behavior or associated with other behavioral changes such as grooming or locomotor activity as induced by i.c.v. injection of somatostatin.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (Harlan Laboratories, San Diego, CA) weighing 280–350 g were housed 4/cage under controlled illumination (6:00 AM to 6:00 PM) and temperature (21–23°C). Animals had free access to standard rodent chow (Prolab RMH 2500; LabDiet, PMI Nutrition, Brentwood, MO) and tap water. Protocols were approved by the Veterans Administration Institutional Animal Care and Use Committee (# 99-127-07). All experiments unless otherwise stated were started between 9:00 and 10:00 AM.

Peptides

The sst_2 agonist, des-$AA^{1,4–6,11–13}$-[$DPhe^2$,Aph^7(Cbm),$DTrp^8$]-Cbm-SST-Thr-NH_2, MW: 1132.5, compound #3 in (24) and the sst_2 antagonist, des-$AA^{1,4–6,11–13}$-[pNO_2-Phe^2,$DCys^3$,Tyr^7,$DAph(Cbm)^8$]-SST-2Nal-NH_2, MW: 1208.4, compound #4 in (31) (Clayton Foundation Laboratories, Salk Institute, La Jolla, CA) were synthesized as previously described (24, 31) and purity characterized by high pressure liquid chromatography, capillary zone electrophoresis and mass spectrometry. In all cases, peptide content (expressed in pmol and nmol) corresponds to actual weight minus TFA counter-ion and water of lyophilization estimated to be 20% lower than peptide weight expressed in μg. Peptides were kept in powder form at −80°C and dissolved in pyrogen-free distilled water (vehicle) immediately before administration except otherwise stated.

Intracerebroventricular injection

Intracerebroventricular cannulation and injections were performed as previously described (32). Rats were anesthetized with an i.p. injection of a mixture of ketamine hydrochloride

(75 mg/kg bw, Ketanest, Fort Dodge Laboratories Inc., Fort Dodge, IA) and xylazine (5 mg/kg, Rompun, Mobay Corporation, Shawnee, KS), placed in a stereotaxic apparatus, and implanted with a chronic guide cannula (22-gauge, Plastic One Products, Roanoke, VA) into the right lateral brain ventricle. Stereotaxic coordinates obtained from the Paxinos and Watson brain atlas (33) were (from skull surface) 0.8 mm posterior, 1.5 mm right lateral, and 3.5 mm ventral to the bregma. The guide cannula was secured by dental cement and anchored by four stainless steel screws (Plastics One Inc.) fixed to the skull with dental cement (Stoelting Co., Wood Dale, IL) and occluded. After surgery, animals were allowed to recover for 7 days and housed individually. During that time rats were handled for 5 days to become accustomed to i.c.v. injection through the guide cannula. For i.c.v. injections, a 28-gauge cannula (1 mm longer than the guide cannula) connected to a 25 μl Hamilton syringe by a PE-50 tube (Intramedic Polyethylene Tubing, Clay Adams, NJ) was filled with injection solution and inserted into the guide cannula and 10 μl except otherwise stated were delivered by pressure injection over 1 min in lightly hand restrained conscious rats. At the end of the experiments, the correctness of injection into the lateral ventricle was verified by injecting 10 μl dye (0.1% toluidine blue) under similar conditions and assessing selective dye distribution in the ventricle thereafter. No animals were excluded from data analysis.

Food intake experiments

Singly housed, *ad libitum* fed, chronically i.c.v. cannulated rats were injected i.c.v. during the light phase with the sst_2 agonist or vehicle and thereafter maintained in their familiar single housing cages with a reduced amount of bedding. Food intake was monitored up to 24 hours and water intake simultaneously for 9 hours. The i.c.v. doses of the sst_2 agonist were based on previous reports showing no barrel rotation after i.c.v. injection of somatostatin or SMS 201–995 at doses ≤1 μg (34). Based on dose response results, 1 μg/rat i.c.v. was selected for all further studies. In subsequent experiments, the i.c.v. injection (5 μl) of the sst_2 antagonist (1 μg/rat) or vehicle was followed by i.c.v. injection (5 μl) of the sst_2 agonist (1 μg/rat) or vehicle. Cumulative food intake was measured over a period of 9 hours. To assess the influence of sst_2 receptors on dark phase food intake, the sst_2 antagonist (1 μg/rat) or vehicle was injected i.c.v. at the onset of the dark phase. The dose of the sst_2 antagonist was based on pilot study data showing the blockade of the central orexigenic response induced by an oligosomatostatin agonist by the sst_2 antagonist injected at 1 μg/rat i.c.v. In other studies, freely fed rats without i.c.v. cannula and housed 2/cage were accustomed to single housing 8 hours/day for three days prior to the experiment, then they were injected intraperitoneally (i.p., 0.3 ml) with vehicle (saline) or sst_2 agonist (30 μg/kg in saline). Rats were singly housed after injection and food intake was monitored for 24 hours.

All food intake experiments except for one, as mentioned above, were started during the light phase between 9:00 AM and 10:00 AM and repeated in a crossover design. Food intake was assessed by calculating the difference between pre-weighed chow and remaining chow and spillage at different time intervals post injection and expressed as cumulative or non-cumulative food intake for time-related intervals in g/300g body weight (bw).

Behaviors

Ad libitum fed, singly housed, chronically i.c.v. cannulated rats were injected i.c.v with sst_2 agonist (1 μg/rat) or vehicle and placed in their home cage (42.9 × 22.4 cm) with the bottom covered with a paper divided into 6 equal squares (14.3 × 11.2 cm). Animals had free access to food and water post injection. During the 2nd hour post injection, at the time of a significant increase in food intake based on previous experiments, the specific behaviors consisting of grooming (including scratching, licking and washing), locomotor activity (total number of squares crossed), food intake (including food approach) and water intake (including water approach) were monitored by two observers which sat motionless in the room. Each behavioral component was counted again when lasting longer than 5 sec. The investigators were blinded to the animals' treatment.

To control for pica behavior, i.c.v. cannulated rats were injected with sst_2 agonist (1 μg/rat) or vehicle, and thereafter maintained in their single housing cages without access to food for 2 hours, and possible ingestion of bedding material was monitored.

Rectal temperature

Ad libitum fed, singly housed, chronically i.c.v. cannulated rats were injected i.c.v. with sst_2 agonist (1 μg/rat) or vehicle and had access to food and water after injection. Rectal temperature was measured before and at 1, 2, 4, 10 and 22 hours post injection using a digital thermometer (Lumiscope Co., Inc., Piscataway, NJ). For each measurement, the thermometer lubricated with chlorhexidine gluconate (Surgilube, E. Fougera & Co., Atlanta Inc., NY) was inserted into the rectum of lightly hand restrained rats and left for 10 sec to obtain a stable reading. Rats were handled for three days before the experiment to get accustomed to the temperature measurement procedure.

Gastric emptying

Gastric emptying of a standard rodent chow meal was performed as described previously (35). Rats were fasted for 20 hours and re-fed starting at 7:00 AM for 2 hours, then food and water were removed and vehicle (pyrogen-free distilled water i.c.v. or saline i.p.) or sst_2 agonist (1 μg/rat i.c.v. or 30 μg/kg i.p.) was injected. Gastric emptying was assessed 2 hours later. Animals were anesthetized by CO_2 inhalation, the abdominal and thoracic cavities opened and rats euthanized by cardiac incision. Afterwards, pylorus and cardia were clamped, and the stomach removed. The gastric content was collected, dried for 24 hours at 37°C and weighed. The solid food ingested by the animals during re-feeding before treatment was determined by the difference between the weight of the chow before re-feeding and the weight of the remaining chow and spill at the end of the re-feeding period. The 2-h gastric emptying rate was calculated as (1-dry gastric content/food intake) × 100.

Statistical analysis

Data are expressed as mean±S.E.M. and were analyzed by one-way analysis of variance (ANOVA) followed by Tukey *post hoc* test or two-way ANOVA followed by Holm-Sidak method. Differences between groups were considered significant when $P<0.05$.

RESULTS

Intracerebroventricular injection of the sst_2 agonist increased light phase food intake in ad libitum fed rats

The sst_2 agonist injected i.c.v. (0.1 or 1 μg/rat) during the light phase induced a dose-related increase in food intake starting at 2 hours post injection in *ad libitum* fed rats chronically i.c.v. cannulated and singly housed thereafter (Fig. 1A). The sst_2 agonist at 0.1 μg/rat compared with 1 μg/rat caused a similar magnitude of food intake increase at 2 hours, however, at 4 hours post injection values were 42% lower and no longer different from vehicle. By contrast, the sst_2 agonist injected at 1 μg/rat resulted in a significant increase of cumulative food intake from the 2nd hour ($P<0.05$) up to 9 hours post injection ($P<0.001$) while the 24-hours value was not different compared to vehicle-treated rats (Fig. 1A). Moreover, only the dose of 1 μg/rat significantly increased food intake during the 1st to 2nd hour ($P<0.05$), 2nd to 4th hour ($P<0.01$) and 4th to 6th hour ($P<0.001$) while reducing the dark phase food intake by 49% for the 9–24 hours period post injection ($P<0.001$) compared to vehicle (Fig. 1B). We also observed that the sst_2 agonist injected at 1 μg/rat i.c.v. induced a 4.2-fold increase in cumulative water intake over the 9 hours post injection compared to vehicle ($P<0.001$; Fig. 1C). Body weight changes during the 24 hours post injection were not significantly different between vehicle and the sst_2 agonist at both doses, although there was a trend towards a decrease (% of body weight, vehicle: −0.9±0.6, n=8, *vs*. sst_2 agonist 0.1 μg/rat: −2.5±1.2, n=9 and 1 μg/rat: −3.1±0.9, n=10 respectively, $P>0.05$). When rats were injected with sst_2 agonist (1 μg/rat, i.c.v.) without access to food thereafter, no consumption of bedding material was observed (data not shown).

In contrast to i.c.v. injection, the sst_2 agonist (30 μg/kg) injected i.p. in naive rats did not alter food intake compared to rats injected i.p. with saline as monitored at 1, 2, 4, 6, 9 and 24 hours post injection and expressed as cumulative values (Table 1) or independently at each time period (data not shown).

Intracerebroventricular injection of the sst_2 antagonist blocked the i.c.v. sst_2 agonist-induced eating and decreased dark phase food intake

The sst_2 antagonist (1 μg/rat, i.c.v.) completely blocked the sst_2 agonist (1 μg/rat, i.c.v.)-induced increase in food intake during the 9-hours measurement period ($P<0.01$) while alone having no effect on food intake during the light period (Fig. 2). However, the sst_2 antagonist injected i.c.v. (1 μg/rat) at the beginning of the dark phase in *ad libitum* fed rats significantly reduced cumulative dark phase food intake at 5 h ($P<0.05$) and 14 h ($P<0.05$) post injection compared to vehicle-treated animals (Fig. 3).

Intracerebroventricular injection of the sst_2 agonist induced grooming and drinking behaviors and locomotor activity in ad libitum fed rats

The sst_2 agonist (1 μg/rat, i.c.v.) induced eating behavior including food approach ($P<0.05$) and drinking behavior including water approach ($P<0.05$) compared to vehicle as monitored during the 2nd hour post injection in the light phase (Fig. 4). Furthermore, sst_2 agonist injected rats displayed increased grooming behavior and locomotor activity ($P<0.001$; Fig. 4). No barrel rotations, reported after central injection of somatostatin at doses ≥1 μg/rat (36,

37), were observed in the present study. When injected i.p., the sst_2 agonist (30 μg/kg) did not induce changes in these behavioral parameters compared to vehicle (data not shown).

Intracerebroventricular injection of the sst_2 agonist increased rectal temperature

The rectal temperature rose significantly ($P<0.05$) at 1 hour after i.c.v. injection of vehicle in rats lightly hand restrained during the injection. Values decreased at 2 hours and thereafter, there was a linear time-related significant rise reaching a peak in the dark phase feeding period ($P<0.001$; Fig. 5). I.c.v. injection of the sst_2 agonist (1 μg/rat) caused a significant rise in rectal temperature compared to vehicle starting at 1 hour ($P<0.01$) which was maintained at 2 hours ($P<0.001$) and 4 hours ($P<0.05$; Fig. 5) post injection, whereas in the dark phase after 10 h and in the next light phase after 22 hours post injection, no differences were detectable between groups ($P>0.05$; Fig. 5).

The sst_2 agonist injected i.c.v. or i.p. did not alter gastric emptying of rats

After an overnight fast, the 2 hours food intake in the re-feeding period prior treatment was similar in the two groups (data not shown). The sst_2 agonist injected i.c.v. (1 μg/rat) did not modify the 2-hours gastric emptying of the ingested food compared to vehicle-treated rats ($P>0.05$; Table 2). Likewise, peripheral injection of the sst_2 agonist (30 μg/kg) did not change the 2-hours gastric emptying compared to vehicle ($P>0.05$; Table 2).

DISCUSSION

The present study established that the sst_2 agonist, des-$AA^{1,4–6,11–13}$-[$DPhe^2$,Aph^7(Cbm),$DTrp^8$]-Cbm-SST-Thr-NH_2 (24) injected i.c.v. acts in the brain to induce an increase in food intake, grooming and drinking behavior, locomotor activity and rectal temperature in *ad libitum* fed rats while not influencing gastric emptying. Such responses represent a selective activation of brain sst_2 receptors since the peptide displays high affinity to sst_2 (IC_{50} 7.5–20 nM), low affinity to sst_5 (IC_{50}: 109–260 nM) and no affinity to the other somatostatin receptor subtypes (IC_{50}: sst_1 > 1000 nM, sst_3 942–1094 nM, sst_4 872–957 nM) (24) and its orexigenic action was prevented by a sst_2 antagonist.

Peptides showing selectivity for recombinant somatostatin receptors have been largely unexplored regarding their central actions to influence food intake. The only previously used somatostatin sst_2/sst_3/sst_5 receptor agonist, SMS 201–995 (octreotide) (38), stimulated the 24-hours food intake during the 4 to 6 day period after continuous third ventricular infusion at 5 μg/rat/day (4). In the present study, we demonstrated that the selective sst_2 agonist injected acutely i.c.v. potently stimulates food intake in *ad libitum* fed rats. This is supported by a) the low doses (70 or 700 pmol/rat), b) the sustained orexigenic effect from 2 to 6 hours post injection and c) the ability of the peptide to induce eating in the light phase which is associated with low feeding drive (39). In addition, we established that the sst_2 agonist action is not related to an induction of pica as ingestion of bedding material in the absence of food was not stimulated. However, there was a 1-hour delay in the onset of sst_2 agonist's action which differs from that of other orexigenic peptides such as ghrelin, galanin or galanin-like peptide which upon i.c.v. injection in the light phase induced a rapid onset and short lasting food intake response in rats (40–42). We have ruled out that such a delay is

related to the time required for peptide leakage from the cerebrospinal fluid to the peripheral circulation (13), where activation of sst_2 receptors is known to inhibit gut hormones including those involved in satiety signaling, *e.g.* cholecystokinin (43). This is supported by the demonstration that the orexigenic effect of the sst_2 agonist was not reproduced by i.p. injection of the peptide at a 100-fold higher dose (~6 nmol/rat) than the maximal effective dose given i.c.v. The observed significant reduction of the nocturnal food consumption leading to similar 24-hours cumulative food intake observed in i.c.v. sst_2 agonist injected rats may be related to the satiety effect of the ingested food during the light phase reducing the drive of dark phase feeding as well as the loss of efficacy of the peptide after 9 hours post injection.

The central and peripheral mechanisms underlying the orexigenic action of the i.c.v. sst_2 agonist are still to be unraveled. The peptide may act centrally to inhibit repressing orexigenic mechanisms in place during the light phase leading to activation of downstream mediators such as agouti-related peptide (AgRP) known to have a delayed and long-lasting orexigenic effect (44). Alternatively, the sst_2 agonist could activate the hypothalamic neuropeptide Y (NPY) pathway, a well established orexigenic signal (45). In line with this assumption, somatostatin derived from the periventricular nucleus (23) was reported to activate hypothalamic NPY neurons bearing the sst_2 receptor (46). Peripheral release of ghrelin is one of the main established physiological initiators of food intake (47). Whether changes of circulating ghrelin levels contribute to the orexigenic action of the sst_2 agonist, remains to be investigated. However, existing evidence does not support a role in the initiation of the feeding response. Our recent studies using a pan-somatostatin receptor agonist injected i.c.v. at 1 μg/rat showed a sst_2 mediated rapid increase in light phase food intake in *ad libitum* fed rats without alterations of acyl ghrelin levels up to 2 hours post injection while a significant rise occurred at 3 hours post injection compared to vehicle (48). In addition, while direct stimulation of sympathetic nerves induces a rise in circulating ghrelin in rats (49), i.c.v. injection of somatostatin or ODT8-SST results in a rapid decrease in gastric sympathetic outflow (50) and blockade of stimulated circulating catecholamine release (51) in rats consistent with the lack of rise of acyl ghrelin for 2 hours post i.c.v. injection of ODT8-SST.

The physiological role of the brain somatostatin-sst_2 signaling system in the regulation of food intake in rats is supported by the low doses of somatostatin or sst_2 agonist injected either i.c.v. or into the anterior piriform cortex inducing an orexigenic response in rats (5, 12, present study). In addition, there is evidence that chronic third ventricular infusion of somatostatin antiserum (50 μl/d) over two days significantly decreased daily cumulative food intake (4). In the present study, the sst_2 antagonist injected i.c.v. at the onset of the dark phase significantly attenuated spontaneous food intake occurring in the middle of the dark phase (39). Collectively, these studies combined with reported circadian variations of hypothalamic somatostatin peaking in the early dark phase when rats show their maximum food intake and lowest levels in the early light phase when the drive to eat is low (52) suggest a contribution of hypothalamic somatostatin-sst_2 signaling in nocturnal feeding.

Somatostatin injected i.c.v. at different doses exerts opposite effects on behavior with low doses (0.01 and 0.1 μg/rat) stimulating exploration, while higher doses (1 and 10 μg/rat)

result in transient excitation followed by decreased reactivity (27). A role of the brain sst_2 receptors in regulating behavioral activity is supported by the report that sst_2 receptor deficient mice displayed reduced locomotor activity (53, 54) and conversely by the overall increased locomotor activity in response to i.c.v. sst_2 agonist (present study). Such enhanced motor activity may have a bearing with the observed trend towards a decrease in body weight at 24 hours post sst_2 i.c.v. injection. The increased locomotor activity along with food intake in response to the sst_2 agonist administered during the circadian light photoperiod reproduces features of the dark phase when rats are most active and consume the majority of their daily food intake (39). Drinking behavior was also increased by i.c.v. injection of sst_2 agonist although it cannot be dissociated whether this reflects a direct thirst-related behavior or a result of increased food ingestion.

Another central action of somatostatin in rats that was well established early on, is the induction of hyperthermia with somatostatin-28 being more potent than somatostatin-14 (55). As somatostatin-28 binds to $sst_{1–5}$ (56), the receptor subtype(s) mediating its thermoregulatory effect was yet to be delineated. The present data provide the first evidence that selective activation of central sst_2 receptors results in a thermogenic response without behavioral manifestations of sickness. The increase in rectal temperature does not seem to be secondary to the increase in food intake because the peak rise in rectal temperature occurred during the first hour post injection in the absence of changes in food intake. However, the thermogenic effect could be related, at least in part, to the increased activity. Although the hyperthermic response was similar in intensity and pattern to that produced by central injection of cytokines such as interleukin-1 (57), the other central actions induced by activation of sst_2 (increase in food intake, no change in gastric emptying) opposite to those of central interleukin-1 (suppression of food intake and gastric emptying) (57, 58), do not support the involvement of cytokines. A physiological role for hypothalamic somatostatin in thermoregulation is supported by a correlation between hypothalamic somatostatin levels and thermoregulation associated with changes in ambient temperature (28). In addition, hypothermia developed in response to central administration of a somatostatin antagonist in rats (59).

Peptides that stimulate food intake following injection into the brain such as thyrotropin-releasing hormone (TRH) (60) or ghrelin (41) also accelerate gastric transit (41, 61). However, in the present study, the i.c.v. sst_2 agonist-induced stimulation of ingestive behavior was not accompanied by changes in gastric emptying. These data extend our previous findings showing that the injection of NC-8-12, a selective sst_2 and sst_3 agonist, into the cisterna magna had no effect on gastric emptying of a non-nutrient solution, while somatostatin-28 or a sst_5 agonist under the same conditions stimulated gastric emptying (62).

In summary, this study is the first to use the recently developed selective peptide sst_2 agonist and sst_2 antagonist to provide evidence for a role of brain sst_2 receptors in the stimulation of food intake in rats. Whether the sustained feeding response to i.c.v. injection of the sst_2 agonist in the light phase reflects the activation of brain orexigenic circuitries or inhibition of inhibitory mechanisms in place during the light phase will need to be delineated. The present data also support a pleiotropic effect of somatostatin acting *via* sst_2 receptors

resulting in orexigenic, behavior and thermogenic responses. This suggests that activation of brain sst_2 receptors may play a primary role in mediation of brain somatostatin actions consistent with the distribution of the sst_{2A} receptor in the rat hypothalamus and other brain areas.

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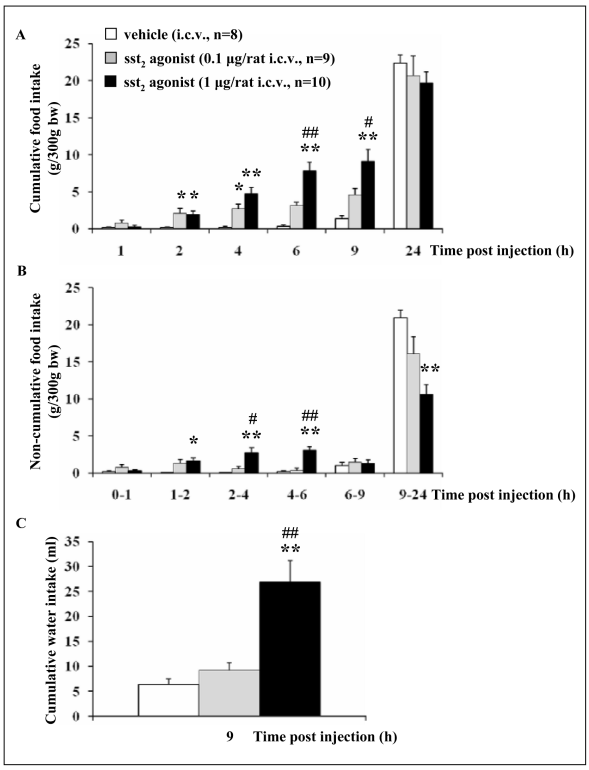

Fig. 1.

The sst_2 agonist injected into the lateral brain ventricle increases light phase food and water intake in *ad libitum* fed rats. The sst_2 agonist (0.1 or 1 µg/rat) or vehicle (pyrogen-free distilled water) was injected i.c.v. during the light phase in *ad libitum* fed rats chronically implanted with cannula. Food intake was monitored for 24 hours post injection and expressed as cumulative (A) and non-cumulative (B) food intake. Cumulative water intake was monitored for 9 hours post injection (C). The first 9 hours post injection are within the light phase, the 9–24 hours period encompasses the dark phase as well as the first 3 hours of the next light phase. Each bar represents the mean ± S.E.M. of 8–10 rats/group. * $P<0.05$, ** $P<0.01$ *vs*. vehicle; $^{\#}$ $P<0.05$, $^{\#\#}$ $P<0.005$ *vs*. 0.1 µg sst_2 agonist.

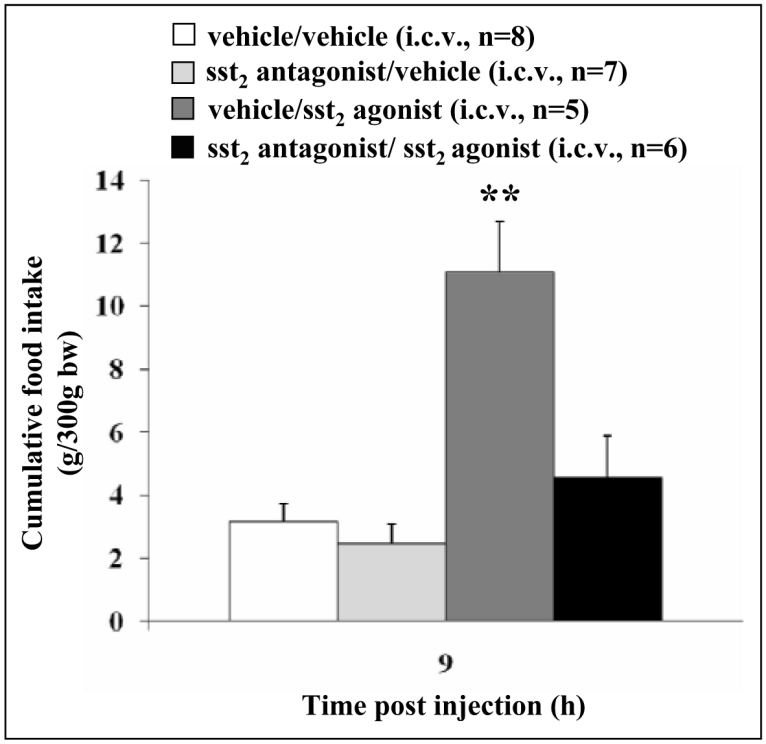

Fig. 2.

The sst_2 antagonist injected into the lateral brain ventricle blocks the sst_2 agonist-induced food intake in rats. The i.c.v. injection of the sst_2 antagonist (1 μg/rat) or vehicle (pyrogen-free distilled water) was followed by that of sst_2 agonist (1 μg/rat) or vehicle during the light phase in *ad libitum* fed rats chronically implanted with cannula. Food intake was monitored for 9 hours post injection. Each bar represents the mean ± S.E.M. of 5–8 rats/group. ** $P<0.01$ *vs*. vehicle/vehicle, sst_2 antagonist/vehicle and sst_2 antagonist/sst_2 agonist, respectively.

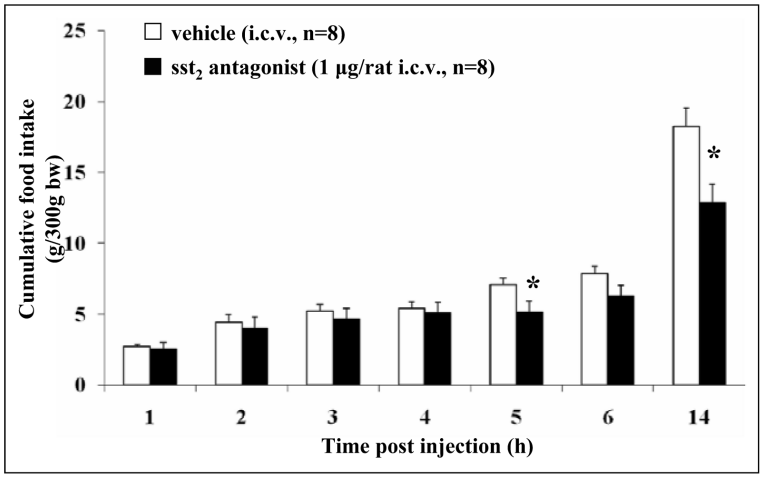

Fig. 3.

The sst_2 antagonist injected into the lateral brain ventricle decreases dark phase food intake in *ad libitum* fed rats. The sst_2 antagonist (1 µg/rat) or vehicle (pyrogen-free distilled water) was injected i.c.v. at the beginning of the dark phase in *ad libitum* fed rats chronically implanted with cannula. Cumulative food intake was monitored for 14 hours post injection. The first 12 hours post injection are within the dark phase and the 14 hours period also encompasses the first two hours of the next light phase. Each bar represents the mean ± S.E.M. of 8 rats/group. * $P<0.05$ *vs*. vehicle.

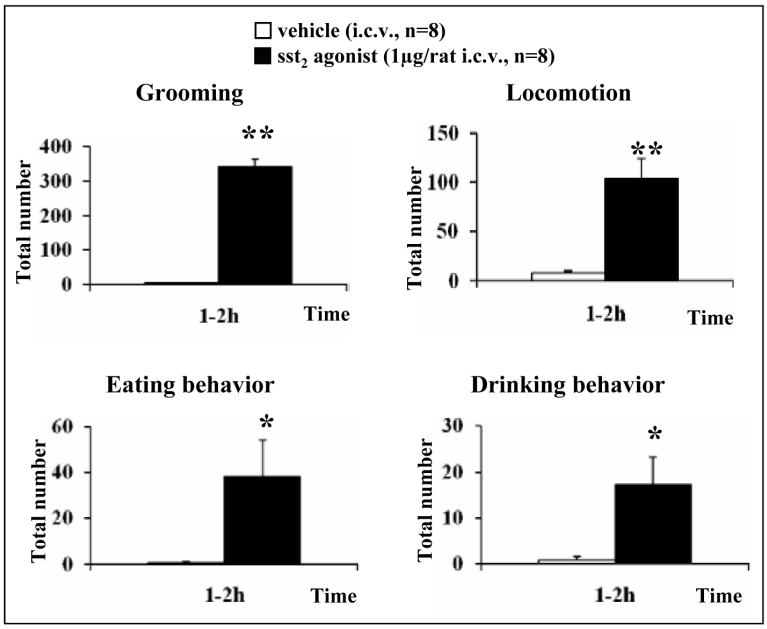

Fig. 4.

The sst_2 agonist injected intracerebroventricularly induces behavioral changes in *ad libitum* fed rats. The sst_2 agonist (1 µg/rat) or vehicle (pyrogen-free distilled water) was injected during the light phase in *ad libitum* fed chronically cannulated rats. Animals were placed in their home cage with access to food and water and behaviors consisting of grooming (including scratching, licking and washing), locomotor activity (total number of squares crossed), food intake (including food approach) and water intake (including water approach) were monitored during the second hour post injection. Each behavioral component was counted when lasting longer than 5 sec. Each bar represents the mean S.E.M. of 8 rats/ group. * $P<0.05$, ** $P<0.001$ *vs*. vehicle.

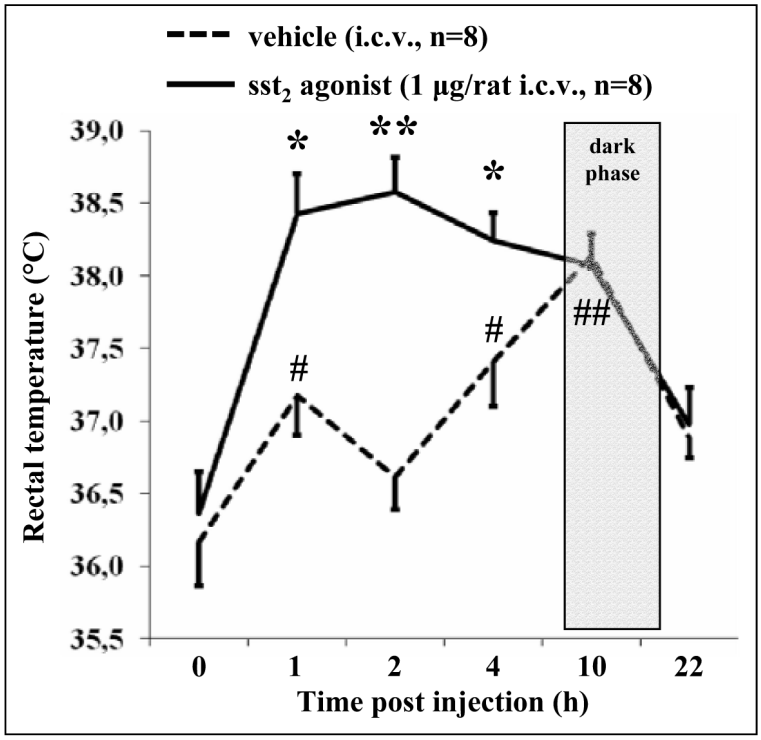

Fig. 5.

The sst_2 agonist injected into the lateral brain ventricle increases body temperature in rats. The sst_2 agonist (1 µg/rat) or vehicle (pyrogen-free distilled water) was injected during the light phase in *ad libitum* fed chronically cannulated rats and rectal temperature was measured in conscious lightly hand-restrained rats before and at various time intervals after injection. Data are presented as mean ± S.E.M. of 8 rats/group. * $P<0.05$, ** $P<0.001$ *vs*. vehicle at the respective time point; $^{\#}$ $P<0.05$, $^{\#\#}$ $P<0.001$ *vs*. vehicle at time point 0.

Table 1

The sst_2 agonist injected intraperitoneally does not modify food intake in rats.

Time post injection (h)	Treatment	
	vehicle (n=9)	sst_2 agonist (n=9)
1	0.2 ± 0.1	0.5 ± 0.2
2	0.5 ± 0.2	0.9 ± 0.3
4	0.5 ± 0.2	1.0 ± 0.3
6	0.9 ± 0.3	1.2 ± 0.3
9	2.9 ± 0.5	3.2 ± 0.5
24	21.0 ± 1.0	21.5 ± 0.5

Data are expressed as g/300 g body weight as mean ± S.E.M. of number of rats indicated in parentheses; $P>0.05$. The sst_2 agonist (30 μg/kg in saline) or vehicle (saline) was injected i.p. during the light phase in *ad libitum* fed non-cannulated rats and cumulative food intake was monitored for 24 hours post injection. The first 9 hours post injection are within the light phase, the 9–24 hours period encompasses the dark phase as well as the first 3 hours of the next light phase.

Table 2

The sst_2 agonist injected intracerebroventricularly or intraperitoneally does not modify gastric emptying of solid food after a fast.

Gastric emptying (% in 2h)[a]		Gastric emptying (% in 2h)[a]	
vehicle, i.c.v.[b] (n=8)	**sst_2 agonist, i.c.v.[b] (n=6)**	**vehicle, i.p.[b] (n=10)**	**sst_2 agonist, i.p.[b] (n=8)**
67.6 ± 4.5	70.6 ± 5.0	59.5 ± 4.9	61.6 ± 3.0

[a] Values are mean±S.E.M. of number of rats indicated in parentheses; $P>0.05$ compared with vehicle.

[b] Rats were fasted for 20 hours, then re-fed for 2 hours and sst_2 agonist (1 μg/rat, i.c.v. or 30 μg/kg, i.p.) or vehicle (i.c.v. or i.p. respectively) was injected during the light phase. Food and water were removed and gastric emptying was assessed 2 hours after injection.