Title

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Authors

Karasawa, Hiroshi
Yakabi, Seiichi
Wang, Lixin
et al.

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Orexin-1 receptor mediates the increased food and water intake induced by intracerebroventricular injection of stable somatostatin pan-agonist, ODT8-SST in rats

Hiroshi Karasawa, **Seiichi Yakabi**, **Lixin Wang**, and **Yvette Taché**
Department of Medicine, CURE/Digestive Diseases Center, Digestive Diseases Division, University of California at Los Angeles, and Veterans Affairs Greater Los Angeles Healthcare System, Los Angeles, CA, USA

Abstract

Intracerebroventricular (icv) injection of the stable somatostatin pan-agonist, ODT8-SST induces a somatostatin 2 receptor (sst_2) mediated robust feeding response that involves neuropeptide Y and opioid systems in rats. We investigated whether the orexigenic system driven by orexin also plays a role. Food and water intake after icv injection was measured concomitantly in non-fasted and non-water deprived rats during the light phase. In vehicle treated rats (100% DMSO, icv), ODT8-SST (1 μg/rat, icv) significantly increased the 2-h food and water intake compared to icv vehicle plus saline (5.1 ± 1.0 g vs. 1.2 ± 0.4 g and 11.3 ± 1.9 mL vs. 2.5 ± 1.2 mL, respectively). The orexin-1 receptor antagonist, SB-334867 (16 μg/rat, icv) completely inhibited the 2-h food and water intake induced by icv ODT8-SST. In contrast, the icv pretreatment with the selective somatostatin sst_2 antagonist, S-406-028, established to block the orexigenic effect of icv ODT8-SST, did not modify the increased food and water intake induced by icv orexin-A (10.7 μg/rat). These data indicate that orexin-1 receptor signaling system is part of the brain neurocircuitry contributing to the orexigenic and dipsogenic responses induced by icv ODT8-SST and that orexin-A stimulates food intake independently from brain sst_2 activation.

Keywords

food intake; orexin-A; ODT8-SST; SB-334867; somatostatin 2 receptor; water intake

Address correspondence to: Yvette Taché, Ph.D. Mailing address: Bldg. 115, Rm. 117B, 11301 Wilshire Blvd, Los Angeles, CA, 90073, USA YTache@mednet.ucla.edu Phone: +1 (310) 478 3711, Ex. 41831 / FAX: +1 (310) 268 4963.

Contributors Hiroshi Karasawa designed, performed the experiments and wrote the manuscript; Seiichi Yakabi and Lixin Wang participate in the experiments and reviewed the manuscript; Yvette Taché designed the experiments and wrote the manuscript.

Conflict of interest Nothing to declare.

1. Introduction

Somatostatin is a pleiotropic neuropeptide that is expressed in the central nervous systems [1,2]. In addition to its originally described physiological action to inhibit growth hormone release, somatostatin exerts several extrapituitary actions [3] in keep with its widespread brain distribution and the binding to five receptor subtypes named sst_1–sst_5 [4]. In particular, the somatostatin system in the brain may play an important role in controlling ingestive behavior. Recently, we reported that acute intracerebroventricular (icv) injection of the stable somatostatin pan-agonist, ODT8-SST [5] induces a rapid in onset and robust stimulation of food intake in non-fasted rats during the light phase through activation of brain sst_2 signaling pathway [6,7]. Furthermore, icv treatment of the selective sst_2 antagonist S-406-028 [8], at a dose that blocked the stimulation of food consumption induced by icv ODT8-SST also dampened the spontaneous food intake during the dark phase [6]. Previous pharmacological studies indicate that the orexigenic action of icv ODT8-SST involves the recruitment of neuropeptide Y (NPY) Y_1 receptor systems [6].

Orexins are orexigenic peptides expressed in two isoforms, orexin-A and -B both derived from the same precursor gene prepro-orexin [9]. Orexin-synthetizing neurons are mainly expressed in the lateral hypothalamic area (LHA) and send widespread intra- and extra-hypothalamic monosynaptic projections to cerebral cortex, circumventricular organs, limbic system and brainstem [10]. These peptides bind to two related G-protein-coupled receptors called orexin-1 receptor (OX_1R) and orexin-2 receptor (OX_2R) [11] which are widely distributed in the brain with a robust expression on regions integrating primarily feeding (lateral, arcuate, and paraventricular hypothalamus), sleep/wake and arousal behaviors (locus coeruleus and dorsal raphe) [11–13]. Convergent studies established that orexin-A which has one order of magnitude greater affinity to OX_1R than orexin-B [11], is a more effective appetite and drinking stimulant compared to orexin-B [11,14] and these effects are mainly mediated by the OX_1R as shown by the use of the selective antagonist, SB-334867 [15,16]. Of interest, previous neuroanatomical and functional studies established a reciprocal interaction between orexin-A and NPY [17,18]. However whether there is an interdependence between orexin-A and somatostatin to promote food intake in rats is so far unknown.

Therefore, in the present study, we investigated the possible functional linkage between these two orexigenic peptides by examining the effects of icv pretreatment of the orexin OX_1R antagonist, SB-334867 [15] and somatostatin sst_2 receptor antagonist, S-406-028 [8] on the orexigenic effect induced by icv ODT8-SST and icv orexin-A, respectively. Moreover, we monitored water intake concomitantly since both orexin-A and the somatostatin stable analogs, octreotide or ODT8-SST injected icv have been reported to increase water intake [7,14,19].

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats weighing 230–250 g were purchased from Harlan Laboratories (San Diego, CA). Animals were kept under controlled illumination (12/12 h

light/dark cycle, lights on/off: 6:00/18:00) and temperature (22 ± 2°C). Animals were fed standard rodent diet (Prolab RMH 2500; LabDiet, PMI Nutrition, Brent wood, MO) and tap water *ad libitum*. Animal care and experimental procedures followed institutional ethic guidelines and conformed to the requirements of the federal authority for animal research conduct. All procedures were approved by the Animal Research Committee at Veterans Affairs Greater Los Angeles Healthcare System (animal protocol #11047-09).

2.2. Peptides and compounds

ODT8-SST (des-AA^{1,2,4,5,12,13}-[DTrp^{8}]-somatostatin , MW 1078.5, compound 1 in [5]), the selective sst$_2$ receptor antagonist, S-406-028 (des-AA$^{1,4–6,11–13}$-[pNO$_2$-Phe^{2},DCys^{3},Tyr^{7},DAph(Cbm)^{8}]-SST-2NaI-NH$_2$, MW 1208.5, compound 4 in [8]), and orexin-A were synthetized and purity-checked (Clayton Foundation Laboratories for Peptide Biology, La Jolla, CA). Peptides were dissolved in saline. The OX$_1$R antagonist, SB-334867 [15] was purchased from Tocris Bioscience (Bristol, United Kingdom). In view of the low water solubility of SB-334867, the compound was dissolved in 100% dimethylsulfoxide (DMSO) purchased from Sigma-Aldrich Co. (St. Louis, MO) as in other studies [20]. The corresponding vehicle was also 100% DMSO.

2.3. Icv cannulation and injection

Icv cannulation and injections were performed as previously described [21]. Rats were anesthetized by an intramuscular injection of ketamine hydrochloride (75 mg/kg, Ketanest; Fort Dodge Laboratories, Fort Dodge, IA) and xylazine (5 mg/kg, Rompun; Mobay Corporation, Shawnee, KS). They were placed in a stereotaxic apparatus and implanted with a chronic guide cannula (22-gage, Plastics One Inc., Roanoke, VA) into the right lateral brain ventricle. Stereotaxic coordinates were selected according to the Paxinos and Watson's brain atlas [22]: 0.8 mm posterior, 1.5 mm right lateral, and 4.0 mm ventral from the bregma. The guide cannula was secured by dental cement and anchored by screws fixed into the skull and occluded. After surgery, animals were housed individually and allowed to recover for 1 week. During this time, rats were handled 2–3 min each day for 3 days. The icv injection was performed in lightly hand-restrained conscious rats in a 10 μL delivered over 1 min for single injection or 5 μL over 30 sec for each of two consecutive injections with the cannula remaining in place for 1 min after each injection. The correct location of the cannula into the right lateral ventricle was verified at the end of the experiment. No animal had to be excluded from the data analysis.

2.4. Food and water intake measurement

Food intake and water intake were determined by weighing food and water bottle before and after each time period and calculating the amount consumed. To avoid spill of water, we used water bottles with a ball-pointed sipper tube. Spillage of food was recovered and weighed. Food and water intake are expressed as gram and milliliter per rat, respectively. The mean body weight (327 ± 28 g, mean ± SD) before the experiments were not significantly different between groups.

2.5. Experimental protocols

Before each experiment, rats were placed in a cage with a grid floor with free access to food and water overnight. The icv treatment started between 9 am and 10 am.

Effects of the icv orexin OX_1R antagonist on food and water intake induced by icv ODT8-SST—Rats were injected icv (5 μL) with vehicle (100% DMSO) or the OX_1R antagonist SB-334867 (16 μg = 50 nmol/rat) immediately followed by second icv injection (5 μL) of saline or ODT8-SST (1 μg = 0.93 nmol/rat). Then, rats were placed back in the home cage with a grid floor and paper under the grid to facilitate collecting the spillage of food. Food and water intake were measured for 2 h post injection. The dose of SB-334867 and ODT8-SST was based on previous studies [6,23].

Effects of the icv somatostatin sst_2 receptor antagonist on food and water intake induced by icv orexin-A—Rats received icv injection (5 μL) of vehicle (saline) or the sst_2 antagonist S-406-028 (1 μg = 0.83 nmol/rat) immediately followed by the second icv injection (5 μL) of saline or orexin-A (10.7 μg = 3 nmol/rat). Rats were placed back in the home cage and food and water intake were measured for 2 h post injection as described above. The dose of S-406-028 and orexin-A was based on the previous studies [6, 24].

2.6. Statistical analysis

Data are shown as mean ± SEM. Comparisons between multiple groups were performed by 2-way ANOVA followed by *post hoc* multiple comparisons. *P* values of less than 0.05 were considered statistically significant.

3. Results

3.1. The orexin OX_1R antagonist, SB-334867 injected icv blocked food and water intake induced by icv ODT8-SST

When combined with icv vehicle pretreatment (100% DMSO, 5 μL), icv injection of ODT8-SST (1 μg/rat) significantly increased the 2-h food intake compared to vehicle plus saline (5.1 ± 1.0 vs. 1.2 ± 0.4 g, $P < 0.01$; Fig. 1A) as monitored in the light phase in freely fed rats. Pretreatment of the OX_1R antagonist, SB-334867 (16 μg/rat, icv) completely abolished the orexigenic effect of icv ODT8-SST (1.2 ± 0.3 vs. 5.2 ± 1.0 g, $P<0.01$) while having no effect on food intake in saline-treated animals (1.2 ± 0.3 g/rat). Two-way ANOVA showed a significant influence of ODT8-SST ($F_{1,16} = 11.4$, $P < 0.01$), SB-334867 ($F_{1,16} = 11.3$, $P < 0.01$), and ODT8-SST × SB-334867 ($F_{1,16} = 11.9$, $P < 0.01$). The water intake monitored simultaneously showed a similar response to treatments. ODT8-SST combined with vehicle significantly increased water intake (11.3 ± 1.9 vs. 2.5 ± 1.2 mL of vehicle plus saline, $P < 0.01$; Fig. 1B) and SB-334867 pretreatment also ablated this effect (3.6 ± 1.3 mL, $P < 0.01$ vs. vehicle plus ODT8-SST). Two-way ANOVA showed a significant influence of ODT8-SST ($F_{1,16} = 14.6$, $P < 0.01$), SB-334867 ($F_{1,16} = 9.0$, $P < 0.01$), and ODT8-SST × SB-334867 ($F_{1,16} = 9.8$, $P < 0.01$).

3.2. The somatostatin sst_2 antagonist, S-406-028 injected icv had no effect on food and water intake induced by icv orexin-A

Orexin-A (10.7 μg/rat, icv) combined with icv vehicle (saline) pretreatment induced a modest but significant increase of the 2-h food intake during the light phase compared to that in rats treated with vehicle plus saline (2.3 ± 0.2 vs. 1.1 ± 0.2 g, $P < 0.01$; Fig. 2A). The sst_2 antagonist S-406-028 (1 μg/rat, icv) had no effect on the orexigenic effect induced by orexin-A (2.1 ± 0.3 vs. 2.3 ± 0.2 g, $P = 0.52$). Two-way ANOVA showed a significant influence of orexin-A ($F_{1,24} = 19.1$, $P < 0.001$). Likewise the 2-h water intake showed a similar trend to that in food intake although the effect of icv orexin-A combined with vehicle vs. icv saline with vehicle did not reach the statistical significance (4.6 ± 0.9 vs. 2.4 ± 0.4 mL, $P = 0.052$; Fig. 2B), probably because of relatively larger inter-individual differences. Pretreatment of the sst_2 antagonist had no effect on water intake either when combined with saline or orexin-A.

4. Discussion

In the present study, we provided pharmacologic evidence that the rapid increase in food and water intake induced by the somatostatin agonist, ODT8-SST injected icv during the light phase involves downstream the activation of OX_1R signaling system in freely fed rats. In addition, we showed the feeding and drinking responses to icv orexin-A are independent from the established sst_2 orexigenic and dipsogenic pathways [6,7].

The icv injection of the pan-somatostatin agonist ODT8-SST at 1 μg (0.93 nmol) resulted in a 4.2-fold increase in the 2-h food intake monitored in freely fed rats during the light phase consistent with our previous findings [6]. We reported that under these conditions, ODT8-SST action is blocked by the icv injection of the sst_2 antagonist, S-406-028 and mimicked by the icv injection of sst_2 agonist, indicative that activation of sst_2 signaling is mainly involved in the feeding response to icv ODT8-SST [6,7]. In the present study, the robust stimulation of food intake elicited by icv ODT8-SST was completely prevented by the OX_1R antagonist, SB-334867 injected icv. The blockade occurred at an icv dose of SB-334867 devoid of intrinsic effect on food intake when tested under the same conditions. Previous studies indicate that the effects of SB-334867 on basal food intake is dependent upon the endogenous levels of orexin-A as shown by the reduction of feeding mainly in the nocturnal phase associated with high endogenous levels of hypothalamic orexin-A [16,25,26]. Therefore the lack of icv SB-334867 effect during the light phase corresponding to the nadir of hypothalamic orexin levels [25] further supports this contention.

We previously found that icv ODT8-SST orexigenic action was prevented by the icv NPY_1 antagonist, BIBP-3226 [6]. Compelling evidence indicates that arcuate NPY_1 signaling serves as one of the downstream effector of the LHA orexin-induced feeding response. This is supported by the defined direct projections from LHA orexin-synthetizing neurons onto NPY immunoreactive arcuate neurons which also express OX_1R [17,27]. Furthermore icv orexin activates NPY neurons located in the arcuate nucleus [18,28] and selectively increases hypothalamic NPY gene expression [24]. Lastly, the icv orexigenic effect of orexin-A is attenuated by icv injection of NPY_1 antagonist [18,29]. Collectively, these pharmacologic evidences would be consistent with icv ODT8-SST involving downstream

orexin-OX_1R signaling pathway which subsequently activates the NPY-NPY_1 orexigenic response. However neuroanatomical circuitries that underlie the link between brain sst_2 receptor mediated orexigenic action of icv ODT8-SST [6,7] and the recruitment of brain orexin neurons are still to be established. The sst_2 immunoreactivity is diffusely distributed throughout the hypothalamus [30] on both somatodendritic and axonic elements allowing transduction at the pre- and postsynaptic levels [31]. Our previous report [32] showed that the icv injection of ODT8-SST at the orexigenic dose used in the present study, induced Fos immunoreactivity mainly in the supraoptic nucleus and paraventricular nucleus unlike orexin-producing neurons located in caudal hypothalamus including the LHA, perifornical region or the dorsomedial nucleus in rats suggestive of an indirect action. Based on the postsynaptic and presynaptic GABAergic strong inhibitory input to orexin neurons [33] and the inhibitory effect of somatostatin on GABA transmission [34,35], it may be speculated that the stimulation of sst_2 receptor may act by suppressing tonic inhibition on orexin neurons which needs to be ascertained.

The present study also showed that orexin-A injected icv at 3 nmol/rat induces a modest 2.3 fold increase in food intake, which was relatively smaller than that induced by ODT8-SST injected icv at 0.93 nmol/rat, in freely fed rats during the light phase. Likewise, other studies established that the feeding response to icv orexin-A was weak compared to that of NPY in rats [36]. Orexin-A action is also time sensitive with a lower stimulatory feeding response when the peptide was injected icv in the morning vs. the afternoon of the light phase [37] and a minimal effect on food or water intake when administered before the dark phase [37,38]. Based on previous reports that icv injection of NPY_1 antagonist did not induce a complete suppression of orexin feeding response [18,29] and OX_1R immunoreactivity is expressed in somatostatin neurons of the periventricular hypothalamic nucleus [27], we postulated that the somatostatin sst_2 system may also contribute to orexin-A action. However the sst_2 antagonist injected icv at dose blocking the stimulation of food intake evoked by icv ODT8-SST [6] did not alter the feeding stimulation induced by icv orexin-A. These data established that the orexigenic action of orexin-A is not linked with the activation of sst_2 pathway. There is evidence that orexigenic action of orexin-A is independent of other orexigenic peptides namely agouti-related peptide and melanin-concentrating hormone while being linked to NPY systems [24].

Lastly, we showed parallel findings between the dipsogenic and the feeding response whereby the icv injection of OX_1R antagonist completely blocked the 4.5-fold increase in water intake induced by icv ODT8-SST while the 1.9-fold increase in water intake induced by icv orexin-A was not influenced by icv sst_2 antagonist. Drinking and feeding are closely related behaviors [39,40] although each can be controlled by independent mechanisms [41]. Physiologically, rats are known to drink 1–2 mL of water for each gram of food eaten to compensate for the introduced solutes into blood and water shifts from body fluids into the gut [42]. Therefore, the paralleled changes in water with food intake observed in this study are consistent with this physiologic response. However somatostatin analog and orexin-A as well as NPY can exert dipsogenesis in the absence of food upon icv injection [14,19,43]. Taken together these data point to the involvement of OX_1R-NPY_1 signaling in mediating the feeding and drinking stimulation by icv injection of ODT-8SST which needs to be further delineated at the cellular level.

5. Conclusion

In conclusion, we showed the involvement of the OX_1R signaling in addition to the previously reported NPY_1 systems [6] in the orexigenic and dipsogenic effects of icv ODT8-SST in freely fed rats during the light phase. These findings and the established downstream linkage between hypothalamic orexin-OX_1R and NPY-NPY_1 signaling system to induce food intake support a linkage between these orexigenic circuits. By contrast, the feeding and drinking responses to icv orexin-A is independent from the brain sst_2 pathways.

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Abbreviations

DMSO	dimethylsulfoxide
icv	intracerebroventricular (ly)
LHA	lateral hypothalamic area
NPY	neuropeptide Y
OX_1R	orexin-1 receptor
OXR-2	orexin-2 receptor
sst	somatostatin receptor

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Highlights

- Somatostatin agonist, ODT8-SST exerts dipsogenic and orexigenic effects.
- Orexin-1 receptor antagonist, SB-334867 blocked ODT8-SST effects in rat brain.
- Somatostatin 2 receptor antagonist did not influence orexigenic effect of orexin-A.
- Orexin-1 receptor signaling system is downstream of ODT8-SST feeding behavior.

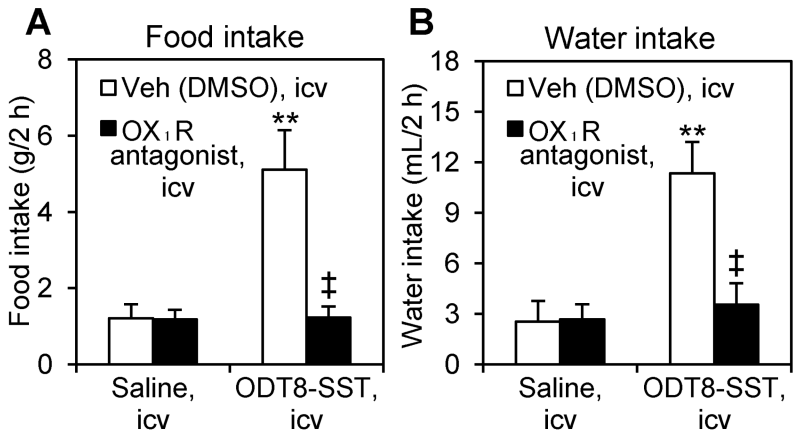

Figure 1.

The OX_1R antagonist, SB-334867 blocked ODT8-SST-induced stimulation of food intake (A) and water intake (B) in freely fed rats. Vehicle (DMSO, 5 μL/rat) or SB-334867 (16 μg/rat) was injected intracerebroventricularly (icv) immediately followed by icv saline or ODT8-SST (1 μg/rat) during the light phase. Food and water intake was measured for 2-h post injection. Data are mean ± SEM of n = 5 rats/group. $^{**}P < 0.01$ icv vehicle + icv ODT8-SST vs. icv vehicle + icv saline. $^{‡}P < 0.01$ icv OX_1R antagonist + icv ODT8-SST vs. icv vehicle + icv ODT8-SST.

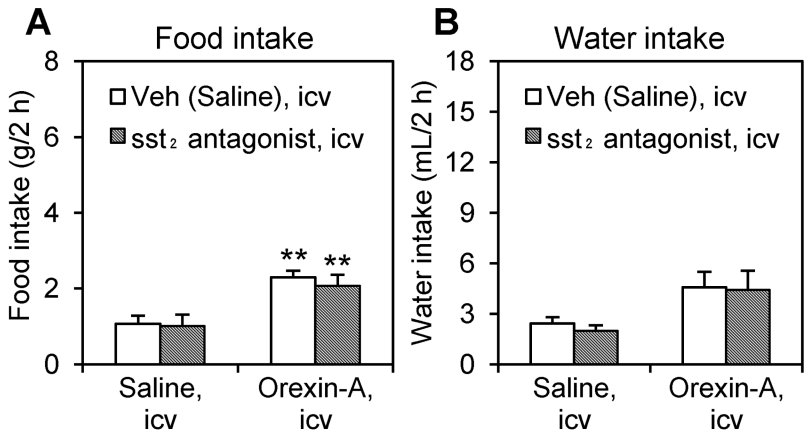

Figure 2.

The sst_2 antagonist, S-406-028 did not influence orexin-A-induced stimulation of food intake (A) and water intake (B) in freely fed rats. Rats received icv pretreatment of vehicle (saline, 5 μL/rat) or sst_2 antagonist (1 μg/rat) immediately followed by icv saline or orexin-A (10.7 μg/rat) during the light phase. Food and water intake was measured for 2-h post injection. Data are mean ± SEM of n = 7 rats/group. $^{**}P < 0.01$ icv vehicle or sst_2 antagonist + icv orexin-A vs. icv vehicle or sst_2 antagonist + icv saline