ORIGINAL ARTICLE

Orexin reverses cholecystokinin-induced reduction in feeding

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Aim: This study was designed to investigate the effect of orexin on anorexia induced by cholecystokinin (CCK), a peripheral satiety signal.
Methods: We administered orexin A (0.01–1 nmol/mouse) and CCK-8 (3 nmol/mouse) to mice. Food intake was measured at different time-points: 20 min, 1, 2 and 4 h post-intracerebroventricular (i.c.v.) or intraperitoneal (i.p.) administrations.
Results: Intracerebroventricular-administered orexin significantly increased food intake in a dose-dependent manner. The inhibitory effect of i.p.-administered CCK-8 on food intake was significantly negated by the simultaneous i.c.v. injection of orexin in a dose-dependent manner.
Conclusions: Orexin reversed the CCK-induced loss of appetite. Our results indicate that orexin might be a promising target for pharmacological intervention in the treatment of anorexia and cachexia induced by various diseases.
Keywords: orexin, cholecystokinin, mice, food intake, anorexia
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Introduction

Orexin is a hypothalamic neuropeptide identified as a ligand for an orphan G-protein coupled receptor [1–3]. The peptide is localized in neuronal cell bodies of the lateral hypothalamic area (LHA) and its adjacent area, which contribute to the regulation of food intake and energy balance [4–6]. Orexin-containing neurones project to numerous hypothalamic and extrahypothalamic sites including the cerebral cortex, circumventricular organs and brainstem [4,5]. Therefore, orexin is likely to be involved in regulating various functions including emotion, arousal, feeding and drinking [3,7–10]. Previous studies have shown that orexin increases food intake in rats and sheep when administered centrally. However, the relationship between orexin and peripheral satiety systems still remains to be determined. We investigated the effect of orexin on anorexia induced by cholecystokinin (CCK), a potential peripheral satiety signal [9,10].

Materials and Methods

Male mice (32–35 g) of the ddy strain were purchased from JAPAN SLC (Shizuoka, Japan) at 7 weeks of age. They were individually housed in a regulated environment ($22 \pm 2\,^{\circ}C$, $55 \pm 10\%$ humidity, 12:12 hours light:dark cycle with light on at 07.00 hours). Food and water were available *ad libitum* unless otherwise indicated.

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For i.c.v. injection, the mice were anaesthetized with sodium pentobarbital (80–85 mg/kg i.p.) and placed in a stereotaxic instrument (SR-6, Narishige, Tokyo, Japan) 7 days before the experiments. A hole was made in each skull by using a needle inserted 0.9 mm lateral to the central suture and 0.9 mm posterior to the bregma. A 24-gauge cannula bevelled at one end over a distance of 3 mm (Safelet-Cas, Nipro, Osaka, Japan) was implanted into the third cerebral ventricle for i.c.v. injection. The cannula was fixed to the skull with dental cement and capped with silicon without an obtruder. A 27-gauge injection insert was attached to a microsyringe by PE-20 tubing. Using tweezers, this was easily inserted into a fixed cannula without holding the mouse and thus without greatly disturbing the animal's behaviour. After completion of the experiment, the location of the cannula tip was confirmed by injection of dye (Evans blue 0.5% and Zelatin 5%) and histological examination of frozen brain sections.

Orexin-A and CCK-8 were purchased from Peptide Institute (Osaka, Japan). Just before administration, each drug was diluted in 4 μl of artificial cerebrospinal fluid (ACSF) for i.c.v. injection, or in 100 μl of physiological saline for i.p. injection, both of which also served as the control solutions.

Experiments were started at 10.00 hours. Before feeding tests, mice were given free access to food and water, except for experiment on the effect of co-administration of orexin and CCK on food intake, in which mice were food deprived for 16 h with free access to water. Food intake was measured by subtracting uneaten food from the initially premeasured food at different time-points: 20 min, 1, 2 and 4 h post administration.

Results were expressed as mean ± s.e.m. Analysis of variance (ANOVA; STATVIEW, version 4.5, Abacus Concepts, Berkeley, CA, USA), followed by Bonferroni's *t*-test, were used to assess the differences among groups. $p < 0.05$ was considered to be statistically significant. The mice were used only once in each experiment. All experiments were approved by our university animal care committee.

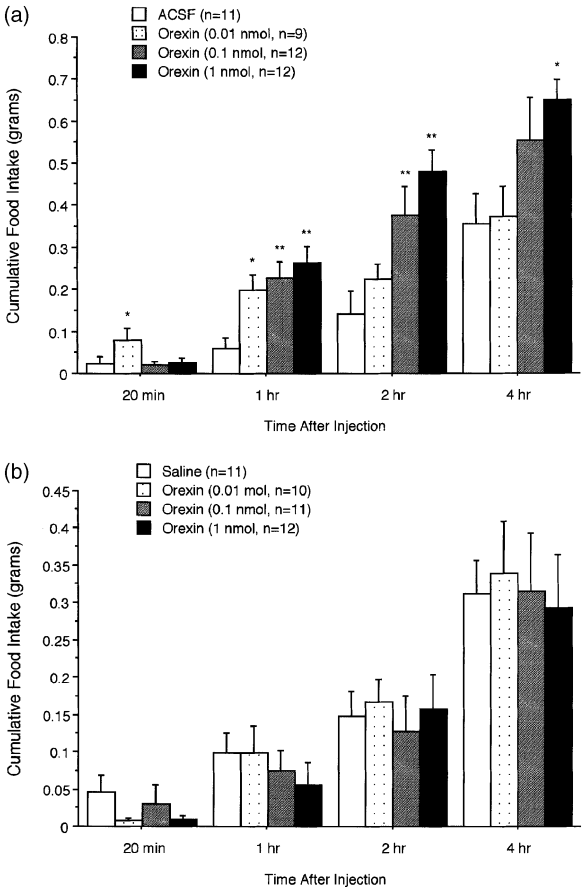

Fig. 1 (a) Effects of i.c.v.-administered orexin (0.01–1 nmol/mouse) on cumulative food intake in non-food deprived mice. Results are expressed as mean ± s.e.m; 'n' indicates the number of mice used. *$p < 0.05$; **$p < 0.01$ compared with the control group by Bonferroni's *t*-test.(b) Effects of i.p.-administered orexin (0.01–1 nmol/mouse) on cumulative food intake in non-food deprived mice.

Results

The effects of orexin (0.01–1 nmol/mouse) on food intake are shown in figure 1. Intracerebroventricular-administered orexin significantly increased food intake in a dose-dependent manner, compared with ACSF-treated controls (figure 1a); i.p. administration of orexin showed no significant effect on feeding behaviour (figure 1b). The effects of orexin on CCK-induced reduction in feeding are shown in figure 2. The inhibitory effect of i.p.-administered CCK-8 (3 nmol/mouse) on food intake was significantly negated by the simultaneous i.c.v. injection of orexin (0.1–1 nmol/mouse) in a dose-dependent manner.

Discussion

Anorexia and body weight loss are accompanied by various diseases, including anorexia nervosa, cancer and acquired immunodeficiency syndrome (AIDS) [11,12]. However, there is no definitive therapy for the anorexia and cachexia. Physiological mechanisms regulating feeding and energy metabolism are complex and remain to be elucidated [6,9,10,13]. It is now recognized that

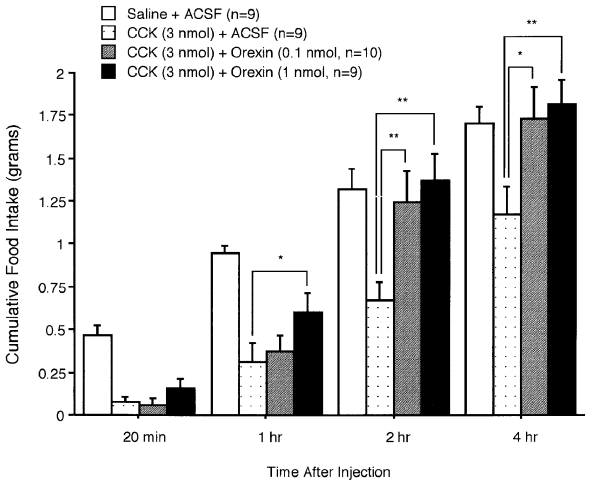

Fig. 2 Effects of i.c.v.-administered orexin (0.1–1 nmol/mouse) on anorexia induced by i.p. injection of CCK-8 (3 nmol/mouse) in food-deprived mice. Results are expressed as mean ± s.e.m; 'n' indicates the number of mice used. *$p < 0.05$; **$p < 0.01$ compared with the control group by Bonferroni's *t*-test.

neuropeptides in the hypothalamus play a pivotal role in the regulation of energy balance. A few neuropeptides are thought to increase feeding, including neuropeptide Y (NPY), agouti-related protein (AGRP), melanin-concentrating hormone (MCH), opioid peptides and galanin [6,9,10,13]. In the present study, centrally administered orexin produced a feeding-stimulatory effect in mice. This confirms that orexin also influences energy balance as a component of feeding-stimulatory systems.

On the other hand, several peptides are thought to decrease food intake, including leptin, corticotropin-releasing factor (CRF), urocortin, CCK, bombesin, cocaine- and amphetamine-regulated transcript (CART) and glucagon [6,9,10,14]. CCK, a well known gastrointestinal peptide, is known to be a potential peripheral satiety agent and affect energy balance through a negative feedback loop [6,9,10]. Previous studies have shown that administered interleukin 1 increases CCK levels including CCK-8, resulting in anorexia and delayed gastric emptying [15]. It has also been reported that CCK responses to meals are increased in patients with anorexia nervosa [11,16]. It was recently demonstrated that administered CCK mobilized gastric leptin, increased plasma leptin levels and potentiated satiety effect [17]. In the present study, we demonstrated that i.c.v. injection of orexin significantly reversed the CCK-induced loss of appetite. These observations indicate that orexin might be a promising target for pharmacological intervention in the treatment of anorexia and cachexia induced by various diseases.

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