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The Role of Orexigenic Neuropeptides in the Ingestion of Sweet-tasting Substances in Rats

Yuichi Furudono[1], Chiho Ando[2], Motoi Kobashi[3], Chizuko Yamamoto[2] and Takashi Yamamoto[2]

[1]Tobacco Science Research Center, Japan Tobacco Inc., Yokohama 227-8512, Japan, [2]Department of Behavioral Physiology, Graduate School of Human Sciences, Osaka University, Suita, Osaka 565-0871, Japan and [3]Department of Oral Physiology, Okayama University Graduate School of Medicine and Dentistry, Okayama 700-8525, Japan

Correspondence to be sent to: Takashi Yamamoto, e-mail: yamamoto@hus.osaka-u.ac.jp

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Introduction

Palatability is one of the most important factors that regulate food and fluid intake in animals. Animals usually prefer sweet-tasting substances and consumption often exceeds the need for homeostatic repletion. Recently, new chemical mediators that regulate appetite have been identified; for example, orexins (orexin-A and B), melanin-concentrating hormone (MCH), agouti-related protein (AgRP), ghrelin and neuropeptideY (NPY) all are known to stimulate feeding and, thus, to function as orexigenic peptides (Inui, 2000; Schwartz *et al.*, 2000). On the other hand, leptin, alpha-melanocyte-stimulating hormone (α-MSH), urocortin and cocaine- and amphetamine-regulated transcript (CART) are feeding-inhibiting or anorexigenic peptides (Inui, 2000; Schwartz *et al.*, 2000). Many peptides are produced in the hypothalamus, and are well studied in terms of feeding, energy homeostasis and obesity. However, little is known about the role of these peptides in palatability-induced ingestion. We therefore investigated the involvement of hypothalamic neuropeptides in palatability-induced ingestion. We also examined the role of orexin-A in gastric functions to elucidate how this peptide increases food intake.

Methods and results

mRNA levels of hypothalamic neuropeptides after the drinking of saccharin solution or water

The time-courses of changes in mRNA levels for orexin, NPY and MCH after drinking a 5 mM solution of saccharin or water were examined. Wistar male rats were used in this and all other experiments in the present study. Each rat was given 10 ml of saccharin solution or water. The hypothalamus was extracted at different time points after drinking. Total RNA was extracted and mRNA levels of neuropeptides were measured using the conventional RT-PCR method. Drinking of saccharin facilitated an increase in the orexin mRNA level compared to that of water. Orexin levels showed a peak at 60 min, and then decreased gradually to levels obtained by water drinking within 210 min. NPY mRNA levels increased after 30 min compared with the 0 min control point and remained stable at high levels until 210 min. MCH mRNA levels did not differ between saccharin and water intakes, and remained stable throughout the time examined.

Effects of hypothalamic neuropeptides on the intake of saccharin solution and water

We examined the effects of the hypothalamic peptides on fluid intake. Orexin-A (3 nmol), MCH (3 nmol), NPY (0.6 nmol), ghrelin (0.3 nmol) or AgRP (0.15 nmol) was directly administered into the lateral ventricle to examine their effects on fluid intake. The doses used were sufficient to induce food intake. The amount of drinking was measured for 2 h after injection. Orexin-A administration increased both water and saccharin intake compared to vehicle administration (saccharin, 27.9 ± 0.9 versus 19.8 ± 0.8; water, 18.8 ± 1.2 versus 14.2 ± 0.8, $P < 0.05$ test). Similarly, administration of MCH increased the intake of both fluids (saccharin, 26.2 ± 0.9 versus 19.0 ± 0.4; water, 19.3 ± 1.3 versus 15.0 ± 0.5, $P < 0.05$). However, NPY injections enhanced only the intake of saccharin (25.9 ± 0.9 versus 20.0 ± 1.1, $P < 0.05$). These results suggest that orexin-A, MCH and NPY are related to the facilitation of drinking. On the other hand, ghrelin and AgRP had no effects on fluid intake at the doses used here. High doses of ghrelin (3 nmol) and AgRP (1 nmol) had no significant effects either, suggesting that ghrelin and AgRP have little influence on drinking behavior.

Effects of naloxone on the orexigenic actions of orexin-A, MCH and NPY

Opioids are known to be associated with the palatability of foods (especially that of sweet-tasting substances) and to stimulate feeding. Lynch (1986) reported that naloxone, an opioid antagonist, was effective in blocking the intake of and preference to saccharin. We examined the possible interactions of orexin-A, MCH and NPY with the opioid system. For this purpose, naloxone (0.3 mg/kg) or saline was injected i.p. 20 min before the intracerebroventricular (i.c.v.) administration of the hypothalamic neuropeptides or vehicle. The intake of 5 mM saccharin was measured 2 h after peptide administration. When saline was injected in advance, orexin-A facilitated the intake of saccharin solution (26.3 ± 0.3 g). However, when naloxone was injected in advance, orexin-A did not increase saccharin intake at all (16.4 ± 1.0 g). In a pilot study, we confirmed that 0.3 mg/kg naloxone itself had no effects on drinking behavior. Naloxone also blocked the effects of NPY. On the other hand, naloxone had no effect on the orexigenic action of MCH. These results suggest that the opioid system is essential for the orexigenic actions of orexin-A and NPY, but not of MCH. Since opioids are associated with palatability, orexin-A and NPY may exert their palatability-induced ingestion influence via the opioid system.

The role of orexin-A in gastric functions

Increased ingestion may be closely related to enhanced digestive functions. To address this issue, we examined the effects of i.c.v. injection of orexin-A on gastric motility. The motility changes were measured as alterations in intragastric pressure, measured by means of two balloons in two distinct parts of the stomach, i.e. in the proximal and distal part (Kobashi *et al.*, 2002). The proximal stomach is known to function as a reservoir, whereas the distal stomach's main function is to stir and drain gastric contents. Orexin-A (3 nmol) administered intracisternally induced strong rhythmical gastric motility, while the vehicle had no effects. Since the function of the distal stomach is to stir and discharge gastric contents, gastric

motilities induced by orexin-A may facilitate digestive function. In the proximal stomach, orexin-A induced relaxation, while the vehicle had no effects. It is suggested that the relaxation of the proximal stomach enables it to accept more food, and the facilitation of phasic contractions of the distal stomach accelerates draining of the increased gastric contents.

Thus, it is plausible that the orexigenic action of orexin-A accompanies the activation of digestive function. To confirm that the increased activity of the stomach by orexin-A administration is related to increased consumption of food, we investigated the effects of orexin-A on the draining of gastric contents. The rats were given a mash made of 4 g powdered chow mixed with an equal amount of water. Orexin-A (3 nmol) or the vehicle was administered after the rats ate all the mash. We extirpated the stomach and took out the gastric contents 150 min after the rats ate all the mash. Gastric contents were dried and the weight of the residual chow was measured. Orexin-A significantly increased draining of the mash (2.52 ± 0.1 g) compared with vehicle administration (1.99 ± 0.1 g). This result suggests that stomach movements induced by orexin-A administration are physiological and related to digestive functions.

Effects of taste on the draining of gastric contents

As mentioned above, palatability of the sweetener, saccharin, increased orexin levels, and orexin-A, in turn, facilitated draining of the gastric contents. It is therefore probable that a sweet-tasting food is drained out of the stomach faster than a food of bitter taste. To examine this, we measured the drained amount 150 min after rats ate taste adulterated mash. Mash foods were flavored with either 0.1 M saccharin or 0.01 M quinine. Control mash had no additional taste. Saccharin flavor increased the draining of mash from the stomach compared with that of control mash (2.61 ± 0.2 versus 1.96 ± 0.1, $P < 0.05$). On the other hand, quinine flavor suppressed draining of the mash (1.41 ± 0.1 versus 1.96 ± 0.1, $P < 0.05$).

Discussion

In the present series of experiments we investigated the possible involvement of hypothalamic neuropeptides in palatability-induced ingestion. Drinking of saccharin increased the mRNA levels of orexin and NPY. Administration of orexin-A and NPY stimulated drinking behavior. These findings suggest that the palatability of sweeteners activates hypothalamic peptides such as orexin-A and NPY, and in turn, the activation of these hypothalamic peptides facilitates ingestion. Naloxone inhibited increased saccharin intake induced by orexin-A and NPY. Therefore, orexin-A and NPY might be related to palatability-induced ingestion via the opioid system. We then examined the effects of orexin-A on gastric motility, and found that orexin-A induced phasic contractions in the distal stomach, but relaxation in the proximal stomach. These results indicate that the orexigenic action of orexin-A is accompanied by activation of the digestive function. In the next experiment, we investigated the effects of orexin-A on the draining of gastric contents. Since orexin-A administration increased the draining of gastric contents, gastric motor responses induced by orexin-A can be considered physiological and related to digestive functions. In accordance with the above-mentioned findings, saccharin increased orexin levels, and orexin-A facilitated the draining of gastric contents. The amount of drained mash from the stomach was larger when the mash was flavored with saccharin compared to mash with quinine flavoring. These results may answer a common question why you can eat sweet-tasting, palatable foods faster and in larger volumes than bitter-tasting aversive foods.

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