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ORIGINAL PAPER

Centrally administered ghrelin potently inhibits water intake induced by angiotensin II and hypovolemia in rats

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Abstract Ghrelin is a potent, centrally acting orexigenic hormone. Recently, we showed that centrally administered ghrelin is a potent antidipsogenic hormone in 24-h water deprived rats. In this study, we examined the effect of intracerebroventricular (icv) injection of ghrelin on angiotensin II (AII)-induced water intake in rats. We also examined the effects of icv injection of ghrelin on drinking induced by intraperitoneal injection of an isotonic polyethylene glycol (PEG) solution that causes isotonic hypovolemia. Water intake induced by the icv injection of AII or ip injection of PEG was significantly reduced after icv injection of ghrelin, although food intake was stimulated by the hormone. The drinking induced by AII was also inhibited by the icv administration of 4α-phorbol 12, 13-didecanoate, an agonist of the osmosensitive TRPV4 channel. This study showed that ghrelin is a potent antidipsogenic peptide by antagonizing general dipsogenic mechanisms including those activated by AII and hypovolemia in rats.

Keywords Angiotensin II · Desacyl ghrelin · Drinking · Ghrelin · Hypovolemia · TRPV4

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Introduction

Homeostasis of body fluids is regulated by a balance between water intake and excretion. In the central nervous system (CNS), osmoreceptors, which detect changes in osmotic pressures of body fluids, are important in osmoregulation. Osmoreceptors are suggested to be distributed in the circumventricular organs (CVOs), including the organum vasculosum of the lamina terminalis (OVLT) and subfornical organ (SFO) [1]. However, molecular identification of this osmoreceptor has not yet been achieved.

The transient receptor potential (TRP) superfamily of non-selective cation channels is known to be present in many species [2]. Of six subfamilies, some members of the TRPV and TRPM subfamilies are temperature sensitive, and each has a different range of temperature sensitivity. Previous study showed that TRPV4, one of the members of TRPV subfamily, is sensitive to osmotic pressure, as well as temperature [3]. They showed that TRPV4 was activated by hypotonic stimuli, resulting in an increase in intracellular Ca^{2+} concentration. Recently, Tsushima and Mori [4] showed the TRPV4 agonist, 4α-phorbol 12, 13-didecanoate (4α-PDD), decreased angiotensin II (AII)-induced water intake. These reports suggested that the TRP family might be involved in central regulation of body fluid balance, including the osmosensing mechanism and water drinking behavior.

Most species show a close relationship between drinking and feeding [5, 6]. Approximately 80% of spontaneous daily water intake is temporally associated with feeding in rats [7]. Ghrelin, a 28-amino-acid neuropeptide and an endogenous ligand for the growth hormone secretagogue receptor (GHS-R), was first isolated from stomach [8]. However, ghrelin is found in the brain and now is recognized as a neuropeptide. In addition to the stimulation of

growth hormone secretion from the anterior pituitary, central and peripheral administration of ghrelin strongly stimulates feeding in mammals [9, 10]. Ghrelin is established as a major orexigenic hormone acting not only from the periphery but also locally in the brain. Ishizaki et al. [11] reported that intracerebroventricular (icv) and intravenous (iv) injection of ghrelin increased plasma arginine vasopressin (AVP) levels in conscious rats. AVP is well known as an important hormone involved in body fluid balance [12]. Thus, it is possible that ghrelin may have a potent effect on drinking behavior and body fluid balance in mammals. Our previous study demonstrated that dehydration-induced drinking was significantly inhibited by icv administration of ghrelin in conscious rats [13]. Recently, Mietlicki et al. [14] showed that centrally administered AII-induced drinking was reduced by icv administration of ghrelin.

In the present study, we examined the effects of centrally administered ghrelin, 4α-PDD, and desacyl ghrelin on AII- and/or hypovolemia-induced drinking behavior in conscious rats. Icv administration of AII and intraperitoneal (ip) injection of polyethylene glycol (PEG) caused marked increase of water intake in euhydrated rats. Peripheral administration of PEG is known to produce a gradual and progressive reduction in plasma volume without any change of plasma osmolality and sodium concentration [15].

Materials and methods

Animals

Adult male Wistar rats, weighing 200–300 g, were housed individually in plastic cages in an air-conditioned room (24 ± 1°C) under a 12-h light (0700–1900)/12-h dark cycle.

All procedures in the present study were done in accordance with guidelines on the use and care of laboratory animals as set out by the Physiological Society of Japan and under the control of the Ethics Committee of Animal Care and Experimentation, University of Occupational and Environmental Health, Japan.

Surgical procedures

For icv injection of solutions, animals were implanted with stainless steel cannulae in the lateral ventricle. Animals were anesthetized (sodium pentobarbital, 50 mg/kg body weight, ip injection) and then placed in a stereotaxic frame. A stainless steel guide cannula (550 μm outer diameter, 10 mm length) was implanted stereotaxically at the following coordinates: 0.8 mm posterior to the bregma, 1.4 mm lateral to midline, and 2.0 mm below the surface of the left cortex such that a tip of the cannula was 1.0 mm above the left cerebral ventricle [16]. Two stainless steel anchoring screws were fixed to the skull, and the cannula was secured in place by acrylic dental cement.

The animals were then returned to their cages and allowed to recover for at least 5 days. During the recovery period, the animals were handled daily.

Central administration of ghrelin, 4a-PDD, desacyl ghrelin, and AII

For icv injection of ghrelin, desacyl ghrelin, 4α-PDD, AII, or vehicle (sterile 0.9% saline), a stainless steel injector (300 μm, outer diameter) was introduced through the cannula at a depth of 1.0 mm beyond the end of the guide. The total volume of injected solution of ghrelin, desacyl ghrelin, 4α-PDD, AII, and saline into the lateral ventricle was 5 μl. Rat ghrelin and desacyl ghrelin was purchased from the Peptide Institute (Minoh, Japan). 4α-PDD and AII were purchased from the Sigma-Aldrich (Tokyo, Japan). Ghrelin, desacyl ghrelin, 4α-PDD, and AII were dissolved in sterile 0.9% saline.

Experimental procedures

In the first experiment, a dose-response study was performed to confirm the effects of 4α-PDD on AII-induced water intake. Thirty minutes after icv injection of 4α-PDD (1, 3, or 10 nmol) or vehicle, we administered AII (96 pmol) or vehicle. After icv injection of AII or vehicle, the animals were put into metabolic cages. We measured the cumulative water intake 30 and 60 min after icv injection of AII. The number of rats was 6–7 in each group.

In the second experiment, 30 min after icv injection of ghrelin (1 nmol), 4α-PDD (10 nmol), or vehicle, we administered AII (96 pmol) or vehicle. After icv injection of AII or vehicle, the animals were put into metabolic cages. We measured the cumulative water intake and food intake 15–180 min after icv injection of AII. Number of rats was 6–9 in each group. Moreover, 30 min after icv injection of desacyl ghrelin (200 nmol) or vehicle, we administered AII (96 pmol) or vehicle. After icv injection of AII or vehicle, the animals were put into metabolic cages. We measured the cumulative water intake and food intake 30 and 60 min after icv injection of AII. The number of rats was 6 in each group.

In the third experiment, after ip injection of PEG (MW 2700–3500, 20 ml/kg) or vehicle, the animals were deprived of water for 6 h. Six hours later, we administered ghrelin (1 nmol), 4α-PDD (10 nmol), or vehicle. After icv injection of solutions, the animals were put into metabolic cages. We measured the cumulative water intake and food

intake 15–180 min after icv injection of solutions. The number of rats was 6 in each group.

Measurement of total protein, sodium, and hematocrit in plasma

Plasma concentration of total protein and sodium were measured by the automatic analyzer (Hitachi 710, Hitachi, Ibaraki, Japan) to confirm the isotonic hypovolemia caused by ip administration of PEG. Hematocrit (Hct) was measured by using a Hct capillary tube (Terumo Co. Ltd, Tokyo, Japan).

Statistical analysis

A mean deviation from control (percentage) ± SEM was calculated from data obtained from measurement of the cumulative water drinking and food intake, Hct, plasma total protein, and sodium. Each group within an experiment was compared with the vehicle-treated group. The data were analyzed using a one-way fractional ANOVA followed by a Bonferroni correction for multiple comparisons. The statistical significance was set at $P < 0.05$.

Results

Dose response study of icv injection of 4α-PDD on AII-induced water intake

In the dose response study using 4α-PDD, water intake was significantly inhibited 30 and 60 min after icv injection of 4α-PDD (10 nmol) in comparison with vehicle (Fig. 1). We could confirm the antidipsogenic effect of 4α-PDD on AII-induced water intake, as previously reported [4]. However, the effects of icv injection of 4α-PDD (1 and 3 nmol) were not different from that of vehicle-injected controls (Fig. 1). Therefore, we used a dose of 10 nmol 4α-PDD in subsequent experiments.

Effects of icv injection of ghrelin on AII-induced water intake

Water intake was significantly increased after icv injection of AII (96 pmol) in comparison with control rats (Fig. 2a). AII-induced water intake was significantly inhibited 15–120 min after icv injection of ghrelin (1 nmol) or 4α-PDD (10 nmol) in comparison with vehicle (Fig. 2a), as previously reported [4, 14]. The effect of ghrelin was similar to 4α-PDD at 15–120 min (Fig. 2a). However, the effect of ghrelin was relatively transient compared with 4α-PDD, and 180 min after icv injection, the effect of ghrelin was no longer different from that of vehicle-injected controls

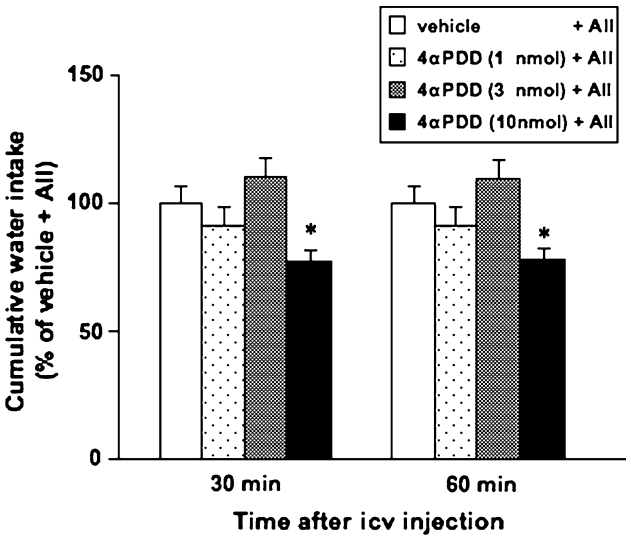

Fig. 1 Effects of intracerebroventricular (*icv*) injection of 4α-PDD (1, 3, and 10 nmol) or vehicle on cumulative water intake for 30 and 60 min after icv injection of angiotensin II (*AII*) (100 ng) in conscious rats. Data for cumulative water intake are expressed as the mean ± SEM ($n = 6$–7). *$P < 0.05$, compared with vehicle-injected rats

(Fig. 2a). Thus, the inhibition with 4α-PDD was greater than that of ghrelin 180 min after injection (Fig. 2a).

Food intake was significantly increased 120 min after icv injection of ghrelin in comparison with vehicle (Fig. 2b). This effect lasted 180 min after icv injection of ghrelin (Fig. 2b). Food intake did not change after injection of 4α-PDD or AII in comparison with vehicle (Fig. 2b).

We also examined the effects of centrally administered desacyl ghrelin on AII-induced water intake. Water intake did not change 30 and 60 min after icv injection of desacyl ghrelin (200 nmol) in comparison with vehicle (Fig. 3).

Effects of icv injection of ghrelin on PEG-induced water intake

The Hct and concentrations of total protein and sodium in plasma after ip injection of PEG are shown in Table 1. Hct and plasma concentrations of total protein were significantly increased after ip injection of PEG ($P < 0.01$) in comparison with control (euhydrated) rats and vehicle-treated rats. In all groups, plasma concentration of sodium did not change significantly.

Water intake was significantly increased after ip injection of PEG (20 ml/kg) in comparison with control rats (Fig. 4a). PEG-induced water intake was significantly inhibited 15–60 min after icv injection of ghrelin (1 nmol) in comparison with vehicle and 4α-PDD (10 nmol) (Fig. 4a). However, 120 min after icv injection, the effect of ghrelin was not different from that of vehicle-injected controls (Fig. 4a).

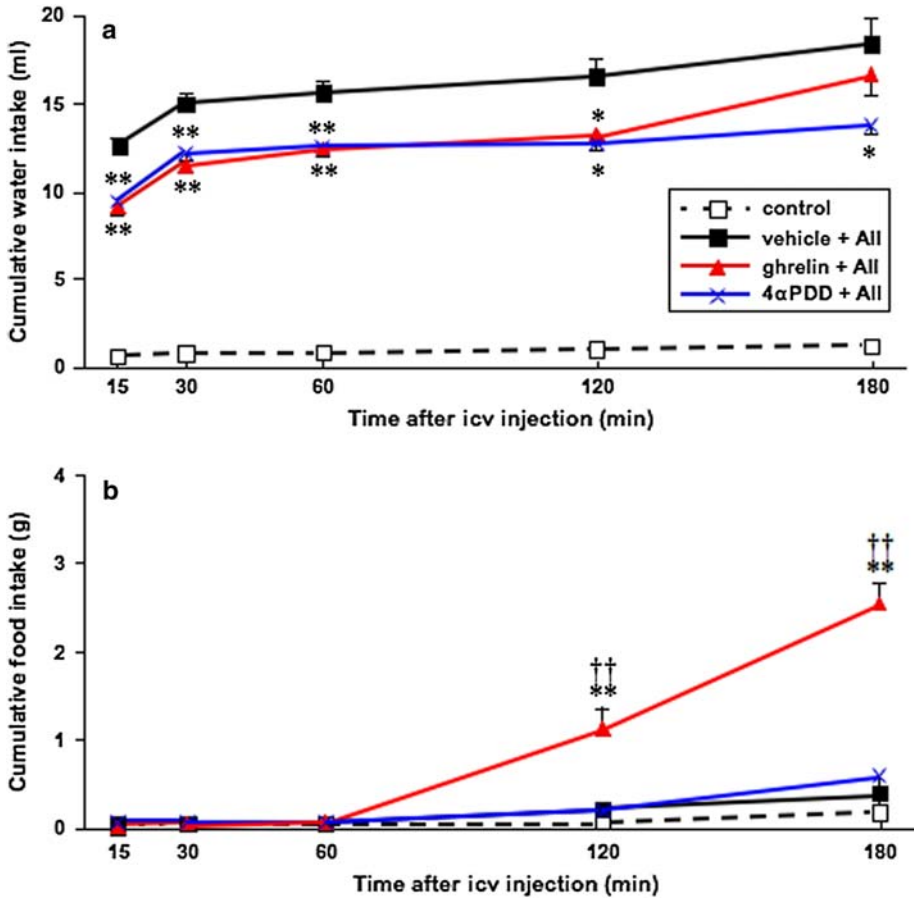

Fig. 2 Effects of intracerebroventricular (*icv*) injection of ghrelin (1 nmol), 4α-PDD (10 nmol) or vehicle on cumulative water intake (**a**) and food intake (**b**) for 15–180 min after icv injection of angiotensin II (*AII*) (96 pmol) in conscious rats. Data for cumulative water intake and food intake are expressed as the mean ± SEM ($n = 7$–15). $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with vehicle-injected rats. $^{\dagger}P < 0.05$ and $^{\dagger\dagger}P < 0.01$, compared with 4a-PDD-injected rats

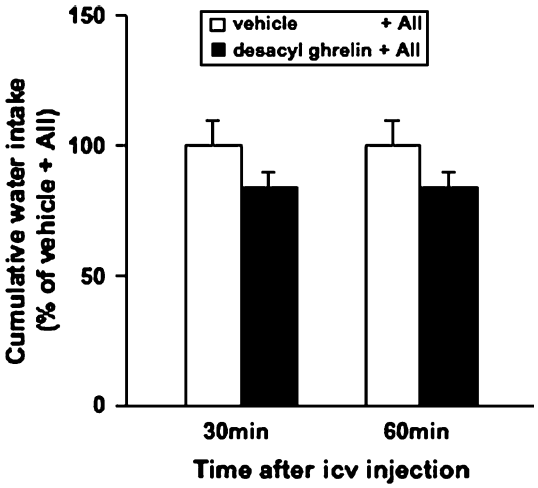

Fig. 3 Effects of intracerebroventricular (*icv*) injection of desacyl ghrelin (200 nmol) or vehicle on cumulative water intake for 30 and 60 min after icv injection of angiotensin II (*AII*) (96 pmol) in conscious rats. Data for cumulative water intake are expressed as the mean ± SEM ($n = 6$–7)

Food intake was significantly increased 60 min after icv injection of ghrelin in comparison with vehicle (Fig. 4b). This effect lasted for 180 min after icv injection of ghrelin (Fig. 4b). Food intake did not change after injection of 4α-PDD in comparison with vehicle (Fig. 4b).

Discussion

The present study demonstrates that centrally administered ghrelin causes the inhibition of PEG-induced as well as AII-induced water intake in rats. We measured water intake together with food intake using metabolic cages after icv injection of ghrelin or 4α-PDD. In both AII-induced and PEG-induced water intake, icv injection of ghrelin potently decreased water intake, while icv injection of 4α-PDD decreased AII-induced water intake as previously reported [4], and did not change PEG-induced water intake. Moreover, AII-induced water intake was not significantly affected by centrally administered desacyl ghrelin.

Central administration of AII is known to have rapid and large dipsogenic effect in rats [17]. It has been hypothesized that AII acts on the CVOs, including the OVLT and SFO. The neurons in the OVLT and SFO are osmosensitive and AII sensitive in the rats [1, 18, 19]. Centrally administered AII induced c-*fos* protein (Fos) in the OVLT and

Table 1 Effects of ip injection of polyethylene glycol on hematocrit (Hct), plasma concentration of total protein, and sodium 6 h after treatment

	Hct (%)	Total protein (g/dl)	Plasma Na^+ (mEq/l)
Control	41.7 ± 0.49	6.0 ± 0.17	142 ± 0.7
Vehicle (saline ip + water deprivation)	41.1 ± 0.30	5.7 ± 0.10	140 ± 0.9
PEG (PEG ip + water deprivation)	48.6 ± 1.08**	7.0 ± 0.24**	140 ± 0.7

PEG Polyethylene glycol. Values represent mean ± SEM ($n = 6$–8)

**$P < 0.01$, compared with other groups

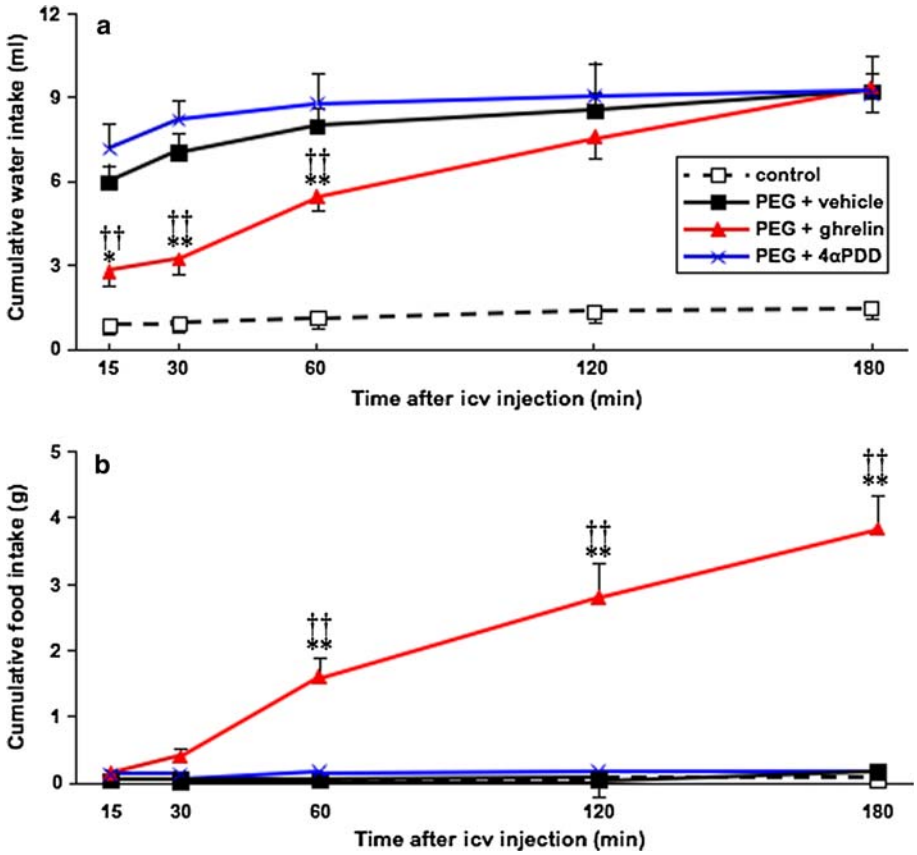

Fig. 4 Cumulative water intake (**a**) and food intake (**b**) for 15–180 min after intracerebroventricular (*icv*) injection of ghrelin (1 nmol), 4α-PDD (10 nmol), or vehicle in hypovolemia rats induced by ip injection of polyethylene glycol (*PEG*). Data for cumulative water intake and food intake are expressed as the mean ± SEM ($n = 6$–8). *$P < 0.05$ and **$P < 0.01$, compared with vehicle-injected rats. †$P < 0.05$ and ††$P < 0.01$, compared with 4a-PDD-injected rats

SFO in rats [20]. Our previous study showed that centrally administered ghrelin did not induce Fos in the OVLT and SFO [13]. It is possible that centrally administered ghrelin may excite inhibitory neurons directly or indirectly to attenuate AII-induced water intake in rats. This point should be clarified by further study.

Two major molecular forms of ghrelin were found in the stomach and plasma: acylated ghrelin, which has *n*-octanoylated serine in position 3, and desacyl ghrelin [21]. Acylation is essential for the binding of ghrelin to the GHS-R, and although desacyl ghrelin does not bind to the GHS-R, it may be biologically active [21–23]. Previous studies showed that centrally administered desacyl ghrelin can increase [24] or decrease [25] food intake. Matsuda et al. [26] showed that desacyl ghrelin inhibits acyl ghrelin-induced orexigenic activity in goldfish. They suggested that desacyl ghrelin might have an unknown receptor, which was different from ghrelin-binding receptor. We showed that centrally administered ghrelin inhibited AII-induced water intake, while desacyl ghrelin did not have the effect on AII-induced water intake (Fig. 3). These results suggested that GHS-R might hold the key to clarifying the mechanism of antidipsogenic effect of ghrelin. Thus, further studies are required to demonstrate the relationship of ghrelin and GHS-R in the regulatory system of drinking in the CNS.

Peripheral administration of PEG is known to produce a reduction in plasma volume without any change of plasma osmolality [15]. In PEG-administered rats, we observed a significant increase in Hct and plasma concentration of

total protein, but not in plasma concentration of sodium compared with controls (Table 1). Hct and plasma concentrations of total protein were significantly increased after ip injection of PEG ($P < 0.01$) in comparison with control (euhydrated) rats and vehicle-treated rats. In all groups plasma concentration of sodium did not change significantly. Therefore, isotonic hypovolemia occurred in PEG-administered rats. Centrally administered 4α-PDD did not change PEG-induced water intake (Fig. 4). As TRPV4 is sensitive to osmotic pressure [3], the lack of effect of 4α-PDD on PEG-administered rats was expected. On the other hand, centrally administered ghrelin did inhibit PEG-induced water intake. These results suggest that ghrelin and 4α-PDD have different sites of action related to water drinking in the CNS in rats and the inhibition due to centrally administered ghrelin is caused not only by osmosensitive sites in the CNS but by other areas, including unknown centers that regulates water intake.

In the present study, although AII-induced water intake was increased 120–180 min after icv injection of ghrelin (1 nmol/rat), these increases may be caused by the feeding-associated drinking (prandial drinking) as food intake markedly increased after 60 min of injection (Fig. 2a, b). The prandial drinking was also observed 60–180 min after icv injection of ghrelin in the PEG-treated rats (Fig. 4a, b). It is interesting that antidipsogenic effects of ghrelin precede the orexigenic effect. Previously our study also showed this same phenomenon in dehydrated rats [13]. However, this phenomenon was not shown in the report by Mietlicki et al. [14]. Although it is difficult to explain this difference, differences between Wistar and Sprague-Dawley rats might account for the apparent discrepancy.

Generally, it is recognized that water intake is controlled by two major systems, osmolality and water volume [27]. Moreover, AII acts as a major system regulating water intake independent of osmolality and extracellular volume [28]. Centrally administered 4α-PDD inhibited AII-induced water intake and did not change hyperosmolality-induced water intake [4]. We showed that centrally administered 4α-PDD did not change PEG-induced water intake, which is not related to osmolality. These results suggested that 4α-PDD may have an important role on AII-induced water intake. A recent study showed that AII-induced drinking was reduced by icv injection of ghrelin [14]. In our previous and present studies, we showed that centrally administered ghrelin inhibited water intake in dehydrated, AII-induced, PEG-induced thirsty rats [13]. These results suggested that ghrelin and 4α-PDD may have different action sites related to water drinking in the CNS in rats. Especially ghrelin is a potent antidipsogenic peptide and may be an important key to clarify the general dipsogenic mechanisms in the CNS.

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References

1. Johnson AK, Cunningham JT, Thunhorst RL (1996) Integrative role of the lamina terminalis in the regulation of cardiovascular and body fluid homeostasis. Clin Exp Pharmacol Physiol 23:183–191
2. Clapham DE (2003) TRP channels as cellular sensors. Nature 426:517–524
3. Liedtke W, Choe Y, Martí-Renom MA, Bell AM, Denis CS, Sali A, Hudspeth AJ, Friedman JM, Heller S (2000) Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. Cell 103:525–535
4. Tsushima H, Mori M (2006) Antidipsogenic effects of a TRPV4 agonist, 4alpha-phorbol 12, 13-didecanoate, injected into the cerebroventricle. Am J Physiol Regul Integr Comp Physiol 290:R1736–R1741
5. de Castro JM (1988) A microregulatory analysis of spontaneous fluid intake by humans: evidence that the amount of liquid ingested and its timing is mainly governed by feeding. Physiol Behav 43:705–714
6. Morgan CA, Emmans GC, Tolkamp BJ, Kyriazakis I (2000) Analysis of the feeding behavior of pigs using different models. Physiol Behav 68:395–403
7. Kissileff HR (1969) Food-associated drinking in the rat. J Comp Physiol Psychol 67:284–300
8. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K (1999) Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402:656–660
9. Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DG, Ghatei MA, Bloom SR (2000) The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology 141:4325–4328
10. Cowley MA, Smith RG, Diano S, Tschop M, Pronchuk N, Grove KL, Strasburger CJ, Bidlingmaier M, Esterman M, Heiman ML, Garcia-Segura LM, Nillni EA, Mendez P, Low MJ, Sotonyi P, Friedman JM, Liu H, Pinto S, Colmers WF, Cone RD, Horvath TL (2003) The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. Neuron 37:649–661
11. Ishizaki S, Murase T, Sugimura Y, Kakiya S, Yokoi H, Tachikawa K, Arima H, Miura Y, Oiso Y (2002) Role of ghrelin in the regulation of vasopressin release in conscious rats. Endocrinology 143:1589–1593
12. Caldwell HK, Young WS III (2006) Oxytocin and vasopressin: genetics and behavioral implications. In: Lajtha A, Lim R (eds) Handbook of neurochemistry and molecular neurobiology: neuroactive proteins and peptides, 3rd edn. Springer, Berlin, pp 573–607
13. Hashimoto H, Fujihara H, Kawasaki M, Saito T, Shibata M, Otsubo H, Takei Y, Ueta Y (2007) Centrally and peripherally administered ghrelin potently inhibits water intake in rats. Endocrinology 148:1638–1647
14. Mietlicki EG, Nowak EL, Daniels D (2009) The effect of ghrelin on water intake during dipsogenic conditions. Physiol Behav 96:37–43

15. Stricker EM, Verbalis JG (1986) Interaction of osmotic and volume stimuli in regulation of neurohypophyseal secretion in rats. Am J Physiol 250:R267–R275
16. Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates, 4th edn. Academic Press, Sydney
17. Epstein AN, Fitzsimons JT, Rolls BJ (1970) Drinking induced by injection of angiotensin into the rain of the rat. J Physiol 210:457–474
18. Tanaka J, Kaba H, Saito H, Seto K (1985) Electrophysiological evidence that circulating angiotensin II sensitive neurons in the subfornical organ alter the activity of hypothalamic paraventricular neurohypophyseal neurons in the rat. Brain Res 342:361–365
19. Nelson DO (1988) Development of angiotensin II-sensitive OVLT neurons in SHR and WKY rats. Brain Res 467:105–110
20. Zhu B, Herbert J (1996) Central antagonism of atrial natriuretic peptides on behavioral and hormonal responses to angiotensin II: mapping with c-fos. Brain Res 734:55–60
21. Hosoda H, Kojima M, Matsuo H, Kangawa K (2000) Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue. Biochem Biophys Res Commun 279:909–913
22. Heijboer AC, van den Hoek AM, Parlevliet ET, Havekes LM, Romijn JA, Pijl H, Corssmit EP (2006) Ghrelin differentially affects hepatic and peripheral insulin sensitivity in mice. Diabetologia 49:732–738
23. Muccioli G, Baragli A, Granata R, Papotti M, Ghigo E (2007) Heterogeneity of ghrelin/growth hormone secretagogue receptors. Toward the understanding of the molecular identity of novel ghrelin/GHS receptors. Neuroendocrinology 86:147–164
24. Toshinai K, Yamaguchi H, Sun Y, Smith RG, Yamanaka A, Sakurai T, Date Y, Mondal MS, Shimbara T, Kawagoe T, Murakami N, Miyazato M, Kangawa K, Nakazato M (2006) Des-acyl ghrelin induces food intake by a mechanism independent of the growth hormone secretagogue receptor. Endocrinology 147:2306–2314
25. Asakawa A, Inui A, Fujimiya M, Sakamaki R, Shinfuku N, Ueta Y, Meguid MM, Kasuga M (2005) Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. Gut 54:18–24
26. Matsuda K, Miura T, Kaiya H, Maruyama K, Shimakura S, Uchiyama M, Kangawa K, Shioda S (2006) Regulation of food intake by acyl and des-acyl ghrelins in the goldfish. Peptides 27:2321–2325
27. Johnson AK (2007) The sensory psychobiology of thirst and salt appetite. Med Sci Sports Exerc 39:1388–1400
28. McKinley MJ, Walker LL, Alexiou T, Allen AM, Campbell DJ, Di Nicolantonio R, Oldfield BJ, Denton DA (2008) Osmoregulatory fluid intake but not hypovolemic thirst is intact in mice lacking angiotensin. Am J Physiol Regul Integr Comp Physiol 294:R1533–R1543