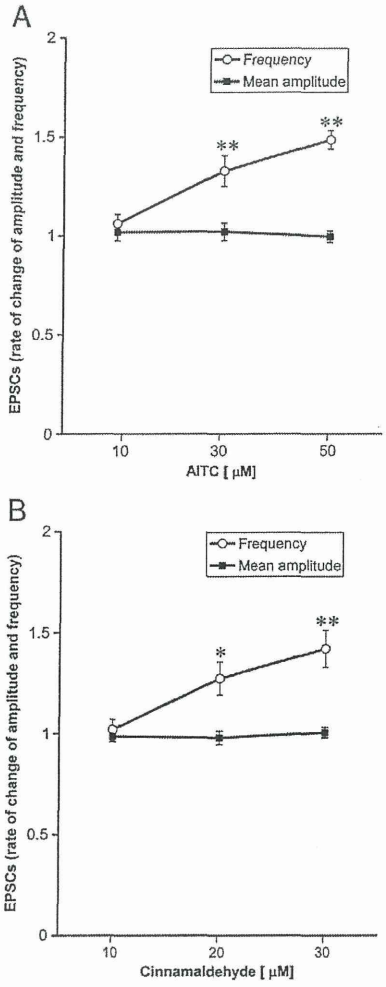

Fig. 3. Concentration–response relationship of AITC- and cinnamaldehyde-induced potentiation of miniature excitatory postsynaptic currents (mEPSCs). Supraoptic neurones were exposed to AITC at three different concentrations (10 μM, 30 μM and 50 μM; $n=5$–9) (A) or cinnamaldehyde at three different concentrations (10 μM, 20 μM and 30 μM; $n=5$–9), and the effects were expressed as rate of change (±S.E.M.) of frequency and amplitude of mEPSCs recorded for 3-min periods (after the start of AITC or cinnamaldehyde application) from value recorded in control periods before AITC application. Open circle, rate change of frequency; closed square, rate change of amplitude. *$P<0.05$ versus control and **$P<0.01$ versus control.

and a high concentration of menthol attenuates the effect of activation of TRPA1 (Karashima et al., 2007; Macpherson et al., 2006). Exposure to a high concentration of menthol (300 μM) did not affect the mEPSCs (frequency 99.0±4.9%, amplitude 99.1±1.9% of control. $n=6$). Pre-exposure to a high concentration of menthol attenuated the potentiation of mEPSCs by AITC (50 μM) and cinnamaldehyde (30 μM). High concentration of menthol almost abolished the AITC- and cinnamaldehyde-induced increase in the mEPSCs frequency, but had no effect on the amplitude of the mEPSCs (AITC: frequency 106± 8.6%, amplitude 103±3.4% of control, $n=6$; cinnamaldehyde: frequency 103±4.9%, amplitude 103±1.6% of control, $n=3$) (Figs. 4C, D and 5C, D). HC-030031$_{(10\ \mu M)}$ also attenuated the AITC- and cinnamaldehyde-induced potentiation of mEPSCs (AITC: frequency 107±4.1%, amplitude 102±1.6% of control, $n=6$; cinnamaldehyde: frequency 107±4.9%, amplitude 96.9±1.6% of control, $n=6$) (Figs. 4E, F and 5E, F). These results suggest the possible involvement of TRPA1 channels in both AITC- and cinnamaldehyde-induced potentiation of the mEPSCs.

3.5. Effect of AITC on Ca^{2+}-free perfusion medium

To examine whether the potentiation of mEPSC by AITC is dependent on extracellular Ca^{2+}, we used Ca^{2+}-free solution. The frequency and amplitude of mEPSC in the Ca^{2+}-free solution were significantly smaller than that in normal perfusion solution (normal versus Ca^{2+}-free: frequency 1.67±0.2 Hz versus 0.97±0.1 Hz, $n=8$–9, $P<0.01$, amplitude 24.4±0.4 pA versus 22.9±0.3 pA, $n=8$–9, $P<0.05$). Under this condition, AITC did not increase the frequency and amplitude of mEPSC (frequency 105±6.1% of control, amplitude 104±1.8% of control, $n=8$) (Fig. 6). Thus, the AITC-induced potentiation of mEPSC was extracellular Ca^{2+}-dependent.

4. Discussion

In the present study, we provided the first evidence that AITC and cinnamaldehyde are well known as TRPA1 agonists potentiate excitatory synaptic inputs to the supraoptic MNCs in rats using a whole-cell patch-clamp technique. Because glutamate and GABA are two major synaptic inputs into the SON neurones (Meeker et al., 1993; Wuarin and Dudek, 1993), the potentiation of mEPSCs by TRPA1 agonists, AITC and cinnamaldehyde may, at least in part, account for the excitatory action on electrical activity. The mEPSCs recorded in the SON slice preparations that we employed in this study were virtually insensitive to TTX. This result suggests that TRPA1 modulates glutamate release from the presynaptic terminal, and that increases of EPSCs do not depend upon action potential. The neurones were recorded in thin SON slices containing only the SON and the perinuclear zone, and the mEPSCs and mIPSCs probably reflect spontaneous transmitter release from the terminals of the cut axons, disconnected from their cell origin.

TRPA1 is a non-selective cation channel and is activated by noxious cold, pungent natural compounds such as AITC and cinnamaldehyde, mechanosensation and alkaline pH (Story et al., 2003; Bandell et al., 2004; Fujita et al., 2008; Jordt et al., 2004; Kwan et al., 2006, 2009). Activation of the TRPA1 channel is reversibly blocked by a high concentration of menthol (Macpherson et al., 2006; Karashima et al., 2007; Xiao et al., 2008). Moreover, a recent study demonstrated that hypertonic solution activates TRPA1 channels in human embryonic kidney 293 cells transiently expressing rat TRPA1 (Zhang et al., 2008).

Plasma osmolality is well known to regulate the activity of MNCs (Mason, 1980; Leng et al., 1982; Bourque, 1989). The supraoptic MNCs receive synaptic inputs from the organum vasculosum lamina terminals, the median nucleus of the preoptic area and the subfronical organ (Chaudhry et al., 1989; Honda et al., 1990; Richard and Bourque, 1992; 1995). These areas are very sensitive to osmotic changes and regulate body fluid and drinking behaviour (Bourque et al., 1994). In addition to integrative information from the osmosensitive areas, the MNCs are themselves also osmosensitive (Mason, 1980; Oliet and Bourque, 1992, 1993). Hyperosmotic stimuli also directly modulate glutamatergic inputs to the supraoptic MNCs by acting on the presynaptic terminals (Inenaga et al., 1997). A recent finding has indicated that an N-terminal variant of TRPV1 channel is required for osmosensory transduction in mouse SON neurones (Sharif Naeini et al., 2006). TRPA1 is present in TRPV1-expressing sensory neurones (Story et al., 2003; Kobayashi et al., 2005). However, it is unknown whether TRPA1 expresses in the SON. Our present study demonstrated that TRPA1 agonists potentiate excitatory synaptic inputs and these effects were attenuated by a high concentration of menthol and HC-030031. The results of our studies did not contradict previous reports which provide the relationship with TRPA1 channel and menthol (Karashima et al., 2007; Macpherson et al., 2007a; Xiao et al., 2008). These results suggest that activation of the TRPA1 channel possibly potentiates the excitatory synaptic inputs to the supraoptic MNCs neurones. Formalin, known as TRPA1 agonist-induced nociceptive stimulation, causes a rapid elevation of AVP levels (Suzuki

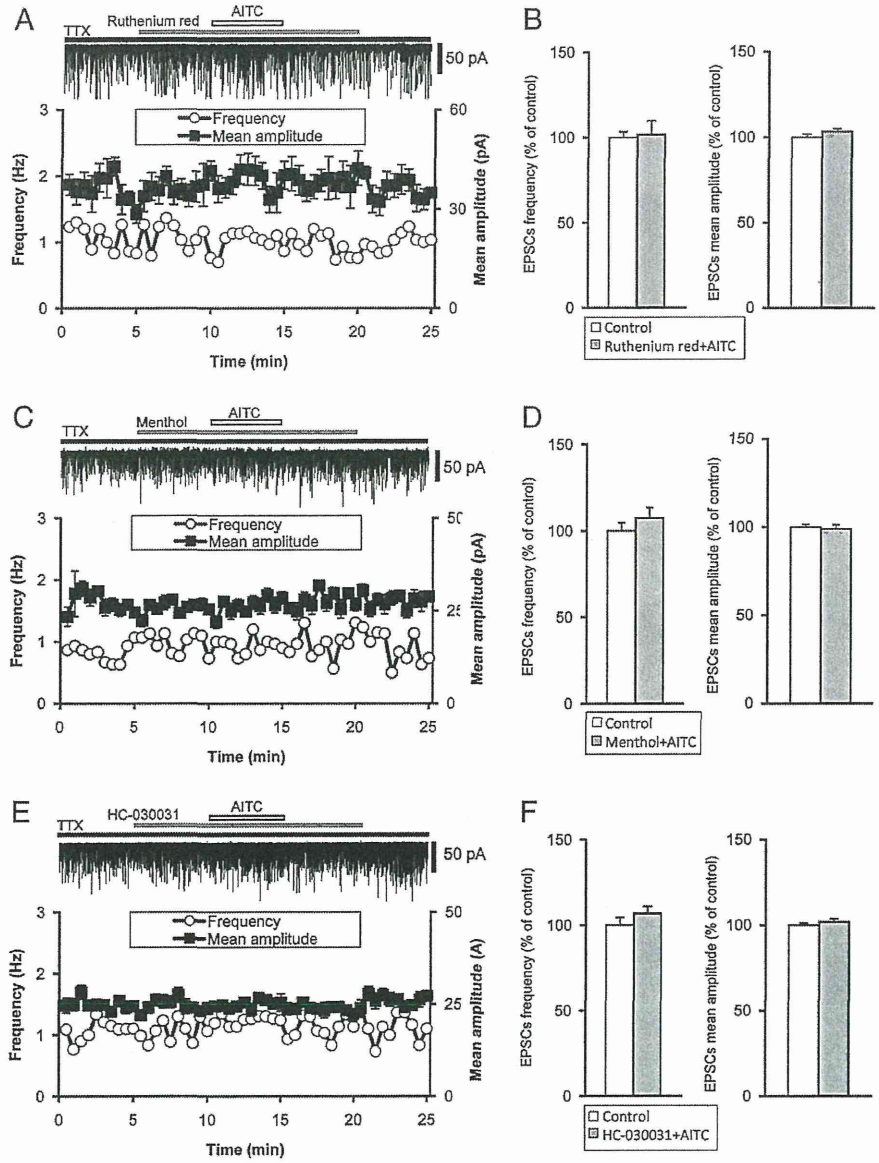

Fig. 4. Characterization of AITC-induced potentiation of mEPSCs. (A, C and E) Representative examples of the effects of ruthenium red (10 μM), non-specific TRP channels blocker (A), high concentration of menthol (300 μM) (C) and HC-030031 (10 μM) (E), TRPA1 selective channel blockers, on AITC-induced potentiation of mEPSCs. EPSCs were recorded in the presence of TTX (1 μM). The holding potential was −70 mV. Plots of frequency are single measurement, whereas plots of amplitude are mean ± S.E.M. over 30 s. (B, D and F) Summary data for characterization of mEPSCs under AITC (50 μM) application. Frequency (left) and amplitude (right) of mEPSCs. Ruthenium red plus AITC ($n=6$) (B), high concentration of menthol plus AITC ($n=6$) (D) and HC-030031 plus AITC ($n=6$) (F), respectively. Data are mean ± S.E.M.

et al., 2009). TRPA1 recognizes temperature and a chemical sense in the brain of the snake (Gracheva et al., 2010). The potentiation of excitatory synaptic transmission by the activation of presynaptic terminals TRPA1 may have an important role in neuronal activity and the secretion of neurohypophysial hormones (AVP and oxytocin (OXT)). It cannot be denied that extracellular matrixes around the MNCs, such as protein, glial cell and other mechano-channels, may participate in the regulation of glutamatergic release by TRPA1 agonists.

Previous studies have reported that noxious compounds such as AITC are activated through covalent modification of cysteine residues in the intercellular N-terminal domain (Hinman et al., 2006; Macpherson et al., 2007b), whereas activation by intercellular calcium appears to be dependent on the N-terminal EF-hand calcium-binding domain (Doerner et al., 2007; Zurborg et al., 2007). In the present study, AITC-induced potentiation of mEPSCs was attenuated under extracellular Ca^{2+} free solution. A recent study demonstrated that transmembrane domain 5 is a critical molecular determinant of menthol sensitivity in mammalian TRPA1 channels (Xiao et al., 2008). These results indicate that TRPA1 in the SON may be activated through covalent modification of cysteine residues in the N-terminal domain and by extracellular Ca^{2+}-dependent. However, it remains obscure what kind of physiological role TRPA1 mediates.

The MNCs (AVP- or OXT-producing neurones) in the SON can be divided into two groups based on their firing pattern. By combining immunohistochemical and electrophysiological techniques, most of the phasically firing neurones contain AVP (phasic neurones), whereas the other neurones that do not fire phasically (nonphasic neurones) contain OXT (Yamashita et al., 1983; Cobbett et al., 1986; Armstrong et al., 1994). A previous immunohistochemical study

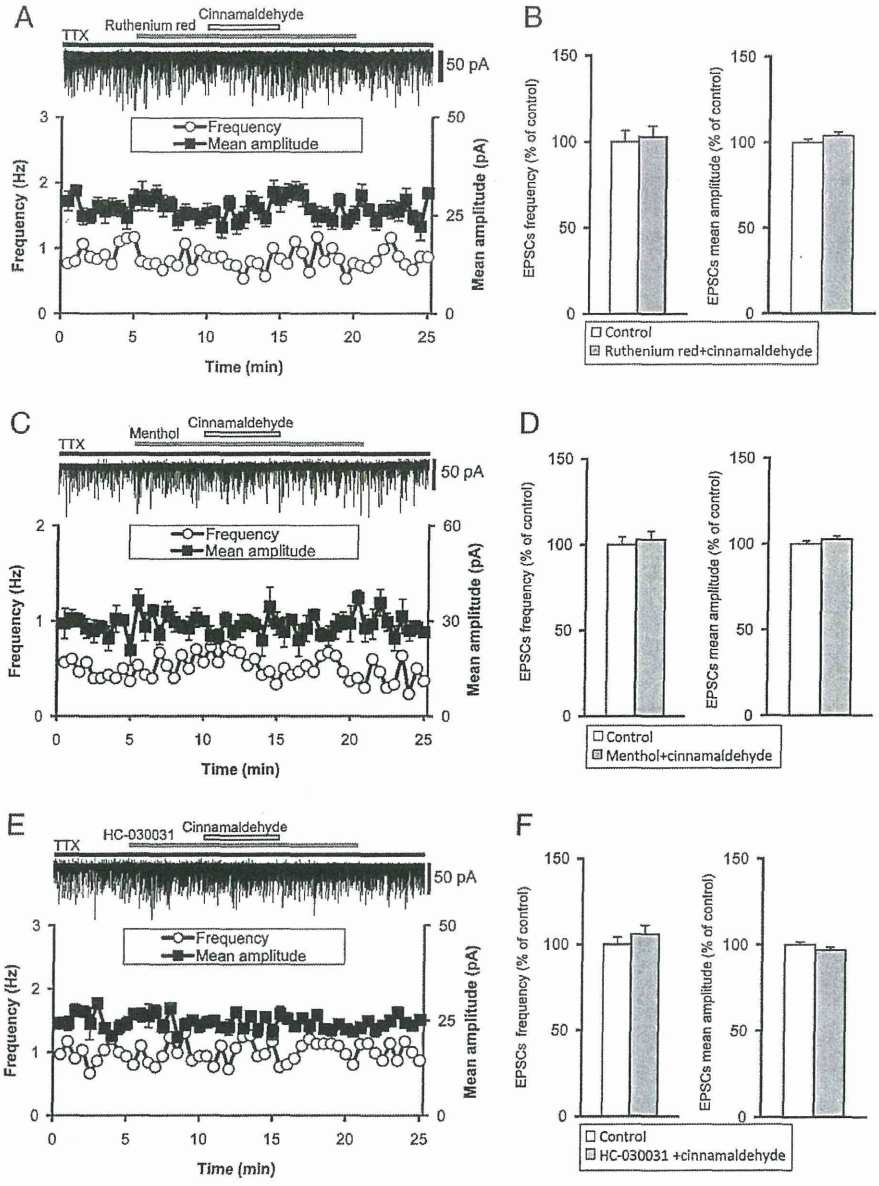

Fig. 5. Characterization of cinnamaldehyde-induced potentiation of mEPSCs. (A, C and E) Representative examples of the effects of ruthenium red (10 μM), non-specific TRP channels blocker (A), high concentration of menthol (300 μM) (C) and HC-030031 (10 μM) (E), TRPA1 selective channel blockers, on cinnamaldehyde-induced potentiation of mEPSCs. EPSCs were recorded in the presence of TTX (1 μM). The holding potential was −70 mV. Plots of frequency are single measurement, whereas plots of amplitude are mean ± S.E.M. over 30 s. (B, D and F) Summary data for characterization of mEPSCs under cinnamaldehyde (30 μM) application. Frequency (left) and amplitude (right) of mEPSCs. Ruthenium red plus cinnamaldehyde ($n=6$) (B), high concentration of menthol plus cinnamaldehyde ($n=3$) (D) and HC-030031 plus cinnamaldehyde ($n=6$) (F), respectively. Data are mean ± S.E.M.

demonstrated that AVP neurones are more common in the caudal and ventral parts of the SON, while OXT neurones tend to be found rostrally and dorsally (Rhodes et al., 1981). Subsequent topographic analysis revealed the majority of Fos-expressing AVP neurones occupy the ventral part of the SON, while Fos-OXT neurons are mainly in the dorsal part on hyperosmotic stimulation (Pirnik et al., 2004). We recorded mEPSCs in the ventral part of the SON. In the present study, approximately 75% of the tested supraoptic MNCs were sensitive to AITC and cinnamaldehyde. Taken together, and although we identified the cell types electrophysiologically, the possibility that the action of AITC and cinnamaldehyde is restricted to a single cell type (AVP- or OXT-producing neurones) is unlikely.

In conclusion, AITC and cinnamaldehyde potentiate excitatory synaptic inputs to the MNCs in the SON on electrophysiolgy. Additional investigations will be required to clarify the physiological role of TRPA1 in glutamatergic excitatory synaptic transmission in supraoptic MNCs.

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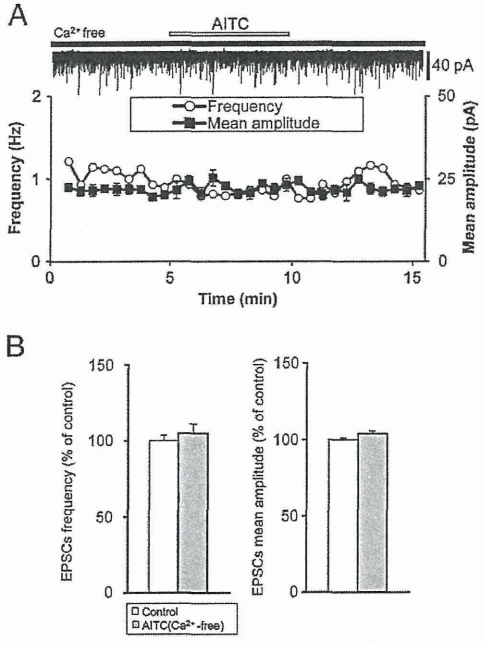

Fig. 6. AITC-induced potentiation of mEPSCs is extracellular Ca^{2+} dependent. (A) A representative example of the effect of AITC (50 μM) on AITC-induced potentiation of mEPSCs in the Ca^{2+}-free perfusion medium. (B) Summary data for the effects of AITC on frequency and amplitude of mEPSCs in Ca^{2+}-free solution ($n=8$). Data are mean ± S.E.M.

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Central Effects of Ghrelin, a Unique Peptide, on Appetite and Fluid/Water Drinking Behavior

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Abstract: Ghrelin is a stomach-derived peptide discovered as a ligand of the orphan G-protein coupled receptor. Ghrelin is now recognized as a major orexigenic neuropeptide. Immunohistochemical studies demonstrated that centrally administered ghrelin induced c-*fos* protein expression in many areas in the brain. Indeed, centrally administered ghrelin has various effects such as stimulating feeding, arousal, increasing gastric acid secretion, release of hormones from the pituitary, and inhibition of water intake. In particular, we recently showed that ghrelin was an antidipsogenic peptide with a simultaneous orexigenic effect. This may be of important, because most spontaneous daily water intake is temporally associated with feeding. Here, we summarise recent findings on the integration of central effects of ghrelin that regulate feeding, release hormones from the pituitary and inhibit fluid/water intake.

Keywords: Angiotensin II, feeding, hypothalamus, intracerebroventricular, neuroendocrine, pituitary, polyethylene glycol, fluid/ water intake.

INTRODUCTION

Ghrelin, consisting of 28 amino acids, is a peptide discovered in the stomach as a ligand of the orphan G-protein coupled receptor and participates in the regulation of growth hormone (GH) release via interactions with the GH secretagougue (GHS) type 1a receptor (GHS-R1a), the functionally active form of the GHS-R [1]. Ghrelin is also found in the brain and is now recognized as a neuropeptide released not only from the periphery but also locally in the brain. Ghrelin-immunoreactive neurons are present in the hypothalamus, especially in the arcuate hypothalamic nucleus (Arc), the paraventricular nucleus (PVN), the dorsomedial hypothalamus (DMH), and lateral hypothalamus [1, 2]. Intracerebroventricular (icv) administration of ghrelin induced c-*fos* protein (Fos) expression in various areas in the central nervous system (CNS) in rats, including the organum vasculosum of the lamina terminalis (OVLT), the median preoptic nucleus (MnPO), the subfornical organ (SFO), the supraoptic nucleus (SON), the PVN, the Arc, the area postrema (AP) and the nucleus of the tractus solitarius (NTS) [3-5]. The expression of the c-*fos* gene has been widely used to detect neuronal activity in the CNS [6]. Indeed, central administration of ghrelin has various effects (Table **1**), including 1) stimulation of not only GH secretion, but also secretion of prolactin (PRL) and adrenocorticotrophic hormone (ACTH), 2) an increase in appetite and energy intake, 3) influences on sleep and behavior, and 4) stimulation of gastric motility and gastric acid secretion. Moreover, recently, we found inhibitory effects of centrally administered ghrelin in dehydration-, angiotensin II (AII) and isotonic hypovolemia-induced water intake in rats [3, 7]. Centrally administered ghrelin also inhibited hypertonic-induced water intake [8].

In this review, we focus on the representative roles of ghrelin in the CNS in 1) the physiological, 2) neuroendocrinological, 3) hypothalamo-neurohypophysial, and 4) body fluid homeostatic effects.

CENTRAL EFFECTS OF GHRELIN

Although ghrelin was initially identified by virtue of its ability to elicit GH secretion from stomach [1, 9], central administration of ghrelin strongly stimulates feeding and an increase in body weight in mammals [10, 11] and non-mammals [12], except for bird [13-15]. Chronic icv administration of ghrelin strongly stimulated feeding and increased body weight gain in rats [5, 16]. Therefore, ghrelin has been established as a major orexigenic hormone acting not only from the periphery but also locally in the brain [1]. In addition, centrally administered ghrelin has various physiological effects (Table **1**), including regulation of sleeping, gastric acid secretion and body temperature. In this context, we describe recent finding on the central physiological effects of ghrelin.

It is well known that brain controls appetite and that feeding behavior is regulated by the hypothalamus [17]. Ghrelin is synthesized in the brain as the peptide was detected by immunohistochemistry in the Arc in colchicine-treated rats [1]. Icv administration of ghrelin induced Fos expression in the Arc [3-5]. The Arc is a critical site for feeding and body weight control because its neurons express the leptin-regulated orexigenic peptides, neuropeptide Y (NPY) and agouti-related protein (AGRP), and leptin-dependent anorexic pro-opiomelanocortin (POMC), and cocaine- and amphetamine-regulated transcript (CART) [18-22]. Icv administration of ghrelin increased both NPY and AGRP gene

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expression in the Arc [5]. Antibodies against and antagonists of NPY and AGRP abolished ghrelin-induced feeding [5, 23]. Moreover, in the Arc ablated rats, icv administration of ghrelin stimulated GH secretion, but did not stimulate food intake [24]. These results indicate that ghrelin may stimulate feeding behavior by exciting NPY and AGRP neurons in the Arc.

Other hypothalamic peptides, apart from ghrelin, are involved with feeding behavior. Leptin is the product of the *ob* gene, and an anorexia-mediated molecule produced from adipose tissue [17]. Most Arc NPY, AGRP, POMC and CART neurons also express leptin receptors and are regulated by leptin [17]. Icv administration of ghrelin blocked leptin-induced feeding reduction [5, 25, 26]. Ghrelin may conflict with leptin in regulating the NPY and AGRP neurons.

Orexin, an orexigenic hypothalamic neuropeptide, is also involved in the regulation of food intake [27]. Icv administration of ghrelin induced Fos expression in orexin-producing neurons [28]. The ghrelin-induced increase of food intake was reduced in orexin knockout mice [28]. The feeding is regulated by ghrelin in co-operating with orexin.

Interestingly, centrally administered ghrelin inhibits food intake in birds [13-15]. In the Japanese quail, Shousha *et al.* showed that high dose peripherally administered ghrelin inhibited food intake whereas a low dose of ghrelin stimulated food intake [15]. Shousha *et al.* also showed that centrally administered ghrelin inhibited food intake [15]. In chicks, centrally administered ghrelin inhibit food intake [13, 14]. Although it is unclear why the effect of ghrelin is opposite in mammals and birds, the role of ghrelin on feeding may have altered during evolution.

The interactions between feeding and sleep are well-known. For example, the starvation-induced sleep loss in rats [29]. Icv administration or microinjection of ghrelin in the CNS increased arousal in rats [30, 31]. Moreover, ghrelin knockout mice have reduced duration of non-rapid-eye movement sleep and increase amount of wakefulness and rapid-eye-movement sleep compared with wild-type mice [31]. Therefore, although the mechanism of ghrelin at sleep and waking rhythm is unclear, ghrelin may have an important role in sleep regulation.

Ghrelin is a rational orexigenic peptide, because of stimulating not only feeding but also gastric acid secretion. Centrally administered ghrelin stimulates gastric acid secretion in urethane-anesthetized rats [32]. Ghrelin-induced gastric acid secretion is completely abolished in both the vagotomized and atropine-treated rats [32]. As icv administration of ghrelin induced Fos in the NTS [3-5, 32], this effect of ghrelin relates to the central regulation of gastric acid secretion by the vagus nerve.

Icv administered ghrelin decrease body temperature in rats [23]. Recently, Mano-Otagiri *et al.* demonstrated that icv administered ghrelin inhibited noradrenaline release in brown adipose tissue of rats [33]. Although the mechanism of ghrelin-induced hypothermia is unclear, it may be related to the metabolic change after icv administration of ghrelin in rodents [10, 34]. Further study will clarify the role of ghrelin on the regulating body temperature.

Table 1. Central Effects of Ghrelin

Hormone Release	
Growth hormone (GH) release	↑[1, 9]
Prolactin (PRL) release	→[37] ? ↓[52]
Adrenocorticotrophic hormone (ACTH) release	↑[37, 40, 41]
Cortisol release	↑[37, 40, 41]
Thyroid stimulating hormone (TSH) release	→[49] ? ↓[41, 48]
Arginine vasopressin (AVP) release	↑[37, 59]
Luteinizing hormone (LH) release	↓[57, 58]
Appetite	↑[10 11] (↓[13-15], in birds)
Adiposity	↑[10]
Gastric Functions	
Gastric acid secretion	↑[32]
Gastric motility	↑[32]
Body Temperature	↓[23]
Water Intake	
Dehydration-induced water intake	↓[3]
Angiotensin II-induced water intake	↓[7, 8]
Hypovolemia induced water intake	↓[7]
Hypertonia-induced water intake	↓[8]
Alcohol Intake	↑[35]
Sleep	↓[30, 31]
Anxiety	↑[38, 39]

↑ increase, ↓ decrease, → no change

Recently, there are some noteworthy evidence that ghrelin may have an important role in alcoholism [35, 36]. Icv administered ghrelin increased and ghrelin receptor antagonists reduced alcohol intake in mice [35]. They suggested that central ghrelin signalling was required for alcohol reward. Ghrelin may be a target for treatment on alcohol-related disorder.

NEUROENDOCRINE EFFECTS OF GHRELIN

Ghrelin is a multi-functional peptide (Table 1). It is well known that ghrelin was discovered in the stomach and stimulates GH release via interactions with the GHS-R1a in human and rat [1, 9]. However, the effects of ghrelin on pituitary hormones are not restricted to GH. Ghrelin stimulates the release of various kinds of hormones, including corticotropin-releasing hormone (CRH), NPY, and arginine vasopressin (AVP), from the rat pituitary *in vitro* [37]. In this context, we describe the neuroendocrine effects of ghrelin on adenohypophyseal hormone release.

The effects of centrally administered ghrelin have been examined on anxiety-like behavior in rodents. Icv administration of ghrelin induced anxious-like behavior in mice and rats [38, 39]. These effects of ghrelin were inhibited by treatment with CRH receptor antagonist [38]. Icv administration of ghrelin stimulated ACTH and corticosterone secretion [37, 40, 41]. Kristensen *et al.* reported that acute psychological stress increased plasma ghrelin level in rats (2). In *in vitro* electrophysiological studies, ghrelin decreased inhibitory postsynaptic currents (IPSCs) in CRH neuron in the PVN [42]. Various kinds of stress cause neuroendocrine response such as CRH or AVP release from the PVN and activation of the hypothalamus-pituitary adrenal (HPA) axis. These data indicate that centrally administered ghrelin may have an important role in the regulation of stress response.

The thyroid hormones are key in regulating metabolism and energy homeostasis [43, 44]. Ghrelin is thought to be a link with metabolism and energy homeostasis [45]. Plasma ghrelin level are reduced in hyperthyroidism and normalized by medical antithyroid treatment [46]. On the other hand, hypothyroidism increases plasma ghrelin level in rat [47]. Icv administered ghrelin suppressed TSH secretion in rats [41, 48]. Ghrelin also suppressed plasma T_4 level, not T_3 level in rats [48]. However, chronic icv administration of ghrelin does not change plasma TSH and T_4 level in rats [49]. The discrepancy between these studies may be explained by different experimental protocols, duration of administration and dose of ghrelin.

Ghrelin may be involved with the lactotrophic axis. Intravenous (iv) administration of ghrelin stimulates PRL secretion in human [50, 51]. Wren *et al.* reported that icv administration of ghrelin did not any effect PRL secretion in rats [37]. However, icv and ip administration of ghrelin inhibited PRL secretion in prepubertal rats [52]. Tana-Sempere *et al.* demonstrated that icv administration of ghrelin inhibited serum PRL level in 23-day-old male and female rats [52]. Moreover, the inhibitory effects of ghrelin were observed in aged hyperprolactinaemic female rats [52]. Interestingly, ghrelin stimulates PRL secretion in 23-day-old male and female rats *in vitro* [52]. Although the mechanism of ghrelin-induced PRL secretion is not clear, the discrepancy between these studies may be explained by different species (human and rat) and ages and dose of ghrelin. Ghrelin appears to have an important role on regulation of PRL secretion in human and rat at least.

Ghrelin may also participate in the modulation of the hypothalamic-pituitary-gonadal (HPG) axis. Previous studies have demonstrated the expression of the ghrelin gene and protein in the testis and ovary in both humans and rodents [53-56]. Icv administration of ghrelin suppressed luteinizing hormone (LH) secretion in ovariectomized rats [57, 58]. Ghrelin increased LH and follicle-stimulating hormone (FSH) secretion and decreased LH responsiveness to gonadotropin-releasing hormone (GnRH) in prepubertal male rats *in vitro* [58]. With limited evidence it is very difficult to explain fully the effects of ghrelin on the HPG axis, but ghrelin may be important in the regulation of LH and FSH secretion.

CENTRAL EFFECTS OF GHRELIN ON THE HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM

Ghrelin has effects not only on the anterior lobe but also on the posterior lobe of the pituitary. Some investigations have been demonstrated excitatory effects of ghrelin on AVP secretion. Firstly, Ishizaki *et al.* reported that icv and iv injection of ghrelin increased plasma AVP levels in conscious rats [59]. GHSs stimulated AVP release from acute hypothalamic explants *in vitro* [60]. The release of AVP from the magnocellular neurosecretory cells (MNCs) in the SON is crucial for body fluid homeostasis. The MNCs project their axons to the posterior pituitary and secrete AVP and oxytocin (OXT) into the systemic blood flow. Thus, there is a possibility that ghrelin may have a potent effect on drinking behavior and body fluid balance in mammals.

Recently, Yokoyama *et al.* examined that the effect of ghrelin on the excitatory synaptic inputs to the MNCs in the SON using whole-cell patch-clamp recordings in rat brain slice preparations *in vitro* (61). Ghrelin (1 μM) increased the firing rate and depolarized the membrane. Application of CNQX (10 μM), a blocker of non-NMDA receptors, significantly decreased the firing rate, and membrane potential reversed the resting potential. The application of ghrelin (1 μM) caused a significant increase in the frequency of the miniature excitatory postsynaptic currents (mEPSCs) without affecting the amplitude. The increased frequency of the sEPSCs persisted in the presence of tetrodtoxin (1 μM). In contrast to the effect on the mEPSCs, the application of ghrelin (1 μM) did not have significant effects on miniature inhibitory postsynaptic currents (mIPSCs). As glutamate and GABA are two major synaptic inputs into the SON neurones [62, 63], the potentiation of mEPSCs by ghrelin may, at least in part, account for the excitatory action of ghrelin on electrical activity.

Two major molecular forms of ghrelin are found in the stomach and plasma; acylated ghrelin, which has n-octanoylated serine in position 3; and desacyl ghrelin [64]. 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 [64-66]. Desacyl ghrelin (1 μM) did not have a significant effect on mEPSCs in the experiments described above [61]. In addition, ghrelin-induced potentiation of mEPSCs was attenuated by pre-exposure to BIM28163, GHS-R1a receptor antagonist [61]. The peptidergic excitation and AVP response are absent in the MNCs of transient receptor potential vanilloid 1 (trpv1)-/- mice [67]. The ghrelin-induced potentiation of the mEPSCs was significantly suppressed by previous exposure to ruthenium red (10 μM), a TRPV blocker, and BIM28163 (10 μM), a GHS type 1a receptor selective antagonist [61]. The effects of ghrelin on the supraoptic MNCs in *trpv1-/-* mice were significantly attenuated compared with those in wild-type mice counterparts [61]. These results suggest that ghrelin participates in the regulation of synaptic inputs to the MNCs in the SON via interaction with the GH secretagogue type 1a receptor, and that the TRPV1 channel may be involved in ghrelin-induced potentiation of mEPSCs to the MNCs in the SON.

OXT is involved in social bonding, reproductive functions and stress [68, 69]. Recently, Olszewski *et al.* reported that icv administered ghrelin increase Fos immunoreactivity in OXT-secreting neurons in the PVN [70]. Thus it is possible that ghrelin may have an important role in social bonding and stress via regulation of OXT neurons. Further studies may find new role for ghrelin in social behavior.

CENTRAL EFFECTS OF GHRELIN ON BODY FLUID HOMEOSTASIS

Many previous studies have shown effects of ghrelin on food intake. Most species show a close relationship between drinking and feeding [71, 72]. Approximately 80 % of spontaneous daily water intake is temporally associated with feeding in rats [73].

Dehydration-induced drinking is significantly inhibited by icv administration of ghrelin in conscious rats [1] Fig. (**1A** and **1B**). Cumulative water intake was significantly inhibited 15- 60 min by icv administration of ghrelin (1 nmol/ rat) in comparison with the vehicle Fig. (**1A**). Water intake that occurred after water deprivation was significantly inhibited by icv injection of ghrelin (0.1, 1 and 10 nmol/rat) in a dose-related manner, although food intake was stimulated by ghrelin. The antidipsogenic effect was as potent as the orexigenic effect. The inhibition of drinking was comparable to, or even more potent than, atrial natriuretic peptide (ANP), an established antidipsogenic hormone, when administered icv, although the antidipsogenic effect of ANP lasted longer. ANP had no effect on food intake.

Cumulative water intake was not significantly inhibited 120-180 min after icv injection of ghrelin Fig. (**1A**). This phenomenon was caused by feeding-associated drinking (prandial drinking). Since drinking usually occurs with feeding, food was withdrawn to prevent prandial drinking. Interestingly, after administering ghrelin centrally in the absence of food, the antidipsogenic effect of ghrelin became more potent than that of ANP. Since drinking is strongly associated with feeding, it is puzzling that a hormone like ghrelin with a strong effect on food intake can, at the same time, inhibit water intake. Further studies are required to demonstrate the relationship between the roles of ghrelin in controlling fluid balance and feeding.

Centrally administered AII-induced drinking was reduced by icv administration of ghrelin [7, 8] Fig. (**1C**). AII-induced water intake was significantly inhibited 15-120 min after icv injection of ghrelin (1 nmol) or 4α-phorbol 12, 13-didecanoate (4α-PDD)(10 nmol) in comparison with vehicle Fig. (**1C**), as previously reported [8, 74]. 4α-PDD, agonist of TRPV4, decreased AII-induced water intake [74]. The inhibitory effects of ghrelin on drinking were similar to 4α-PDD at 15-120 min Fig. (**1C**). 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. (**1C**). Food intake was significantly increased 120 min after icv injection of ghrelin in comparison with vehicle Fig. (**1D**). This effect lasted 180 min after icv injection of ghrelin. Food intake did not change after injection of 4α-PDD or AII in comparison with vehicle Fig. (**1D**).

We showed that centrally administered ghrelin inhibited AII-induced water intake, while desacyl ghrelin (200 nmol) did not have an effect on AII-induced water intake [7]. Previous studies showed that centrally administered desacyl ghrelin can increase [75] or decrease [76] food intake. Matsuda *et al.* showed that desacyl ghrelin inhibits acyl ghrelin-induced orexigenic activity in goldfish [77]. They suggested that desacyl ghrelin might have an unknown receptor, different from the ghrelin binding receptor. 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.

Water intake was significantly increased after ip injection of PEG (20 ml/kg) in comparison with control rats Fig. (**1E**). Intraperitoneal administration of PEG is known to produce a gradual and progressive reduction in plasma volume without any change of plasma osmolality and sodium concentration [78]. 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. (**1E**). However, 120 min after icv injection, the effect of ghrelin was not different from that of vehicle-injected controls. Food intake was significantly increased 60-180min after icv injection of ghrelin in comparison with vehicle Fig. (**1F**). Food intake did not change after injection of 4α-PDD in comparison with vehicle Fig. (**1F**).

Generally, it is recognized that water intake is controlled by two major systems, osmolality and water volume [79]. AII acts to regulate water intake independent of osmolality and extracellular volume [80]. Centrally administered 4α-PDD inhibited AII-induced water intake but did not change hyperosmolality-induced water intake [74]. We showed that centrally administered 4α-PDD did not change PEG-induced water intake, which is not related to osmolality. These results indicated that 4α-PDD might have an important role on AII-induced water intake. Mietlicki *et al.* showed that both AII-induced and hypertonic-induced drinking was reduced by injection of ghrelin [8]. We showed that centrally administered ghrelin inhibited water intake in dehydrated, AII-induced, PEG-induced thirsty rats [3]. Besides rats, centrally administered ghrelin acts as an antidipsogenic peptide in eels and chickens [81, 82]. These results suggested that ghrelin and 4α-PDD maybe have different sites of action related to water drinking in the CNS in rats. Ghrelin is a potent antidipsogenic peptide and may be key in clarifying general dipsogenic mechanisms in the CNS.

CONCLUSION

The importance of ghrelin in feeding is well known. Recently, we showed the antidipsogenic effects of ghrelin in rats. We summarize the central effects of ghrelin on food intake and water intake in Fig. (**2**). Drinking is generally strongly associated with feeding, but ghrelin can inhibit water intake at the same time as increasing food intake. The antidipsogenic effects of ghrelin are puzzling. Why is there a quite unique hormone like ghrelin that has a strong accelerative effect on feeding and at the same time an inhibitory effect on drinking? We showed that the inhibitory effects on

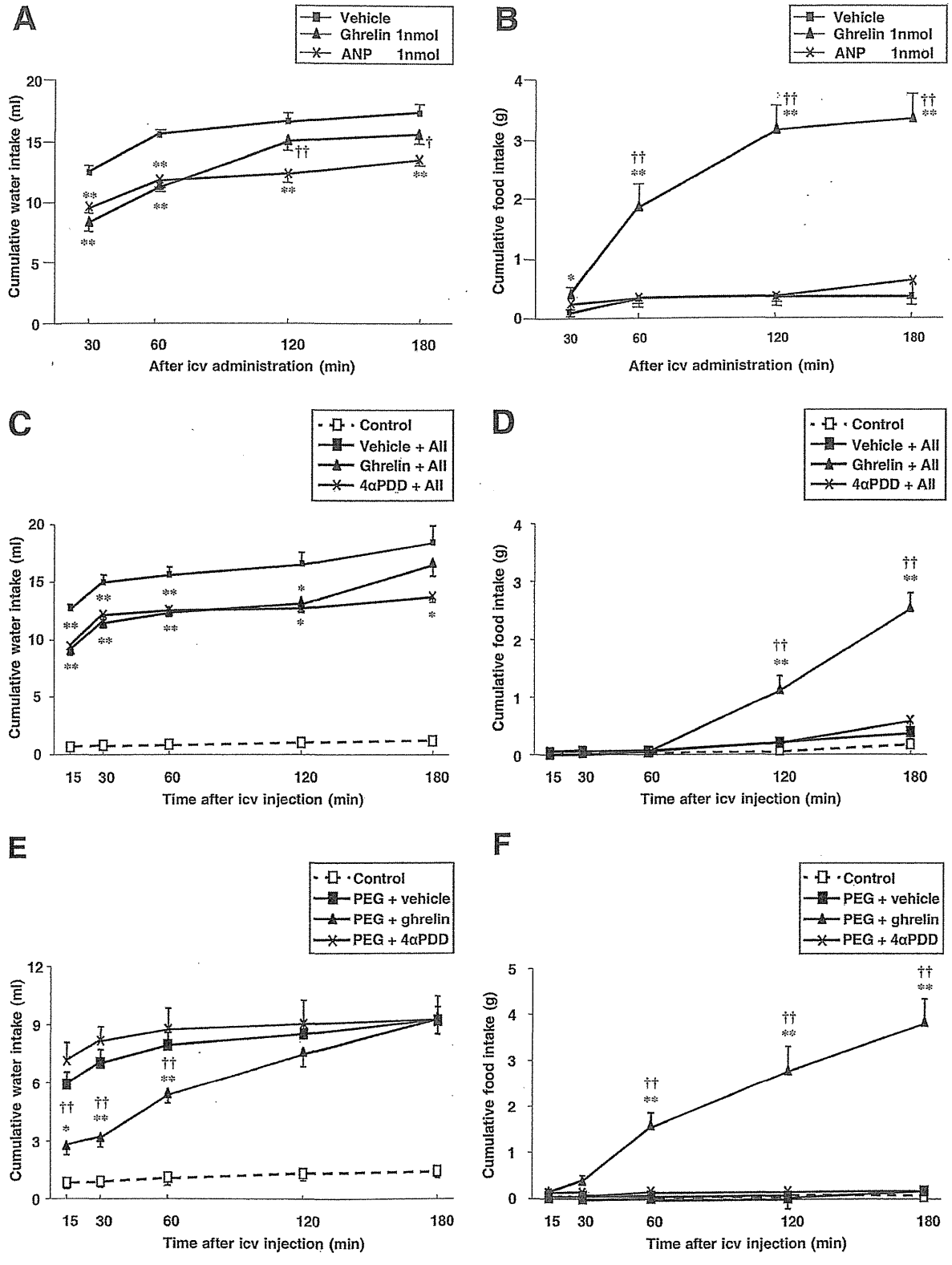

Fig. (1). Effects of intracerebroventricular (icv) injection of ghrelin (1 nmol/rat), ANP (1 nmol/rat) or vehicle on cumulative water intake (**A**) and food intake (**B**) in rats deprived of water overnight (24 h) 30- 180 min after injection of solutions. Effects of intracerebroventricular (icv) injection of ghrelin (1 nmol), 4α-PDD (10 nmol) or vehicle on cumulative water intake (**C** and **E**) and food intake (**D** and **F**) for 15-180 min after icv injection of angiotensin II (AII) (96 p''''''''mol) (**C** and **D**) or ip injection of polyethylene glycol (PEG) (**E** and **F**). Data for cumulative water intake and food intake are expressed as the mean ± SEM (n=6-15). *P<0.05 and **P<0.01, compared with vehicle- injected rats. †P<0.05 and ††P<0.01, compared with ANP-injected rats (**A** and **B**) or 4α-PDD-injected rats (**D**, **E** and **F**). Modified with permission from Fig. 1 and 4 in ref. [2]. and from Fig. 2 and 4 in ref. [6]

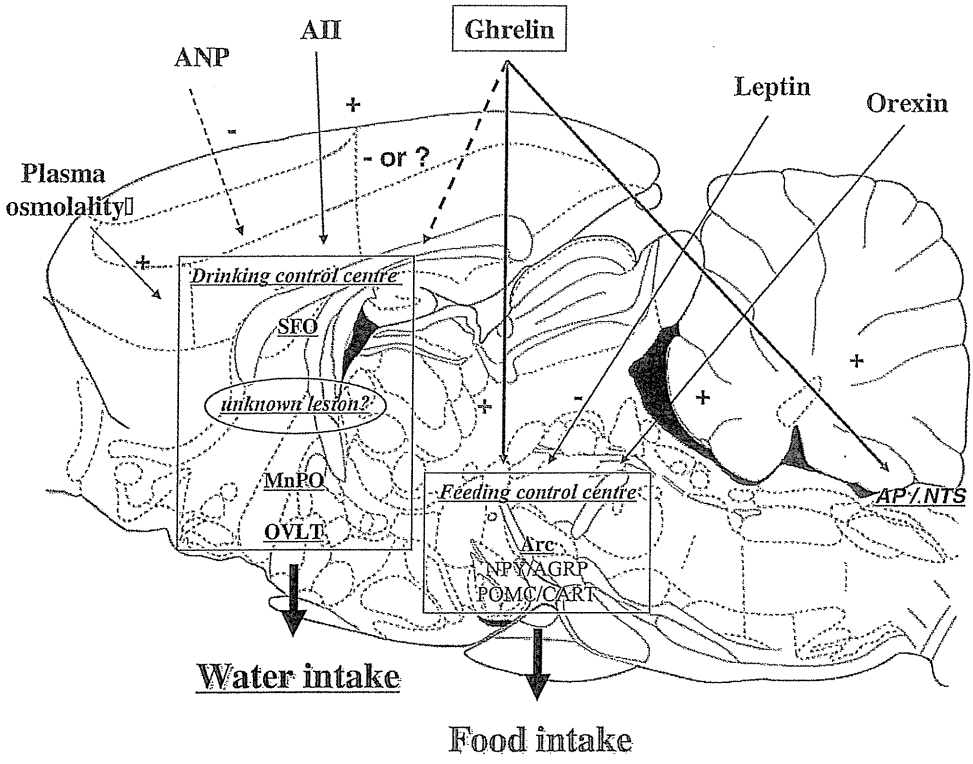

Fig. (2). Central effects of ghrelin on food intake and water intake. Ghrelin activates neuropeptide Y (NPY) and agouti-related protein (AGRP) in the arcuate hypothalamic nucleus (Arc) and stimulated feeding. Ghrelin may compete with leptin and cooperate with orexin in regulating feeding behavior. Ghrelin inhibit water intake in dehydrated, angiotensin II (AII)–induced and hypertonic-induced drinking in rats. The antidipsogenic effect of ghrelin may be morepotent that arterial natriuretic peptide (ANP), an established antidipsogenic hormone. Ghrelin may stimulate circumventricular organs, which include the organum vasculosum of the lamina terminalis (OVLT), the median preoptic nucleus (MnPO), the subfornical organ (SFO) and the area postrema (AP). These are well known to be involved in the regulation of water and electrolyte balance and blood pressure. Particularly, the neurons in the OVLT and SFO are osmosensitive in rats.

water intake appeared earlier than those on feeding [3]. If ghrelin would have a dipsogenic effect, not antidipsogenic effect, ghrelin could not have shown such a strong feeding effect because of the stomach full of drunken water. First, ghrelin may inhibit water intake to prepare for accelerated feeding, burst to cause a voracious appetite, and continuously cause prandial drinking. The antidipsogenic effects of ghrelin may be independent of the orexigenic effects of ghrelin. Therefore, central administered ghrelin would induce the water retention simply by the exciting AVP neuron and release of AVP. Although we do not have any evidence to evaluate this speculation, the action of ghrelin may be an important clue to clarify the mechanism of water intake and interaction between drinking and feeding. We anticipate that further studies can clarify the relationship with between the antidipsogenic and orexigenic effects of ghrelin.

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