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TOPIC HIGHLIGHT

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Different effects of ghrelin, des-acyl ghrelin and obestatin on gastroduodenal motility in conscious rats

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Abstract

Three peptides, ghrelin, des-acyl ghrelin and obestatin are derived from a common prohormone, preproghrelin by posttranslational processing, originating from endocrine cells in the stomach. To examine the effects of these peptides, we applied the manometric measurement of gastrointestinal motility in freely moving conscious rat models. Ghrelin exerts stimulatory effects on the motility of antrum and duodenum in both fed and fasted state of animals. Des-acyl ghrelin exerts inhibitory effects on the motility of antrum, but not on the motility of duodenum in the fasted state of animals. Obestatin exerts inhibitory effects on the motility of antrum and duodenum in the fed state, but not in the fasted state of animals. NPY Y2 or Y4 receptors in the brain may mediate the action of ghrelin, CRF type 2 receptors in the brain mediate the action of des-acyl ghrelin, whereas CRF type 1 and type 2 receptors in the brain mediate the action of obestatin. Vagal afferent pathways might be involved in the action of ghrelin, but not involved in the action of des-acyl ghrelin, whereas vagal afferent pathways might be partially involved in the action of obestatin.

Key words: Ghrelin; Des-acyl ghrelin; Obestatin; Gastrointestinal motility; Hypothalamus

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INTRODUCTION

Ghrelin, des-acyl ghrelin and obestatin are derived from a prohormone, preproghrelin by posttranslational processing. Ghrelin was first identified as endogenous ligand for growth hormone secretagogue receptors (GHS-R) with O-n-octanoyl acid modification at serine 3 position[1]. On the other hand, des-acyl ghrelin has no O-n-octanoyl acid modification[1]. Obestatin was predicted to be formed from preproghelin by a bioinformatic approach[2]. Obestatin was initially reported to be endogenous ligand for orphan G protein-coupled receptor GPR39[2]; however, recent studies have found no specific binding of obestatin to various types of GPR39-expressing cells[3-5]. Ghrelin is a potent stimulator of food intake and gastrointestinal motility[6], while des-acyl ghrelin exerts opposite effects on food intake and gastrointestinal motility[7]. The effects of obestatin on food intake and gastrointestinal motility have been controversial[8-13]. Very recently we have reported that obestatin exerts inhibitory action on gastroduodenal motility in the fed state of conscious rats[14]. Previous studies have shown that food intake and gastroduodenal motility are tightly related. For example, feeding stimulatory peptides such as NPY and ghrelin stimulate gastroduodenal motility[15,16], while feeding inhibitory peptides such as CRF and urocortin inhibit the gastroduodenal motility[17]. Here, we overview different effects of ghrelin, des-acyl ghrelin and obestatin on gastroduodenal motility by using freely moving conscious rat models.

MANOMETRIC MEASUREMENT OF GASTROINTESTINAL MOTILITY IN CONSCIOUS RATS

We developed freely moving conscious rat model to

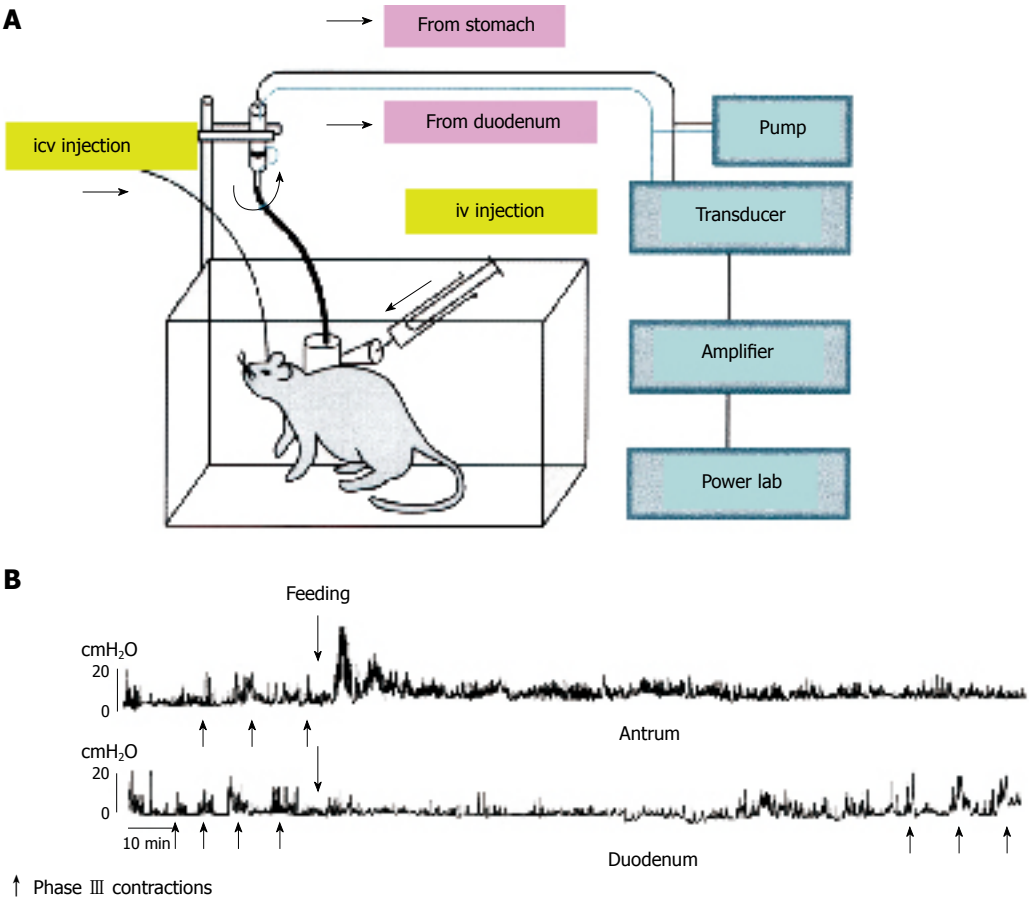
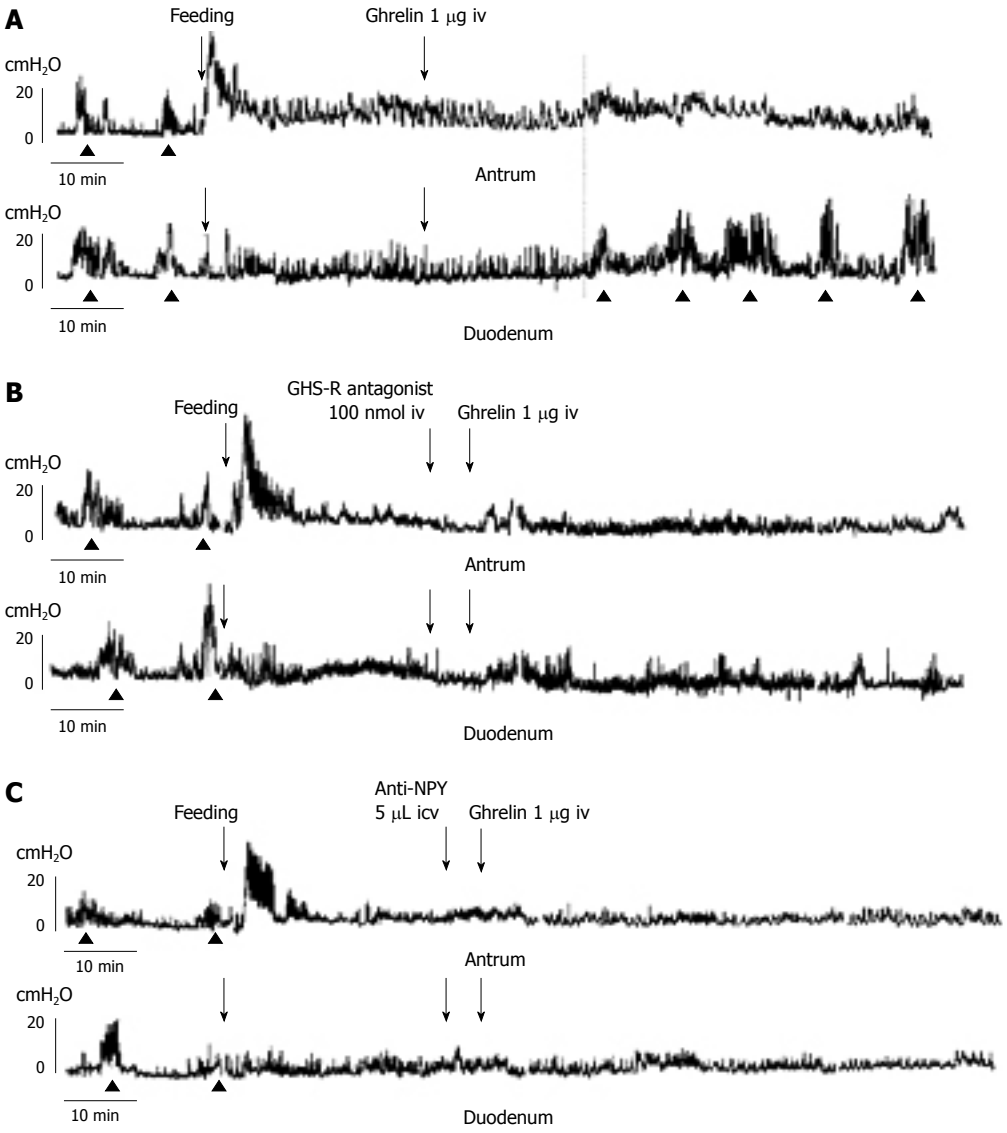


Figure 1 Measurement of gastrointestinal motility. A: Manometric measurement of gastroduo-denal motility in freely moving conscious rats; B: Fasted and fed motor activities in the antrum and duodenum. Phase Ⅲ-like contractions are indicated by arrows.

measure the gastrointestinal motility[15] (Figure 1A). This model permits the measurement of gastrointestinal motility in animals in the physiological fed and fasted states by a manometric method[15]. In the fasted state, the cyclic change of pressure waves were detected in both antrum and duodenum, including the quiescence period during which relatively low amplitude contractions occur (phase I-like contractions), followed by a grouping of strong contractions (phase Ⅲ-like contractions) (Figure 1B). The frequency of the onset of phase Ⅲ-like contractions was 5.3 ± 0.5/h (n = 6) in the antrum and 5.6 ± 0.8/h (n = 6) in the duodenum[16]. After food intake, such fasted motor pattern was disrupted and replaced by a fed motor pattern, which consisted of irregular contractions of high frequency (Figure 1B). The fed pattern continued for 85.7 ± 6.8 min (n = 5) in the duodenum and for more than 240 min in the antrum when rats were given 3 g of chow, and then replaced by the fasted motor pattern[14].

GHRELIN AND GASTRODUODENAL MOTILITY

Intracerebroventricular (icv) and intravenous (iv) injection of ghrelin stimulated the % motor index (%MI) in the antrum and induced the fasted motor activity in the duodenum when given in the fed state of animals[16] (Figure 2A). Icv and iv injection of ghrelin increased the frequency of phase Ⅲ-like contractions in both antrum and duodenum when given in the fasted state of animals[16]. The effects of iv injection of ghrelin on gastroduodenal motility were blocked by iv injection of GHS-R antagonist, but not by icv injection of GHS-R antagonist[16] (Figure 2B). In vagotomized animals, iv injection of ghrelin-induced the fasted motility in both antrum and duodenum when given in the fed state, iv injection of GHS-R antagonist completely blocked phase Ⅲ-like contractions in both antrum and duodenum[16]. Immunoneutralization of NPY in the brain blocked the stimulatory effects of ghrelin on the gastroduodenal motility[16] (Figure 2C). These results indicate that ghrelin released from the stomach may act on the ghrelin receptor on vagal afferent nerve terminals and NPY neurons in the brain may mediate the action of ghrelin on the gastroduodenal motility. *C-Fos* expression in the arcuate nucleus (ARC) in the hypothalamus and in the nucleus tractus solitarius (NTS) induced by intraperitoneal (ip) injection of ghrelin confirmed this effect (Figure 2D). Our previous study showed that immunoneutralization of NPY in the brain completely blocked the phase Ⅲ-like contractions in the duodenum of normal rats, and Y2 and Y4 receptor agonists induced the phase Ⅲ-like contractions in the duodenum when given in the fed state of animals[15]. Combined together, in normal animals ghrelin may stimulate gastroduodenal motility by activating the GHS-R on vagal afferent nerve terminals and affect NPY neurons in the hypothalamus, Y2 and/or Y4 receptors in the brain may mediate the action of ghrelin (Figure 3). Once the brain mechanism is eliminated by truncal vagotomy, ghrelin might be primarily involved in the regulation of fasted molility through GHS-R on the stomach and duodenum.



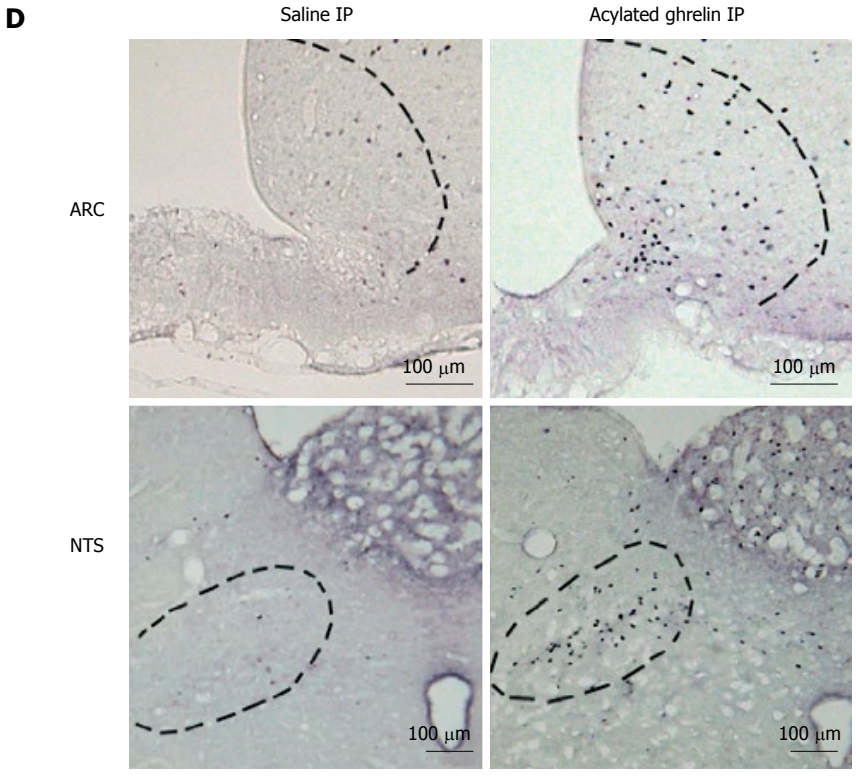
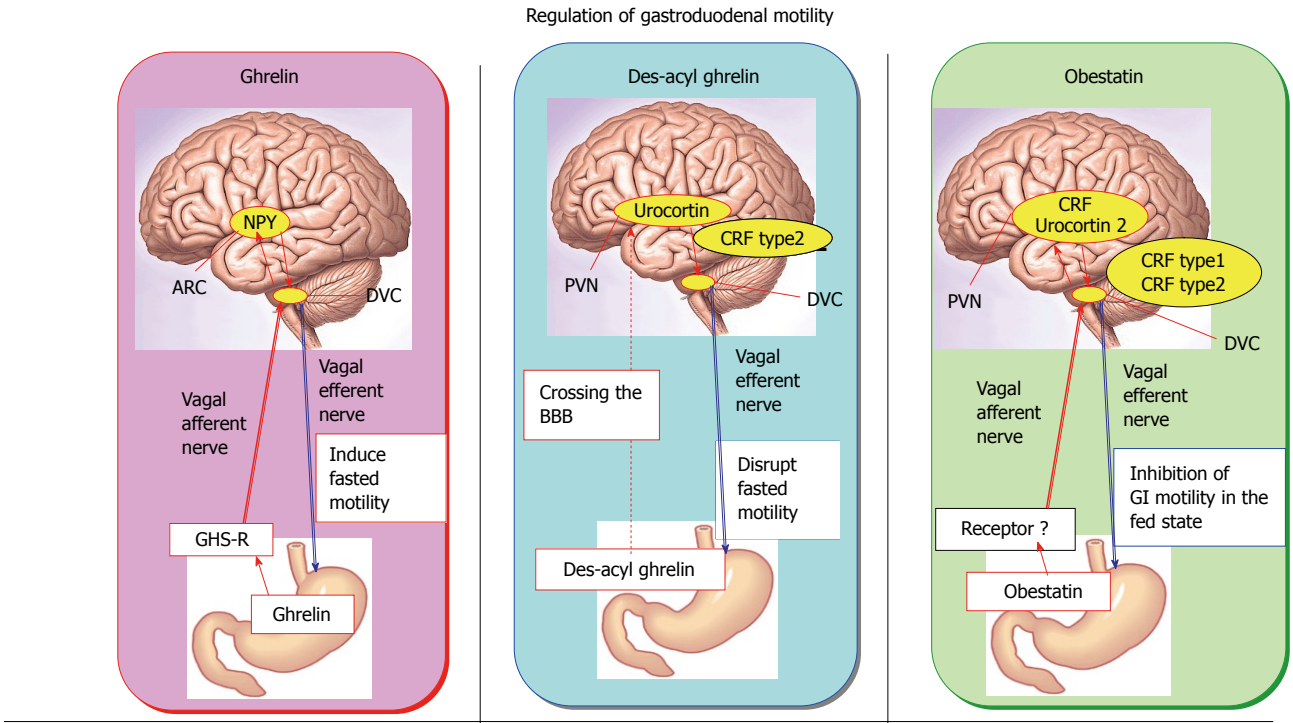


Figure 2 Ghrelin and gastroduodenal motility. A: Effects of iv injection of ghrelin on the fed motor activity of the antrum and duodenum. Iv injection of ghrelin induces the fasted pattern in the duodenum and increases the motor activity in the antrum; B: Iv injection of GHS-R antagonist completely blocks the effect of iv injection of ghrelin; C: Icv injection of NPY antiserum completely blocks the effect of iv injection of ghrelin; D: The density of c-Fos-positive cells in the arcuate nucleus (ARC) and nucleus tractus solitarius (NTS) increases with ip injection of ghrelin compared to saline-injected control.



	Ghrelin		Des-acyl ghrelin		Obestatin	
	Fasted state	Fed state	Fasted state	Fed state	Fasted state	Fed state
Stomach	↑	↑	↓	—	—	↓
Duodenum	↑	↑	↓	—	—	↓
Hypothalamus neuron	NPY		Urocortin		CRF, urocortin	
Brain receptor	Y2, Y4		CRF type 2		CRF type 1, type 2	
Vagal afferent	+		-		+	

Figure 3 Summary diagram of different effects of ghrelin, des-acyl ghrelin and obestatin on the gastroduodenal motility and brain mechanisms mediating the action of these peptide.

Human ghrelin has a structural resemblance to human motilin, and human ghrelin receptors exhibit a 50% identity with human motilin receptors[18]. Therefore, the role of ghrelin in the gastrointestinal motility is comparable with that of motilin[19,20]. Motilin originates from the endocrine cells in the duodenum[19], while ghrelin originates from the endocrine cells in the stomach[21], both of them are involved in the regulation of phase Ⅲ contractions in the gastrointestinal tracts. Motilin induces fasted motility in the stomach and duodenum when it is given peripherally, but not when given centrally[20,22], while ghrelin induces fasted motility in the duodenum when it is given both peripherally and centrally[16]. Since it is known that gastric acidification modulates the action of motilin[23], we examined the relationship between the effects of ghrelin on gastroduodenal motility and intragastric pH. The results showed that within 30 min after feeding, low intragastric pH (pH 2.5 ± 0.2) inhibited the effects iv injected ghrelin on gastroduodenal motility, and that this effect was reversed by an increase of intragastric pH (pH 5.4 ± 0.6) within 60 min after feeding, or by pretreatment of famotidine (intragastric pH 6.0-6.7)[16]. These results suggest that the sensitivity of the GHS-R in the gastrointestinal tract might be inhibited by low intragastric pH.

DES-ACYL GHRELIN AND GASTRODUODENAL MOTILITY

Central and peripheral administration of des-acyl ghrelin has been shown to significantly decrease food intake in food-deprived mice and decrease gastric emptying[6]. Transgenic mice with overexpression of the des-acyl ghrelin gene exhibited a decrease in body weight, food intake and fat mass weight accompanied by moderately decreased linear growth compared with their nontransgenic littermates[6]. In rats, des-acyl ghrelin injected intraperitoneally (ip) effectively decreased food intake in food-deprived rats, and decreased the dark-phase food intake in free-feeding rats, but failed to decrease the light-phase food intake in free-feeding rats[7].

Icv and iv injections of des-acyl ghrelin disrupted fasted motility in the antrum, but not in the duodenum[7] (Figire 4A). The frequencies of fasted motility in the antrum were decreased to 58.9% and 54.5% by des-acyl ghrelin injcted icv and iv, respectively[7]. However icv and iv injections of des-acyl ghrelin did not alter fed motor activity in both the antrum and duodenum[7] (Figure 4A). These data indicate that the dominant role of exogenous des-acyl ghrelin affects fasted motility in the antrum, but not in the duodenum. The results showed that capsaicin

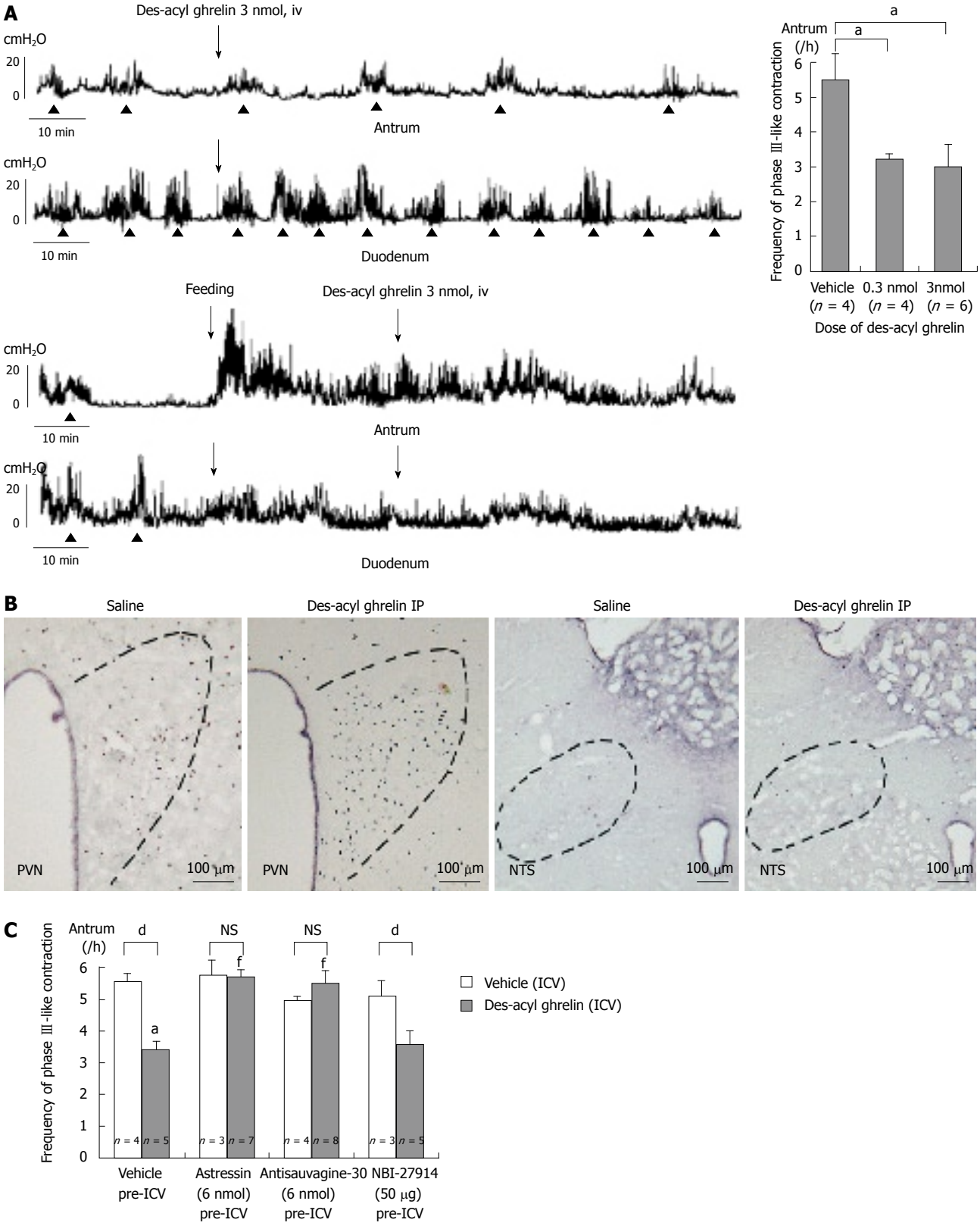


Figure 4 Des-acyl ghrelin and gastroduodenal motility. A: Effects of iv injection of des-acyl ghrelin on the fasted and fed motor activities of the antrum and duodenum. Iv injection of des-acyl ghrelin decreases the frequency of phase Ⅲ-like contractions in the antrum, but not in the duodenum. Iv injection of des-acyl ghrelin does not affect fed motor activity in both antrum and duodenum. [a]$P < 0.05$; B: The density of c-Fos-positive cells in the paraventricular nucleus (PVN) is increased by ip injection of des-acyl ghrelin compared to saline-injected control, whereas that in the NTS is not altered; C: The decreased frequency of phase Ⅲ-like contractions induced by iv injection of des-acyl ghrelin is restored to normal in pretreatment of nonselective CRF receptor antagonist astressin and the selective CRF type 2 receptor antagonist antisauvagine-30, but not CRF type 1 receptor antagonist NBI-27914. [d]$P < 0.01$, [f]$P < 0.001$ compared with a.

treatment did not alter the disruptive effect of iv injection of des-acyl ghrelin on fasted motility in the antrum[7]. These results were consistent with electrophysiological studies, which showed that peripheral administration of

ghrelin suppressed firing of the vagal afferent pathways, whereas des-acyl ghrelin had no effect on vagal afferent pathways[24]. Difference in the involvement of vagal afferent pathways in the action of ghrelin and des-acy ghrelin was confirmed by c-*Fos* expression in the NTS. Ip injection of ghrelin significantly increased the density of c-*Fos*-positive cells in the NTS (Figure 2D), while ip injection of des-acyl ghrelin induced no change in the density of c-*Fos*-positive cells in the NTS compared with vehicle-injected controls[7] (Figure 4B). Taken together, these results suggest that peripherally administered des-acyl ghrelin may cross the blood-brain barrier (BBB) and act directly on the brain receptor and disrupt the fasted motility in the antrum (Figure 3).

The results showed that the centrally administered CRF type 2 receptor antagonist, but not the CRF type 1 receptor antagonist, blocked the effects of centrally and peripherally administered des-acyl ghrelin on gastric motility[7] (Figure 4C). Among two CRF receptor subtypes, CRF type 1 receptor is highly involved in anxiety-related behavior and CRF type 2 receptor is involved in regulating food intake and peripheral functions such as gastric acid secretion or gastric emptying. CRF is a relatively selective ligand for CRF type 1 receptor, whereas urocortin is a ligand more selective for CRF type 2 receptor[25,26]. The density of c-*Fos*-positive cells in the paraventricular nucleus (PVN) was significantly increased by ip injection of des-acyl ghrelin compared to vehicle-injected controls[7] (Figure 4B). These data suggest that peripherally administered des-acyl ghrelin may activate neurons in the PVN by crossing the BBB, and exert inhibitory effects on the antral motility *via* CRF type 2 receptor in the brain (Figure 3).

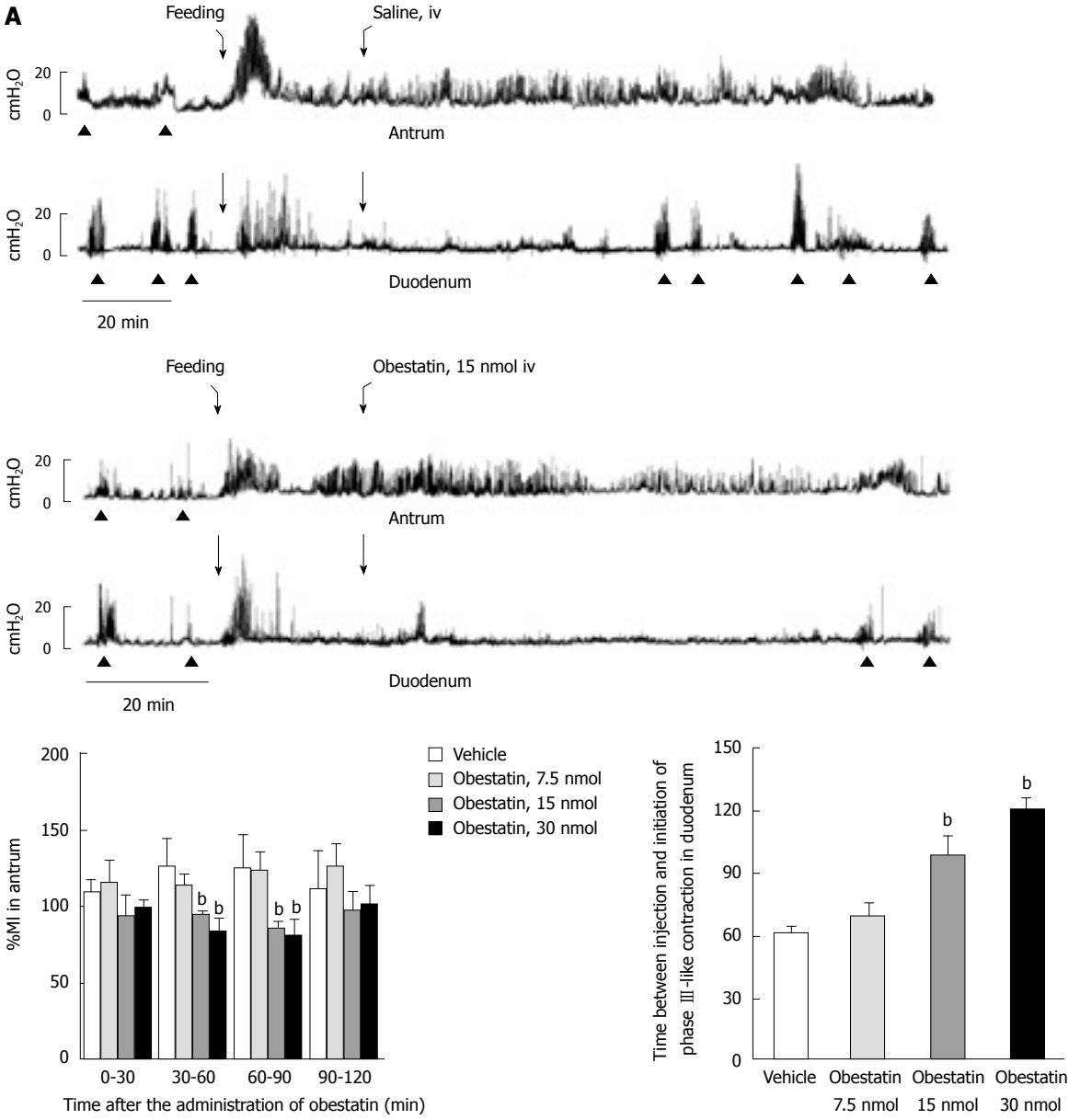
OBESTATIN AND GASTRODUODENAL MOTILITY

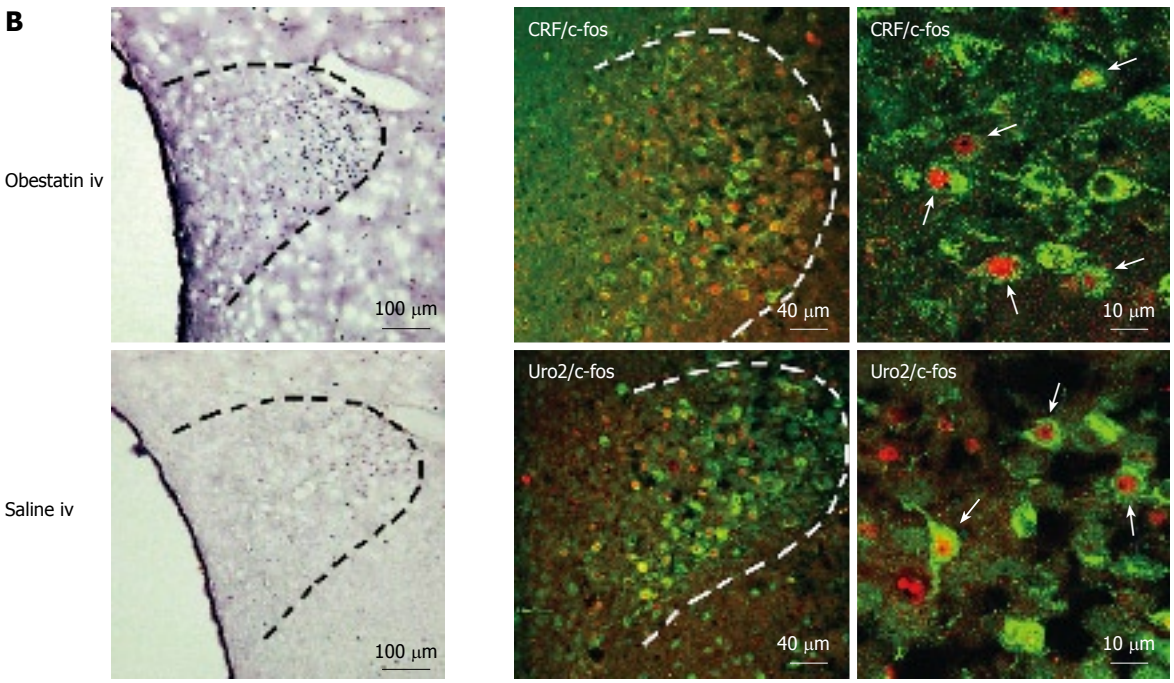
Zhang *et al*[2] first reported that ip injection of obestatin suppressed cumulative food intake, decreased body weight gain, and inhibited gastric emptying and jejunal muscle contraction in mice. Since then, however, the inhibitory effects of obestatin on food intake and gastrointestinal motility have remained controversial[8-13]. Most of the previous studies which showed the negative effects of obestatin on the gastrointestinal motility have only measured the gastric emptying or MMC cycle time as indices for motor activity. In our previous study, for more precise analysis, motor activity in both fed and fasted states was quantified by the %MI, and we measured the time taken to the initiation of phase Ⅲ-like contractions in the antrum and duodenum of conscious rats[14].

Results showed that motor activity in the antrum and duodenum was inhibited when obestatin was given iv to conscious rats in the fed state, but not when it was given in the fasted state[14]. Iv injection of obestatin decreased the %MI of fed motility in the antrum and prolonged the time before the return of fasted motility in the duodenum[14] (Figure 5A). Such inhibitory actions were the opposite of those obtained with ghrelin[16]. The results showed that the inhibitory action of obestatin appeared 30-90 min after iv injection[14] (Figure 5A), which is consistent with the timing of the effects of iv injection of ghrelin (approximately 30 min) on gastroduodenal motility[16] (Figure 2A). Iv injection of obestatin induced a significant increase in the number of c-*Fos*-positive cells in the PVN compared to saline-injected controls[14] (Figure 5B). Immunofluorescence overlap staining showed that the PVN neurons activated by iv injection of obestatin contain CRF or urocortin 2[14] (Figure 5B). The involvement of CRF type 1 and type 2 receptors in the action of obestatin on the gastroduodenal motility was examined[14]. Results showed that the inhibitory action of iv injection of obestatin on the motor activities in the antrum and duodenum were blocked by icv injection of CRF type 1 and type 2 receptor antagonists, suggesting that both types of CRF receptors in the brain may mediate the action of peripherally injected obestatin on gastroduodenal motility[14] (Figure 5C). The results showed that vagal afferent nerve blockade by capsaicin reverses the inhibitory effects of obestatin on duodenal motility, but does not alter the inhibitory effects of obestatin on antral motility[14]. These results suggest that vagal afferent pathways might be involved partially, but not entirely, in the action of obestatin. Involvement of vagal afferent pathways was confirmed by the finding that the number of c-*Fos*-positive neurons in the NTS was increased by iv injection of obestatin[14]. In addition to vagal afferent pathways, it is possible that circulating obestatin acts on brain targets directly by crossing the BBB, because a previous study has shown that there is a rapid influx of iv-injected ^{125}I-labeled obestatin from the blood to the brain[27]. Therefore, the lack of effects of obestatin on antral motility during capsaicin treatment might be explained by direct action of peripherally injected obestatin on brain targets by crossing the BBB, similar to what has been observed for des-acyl ghrelin. We further examined whether obestatin can antagonize the stimulatory effects of ghrelin on gastroduodenal motility[14]. We found that obestatin failed to antagonize the ability of ghrelin either to stimulate the %MI in the antrum or to accelerate the initiation of fasted motility in the duodenum when administrated in the fed state[14]. These results were consistent with previous studies in which obestatin failed to antagonize the ability of ghrelin to stimulate gastric emptying or to shorten the MMC cycle time[8].

GPR39 was initially proposed as the receptor for obestatin[2], and GPR39 expression has been detected in peripheral organs such as the duodenum and kidney, but not in the pituitary or hypothalamus[4]. However recent publications indicate that obestatin is unlikely to be the endogenous ligand for GPR39 on the basis of a lack of specific binding of obestatin to GPR39 receptor-expressing cells[2,4,5,28]. Nevertheless, although binding of obestatin to the receptor GPR39 remains controversial, the functional effect of obestatin on gastrointestinal motility has been clearly demonstrated in our study.

In conclusion, our study indicates that obestatin inhibits gastroduodenal motility in the fed state, but not in


A
Feeding
Saline, iv
cmH2O
20
0
Antrum
Duodenum
20 min
Feeding
Obestatin, 15 nmol iv
Antrum
Duodenum
20 min
%MI in antrum
200
150
100
50
0
Vehicle
Obestatin, 7.5 nmol
Obestatin, 15 nmol
Obestatin, 30 nmol
b
0-30
30-60
60-90
90-120
Time after the administration of obestatin (min)
Time between injection and initiation of phase Ⅲ-like contraction in duodenum
150
120
90
60
30
0
Vehicle
Obestatin 7.5 nmol
Obestatin 15 nmol
Obestatin 30 nmol


B
Obestatin iv
Saline iv
100 μm
CRF/c-fos
40 μm
10 μm
Uro2/c-fos

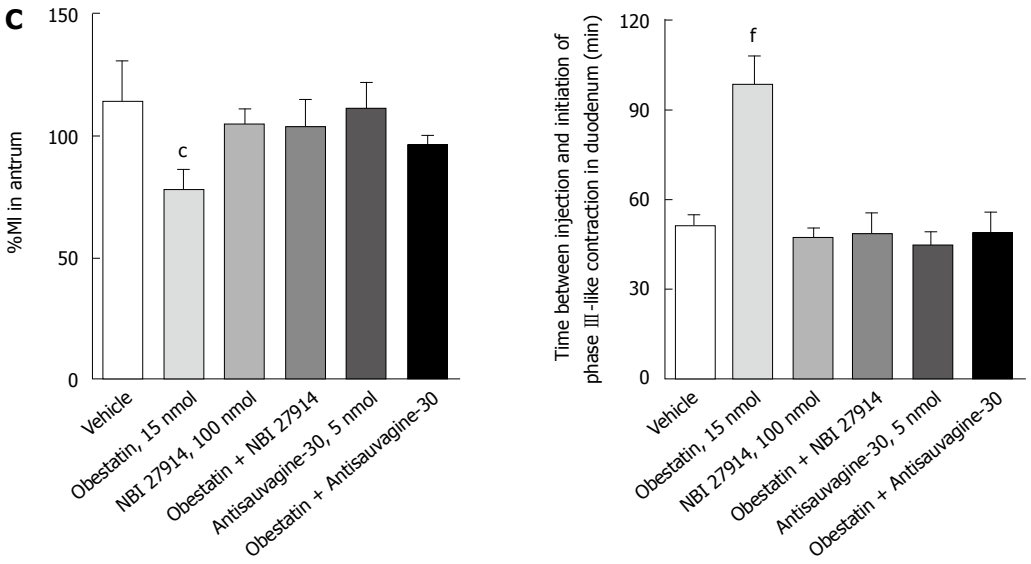


Figure 5 Obestatin and gastroduodenal motility. A: Effects of iv injection of obestatin on the fed motor activity of the antrum and duodenum. Iv injection of obestatin dose dependently decreases the %MI during the 30-90-min period after injection of obestatin in the antrum, and prolongs the time between the initiation of phase Ⅲ-like contractions and injection of obestatin in the duodenum. $^{b}P < 0.01$, compared with vehicle-injected controls; B: The density of c-Fos-positive cells in the PVN is increased by iv injection of obestatin compared to saline-injected control. CRF-positive or urocortin 2-positive neurons are overlapped with c-Fos-positive neurons in the PVN; C: The decrease in %MI that is observed 30-60 min after iv injection of obestatin is reversed by icv injection of the CRF type 1 antagonist NBI-27914 and the CRF type 2 receptor antagonist antisauvagine-30. The elongation of the time between injection of obestatin and initiation of phase Ⅲ-like contractions in the duodenum induced by iv injection of obestatin is also reversed by icv injection of NBI-27914 and antisauvagine-30. $^{c}P < 0.05$, $^{f}P < 0.01$, compared with vehicle-injected controls.

the fasted state of conscious rats. In the brain, CRF- and urocortin 2-containing neurons might be activated by iv injection of obestatin, and at the level, CRF type1 and type 2 receptors might be involved in the inhibitory action of obestatin on antral and duodenal motility (Figure 3). Vagal afferent pathways might be involved partially, but not entirely, in these actions of obestatin (Figure 3).

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