parathyroid surgery for primary hyperparathyroidism (PHPT) at our institution.

Methods

Patients

Between 1983 and 2009, 68 patients from 46 kindreds with PHPT or multiglandular disease underwent parathyroidectomy at our department. To be deemed as having MEN1, they had to meet the following criteria: (1) multiglandular disease with a histopathological diagnosis of parathyroid hyperplasia and (2) satisfying the practical criteria for MEN1 reported by Brandi et al. [2].

Clinical manifestations

All patients had PHPT with serum calcium levels exceeding 10.0 mg/dl (normal 8.5–9.9 mg/dl) and serum intact-PTH levels exceeding 66 pg/ml (normal 10–65 pg/ml). The presence or absence of MEN1-associated diseases was determined by measuring the serum levels of gastrin, insulin, glucagon, prolactin, GH and IGF-1 and by performing imaging studies (MRI, CT) of the pituitary and pancreas.

MEN1 gene

All 68 patients had undergone a *MEN1* gene analysis after providing their informed consent. From each patient, a blood sample was collected and genomic DNA was extracted using a QIA Amp blood kit (Qiagen, Hilden, Germany). All protein-coding regions of exons 2 through 10 of the *MEN1* gene were amplified using polymerase chain reaction (PCR). A PCR assay and sequence analyses were performed, as previously described elsewhere [6]. All identified mutations were confirmed by cloning the PCR products into the pCR vector (Invitrogen, Carlsbad, CA, USA) and nucleotide sequencing. For one case involving a large deletion, the mutation was detected as reported previously [7].

Statistical analysis

The overall survival after initial parathyroidectomy was estimated with the Kaplan–Meier method using the JMP (version 8, SAS Institute Japan Ltd., Tokyo, Japan) statistical software package.

We analyzed genotype–phenotype correlations and the survival outcomes of the MEN1 patients.

Results

Among the 68 patients, 25 did not undergo genetic examinations, while one was negative and 42 were positive for the *MEN1* gene mutation. Ten of the latter 42 patients were excluded from the study due to incomplete clinical data. The remaining 32 patients from 19 kindreds comprised the study population. Five patients were male and 27 were female. The average age and standard deviation at initial parathyroidectomy was 45.6 ± 15 years (range 20–73 years), and the average follow-up period and standard deviation was 153 ± 97 months (range 1–396 months).

A phenotypic analysis of the main MEN1 manifestations among the 32 patients revealed that PHPT was the most common (32 patients, 100 %), followed in order by pancreatic neuroendocrine tumors (PNETs) (16 patients, 50 %) and pituitary tumors (15 patients, 47 %). In the 26 patients without *MEN1* gene confirmation, PHPT was the most common manifestation (26 patients, 100 %), followed in order by pancreatic tumors (10 patients, 38.5 %), pituitary tumors (nine patients, 24.6 %), adrenal tumors (three patients, 11.5 %), thymic tumors (three patients, 11.5 %) and malignant tumors (two patients, 7.7 %) (Table 1).

Genotype–phenotype analysis

The locations and types of *MEN1* mutations are summarized in Table 2. Mutations were most common in exons 2 (10 patients from four kindreds) and 10 (five patients from four kindreds). In exons 3 and 7, five patients (three kindreds) and four patients (three kindreds) had mutations, respectively. The genotype–phenotype associations of tumors of the pancreas and pituitary are shown in Table 3. Sixteen patients developed tumors of the pancreas. Seven

Table 1 Characteristics of the patients without *MEN1 gene* confirmation

Total	26
PHPT	26 (100 %)
Pituitary tumor	9 (34.6 %)
PRL	5
Microadenoma	4
Pancreatic tumor	10 (38.5 %)
Non-functioning	7
Gastrinoma	2
Gastrinoma	1
Adrenal tumor	3 (11.5 %)
Thymic tumor	3 (11.5 %)
Others	2 (7.7 %)
Abdominal desmoids	1
Laryngeal Cancer	1

Table 2 The site and type of the *MEN 1* gene

Location	Type	No. of kindreds	No. of patients
Exon 2			
249_252delGTCT	Frameshift	2	5
358_360delAAG	In-frame deletion	1	4
133G > T	Nonsense	1	1
Exon 3			
455T > A	Nonsense	1	2
512_520delGGGATGTCC	In-frame deletion	2	3
Intron 4			
784 − 9G > A	Splicing site mutation	1	1
824 + 1G > A	Splicing site mutation	1	1
Exon 6			
878delC	Frameshift	1	1
Exon 7			
959C > T	Missense	1	1
955_956insT	Frameshift	1	1
914G > A	Missense	1	2
Exon9			
1324C > T	Nonsense	1	2
Exon 10			
1546_1547insC	Frameshift	3	4
1387G > T	Nonsense	1	1
Large deletion		1	3

patients had PNETs, including four patients with gastrinomas, one patient with an insulinoma, one patient with a glucagonoma and one patient with a malignant tumor. Non-functional pancreatic tumors were observed in nine patients. Mutations in the *MEN1* gene were found at exons 2, 3, 6 and 10, and a large deletion was present in one case. Among 11 patients with functional pituitary adenomas, nine had prolactinomas, one had an ACTH-producing adenoma and one had an adenoma that produced both prolactin and growth hormone. Non-functional pituitary tumors were found in two patients. No particular trends were observed between the sites of mutation and the clinical manifestations in the pancreas or pituitary.

Table 3 also shows the genotype–phenotype correlations of thymic tumors and other malignant diseases. Three patients had lesions in the thymus. One patient with a large deletion mutation had a carcinoma of the thymus, and her son was also found to have a thymic carcinoid. The clinical details of another patient with an exon 3 mutation were not available. Other malignant tumors were found in three patients: one patient with a mutation in exon 2, who also had malignant PNET, died of meningioma; another patient with a mutation in exon 2 developed lung cancer; one patient who had a mutation in exon 10 developed both papillary carcinoma of the thyroid and osteomyelodysplasia.

Survival

Five (16 %) patients died of MEN1-related disease during the follow-up period. The overall survival rates of the patients at 5, 10 and 20 years were estimated to be 99.6 % (95 % CI 88.4–99.9), 95 % (95 % CI 80.8–98.9) and 74.5 % (95 % CI 51.1–89.1), respectively (Fig. 1). The mutations in the patients who died involved exons 2 and 3, and a large deletion. Two patients died of liver metastases from gastrinomas, one patient died of malignant PNET and meningioma and one patient died due to pituitary crisis. The patient with the large deletion died of thymic cancer.

Discussion

The first description of a patient with both parathyroid and pituitary tumors was published in 1903 [8]. Half a century later, in 1952, based on a case series of patients with tumors of the parathyroid, pituitary and pancreatic islets, Underdahl et al. [9] recognized the combination to be a new syndrome. Subsequently, Wermer [6] hypothesized that the syndrome was caused by a mutation in an autosomal gene with a high penetrance.

In our present series, *MEN1* gene mutations were observed in exons 2, 3, 4, 6, 7, 9 and 10, and a large deletion was also recognized. The most frequently observed mutation site was exon 2. The distribution of the mutation sites in our patients was similar to that in a previous report of 24 other Japanese cases [10], although it was different from the results reported by Turner [11]. In our series, frame-shift mutations were the most common form, in agreement with the results of previous reports [12, 13].

Although most manifestations of MEN 1 are thought to be benign, the life expectancy of affected patients has become an important issue. Dean et al. [7] reported that MEN1 patients have a lower 20-year survival rate than normal age- and sex-matched US citizens (64 vs. 81 %). Wilkinson et al. [14] reported the results of a retrospective survey on causes of death in a MEN1 family in Tasmania dating from 1861. Of the 46 family members who had a high probability of having MEN1, 20 (43 %) died due to MEN1-associated conditions, comprising 12 cases of neoplasia and eight cases of hypercalcemia [14]. Wilson et al. [15] observed a dramatic change in the causes of death in their population. Gastrointestinal (GI) hemorrhage was the most common cause of death in the young generation

Table 3 Clinical characteristics of the patients according to the *MEN 1* gene mutation

Location	Pancreatic tumor	Pituitary tumor	Thymic tumor	Other tumors	Number of deaths and cause of death
Exon 2					
249_252delGTCT	5: Gas(1), Glu(1), Malig(1) Non(2)	3: PRL(3)	0	Meningioma (1)	2: Meningioma (1), pituitary crisis (1)
358_360delAAG	2: Non(2)	2: PRL(2)	0	Lung cancer (1)	0
133G > T	1: Non(1)	0	0	–	1: Liver metastases from gastrinoma
Exon 3					
455T > A	2: Gas(1) Non(1)	2: PRL(2)	0	–	1: Liver metastases from gastrinoma
512_520del GGGATGTCC	1: Non(1)	0	Unknown (1)	–	0
Intron 4					
784-9G > A	0	0	0	–	0
824 + 1G > A	0	0	0	–	0
Exon 6					
878delC	1: Non(1)	1: Non(1)	0	–	0
Exon 7					
959C > T	0	1: PRL(1)	0	–	0
955_956insT	0	0	0	–	0
914G > A	0	0	0	–	0
Exon 9					
1324C > T	0	0	0	–	0
Exon 10					
1546_1547insC	2: Gas(1) Non(1)	3: PRL(1), PRL and GH(1), ACTH(1)	0	Papillary carcinoma of the thyroid and osteomyelodysplasia (1)	0
1387G > T	1: Ins(1)	1: Non(1)	0	–	0
Large deletion	1: Gas(1)	0	2: carcinoid and cancer	–	1: Thymic cancer

Gas gastrinoma, *Glu* glucagonoma, *Ins* insulinoma, *PRL* prolactinoma, *GH* growth hormone producing adenoma, *ACTH* ACTH producing adenoma, *Non* non-functioning tumor, *Malig* malignant PNETs. (), number of patients; unknown, histological diagnosis was unknown

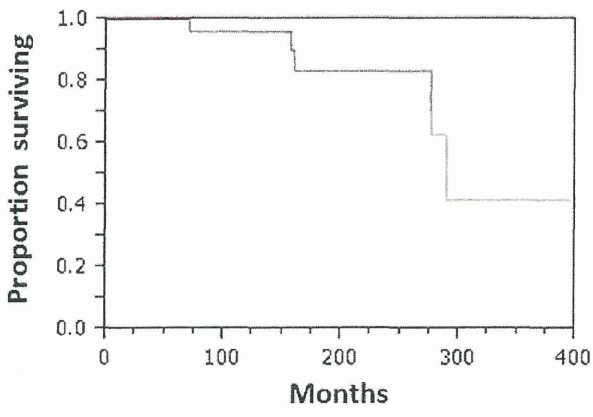

Fig. 1 Overall survival after initial parathyroidectomy in patients with MEN 1

living before the 1960s, whereas metastases of pancreatic carcinoids became the leading cause of death in the 1990s. Goudet et al. [16] also reported similar observations in MEN1 patients: the death rate due to GI hemorrhage was 14.5 % before the 1990s and fell to 2.8 % after the 1990s. Mortality due to tumor progression also changed from 47.8 % before the 1990s to 65.7 % thereafter [16]. Consequently, PNETs and carcinoids have been the predominant causes of death among patients with MEN1.

After the isolation of the *MEN1* gene, many investigators searched for genotype–phenotype or genotype–prognosis correlations. Kouvaraki et al. [17] reported that mutations in exon 2 are frequent in MEN1 patients with PNETs and mutations in exon 3 are frequent in those with thymic carcinoids. Ferolla et al. [18] reported that truncated

mutations are frequently present in patients with MEN1-associated thymic carcinoids. Kouvaraki et al. [17] found no relationship between genotype and prognosis.

The issues addressed in this retrospective study of MEN 1 patients were: (1) whether there are any genotype–phenotype correlations and (2) whether there are any prognostic predispositions depending on the *MEN1* genotype.

With regard to the first issue, we did not find any significant genotype–phenotype correlations. However, our experience is unique from several viewpoints. First, functional PNETs were observed in patients with *MEN 1* mutations in exons 2, 3 and 10 only. Second, thymic tumors were observed in patients with mutations in exon 3 and in the patient with the large deletion. Third, malignant diseases unrelated to MEN1, such as papillary thyroid carcinoma, lung cancer and osteomyelodysplasia, were observed only in patients with mutations in exons 2 and 10. Bartsch et al. [19] reported that patients with mutations in exons 2, 9 or 10 have a significantly higher rate of malignant PNETs (55 %) than those with mutations elsewhere (10 %). Kouvaraki et al. [17] reported that PNETs are frequently observed when the mutation is present in exon 2, whereas carcinoids are frequent when the mutation is in exon 10.

With regard to the second issue, MEN1-associated deaths were identified only among patients with mutations of exons 2 and 3 and in the one patient with a large deletion. However, it is premature to conclude that these mutations are specifically related to mortality. In fact, Vierimaa et al. [20] observed MEN1-related deaths in patients with mutations of exon 10.

Mortality associated with MEN1 in the present series was 16 %, which is quite different from figures reported previously [7, 14, 21]. One of the reasons for this difference may be advances in both knowledge and management of MEN1. In fact, in all of the cohort studies reported previously, the patients had been observed since the early 1900s.

The present study had several limitations. First, the study population included patients who had undergone parathyroidectomy. Such selection may have led to biased estimates of the phenotypic prevalence and disease-specific survival. Second, the number of patients (32) was too small to draw firm conclusions from the results. Third, the follow-up period was limited. Fourth, neither diagnostic nor follow-up examinations were uniform among the patients. The variation in work-up may have compromised the validity of the estimated disease frequency and prognosis.

In conclusion, no significant genotype–phenotype or genotype–prognosis correlations were observed in this study. The fact that the study involved a single institution may also have been a limiting factor. In addition, a longer follow-up period in the patients is needed. In 2008, researchers in Japan established a MEN study group, the "MEN Consortium of Japan," to construct a nationwide database of Japanese MEN patients. It is anticipated that this database will yield data on many unanswered questions related to MEN.

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Review Article

Ghrelin, Des-Acyl Ghrelin, and Obestatin: Regulatory Roles on the Gastrointestinal Motility

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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 regulatory roles of these peptides, we applied the manometric measurement of gastrointestinal motility in freely moving conscious rat or mouse model. 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 and Y4 receptors in the brain may mediate the action of ghrelin, CRF type 2 receptor in the brain may mediate the action of des-acyl ghrelin, whereas CRF type 1 and type 2 receptors in the brain may 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.

1. 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]. Des-acyl ghrelin, on the other hand, has the same amino acid sequence with no O-n-octanoyl acid modification [1]. Obestatin was found by a bioinformatics approach to be encoded by preproghrelin [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].

Recently we developed conscious rat and mouse models to measure physiological fed and fasted motor activities in the gastrointestinal tracts [14–18]. By using these models we succeeded to examine the effects of ghrelin, des-acyl ghrelin, and obestatin on gastroduodenal motility and involvement of hypothalamic peptides mediating the action of these peptides. In this review, we overview the different effects of ghrelin, des-acyl ghrelin, and obestatin on the upper gastrointestinal motility with special attention being paid to brain-gut interactions.

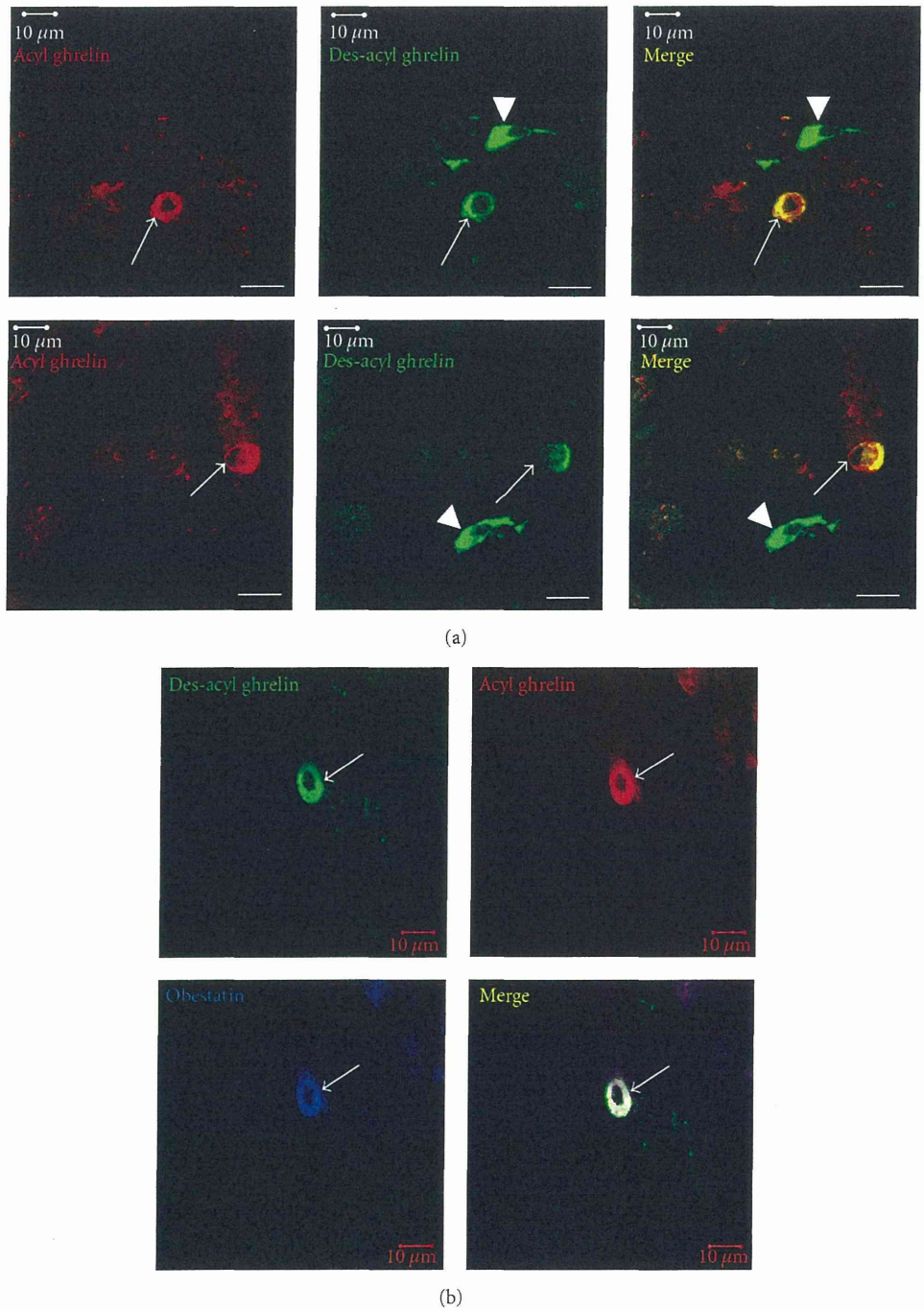

FIGURE 1: Localization of ghrelin, des-acyl ghrelin and obestatin in the rat stomach. (a) Immunofluorescence double staining for acyl ghrelin- (red) and des-acyl ghrelin-positive (green) reaction in the antral mucosa of rat stomach. Acyl ghrelin-positive reaction and des-acyl ghrelin-positive reaction are colocalized in closed-type cells (arrows), whereas des-acyl ghrelin-positive reaction is localized in open-type cells (arrowheads). (b) Immunofluorescence triple staining for des-acyl ghrelin (green), acyl ghrelin (red) and obestatin (blue) in the antral mucosa of rat stomach. Three peptides are colocalized in the closed-type cells (arrows). Bars = 10 μm.

2. Localization of Ghrelin, Des-Acyl Ghrelin, and Obestatin in the Rat Stomach

The localization of ghrelin in the stomach has been studied in various animals by using the specific antibody for ghrelin [19, 20]; however, the localization of des-acyl ghrelin in the stomach has been scarcely examined. We developed antibodies specific for ghrelin (antirat octanoyl ghrelin (1-15) -cys-KLH serum) and for des-acyl ghrelin (antirat des-octanoyl ghrelin (1-15) -cis-KLH serum) and successfully detected the different localization of ghrelin and des-acyl ghrelin in the rat stomach [21].

Both ghrelin- and des-acyl ghrelin-immunoreactive cells were distributed in the oxyntic and antral mucosa of the rat stomach, with higher density in the antral mucosa than oxyntic mucosa. Immunofluorescence double staining showed that ghrelin- and des-acyl ghrelin-positive reactions overlapped in closed-type round cells, whereas des-acyl ghrelin-positive reaction was found in open-type cells in which ghrelin was negative (Figure 1(a)). Ghrelin/des-acyl ghrelin-positive closed-type cells contain obestatin (Figure 1(b)); on the other hand des-acyl ghrelin-positive open-type cells contain somatostatin [21].

The characteristic features of open-type cells that contain des-acyl ghrelin and closed-type cells that contain ghrelin indicate that they may respond differently to intraluminal factors. It is highly possible that open-type cells may react to luminal stimuli more than closed-type cells. Therefore we investigated the effects of different intragastric pH levels on the release of ghrelin and that of des-acyl ghrelin from the ex vivo perfused rat stomach [21]. In a preliminary study we measured the intragastric pH levels in the fasting and fed states of rats and found that intragastric pH in the fasting state was pH 4, whereas that in the fed state was pH 2 [16]. Our results showed that the release of ghrelin was not affected by intragastric pH, whereas the release of des-acyl ghrelin was increased at intragastric pH 2 compared to that at intragastric pH 4 [21]. This result suggests that des-acyl ghrelin-containing cells may sense the intragastric pH via their cytoplasmic processes and release the peptide in accordance with the lower intragastric pH. The fact that the release of des-acyl ghrelin is stimulated by lower intragastric pH seems reasonable because des-acyl ghrelin may act as a satiety signal [6, 7] in the fed state of animals.

3. Manometric Measurement of Gastrointestinal Motility in Conscious Mice and Rats

We developed freely moving conscious animal model to measure the gastrointestinal motility in rats [15] and mice [18]. This model permits the measurement of gastrointestinal motility in animals in the physiological fed and fasted states by a manometric method [15, 18]. In the fasted state, the cyclic changes 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). The frequencies of phase III-like contractions in the fasted motility in the antrum and duodenum in mice (6.0 ± 0.2/h and 6.0 ± 0.3/h, resp.) were significantly ($P < .05$) higher than those in rats (5.3 ± 0.5/h, 5.6 ± 0.8/h, resp.) [15, 18]. After food intake, such fasted motor pattern was disrupted and replaced by a fed motor pattern, which consisted of irregular contractions of high frequency.

4. Ghrelin and Gastroduodenal Motility

Intracerebroventricular (i.c.v.) and intravenous (i.v.) 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, 18] (Figure 2(a)). I.c.v. and i.v. 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 i.v. injection of ghrelin on gastroduodenal motility were blocked by i.v. injection of GHS-R antagonist but not by i.c.v. injection of GHS-R antagonist [16]. Immunoneutralization of NPY in the brain blocked the stimulatory effects of ghrelin on the gastroduodenal motility [16] (Figure 2(b)). 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 (Figures 2(c) and 2(d)). 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, and Y2 and/or Y4 receptors in the brain may mediate the action of ghrelin (Figure 2(d), Table 1). 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 [16].

Human ghrelin has a structural resemblance to human motilin, and human ghrelin receptors exhibit a 50% identity with human motilin receptors [22]. Therefore the role of ghrelin in the gastrointestinal motility is comparable with that of motilin [23, 24]. Motilin originates from the endocrine cells in the duodenum [23], while ghrelin originates from the endocrine cells in the stomach [20]; 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 [24, 25], 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 [26], we examined the relationship between the effects of ghrelin on gastroduodenal motility and intragastric pH. The results showed that within 30 minutes after feeding low intragastric pH (pH 2.5 ± 0.2) inhibited the effects i.v. injected ghrelin on gastroduodenal motility, and that this effect was reversed by an increase of intragastric pH (pH 5.4 ± 0.6) within 60 minutes 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.

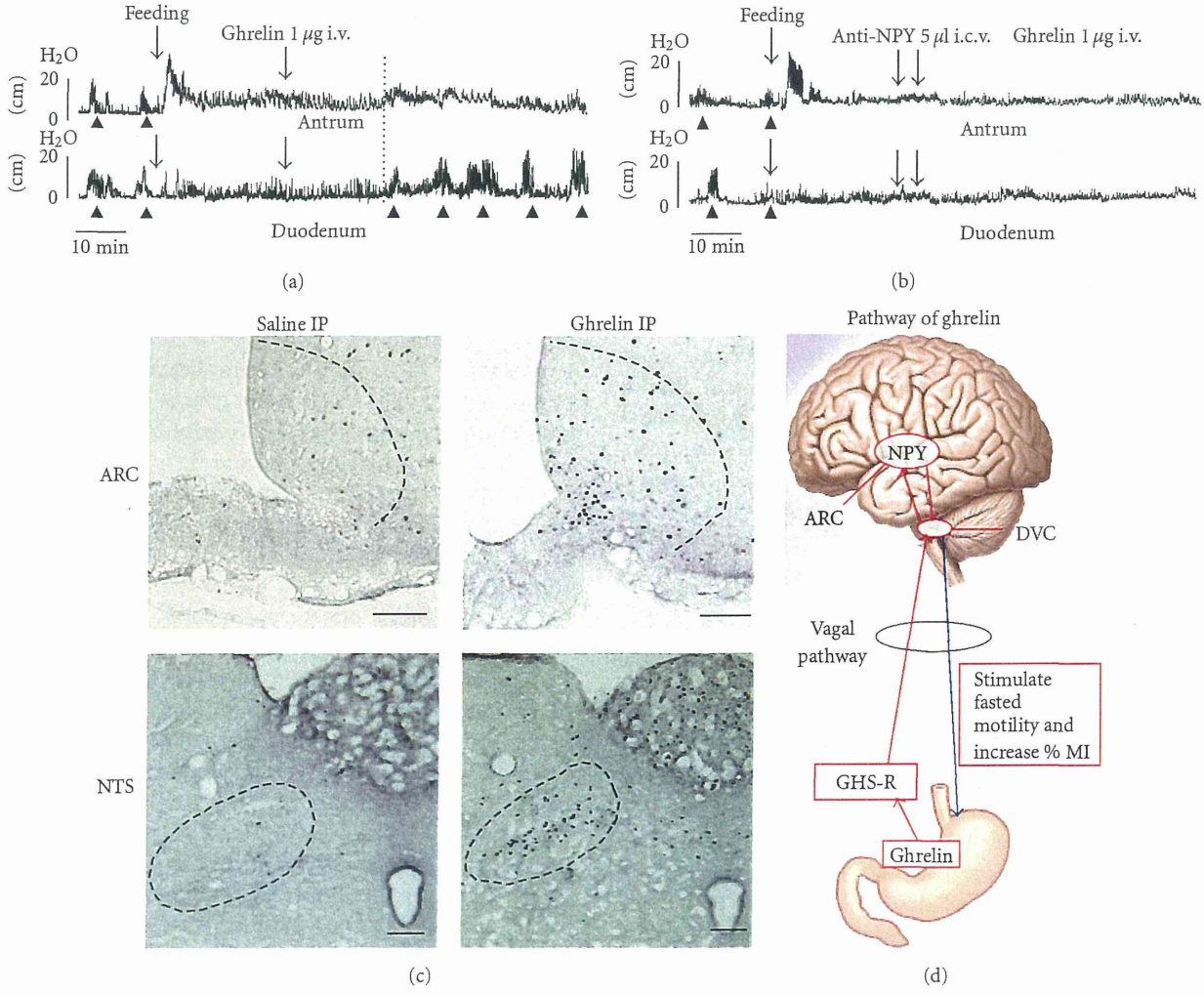

FIGURE 2: Effects of ghrelin on the gastroduodenal motility. (a) Effects of i.v. injection of ghrelin on the fed motor activity of the antrum and duodenum. I.v. injection of ghrelin induces the fasted pattern in the duodenum and increases the motor activity in the antrum. (b) I.c.v. injection NPY antiserum completely blocks the effect of i.v. injection of ghrelin. (c) The density of c-*Fos*-positive cells in the arcuate nucleus (ARC) and NTS is increased by i.p. injection of ghrelin compared to saline-injected control. (d) Summary diagram of the effects of ghrelin on the gastroduodenal motility and brain mechanism mediating its action.

TABLE 1: Summary of the regulatory roles of ghrelin, des-acyl ghrelin and obestatin on the gastroduodenal motility.

	ghrelin		des-acyl ghrelin		obestatin	
	Fasted state	Fed state	Fasted state	Fed state	Fasted state	Fed state
Stomach	↑	↑	↓	—	—	↓
Duodenum	↑	↑	—	—	—	↓
Hypothalamic neuron	NPY		urocortin 2		CRF, urocortin 2	
Brain receptor	Y2, Y4		CRF type 2		CRF type 1, type 2	
Vagal afferent pathway	+		—		+	

5. 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 (i.p.) 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].

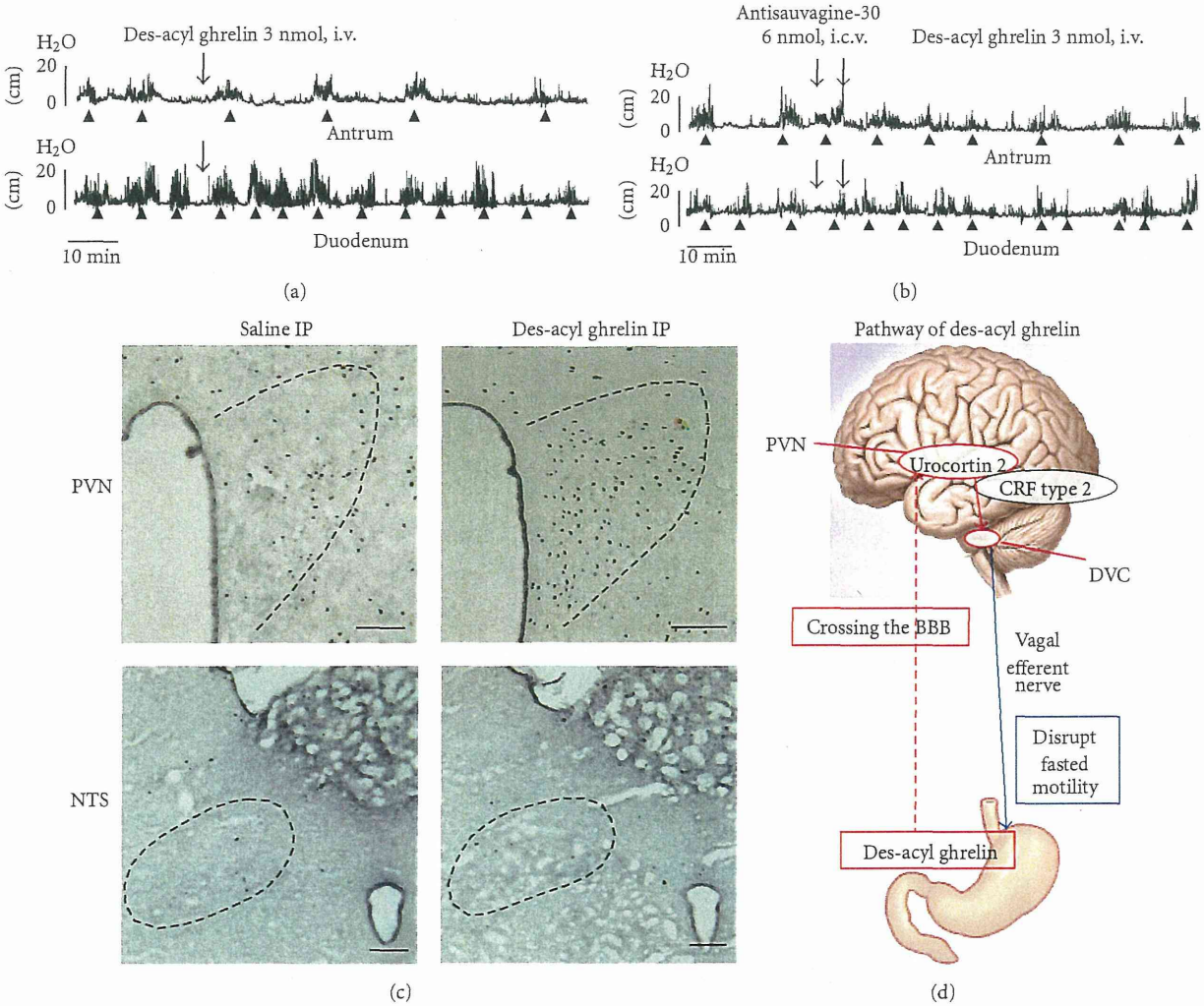

FIGURE 3: Effects of des-acyl ghrelin on the gastroduodenal motility. (a) Effects of i.v. injection of des-acyl ghrelin on the fasted motor activities of the antrum and duodenum. I.v. injection of des-acyl ghrelin decreases the frequency of phase III-like contractions in the antrum but not in the duodenum. (b) The decreased frequency of phase III-like contractions induced by i.v. injection of des-acyl ghrelin is restored to normal in pretreatment of i.c.v. injection of the selective CRF type 2 receptor antagonist antisauvagine-30. (c) The density of c-*Fos*-positive cells in the PVN is increased by i.p. injection of des-acyl ghrelin compared to saline-injected control, whereas that in the NTS is not altered. (d) Summary diagram of the effects of des-acyl ghrelin on the gastroduodenal motility and brain mechanism mediating its action.

I.c.v. and i.v. injections of des-acyl ghrelin disrupted fasted motility in the antrum but not in the duodenum [7] (Figure 3(a)). The frequencies of fasted motility in the antrum were decreased to 58.9% and 54.5% by des-acyl ghrelin injcted i.c.v. and i.v., respectively, [7]. However i.c.v. and i.v. injections of des-acyl ghrelin did not alter fed motor activity in both the antrum and duodenum [7]. 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 treatment did not alter the disruptive effect of i.v. 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 [27]. 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. I.p. injection of ghrelin significantly increased the density of c-*Fos*-positive cells in the NTS (Figure 2(c)), while i.p. 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 3(c)). 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(d)).

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 3(b)). Between