ORIGINAL COMMUNICATION

Abnormal ghrelin secretion contributes to gastrointestinal symptoms in multiple system atrophy patients

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Abstract Patients with multiple system atrophy (MSA) often have evidence of compromised gastrointestinal motility. Ghrelin is a gut hormone that influences gastrointestinal motility in humans. The aim of this study was to determine whether ghrelin secretion is affected in MSA patients, and to investigate the relation between ghrelin secretion and gastrointestinal symptoms. Plasma levels of active ghrelin and unacylated ghrelin were measured in patients with MSA ($n = 30$), other atypical parkinsonian disorders including progressive supranuclear palsy-Richardson syndrome and corticobasal syndrome ($n = 24$), and control subjects ($n = 24$) using enzyme-linked immunosorbent assays. Gastrointestinal symptoms were quantified in all subjects using a self-report questionnaire. The ratio of active ghrelin to total ghrelin in the plasma (active ghrelin ratio) was lower in patients with MSA (mean: 8.0 %) than in patients with other atypical parkinsonian disorders (mean: 13.7 %, $P = 0.001$) and control subjects (mean: 13.9 %, $P = 0.001$). The active ghrelin ratio was correlated with the severity of gastrointestinal symptoms in MSA ($r = -0.5$, $P = 0.004$). Our observations indicate that ghrelin secretion is affected in patients with MSA. The low active ghrelin ratio may contribute to gastrointestinal symptoms in MSA.

Introduction

Nonmotor symptoms have a strong impact on the prognosis and quality of life in patients with multiple system atrophy (MSA). Patients with MSA often have evidence of compromised gastrointestinal motility [1, 2], which may be caused by disruptions in the autonomic nervous system. However, it remains unclear whether neurohormonal function contributes to compromised gastrointestinal motility in MSA.

Ghrelin is a gut hormone that acts as a natural ligand for the growth hormone secretagogue receptor. It plays an important role in the neurohormonal interaction between the gastrointestinal tract and the central nervous system [3]. Ghrelin has been reported to influence gastrointestinal motility in humans [4, 5]. The aim of the present study was to determine whether ghrelin secretion is affected in patients with MSA, and to investigate the relation between ghrelin secretion and gastrointestinal symptoms in these patients.

Methods

Subjects

Subjects were 30 patients with probable MSA (21 patients with MSA-C and 9 patients with MSA-P), 24 patients with other atypical parkinsonian disorders (13 patients with progressive supranuclear palsy-Richardson syndrome and

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11 patients with corticobasal syndrome), and 24 control subjects (16 patients with non-neurodegenerative diseases and 8 healthy individuals). The 16 subjects with non-neurodegenerative diseases consisted of three patients with peripheral neuropathy, three patients with idiopathic normal pressure hydrocephalus, four patients with cervical spondylosis, and six post-stroke patients undergoing rehabilitation. Neurologists specialized in movement disorders clinically diagnosed the patients with MSA and other atypical parkinsonian disorders in accordance with established or proposed diagnostic criteria for the diseases [6–8]. The levodopa-equivalent daily dose of anti-parkinsonian medication was calculated as described previously [9]. None of the patients with MSA or other atypical parkinsonian disorders were receiving anticholinergic therapies. Dementia was not a criterion for exclusion, because patients with other atypical parkinsonian disorders may have associated dementia. Body mass index was evaluated in each subject. No drugs that are known to affect ghrelin secretion in human, namely, olanzapine [10], flutamide [11], estrogen [12], somatostatin [13], growth hormone [14], insulin-like growth factor-I [12], and rikkunshito [15], were administered to the subjects before blood sampling. None of the subjects had alcoholism, diabetes mellitus, inflammatory bowel diseases, or cancer-induced cachexia. Informed consent was obtained from all subjects. The protocol of this study was approved by the Ethics Committee at Niigata University School of Medicine, and was carried out in accordance with the Declaration of Helsinki.

Assessment of gastrointestinal symptoms

The gastrointestinal domain of the scales for outcomes in Parkinson's disease-autonomic (SCOPA-AUT) [16] was used to assess gastrointestinal symptoms. This is composed of seven questions about gastrointestinal symptoms, namely, swallowing/choking, sialorrhea, dysphagia, early abdominal fullness, constipation, straining for defecation, and fecal incontinence. A symptom was considered present (gaining 1 point) if the patient reported that it had occurred regularly or often in the past month, and the severity of gastrointestinal symptoms was rated by the total SCOPA-AUT gastrointestinal score (range 0–7 points).

Blood sampling and hormone assays

On the day prior to blood sampling dinner was served at 1800 h and the time of lights out was set at 2100 h. Subjects were asked to stay in bed from lights out until the time of blood sampling the next morning, except for visits to the toilet. Bedrooms used in this study were in quiet surroundings and set at a comfortable room temperature between 18 and 25 °C throughout the night. These settings were selected to avoid sleep deprivation that could affect ghrelin secretion [17]. Blood samples were obtained from subjects the morning after a 12 h fast. To avoid the influence of food intake [18] and visual or olfactory stimulus of food on ghrelin secretion, subjects were not permitted to eat between meals and breakfast was served after blood sampling. To avoid the effect of alcohol intake on ghrelin secretion, subjects were instructed to abstain from drinking beverages that contained alcohol during the study period [19].

Blood samples were immediately transferred to polypropylene tubes containing Na_2EDTA and aprotinin, and then centrifuged at 3,500 rpm for 10 min. Immediately after centrifugation, one-tenth of the volume of 1 N HCl was added to the separated plasma. There are two circulating forms of ghrelin, acylated (active) ghrelin with n-octanoylation at serine three and unacylated ghrelin. The plasma levels of active ghrelin and unacylated ghrelin were measured using commercially available enzyme-linked immunosorbent assay kits according to the manufacturer's instructions (active ghrelin and desacyl-ghrelin ELISA kit, Mitsubishi Kagaku Iatron Inc., Tokyo, Japan). The sensitivity of the applied methods was 4 fmol/ml for active ghrelin and 14 fmol/ml for unacylated ghrelin. The intra- and interassay coefficients of variation were 5.5 and 4.9 % respectively for active ghrelin, and 4.7 and 8.6 % respectively for unacylated ghrelin. The ratio of active ghrelin to total ghrelin in the plasma (active ghrelin ratio) was obtained using the following formula:

$$\text{Active ghrelin} / (\text{Active ghrelin} + \text{Unacylated ghrelin}) \times 100 \, (\%).$$

Statistical analyses

One-way analysis of variance was used to compare variables across the three groups. Significant main effects of group were followed by post hoc comparisons with Bonferroni corrections. T-tests were used to compare variables across two groups. A χ^2 test was used to compare sex distribution across the three groups. Pearson's correlation coefficient was used to quantify the relation between the SCOPA-AUT gastrointestinal score and the active ghrelin ratio in each group. Values of $P < 0.05$ were considered significant.

Results

Age at the time of evaluation, sex distribution, and body mass index were matched across the three groups, and disease duration and levodopa equivalent daily doses were matched for the two patient groups (Table 1). The SCOPA-AUT gastrointestinal score was significantly higher in the

Table 1 Subjects' demographic characteristics and state of ghrelin secretion

	MSA	Other APD	Control	P value
Number of cases	30	24	24	
Age (years)	67.5 (1.6)	73.1 (1.3)	67.5 (3.0)	0.1
Male:female	16:14	15:9	15:9	>0.5
Disease duration (years)	4.3 (0.5)	3.9 (0.7)	Not applicable	0.64
Levodopa equivalent daily doses (mg/day)	130.6 (40.0)	135.0 (38.4)	Not applicable	0.93
Body mass index (kg/m^2)	21.2 (0.6)	21.5 (0.8)	22.1 (0.6)	0.6
SCOPA-AUT gastrointestinal score	3.8 (0.3)	2.1 (0.2)	0.5 (0.1)	0.001*
Active ghrelin (fmol/ml)	6.9 (0.9)	10.2 (1.3)	11.2 (1.7)	0.05
Unacylated ghrelin (fmol/ml)	102.2 (15.8)	69.9 (10.3)	66.8 (8.1)	0.08
Active ghrelin ratio (%)	8.0 (0.8)	13.7 (1.1)	13.9 (0.9)	0.001**

Data are n or mean (standard error)

MSA multiple system atrophy, *APD* atypical parkinsonism disorder, *SCOPA-AUT* scales for outcomes in Parkinson's disease-autonomic

* Post-hoc P values (after Bonferroni correction): MSA vs. other APD; $P = 0.001$, MSA vs. control; $P = 0.001$, other APD vs. control; $P = 0.002$

** Post-hoc P values (after Bonferroni correction): MSA vs. other APD; $P = 0.001$, MSA vs. control; $P = 0.001$, other APD vs. control; $P = 1.0$

MSA group than in the other two groups (Table 1). Although the levels of plasma active ghrelin and unacylated ghrelin did not differ between the three groups, the active ghrelin ratio was significantly lower in the MSA group than in other two groups (Table 1). The active ghrelin ratio was very similar in the atypical parkinsonian and control groups (Table 1). In the control group, there was no difference in active ghrelin ratio between the 16 patients with non-neurodegenerative diseases (mean: 13.6 %) and 8 healthy individuals (mean: 14.4 %, $P = 0.7$).

In the MSA group, the active ghrelin ratio was significantly correlated with the SCOPA-AUT gastrointestinal score ($r = -0.5$, $P = 0.004$; Fig. 1), such that a lower active ghrelin ratio was associated with more gastrointestinal

Fig. 1 The relation between the ratio of active ghrelin to total ghrelin in the plasma (active ghrelin ratio) and the scales for outcomes in Parkinson's disease-autonomic (SCOPA-AUT) gastrointestinal score in patients with MSA ($r = -0.5$, $P = 0.004$)
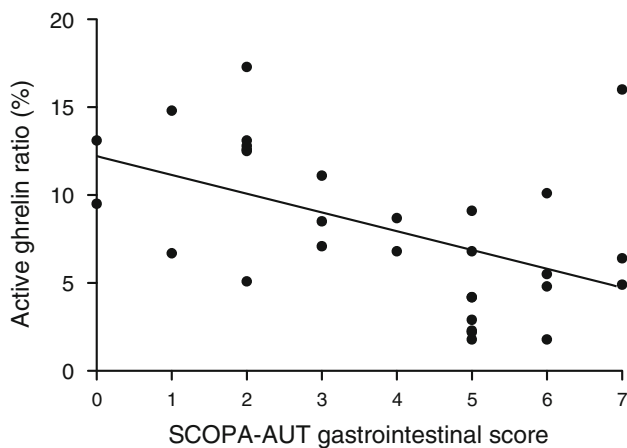
symptoms. There was no correlation between the active ghrelin ratio and the SCOPA-AUT gastrointestinal score in the atypical parkinsonian group ($P = 0.6$) or the control group ($P = 0.9$).

Discussion

In the present study, patients with MSA showed aberrant ghrelin secretion that was characterized by a low active ghrelin ratio. Furthermore, there was a significant correlation between the reduced active ghrelin ratio and the severity of gastrointestinal symptoms in patients with MSA, indicating that a lower active ghrelin ratio was associated with more gastrointestinal symptoms in MSA. This phenomenon was not observed in patients with progressive supranuclear palsy-Richardson syndrome or corticobasal syndrome.

Experimental data have shown that dopamine and dopamine agonists stimulate the release of active ghrelin from a ghrelin-producing cell line in vitro [20], therefore stimulatory effects of these drugs on ghrelin secretion should be considered in patients with MSA and other atypical parkinsonian disorders. However, levodopa equivalent daily doses were matched for MSA and other atypical parkinsonian groups, thereby controlling for the possibility that anti-parkinsonian medications influenced the results of this study.

Previous studies have shown that patients with MSA frequently exhibit delayed gastric emptying [1] and slow colonic transit [2]. Accumulating evidence suggests that active ghrelin facilitates motility of the gastric antrum [4, 5] and colorectum [21], and unacylated ghrelin may

counteract the effects of active ghrelin [22, 23]. Therefore, the reduced active ghrelin ratio presented in this study may contribute to compromised motilities of the gastric antrum and colorectum in patients with MSA.

The pathogenic mechanism of aberrant ghrelin secretion in MSA is not yet known. Cholinergic innervation of the gastrointestinal tract is thought to regulate ghrelin secretion in humans [24]. Postmortem examination of MSA patients has revealed that neurodegeneration occurs in vagal autonomic nuclei [25], which provide extrinsic cholinergic innervation to the gastrointestinal tract. Therefore, there may be a link between disruption of the vagal pathway to the gastrointestinal tract and aberrant ghrelin secretion in patients with MSA. Our observation that the active ghrelin ratio was similar in the atypical parkinsonian and control groups suggests that cholinergic innervation to the gastrointestinal tract is relatively well preserved in patients with progressive supranuclear palsy-Richardson syndrome and corticobasal syndrome.

These findings are the first to show that reduced active ghrelin ratio may contribute to the pathogenic mechanism of compromised gastrointestinal motility in patients with MSA. Pharmacological interventions that have therapeutic value in relation to increasing plasma active ghrelin should be considered for gastrointestinal symptoms in patients with MSA.

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