Ghrelin prevents the development of experimental diabetic neuropathy in rodents

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ABSTRACT

Ghrelin is an acylated peptide discovered in gastric extracts as an endogenous ligand for the growth hormone secretagogue (GHS) receptor. This peptide increases food intake and growth hormone secretion, suppresses inflammation and oxidative stress, and promotes cell survival and proliferation. Our study investigated the pharmacological effect of ghrelin in the prevention of polyneuropathy in streptozotocin-induced diabetes mellitus in C57BL/6N mice, GHS receptor–deficient mice, and growth hormone–deficient rats. Ghrelin or desacyl-ghrelin was administered daily for four weeks immediately after disease onset. The effects of ghrelin on food intake, body weight, blood glucose and plasma insulin levels, nerve conduction velocities, temperature sensation, and 8-isoprostaglandin F2α (8-iso-PGF2α) levels were examined. We found that ghrelin administration did not change food intake, body weight gain, blood glucose levels, or plasma insulin levels in C57BL/6N mice in comparison with mice treated with saline or desacyl-ghrelin administration. Ghrelin administration, but not desacyl-ghrelin, prevented motor and sensory polyneuropathy and reduced the plasma concentrations of 8-iso-PGF2α in C57BL/6N mice. Ghrelin also prevented the reduction in nerve conduction velocities in growth hormone-deficient rats, but not in GHS receptor–knockout mice. In conclusion, ghrelin

administration in a rodent model of diabetes prevented polyneuropathy, and this effect was mediated through the GHS receptor and was independent of growth hormone. The protection against the development of experimental diabetic polyneuropathy by ghrelin could be key in preventing this otherwise intractable disorder.

Keywords: ghrelin, streptozotocin, diabetic polyneuropathy, growth hormone secretagogue receptor

1. Introduction

Diabetic polyneuropathy is a common complication that occurs in large portion of both type 1 and type 2 diabetic patients (Boulton et al., 2005). Typical symptoms of diabetic polyneuropathy include chronic pain, numbness, weakness and difficulties with balance. Hyperglycemia is the definitive cause of polyneuropathy, whereas the vascular, glial, and neuronal damage underlying the progressive axonopathy in diabetic polyneuropathy have complex biochemical etiologies involving oxidative stress, protein glycation, protein kinase C activation, polyol synthesis, and the hexosamine pathway. Although favorable treatments of diabetic polyneuropathy have been suggested as a consequence of various pathogenic mechanisms, these treatments have generally produced disappointing results in clinical trials (Vincent et al., 2011).

Ghrelin, a peptide consisting of 28-amino-acids, was initially isolated from gastric extracts as an endogenous ligand for the growth hormone secretagogue (GHS) receptor (Kojima et al., 1999). Ghrelin acts on the pituitary to stimulate growth hormone release and on the hypothalamus to enhance food intake (Kojima et al., 1999; Nakazato et al., 2001). Ghrelin exist in two major forms, *n*-octanoyl–modified ghrelin and desacyl-ghrelin (a non-acylated form of ghrelin). Acylation at the third amino acid threonine is necessary for the binding of ghrelin to the GHS receptor. Desacyl-ghrelin

can neither bind the GHS receptor nor exhibit growth hormone-releasing activity (Toshinai et al., 2006). The GHS receptor is expressed in the central nervous system, and moreover, in multiple peripheral organs such as the stomach, intestine, pancreas, thyroid, gonads, adrenal, kidney, heart and vasculature, and bone. This widespread expression suggests that ghrelin may have a variety of effects on multiple systems. In fact, ghrelin mediates glucose homeostasis, gastrointestinal, cardiovascular, pulmonary, and immune function, cell proliferation and differentiation, and bone physiology (Kojima and Kangawa, 2006). The diverse actions of ghrelin raise the possibility of its clinical application; indeed, clinical trials with ghrelin for the treatment of anorexia nervosa, chronic respiratory infection, diabetic gastroparesis, and cachexia associated with chronic obstructive pulmonary disease and cancer have commenced (Miki et al., 2012; Miljic et al., 2006; Murray et al., 2005; Neary et al., 2004; Strasser et al., 2008).

Ghrelin has been reported to promote cell proliferation and neurogenesis in the neurons of the hypothalamus, dorsal motor nucleus of the vagus, nucleus of the solitary tract, and spinal cord (Steculorum et al., 2011). However, there has been little data demonstrating an effect of ghrelin on peripheral nerve functions. We have demonstrated that four-week intraperitoneal administration of ghrelin ameliorated the reduction of both motor and sensory nerve velocities, and reduced thermal sensation induced by streptozotocin in mice (Kyoraku et al., 2009). Here we investigate the efficacy of

ghrelin on the prevention of streptozotocin-induced diabetic polyneuropathy in addition to the roles of the GHS receptor and growth hormone in ghrelin's therapeutic mechanisms.

2. Materials and methods

2.1. Animals and induction of diabetes

We used several strains of rodents in these experiments: 6-week-old male C57BL/6N mice weighing 15–17 g (Charles River Japan Inc., Numazu, Japan); 6-week-old male GHS receptor–deficient mice weighing 15–17 g, which were generated by targeted mutation of embryonic stem cells as reported by Sun et al. (2004); and 10-week-old 58–70 g spontaneous dwarf rats (Japan SLC Inc., Hamamatsu, Japan), which were growth hormone deficient due to a point mutation in the *gh* gene (Okuma et al., 1980). Animals were housed individually at constant room temperature (23 ± 1°C) under a 12-h light (08:00–20:00 h)/12-h dark cycle, and were provided standard laboratory chow and water *ad libitum*. After fasting for 24 h, C57BL/6N mice and GHS receptor–deficient mice were given a single intraperitoneal injection of streptozotocin (140 mg/kg body weight; Sigma-Aldrich Japan Inc., Tokyo, Japan),

which was freshly dissolved in sodium citrate buffer (10 mmol/l, pH 5). Spontaneous dwarf rats were given a single intraperitoneal injection of streptozotocin (60 mg/kg body weight). Control animals received an intraperitoneal injection of citrate buffer only. Three days after streptozotocin administration, the animals exhibiting plasma glucose concentrations greater than 16 mmol/l were selected as diabetic animals (Matteucci and Giampietro, 2008; Roussel et al., 2004). Glucose levels were measured with a diagnostic kit (Ascensia Breeze 2, Bayer HealthCare AG, Leverkusen, Germany) using blood samples obtained from tail vein punctures. All experimental procedures were performed in accordance with the Japanese Physiological Society's guidelines for animal care and were approved by the Ethics Committee on Animal Experimentation of the University of Miyazaki.

2.2. Peptide administration

Four groups of 10 C57BL/6N mice were examined: a 'saline' group receiving saline only, 'ghrelin' group receiving 300 nmol/kg body weight/200 µl saline, 'desacyl-ghrelin' group receiving 300 nmol/kg body weight/200 µl saline (Asubio Pharma Co., Tokyo, Japan), and 'control' group (without streptozotocin treatment). Three groups of 6 GHS receptor–deficient mice were examined: saline, ghrelin, and

control groups. Finally, two groups of 6 spontaneous dwarf rats were examined: saline and ghrelin groups. Peptides or saline were administered intraperitoneally twice a day (06:00 and 18:00) for four weeks immediately following streptozotocin or control vehicle administration. We measured body weights, one-day food intake, and blood glucose concentrations of the animals at 10:00–12:00 at weekly or biweekly intervals after streptozotocin treatment.

2.3. Electrophysiology

Animals were anesthetized with pentobarbital (Nembutal, 0.1 ml/mouse, Abbott Co., North Chicago, IL, USA), and their body temperature was maintained at a rectal temperature of 37.5–37.9°C via a heating pad. The right sciatic nerve was stimulated (5–10 V, 0.05 ms single square-wave pulses), proximally at the level of the sciatic notch and distally at the level of the ankle, with paired sub-dermal needle electrodes (NE-2235, NIHON KOHDEN CORP., Tokyo, Japan) as described previously (Stanley et al., 1981). Compound muscle action potentials (CMAPs) were recorded from the interosseous muscles of the ipsilateral foot with two needle electrodes, and were amplified, stored, and displayed on a computer. Sensory nerve conduction velocity (SCV) was determined in a similar manner, using the same stimulating and recording

electrode pairs by measuring the latency difference of the H-reflex (Schratzberger et al., 2001). Averaged distal and proximal motor and sensory latencies from 10 separate recordings, together with the nerve length between the two stimulation sites, were used for determination of the motor nerve conduction velocity (MCV) and SCV. MCVs and SCVs were calculated by dividing the interelectrode distance between the two stimulation sites by the latency difference of the CMAPs. We determined the nerve conduction velocity of the sciatic nerve at weekly intervals for four weeks after streptozotocin treatment.

2.4. Hot plate test

A hot plate test was performed after the last administration of ghrelin at four weeks. Each animal was habituated to the test apparatus for three days before the test. The mice were placed on a hot plate maintained at 55°C, and the latency to lick the front or hind paws was monitored with a video camera and recorded on videotape (Kakinoki et al., 2006). The latency time was then analyzed by two hidden examiners.

2.5. Insulin and 8-iso-prostaglandin F2α (8-iso-PGF2α) measurement

At the end of the experiments, we deprived the mice of food for 8 h and sacrificed them under anesthesia with Nembutal at 21:00–22:00. Blood was obtained for the measurement of plasma insulin with an enzyme immunoassay (EIA) kit (Funakoshi Chemical Co., Tokyo, Japan) and of 8-iso-prostaglandin F2α (8-iso-PGF2α) with an 8-isoprostan EIA kit (Funakoshi Chemical Co., Tokyo, Japan).

2.6. Statistical analysis

Data are expressed as means ± standard error of the mean (S.E.M.). Differences among multiple groups were determined via a one-way or repeated-measures analysis of variance (ANOVA) with Bonferroni post-hoc *t*-tests. When two mean values were compared, the analysis was performed with an unpaired *t*-test. *P*-values less than 0.05 were considered statistically significant.

3. Results

3.1. Body weights, blood glucose levels, and one-day food intake

Body weight gains in the three diabetic groups were suppressed; however, food

intake in all diabetic groups increased one week after streptozotocin treatment and rose to nearly double that in controls two weeks after streptozotocin treatment (Table 1). Neither ghrelin nor desacyl-ghrelin administration to diabetic mice affected body weight gain, food intake, or blood glucose concentrations (Table 1). Plasma insulin concentrations were comparable in the three diabetic groups and markedly reduced in contrast to controls (Table 2). Plasma 8-iso-PGF2α concentrations were significantly increased in the saline and desacyl-ghrelin groups but not in the ghrelin group (Table 2).

3.2. Ghrelin prevented decreases in sciatic MCV and SCV in diabetic mice

The sciatic MCVs (Fig. 1A) and SCVs (Fig. 1B) of the saline and desacyl-ghrelin groups decreased two weeks after streptozotocin treatment in contrast to controls. Ghrelin administration prevented this decrease, and there was no significant difference in MCV or SCV between the ghrelin group and controls over the four-week treatment period.

3.3. Amelioration of reduced sensory perception by ghrelin

The animals in the saline and desacyl-ghrelin groups exhibited significantly longer

latencies to lick the front or hind paws than controls (Fig. 1C), suggesting that the diabetic mice showed thermal hypoalgesia. Ghrelin administration reduced the latencies to control levels.

3.4. No effect of ghrelin in GHS receptor–deficient mice

Body weight, one-day food intake, blood glucose concentration, and plasma insulin concentration of naïve GHS receptor–deficient mice were nearly equal to those of naïve mice (Table 3). Streptozotocin treatment suppressed body weight gains and increased food intake and blood glucose concentrations in diabetic GHS receptor–deficient mice both with and without ghrelin administration (Table 3). With levels similar to naïve mice, we found that the sciatic MCV of naïve 6-week-old GHS receptor deficient mice was 33.0 ± 1.6 m/sec and increased with age by 7.0 ± 1.5 m/sec for four weeks (Fig. 2A). Four-week ghrelin administration to streptozotocin-treated GHS receptor–deficient mice did not reverse the reduction of MCV, and the MCVs were comparable to those of the saline group during the four-week experiment (Fig. 2B). Ghrelin administration in streptozotocin-treated GHS receptor–deficient mice yielded SCVs comparable to those of the saline group during the four-week experiment (Fig. 2C).

3.5. Prevention of nerve conduction velocity reduction in diabetic spontaneous dwarf rats by ghrelin

Body weight change, food intake, and blood glucose concentrations of ghrelin-treated diabetic spontaneous dwarf rats were similar to those of saline-administered spontaneous dwarf rats during the four-week ghrelin administration (Table 4). The sciatic MCV and SCV of the saline group decreased compared with the ghrelin group after streptozotocin treatment, and significant differences in MCV and SCV between the two groups appeared two weeks after the start of ghrelin administration (Fig. 3).

4. Discussion

In the present study, we demonstrated that ghrelin treatment prevented experimental diabetic sensorimotor neuropathy. Ghrelin's effect on diabetic polyneuropathy depended on the GHS receptor but was independent of growth hormone. Ghrelin administration to streptozotocin-treated mice did not alter food intake, body weight, blood glucose levels, or plasma insulin levels compared with streptozotocin-treated mice without ghrelin

administration, suggesting that ghrelin did not affect diabetic conditions.

Although desacyl-ghrelin can neither bind the GHS receptor nor exhibit growth hormone–releasing activity, an accumulating body of evidence has suggested that desacyl-ghrelin has biological interactions with cardiomyocytes, endothelial cells, skeletal myoblasts, adipocytes, cultured fetal skin cells, neuronal precursor cells, and osteoblasts (reviewed in Kojima and Kangawa, 2007; Soares and Leite-Moreira, 2008). Many of these activities are related to cell fate, such as cell survival, inhibiting apoptosis and cell proliferation. Although the molecular mechanisms of desacyl-ghrelin are unresolved, it is possible that it acts through an unidentified GHS receptor–independent alternative pathway (Baldanzi et al., 2002; Muccioli et al., 2004; Thompson et al., 2004; Toshinai et al., 2006). In the present study, however, desacyl-ghrelin had no effect on diabetic neuropathy.

Several factors other than chronic hyperglycemia contribute to the pathogenesis of diabetic polyneuropathy (Vincent et al., 2011) that may be ameliorated by ghrelin treatment. It is known that oxidative stress is present in the diabetic state, and the reduction of antioxidant enzymes in diabetic nerves in part causes diabetic neuropathy (Low et al., 1997). Moreover, activation of microglia in the spinal cord during diabetic neuropathic pain has been reported as a major source of free radicals (Candelario-Jalil et al., 2007). In the present study, we demonstrated that ghrelin treatment significantly

reduced plasma 8-iso-PGF2α in streptozotocin-treated mice, suggesting that ghrelin suppressed oxidative stress in diabetic mice. Previous reports have also shown that ghrelin inhibits inflammatory responses and oxidative stress in peritoneal macrophages and human umbilical vein endothelial cells (Li et al., 2004), and furthermore, that ghrelin inhibits microglial activation and subsequent release of pro-inflammatory cytokines (Lee et al., 2010; Moon et al., 2009).

The endocrine effects of ghrelin are mediated via the GHS receptor. Using GHS receptor–deficient mice, we confirmed here that the GHS receptor was essential for ghrelin's effect on the prevention of diabetic polyneuropathy. It has been reported that GHS receptor–deficient mice were refractory to the stimulatory effects of ghrelin on growth hormone release and feeding behavior; they had normal growth rates, appetite, and body composition under conditions of standard laboratory housing (Sun et al., 2004). This study showed that GHS receptor–deficient mice had normal development of MCV and SCV. Streptozotocin-treated GHS receptor–deficient mice developed hyperglycemia, hyperphagia, and weight loss in a manner similar to C57BL/6N mice. The ineffectiveness of ghrelin administration to diabetic GHS receptor–deficient mice indicated that ghrelin's action to protect against diabetic polyneuropathy required the GHS receptor.

The biological activities of ghrelin through the GHS receptor can be classified into

growth hormone–dependent and –independent pathways, including ghrelin's orexigenic activity independent of growth hormone (Nakazato et al., 2001). The sciatic MCVs and SCVs of naïve growth hormone–deficient rats in this study were similar to those of Wistar and Sprague-Dawley rats (Kato et al., 2005; Schratzberger et al., 2001). Ghrelin administration to streptozotocin-treated spontaneous dwarf rats significantly increased both MCV and SCV, indicating that ghrelin's effect on polyneuropathy was independent of growth hormone.

There are many reports describing the effects of ghrelin administration on glucose metabolism in humans; however, the results have varied according to the dose of ghrelin or the pathophysiological conditions of the subjects (Akamizu et al., 2004; Alvarez-Castro et al., 2006; Broglio et al., 2001; Lucidi et al., 2005; Tassone et al., 2003; Vestergaard et al., 2007). In our preliminary clinical trial, a single intravenous administration of human ghrelin at 0.5 μg/kg to 10 type 2 Japanese diabetic patients did not affect their plasma glucose or insulin levels (unpublished results). Moreover, two-week intravenous ghrelin administration after breakfast at 1 μg/kg to 7 type 2 diabetic patients ameliorated the symptoms associated with diabetic polyneuropathy and significantly increased the tibial MCVs in all patients (unpublished results). However, intravenous administration is not translatable to clinical use. The development of more appropriate dosing methods such as a subcutaneous injection or oral medication is

needed for ghrelin to be clinically applicable.

In conclusion, our results demonstrate that ghrelin administration prevented polyneuropathy in a rodent model of diabetes. The effect of ghrelin on polyneuropathy is mediated through the GHS receptor and is independent of growth hormone. Ghrelin's multifaceted roles suggest a novel preventive treatment for diabetic polyneuropathy, and additional clinical trials for the treatment of early diabetic polyneuropathy may be warranted.

Acknowledgements

This work was supported by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) KAKENHI Grant Number 22126009, Strategic International Research Cooperative Program, Japan Science, Technology Agency (JST) and Takeda Science Foundation to M.N.

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Figure legends

Fig. 1. Effects of ghrelin administration on sciatic nerve MCV (A), and SCV (B), and the latency to lick the paw in the hot plate test (C). (A, B) Data are expressed as means ± S.E.M. $*P < 0.05$ and $^{\dagger}P < 0.001$ versus controls. $^{\#}P < 0.001$ versus streptozotocin + saline and streptozotocin + desacyl-ghrelin. Comparisons at each week were made using a one-way ANOVA with Bonferroni post-hoc *t*-tests. ($^{**}P < 0.001$ versus streptozotocin + saline using repeated-measures ANOVA with Bonferroni post-hoc *t*-test). (C) $*P < 0.01$ versus controls, and $^{\dagger}P < 0.05$ versus streptozotocin + saline and streptozotocin + desacyl-ghrelin using a one-way ANOVA with Bonferroni post-hoc *t*-tests. N = 10 per group.

Fig. 2. Effects of ghrelin administration on nerve conduction velocity in GHS receptor knockout (GHS-receptor$^{-/-}$) mice. (A) Comparison of sciatic MCV of naïve GHS-receptor$^{-/-}$ and naïve GHS-receptor$^{+/+}$ mice. The effects of ghrelin in streptozotocin-treated diabetic GHS-receptor$^{-/-}$ mice on MCV (B), and SCV (C). Data are expressed as means ± S.E.M. $*P < 0.01$ and $^{\dagger}P < 0.001$ versus controls using a one-way ANOVA with Bonferroni post-hoc *t*-tests. N = 6 per group.

Fig. 3. Effects of ghrelin administration on sciatic MCV (A) and sensory nerve SCV (B) in streptozotocin-treated diabetic spontaneous dwarf rats. Data are expressed as means ± S.E.M. $^{*}P < 0.05$ and $^{\dagger}P < 0.01$ versus streptozotocin + saline using a one-way ANOVA with Bonferroni post-hoc *t*-tests. N = 6 per group.

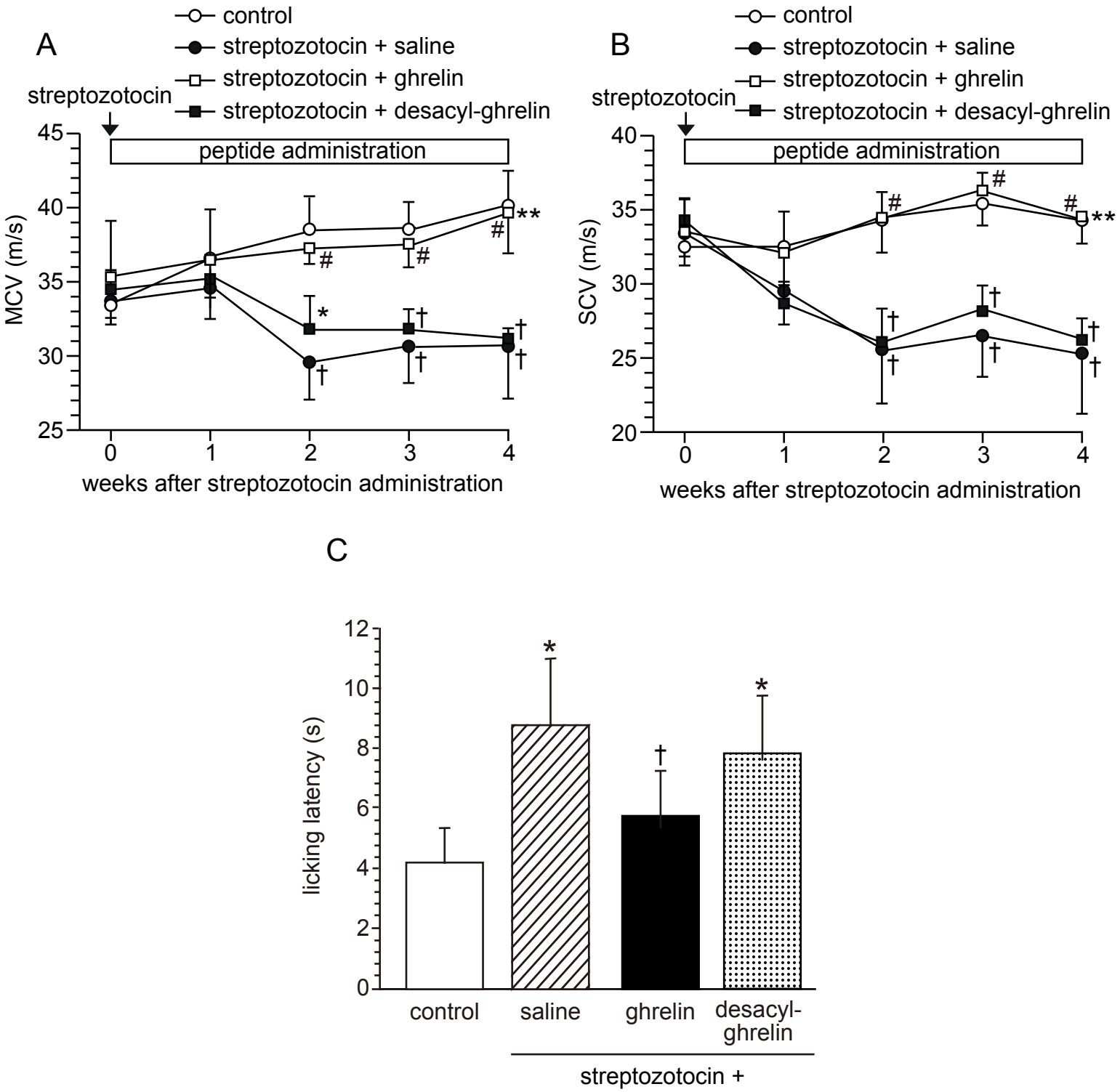
A
control
streptozotocin + saline
streptozotocin + ghrelin
streptozotocin + desacyl-ghrelin
streptozotocin
peptide administration
MCV (m/s)
weeks after streptozotocin administration
B
SCV (m/s)
C
licking latency (s)
control
saline
ghrelin
desacyl-ghrelin
streptozotocin +

Fig. 1.

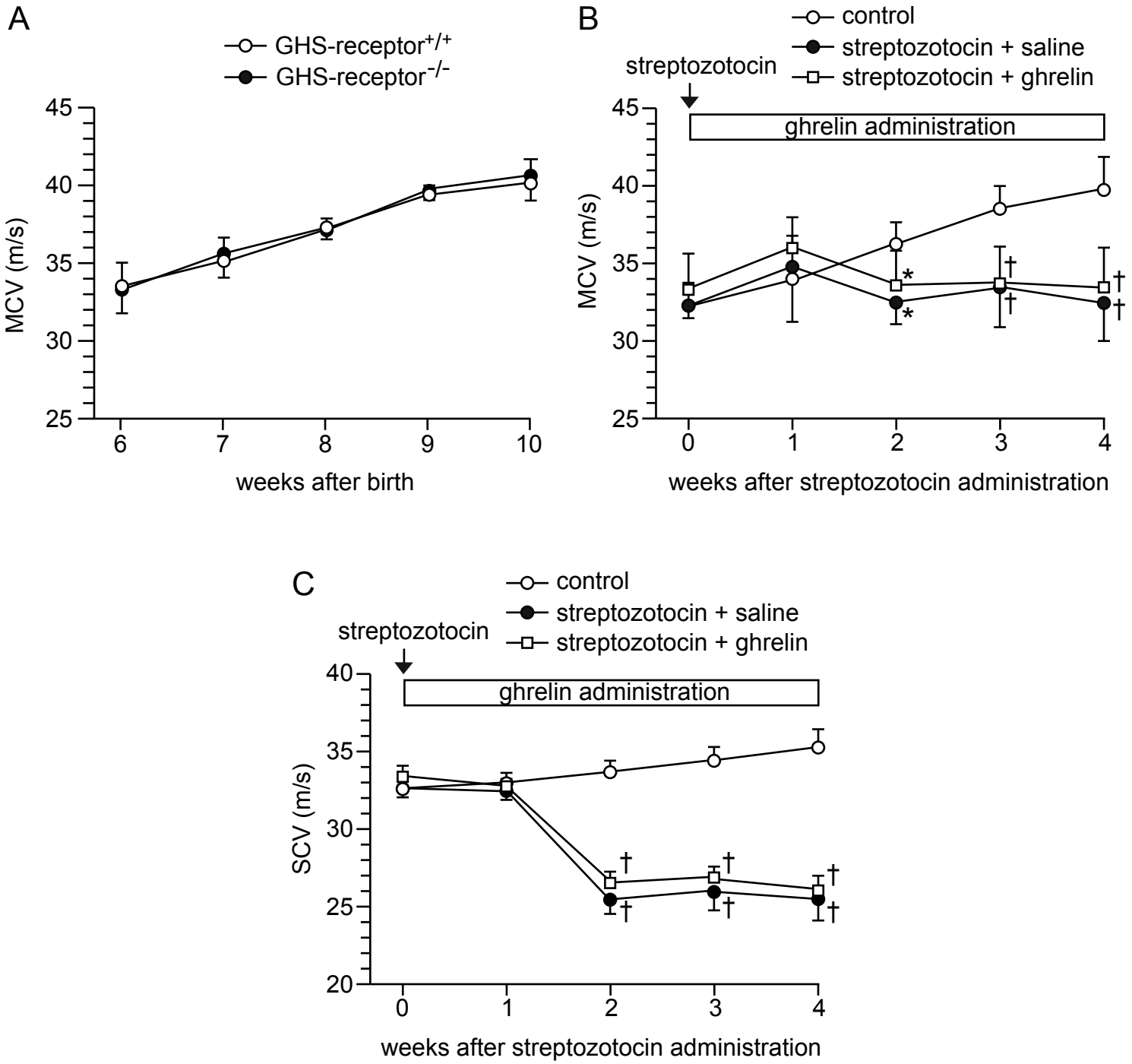
A
GHS-receptor+/+
GHS-receptor-/-
MCV (m/s)
45
40
35
30
25
6
7
8
9
10
weeks after birth
B
control
streptozotocin + saline
streptozotocin + ghrelin
streptozotocin
ghrelin administration
MCV (m/s)
45
40
35
30
25
0
1
2
3
4
weeks after streptozotocin administration
C
control
streptozotocin + saline
streptozotocin + ghrelin
streptozotocin
ghrelin administration
SCV (m/s)
40
35
30
25
20
0
1
2
3
4
weeks after streptozotocin administration

Fig. 2.

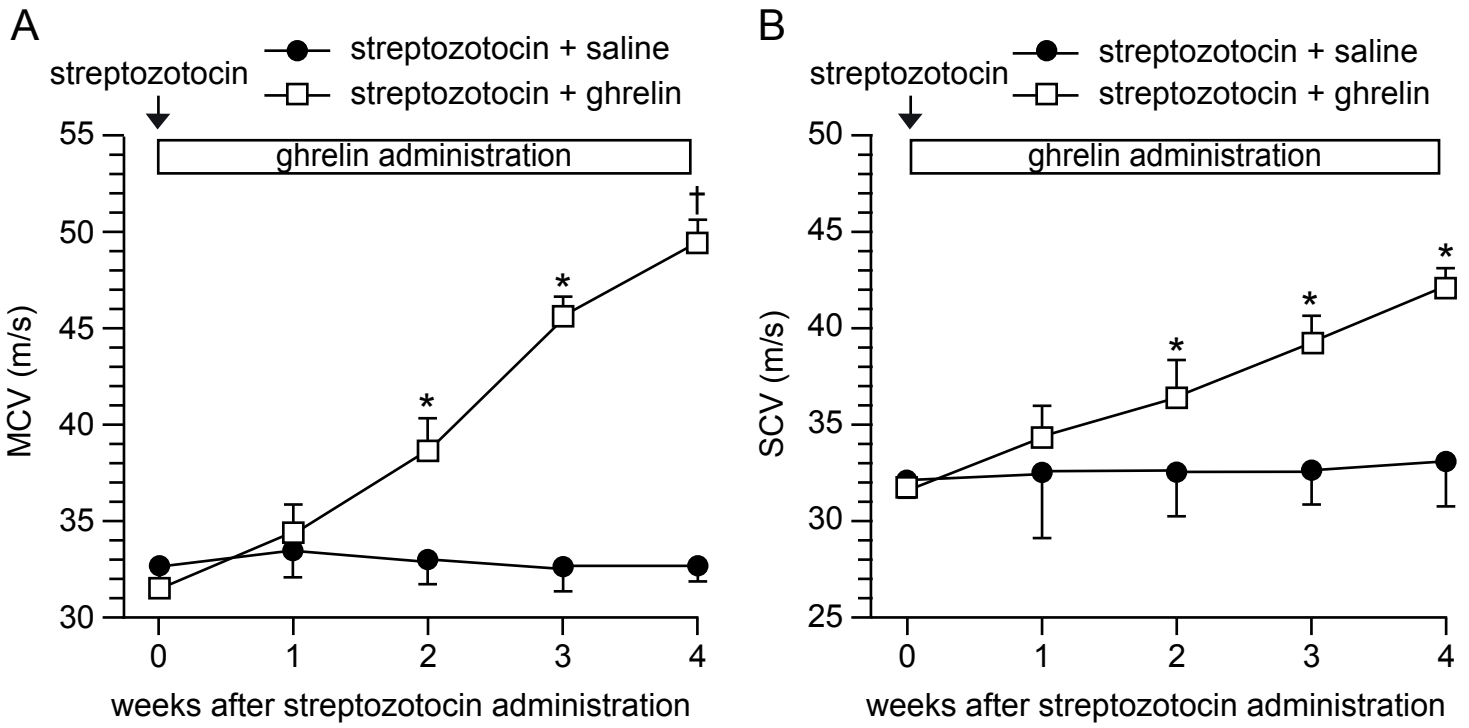
A
streptozotocin
streptozotocin + saline
streptozotocin + ghrelin
ghrelin administration
MCV (m/s)
55
50
45
40
35
30
0
1
2
3
4
weeks after streptozotocin administration
*
*
†
B
streptozotocin
streptozotocin + saline
streptozotocin + ghrelin
ghrelin administration
SCV (m/s)
50
45
40
35
30
25
0
1
2
3
4
weeks after streptozotocin administration
*
*
*

Fig. 3.

Table 1
Body weights, one-day food intake amounts, and blood glucose concentrations of C57BL/6N mice

Weeks after streptozotocin-treatment		Controls	streptozotocin teatment		
			Saline	Ghrelin	Desacyl-ghrelin
0	Body weight (g)	16.5 ± 0.8	17.3 ± 1.2	16.4 ± 2.3	17.1 ± 0.8
	One-day food intake (g)	3.1 ± 0.3	3.0 ± 0.2	3.0 ± 0.2	3.0 ± 0.2
	Blood glucose (mmol/l)	7.2 ± 0.3	7.5 ± 0.3	7.4 ± 0.2	7.3 ± 0.1
1	Body weight (g)	18.6 ± 2.1	16.5 ± 1.4	16.7 ± 2.2	17.7 ± 1.9
	One-day food intake (g)	3.5 ± 0.2	5.6 ± 0.1[a]	5.9 ± 0.4[b]	5.5 ± 0.3[a]
	Blood glucose (mmol/l)	7.5 ± 0.4	27.1 ± 1.1[c]	28.4 ± 1.1[c]	25.0 ± 1.0[c]
2	Body weight (g)	20.4 ± 2.5	16.0 ± 0.8[c]	15.4 ± 1.0[c]	16.1 ± 1.0[c]
	One-day food intake (g)	3.4 ± 0.2	7.1 ± 0.4[c]	6.9 ± 0.5[c]	7.0 ± 0.3[c]
	Blood glucose (mmol/l)	7.6 ± 0.4	30.7 ± 1.7[c]	25.3 ± 2.5[c]	29.0 ± 1.7[c]
3	Body weight (g)	23.0 ± 1.9	16.2 ± 1.7[c]	16.1 ± 1.9[c]	16.4 ± 2.0[c]
	One-day food intake (g)	3.4 ± 0.2	7.1 ± 0.2[c]	6.9 ± 0.4[c]	7.0 ± 0.5[c]
	Blood glucose (mmol/l)	7.2 ± 0.2	28.5 ± 2.1[c]	25.2 ± 1.7[c]	27.6 ± 1.2[c]
4	Body weight (g)	23.4 ± 2.5	17.2 ± 2.2[c]	16.4 ± 1.8[c]	16.5 ± 2.0[c]
	One-day food intake (g)	3.7 ± 0.2	7.0 ± 0.2[c]	6.8 ± 0.3[c]	7.1 ± 0.2[c]
	Blood glucose (mmol/l)	7.4 ± 0.3	27.5 ± 1.3[c]	27.8 ± 1.8[c]	28.9 ± 1.5[c]

[a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ compared with controls. N = 10 per group.

Table 2
Plasma concentrations of insulin and 8-iso-PGF2α of C57BL/6N mice

		streptozotocin treatment		
	Controls	Saline	Ghrelin	Desacyl-ghrelin
Insulin (μU/ml)	16.14 ± 0.32	0.31 ± 0.06[a]	0.33 ± 0.08[a]	0.33 ± 0.05[a]
8-iso-PGF2α (μg/ml)	28.2 ± 6.5	64.7 ± 10.7[a]	24.5 ± 4.6[b]	60.5 ± 10.3[a]

[a] $P < 0.001$ compared with controls. [b] $P < 0.001$ compared with streptozotocin + saline and streptozotocin + desacyl-ghrelin. N = 10 per group.

Table 3

Body weights, food intake, blood glucose and plasma insulin concentrations in naïve GHS-receptor$^{-/-}$ and GHS-receptor$^{+/+}$ mice, and those four weeks after streptozotocin injection in GHS-receptor$^{-/-}$ mice treated with or without ghrelin administration

	naïve GHS-receptor$^{-/-}$	naïve GHS-receptor$^{+/+}$	Streptozotocin-treated GHS-receptor$^{-/-}$ ghrelin administration	
			−	+
Body weight (g)	16.3 ± 1.5	16.7 ± 0.8	14.8 ± 1.9	15.7 ± 1.3
One-day food intake (g)	3.0 ± 0.1	3.0 ± 0.1	6.8 ± 0.3	6.8 ± 0.3
Blood glucose (mmol/l)	7.3 ± 0.2	7.3 ± 0.1	26.8 ± 2.4	28.6 ± 2.1
Insulin (μU/ml)	14.72 ± 0.52	15.26 ± 0.39	0.38 ± 0.16	0.35 ± 0.19

GHS-receptor$^{-/-}$: growth hormone secretagogue receptor–deficient mice. N = 6 per group.

Table 4

Body weights, one-day food intake amounts, and blood glucose concentrations in streptozotocin-treated growth hormone-deficient rats

Weeks after streptozotocin-treatment		ghrelin administration	
		–	+
0	Body weight (g)	66.2 ± 5.4	65.9 ± 5.1
	One-day food intake (g)	7.5 ± 0.8	7.5 ± 0.8
	Blood glucose (mmol/l)	5.4 ± 0.3	5.3 ± 0.3
2	Body weight (g)	63.7 ± 6.3	63.3 ± 6.2
	One-day food intake (g)	7.7 ± 1.1	8.8 ± 1.1
	Blood glucose (mmol/l)	20.7 ± 3.5	19.7 ± 4.0
4	Body weight (g)	61.7 ± 5.8	61.8 ± 6.2
	One-day food intake (g)	9.0 ± 1.6	9.2 ± 2.1
	Blood glucose (mmol/l)	18.6 ± 2.1	19.2 ± 1.2

N = 6 per group