

# Pharmacological profile of a new orally active growth hormone secretagogue, SM-130686

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## Abstract

SM-130686, an oxindole derivative, is a novel orally active GH secretagogue (GHS) which is structurally distinct from previously reported GHSs such as MK-677, NN703 and hexarelin. SM-130686 stimulates GH release from cultured rat pituitary cells in a dose-dependent manner. Half-maximum stimulation was observed at a concentration of  $6.3 \pm 3.4$  nM. SM-130686-induced GH release was inhibited by a GHS antagonist, but not by a GH-releasing hormone antagonist. SM-130686 dose-dependently inhibited the binding of radiolabeled ligand,  $^{35}\text{S}$ -MK-677, to human GHS receptor 1a ( $\text{IC}_{50} = 1.2$  nM). This indicates that SM-130686 stimulates GH release through the GHS receptor. The effect of a single oral administration of SM-130686 on GH release in pentobarbital-anesthetized rats was studied. After treatment with 10 mg/kg SM-130686, plasma GH concentrations measured by radioimmunoassay significantly increased, reaching a peak at 20–45 min, and remained above baseline during the experimental period (60 min). The anabolic effect of repetitive SM-130686 admin-

istration was studied in rats. Rats received 10 mg/kg SM-130686 orally twice a day and were weighed every day for 9 days. At day 9 there was a significant increase in both the body weight and the fat free mass ( $19.5 \pm 2.1$  and  $18.1 \pm 7.5$  g respectively). Serum IGF-I concentration was also significantly elevated 6 h after the last dose of SM-130686. An endogenous GHS ligand for the GHS receptor has recently been identified from stomach extract and designated as ghrelin. The GH-releasing activity *in vitro* relative to ghrelin (100%) was about 52% for SM-130686. It is likely that SM-130686 is a partial agonist for the GHS receptor.

In summary, we describe here an orally active GHS, SM-130686, which acts through the GHS receptor. Repetitive administration of SM-130686 to rats, similar to repetitive administration of GH, significantly increased the fat free mass by an amount almost equal to the gain in body weight.

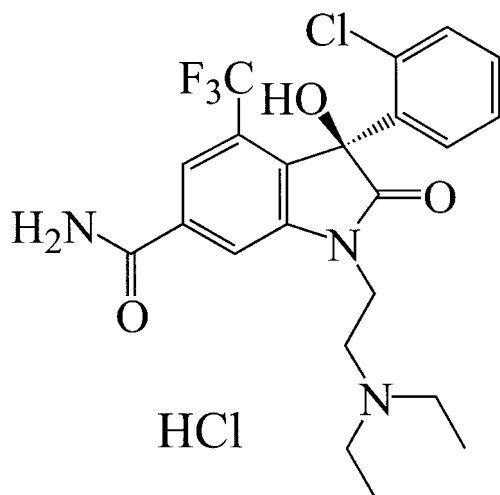
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## Introduction

The secretion of growth hormone (GH) is regulated by the neuroendocrine control system like other anterior pituitary hormones. Two main hypothalamic factors, GH-releasing hormone (GHRH) and somatostatin, are known to be involved as positive and negative regulators, respectively, in GH release (Frohman *et al.* 1992). After GH-releasing peptide (GHRP)-6 and its analogues were found to enhance GH secretion as well as GHRH, a number of new chemical entities with similar characteristics were successfully synthesized. The peptidic factors with GH-releasing activity include GHRP-1, GHRP-2, GHRP-6 and hexarelin; other non-peptidic compounds include L-692,429 and MK-677 (Camanni *et al.* 1998). Among these GH secretagogues (GHSs), MK-677 has been reported to show good oral bioavailability. In addition, chemically synthesized GHSs including GHRP-6 and

MK-677 have been reported to show highly potent GH-releasing activity in several species including humans (Walker *et al.* 1990, Bowers *et al.* 1992, Hartman *et al.* 1992, Chapman *et al.* 1996).

Using the affinity of MK-677 as a clue, a novel GHS receptor was cloned by Howard *et al.* (1996). This receptor is expressed in various kinds of tissues: pituitary, hypothalamus and other areas of the central nervous system (CNS) (Guan *et al.* 1997). Recently, an endogenous GHS ligand for the GHS receptor has been identified from stomach extract and designated as ghrelin, an acylated peptide of 28 amino acids (Kojima *et al.* 1999). Ghrelin mRNA is present in the hypothalamus arcuate nucleus and stomach. Intravenous injection of ghrelin stimulated GH release, suggesting that GH secretion in the pituitary may be regulated not only by ghrelin expressed in the hypothalamus, but also by ghrelin released from the stomach. Since ghrelin mRNA was reported most abundantly in the



**Figure 1** Structure of SM-130686: (S)-1-(2-diethylaminoethyl)-4-trifluoromethyl-6-carbamoyl-3-hydroxy-3-(2-chlorophenyl) oxindole.

stomach, it is very likely that ghrelin has some other physiological functions (Dieguez & Casanueva 2000).

We screened our chemical library for a new GHS using a rat pituitary cell culture system and found an oxindole derivative that showed GH-releasing activity. Modification of this oxindole derivative led to an entirely new class of non-peptidic GHSs represented by SM-130686 (Fig. 1) (Tokunaga *et al.* 2001), which are structurally different from GHRP-6, hexarelin, MK-677 and NN703 (Hansen *et al.* 1999). Here we report the pharmacological profile of a new type of GHS, SM-130686.

## Materials and Methods

### Animals

Adult male Wistar/ST rats and female F344/N rats were purchased from SLC Japan Inc., Sizuoka, Japan. All rats were kept in a temperature ( $23 \pm 2^\circ\text{C}$ )- and humidity ( $55 \pm 10\%$ )-controlled room with lights on between 0800 and 2000 h. Water and standard laboratory chow (CE-2; Cler Japan, Inc., Tokyo, Japan) were freely available. All animal experiments were conducted according to the guidelines of the Sumitomo Pharmaceuticals Committee on Animal Research.

### Materials

Horse and fetal calf sera, non-essential amino acids (100-fold concentrated), penicillin-streptomycin solution and Hank's balanced salt solution (HBSS) were purchased from Gibco, New York, NY, USA. Dulbecco's modified Eagle's medium (DMEM) was from Iwaki, Tokyo, Japan. Collagenase was from Worthington Biochemical,

Lakewood, NJ, USA. DNase type I was from Sigma, St Louis, MO, USA. EDTA, HEPES, NaCl, KCl,  $\text{Na}_2\text{HPO}_4$ ,  $\text{CaCl}_2$  and dimethyl sulfide were from Nacalai Tesque, Kyoto, Japan. Ghrelin was synthesized using the method previously reported by Tschöp *et al.* (2000).

### Rat pituitary cell assay

Anterior pituitaries were obtained from 7-week-old male Wistar/ST rats. The tissue was washed three times with HBSS, cut into small pieces and transferred to the isolation buffer (100  $\mu\text{l}$ /lobe) containing 0.8% collagenase and 0.2% DNase type I. The mixture was incubated for less than 25 min at  $37^\circ\text{C}$ . The cells were collected and washed three times with the culture medium; DMEM was supplemented with 5% horse serum, 2.5% fetal calf serum, 1% non-essential amino acids, 1% penicillin and 1% streptomycin. Cells were resuspended in the above medium and brought to a density of  $1.0 \times 10^5$  cells/ml. The cells (200  $\mu\text{l}$ /well) were seeded onto a 96-well plate (Nunc, Roskilde, Denmark) and cultured for 5 days at  $37^\circ\text{C}$  under 5%  $\text{CO}_2$ .

Following the culture period the cells were washed once with the stimulation buffer (culture medium containing 25 mM HEPES, pH 7.3) and then incubated for 90 min at  $37^\circ\text{C}$  under 5%  $\text{CO}_2$ . This buffer was then replaced with a fresh stimulation buffer containing SM-130686 followed by incubation for 15 min at  $37^\circ\text{C}$  under 5%  $\text{CO}_2$ . The medium was collected and assayed for GH concentration. To study the effect of antagonists, the cells were incubated with an antagonist (final additive concentration 10  $\mu\text{M}$ ) before the SM-130686 solution was added.

### GHS-binding activity

Crude membranes were isolated from CHO cells stably expressing the human GHS receptor 1a. These were suspended in binding buffer (50 mM Tris-HCl, 10 mM  $\text{MgCl}_2$ , 2.5 mM EDTA, pH 7.4) to a concentration of 1 mg protein/ml. These membranes (25  $\mu\text{g}$  protein/well) were mixed with  $^{35}\text{S}$ -MK-677 (100 000 d.p.m./well), with or without different concentrations (0.1–100 nM) of SM-130686 and binding buffer up to a total volume of 250  $\mu\text{l}$ . Non-specific binding was obtained by adding 500 nM cold MK-677. The membranes were incubated at  $25^\circ\text{C}$  for 60 min, and bound radioligand was separated from free radioligand by washing with binding buffer through a GF/B filter (Perkin Elmer, Boston, MA, USA). The radioactivity on the filters was counted in a scintillation counter (Perkin Elmer).

### GH response of SM-130686 in anesthetized rats

Adult male rats (8 weeks old, 250–300 g) were anesthetized by intraperitoneal injection of pentobarbital; a catheter was inserted in the right jugular vein for blood

sampling and drug administration. Ten minutes after surgery, blood samples were withdrawn and different doses of SM-130686 were injected intravenously or administered orally to each animal. Blood samples (200 µl) were drawn at specified times and collected into a tube containing 5 U (50 µl) heparin. Plasma (50 µl) was separated by centrifugation and mixed with 200 µl radioimmunoassay (RIA) buffer. The samples were stored at -40 °C until being assayed for rat GH.

#### *GH response of SM-130686 in conscious rats*

Twenty-seven adult male rats (16 weeks old, 350–400 g) were prepared 7 days before the experiment with indwelling right jugular catheters under pentobarbital anesthesia. After surgery, water was administered orally to the animals to minimize treatment stress. Catheters were kept patent by flushing with 200 µl heparinized saline (50 U/ml) every day. At 0830 h on the day of the experiment, all lines were fitted with extensions filled with heparin. At 1000 h, baseline blood samples were drawn and SM-130686 (10 mg/kg) was administered orally to each rat. Blood samples (100 µl) were drawn every 20 min after administration and collected into a tube containing 5 U (50 µl) heparin. Plasma (50 µl) was separated by centrifugation and mixed with 200 µl RIA buffer. The samples were stored at -40 °C until being assayed for the concentration of rat GH. The blood cells were resuspended in 1 ml saline and returned to the animal after experimentation.

#### *Anabolic effects of SM-130686 in rats*

Female 11-week-old F344/N rats were used in this study. One day before experimentation, all rats were weighed and fat free mass was measured by whole body bioelectrical impedance analysis with a small animal body composition analyzer (SA-2; EM-SCAN Inc., Springfield, IL, USA) (Morbach & Brans 1992). Rats were randomly allocated according to the above two parameters to three treatment groups, each containing six animals. The drug was orally administered twice a day to each rat (1000 h and 1600 h) for 9 days (starting from day 0). The rats were weighed every morning just before administration of the drug, and the fat free mass was measured on day 9.

Six hours (1600 h) after the last dose on day 9, rats were decapitated and trunk blood was collected for hormone analysis. The plasma was separated by centrifugation and stored at -40 °C until being assayed for rat insulin-like growth factor-I (IGF-I) concentration.

#### *GH and IGF-I assays*

Rat GH concentration was measured by the double-antibody RIA method. Rabbit anti-rat GH antibody was

purchased from Biogenesis, Poole, Dorset, UK. GH concentration was expressed in terms of the NIH-RP-2 standard. The intra- and interassay coefficients of variation were below 11% and 13% with a 1 ng/ml limit of quantitation. The IGF-I concentration was measured based on acid-ethanol extraction using a commercial kit (DSL, Austin, TX, USA). The intra- and interassay coefficients of variation were below 6.1% and 9.7% with a 3 ng/ml limit of quantitation.

#### *Calculations*

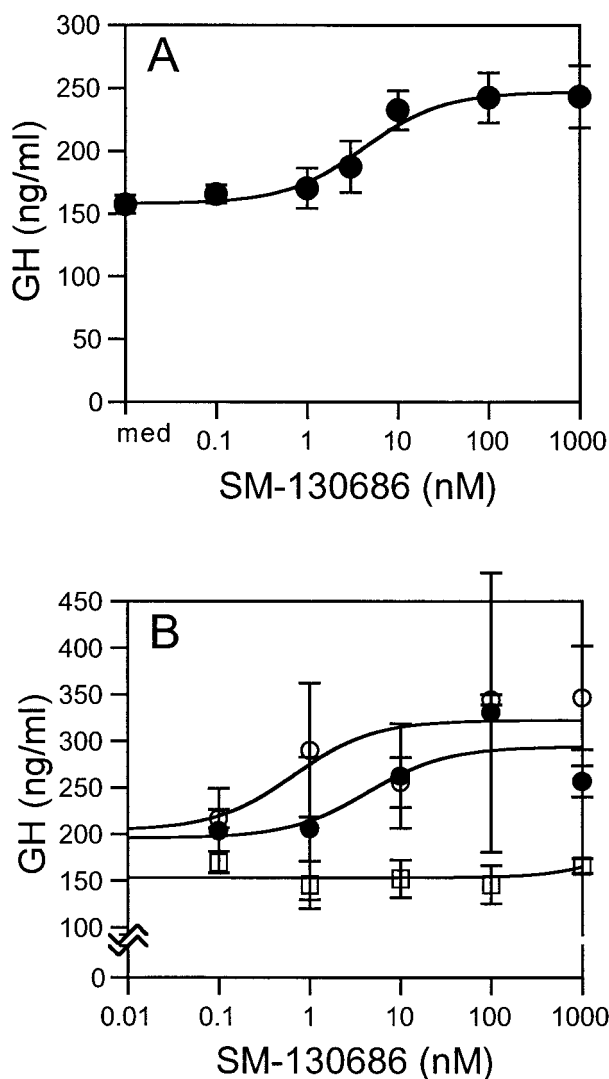
Using the EC<sub>50</sub> value of SM-130686, the potency was calculated as the concentration inducing half-maximal stimulation with the following scheme (GH content = E<sub>max</sub> × concentration of SM-130686 / (EC<sub>50</sub> + concentration of SM-130686) + basal GH content). Area under the concentration (AUC) in the *in vivo* study was calculated using the trapezoidal rule. All data are reported as means ± s.d. except for the EC<sub>50</sub> and E<sub>max</sub> values of GH release in the *in vitro* study (means ± s.e.m.) and GH release in the *in vivo* study. All statistical comparisons were performed using SAS (version 6.12; Cray, NC, USA).

## **Results**

#### *GH-releasing activity of SM-130686 through the GHS receptor*

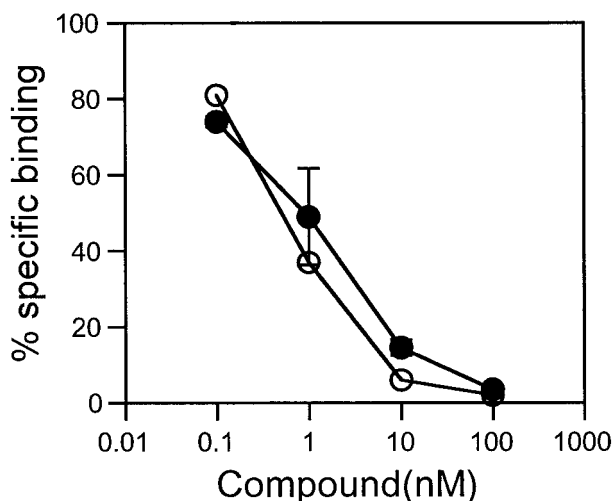
The GH-releasing activity of SM-130686 was studied using cultured rat pituitary cells. GH concentration in the culture medium was determined by RIA 15 min after treatment with SM-130686. SM-130686 enhanced GH release dose-dependently with EC<sub>50</sub> values of 6.3 ± 3.4 nM (means ± s.e.m.) in five different experiments with rat pituitary primary culture (Fig. 2A). To evaluate the pharmacological properties of SM-130686, we investigated the effects of concomitant treatment of GHS antagonist ([D-Arg<sup>1</sup>, D-Phe<sup>5</sup>, D-Trp<sup>7,9</sup>, Leu<sup>11</sup>]-substance P) or GHRH antagonist ([N-acetyl-Trp<sup>1</sup>, D-Arg<sup>2</sup>]-hGHRH(1–29)NH<sub>2</sub>) with SM-130686 on GH-releasing activity (Cheng *et al.* 1997). As shown in Fig. 2B, GHS antagonist inhibited the GH-releasing activity of SM-130686, whereas the GHRH antagonist had neither an additive nor a synergistic effect. These results suggested that SM-130686 increases GH release by acting on the GHS receptor.

We therefore decided to evaluate the binding affinity of SM-130686 for the GHS receptor which was originally identified as MK-677-binding protein and recently found to be the receptor for an endogenous ligand called ghrelin. As shown in Fig. 3, SM-130686 dose-dependently inhibited the binding of radioisotope-labeled ligand, <sup>35</sup>S-MK-677, to recombinant human GHS receptor 1a that was stably expressed in CHO cells. The IC<sub>50</sub> value of



**Figure 2** (A) Dose-response relationship in GH release from rat pituitary cells induced by SM-130686. med, medium; (B) Inhibition of SM-130686-stimulated GH release (●) by [D-Arg<sup>1</sup>,D-Phe<sup>5</sup>,D-Trp<sup>7,9</sup>,Leu<sup>11</sup>]-substance P (GHS antagonist, □), but not by [N-acetyl-Trp<sup>1</sup>,D-Arg<sup>2</sup>]-hGHRH(1-29)NH<sub>2</sub> (GHRH antagonist, ○). GHS or GHRH antagonist concentration was 10 μM. Data are means ± S.D. (n=3).

SM-130686 was calculated to be  $1.2 \pm 0.2$  nM in this assay. In order to investigate whether SM-130686 was specifically bound to the GHS receptor or not, *in vitro* binding activities of SM-130686 were extensively studied on more than 50 other receptors, including opiate, sigma, bradykinin, muscarine, neurokinin, benzodiazepine, serotonin, dopamine, gamma aminobutyric acid, GHRH and somatostatin receptors. The IC<sub>50</sub> values of SM-130686 for these receptors were more than 1 μM (data not shown) indicating that SM-130686 has specific and high affinity for the GHS receptor.



**Figure 3** Inhibition of <sup>35</sup>S-MK-677 binding to CHO membranes expressing the human GHS receptor by MK-677 (○) and SM-130686 (●). Results are means ± S.D. (n=2).

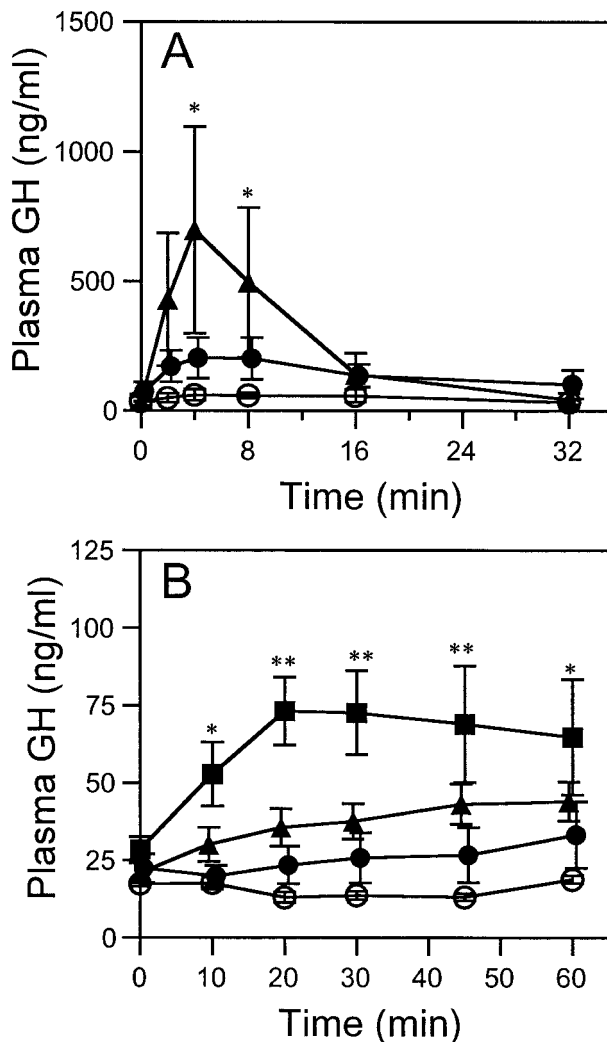
#### Effect of SM-130686 administration on GH release in pentobarbital-anesthetized rats

To study GH-releasing activity *in vivo*, SM-130686 was intravenously injected into pentobarbital-anesthetized rats through a catheter inserted into the right jugular vein. After intravenous injections of 1 and 3 mg/kg SM-130686, the plasma GH concentrations of each group of rats given SM-130686 reached a peak at 4 min and returned to baseline by 16 min (Fig. 4A). The peak plasma GH concentration increased in a dose-dependent manner.

We investigated the pharmacokinetic profile of SM-130686 in rats after oral administration. Bioavailability was found to be 27.6%, indicating that SM-130686 is an orally active GHS. It was therefore decided to investigate the effect of oral administration of SM-130686 on GH release in pentobarbital-anesthetized rats. After oral administration of 1, 3 and 10 mg/kg SM-130686, the peak plasma GH concentrations increased in a dose-dependent manner and remained above baseline during the experimental period of 60 min (Fig. 4B).

#### GH response to oral administration of SM-130686 in conscious male rats

Following the studies using anesthetized rats, the efficacy of SM-130686 oral administration was further assessed by monitoring GH-releasing activity in conscious rats. Rats were implanted with cannulae for blood sampling into the right jugular veins under pentobarbital anesthesia 7 days before the SM-130686 administration experiment. After a single oral administration of SM-130686, plasma GH concentration peaked within 3 h in all four rats (Fig. 5 lower panel). AUC and C<sub>max</sub> values for the plasma



**Figure 4** (A) Time-course of plasma GH levels in male pentobarbital-anesthetized rats after intravenous injection of SM-130686: 0 mg/kg (○,  $n=3$ ), 1 mg/kg (●,  $n=4$ ) and 3 mg/kg (▲,  $n=3$ ). (B) Time-course of plasma GH levels in male pentobarbital-anesthetized rats after oral administration of SM-130686: 10 mg/kg (■,  $n=5$ ), 3 mg/kg (▲,  $n=5$ ), 1 mg/kg (●,  $n=5$ ) and 0 mg/kg (○,  $n=4$ ). Data are means  $\pm$  S.E.M. \* $P<0.025$ , \*\* $P<0.005$  by the Williams test (Williams 1972) (vs group treated with 0 mg/kg).

GH concentration during the first 3 h after 10 mg/kg SM-130686 administration increased compared with vehicle-treated rats (Table 1).

#### Anabolic effect of SM-130686 in normal female rats

The results on GH-releasing activity of SM-130686 *in vitro* and *in vivo* encouraged us to investigate further the efficacy of repetitive administration. SM-130686 was orally administered to normal female rats every day for 9 days

(starting at day 0). The rats were weighed every morning just before administration of the drug, and the fat free mass which was mainly composed of muscle, bone and water, was measured by the bioelectrical impedance method at days -1 and 9. Treatment with 6 mg/kg SM-130686 per day did not significantly affect the body weight gain in rats (Fig. 6). However, rats given 20 mg/kg SM-130686 per day showed a significantly higher body weight gain at day 1 compared with vehicle-treated rats. This enhanced body weight gain in the group given 20 mg/kg per day continued with no apparent sign of attenuation during the experimental period of giving repetitive daily administration. At the end of the treatment (day 9), the gain in fat free mass significantly increased in the SM-130686-treated (20 mg/kg per day) rats (Table 2). Administration of 6 and 20 mg/kg SM-130686 per day increased the plasma IGF-I levels in a dose-dependent manner 6 h after the last administration (day 9) (Table 2).

#### GH-releasing activity of SM-130686 *in vitro* compared with ghrelin, an endogenous ligand for the GHS receptor

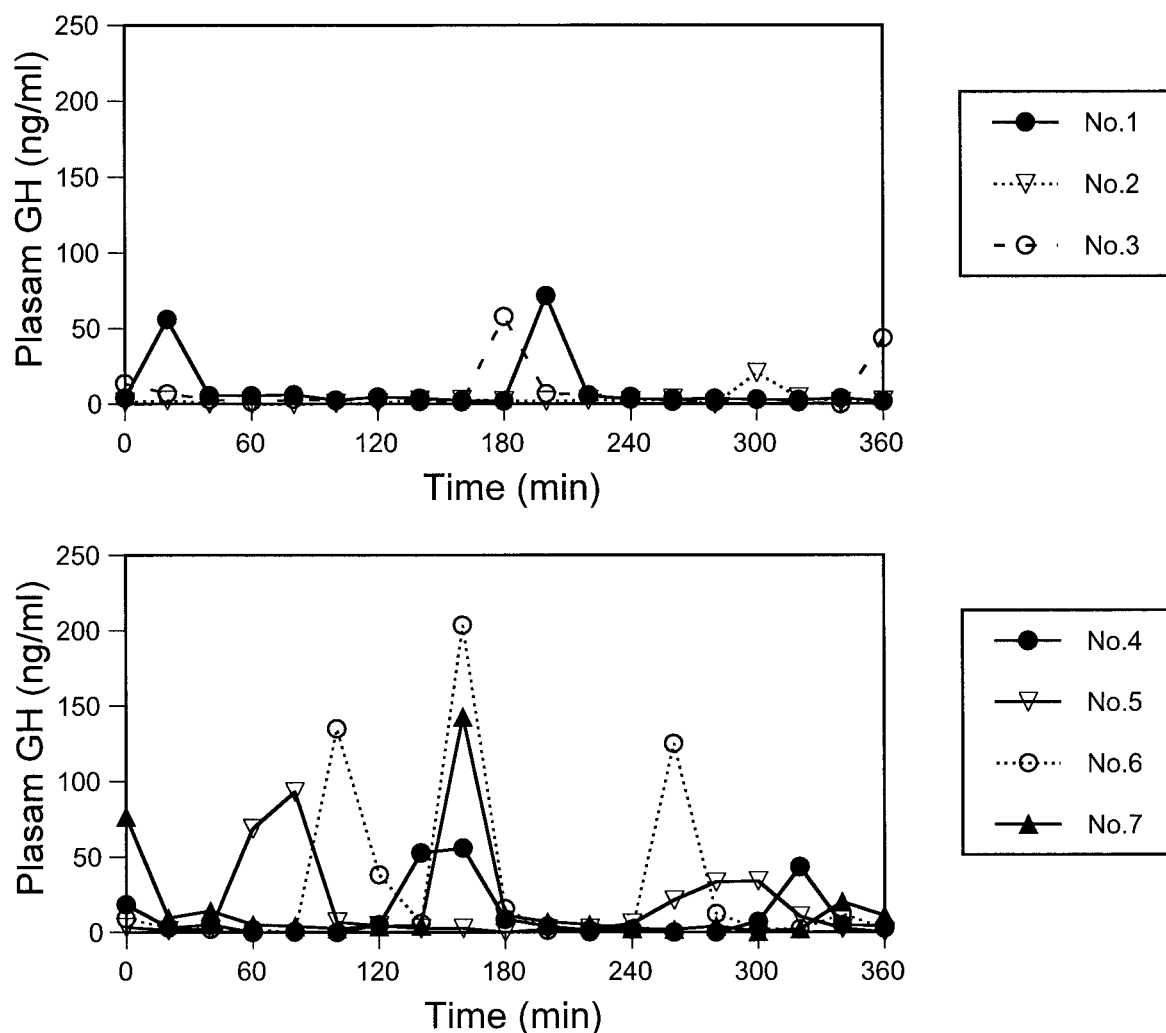
Ghrelin enhanced GH release dose dependently with  $EC_{50}$  values of  $2.4 \pm 0.5$  nM (means  $\pm$  S.E.M.) in three experiments of cultured rat primary pituitary cells (Fig. 7). The  $Emax$  of SM-130686 was  $52 \pm 12\%$  relative to that of ghrelin in the three experiments, indicating that SM-130686 might be a partial agonist for the GHS receptor.

#### Discussion

Among the peptidic and non-peptidic chemical compounds with GH-releasing activity, MK-677 has been reported to show good oral bioavailability (Patchett *et al.* 1995). In this study we have demonstrated a new class of orally active GHSs represented by SM-130686. The  $EC_{50}$  of SM-130686 in enhancing GH release was  $6.3 \pm 3.4$  nM, nearly as potent as MK-677 with an  $EC_{50}$  value of  $2.7 \pm 0.9$  nM as measured in our experiment. The result of a binding assay using  $^{35}S$ -MK-677 as a radioligand also showed that the affinity of SM-130686 with the human GHS receptor 1a ( $IC_{50}=1.2$  nM) was similar to MK-677 ( $IC_{50}=0.5$  nM). The biological activity of SM-130686 in enhancing GH release was blocked by the GHS antagonist, but not by the GHRH antagonist in rat pituitary cells. These pharmacological properties indicate that our SM-130686 enhances GH release by acting on the GHS receptor.

Physiologically, GH is secreted in a pulsatile fashion (Frohman *et al.* 1992, Müller *et al.* 1999). Since the dosing of exogenous GH (once daily or less) does not mimic the normal GH secretion profile, a more natural treatment needs to be developed for patients with GH deficiency. Furthermore, it is known that plasma concentrations of GH and IGF-I wane with aging in the human, and mean





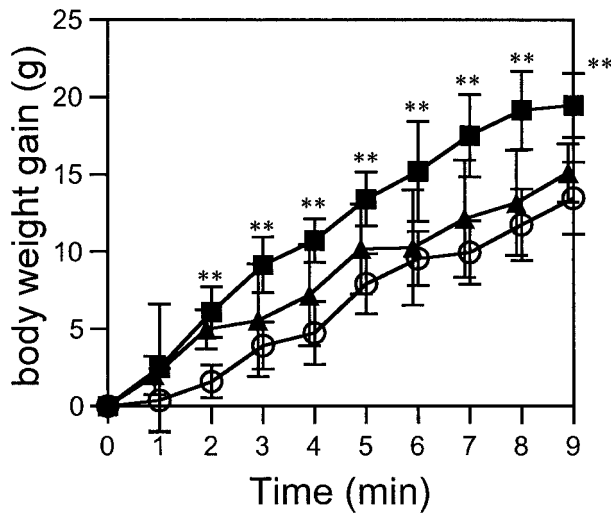
**Figure 5** GH response after oral administration of SM-130686 (lower panel,  $n=4$ ), or vehicle (upper panel,  $n=3$ ) in individual conscious male rats. SM-130686 (10 mg/kg) was administered at 1000 h (0 min). Blood was withdrawn at 0 min before and every 20 min after administration for 360 min.

**Table 1** GH response after oral administration of SM-130686 in conscious male rats. Values are for individual rats<sup>a</sup> or means  $\pm$  s.d.

Treatment	Tmax (min) <sup>a</sup>	Cmax (ng/ml)		AUC (ng/ml · h)	
		0–360 min	180–360 min	0–360 min	180–360 min
Vehicle ( $n=3$ )	180, 200, 300	50 $\pm$ 26	50 $\pm$ 26	42 $\pm$ 23	24 $\pm$ 10
SM-130686 (20 mg/kg per day) ( $n=4$ )	80, 160, 160, 160	124 $\pm$ 64	56 $\pm$ 47	112 $\pm$ 53	33 $\pm$ 17

plasma GH concentration in people over 60 years old is approximately one third to one half that of young adults (Rudman 1985, Zadik *et al.* 1985, Ho *et al.* 1987, Iranmanesh *et al.* 1991). Notably, daily oral administration of MK-677 increases plasma GH concentration in healthy elderly subjects and also increases their plasma IGF-I

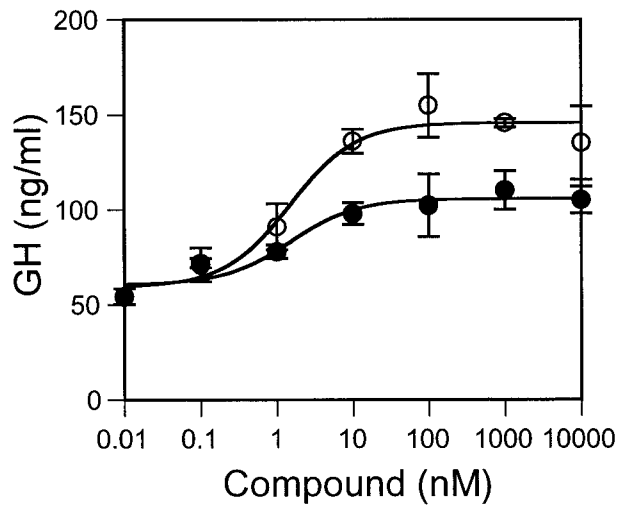
concentration to the normal range of young adults (Chapman *et al.* 1996). Further, the enhancement of pulsatile GH release by MK-677 has been reported to be due to an increase of pulse height (=mass of GH secreted per pulse) rather than the number of pulses in plasma GH secretion profiles. Similarly, the present study



**Figure 6** Time-course of body weight gain in 11-week-old female rats given oral administration of different doses of SM-130686: 0 mg/kg per day (○), 6 mg/kg per day (▲) and 20 mg/kg per day (■). SM-130686 was administered twice a day every day for 9 days. Data are means  $\pm$  s.d. ( $n=6$ ). \*\*\* $P<0.005$  by the Williams test (Williams 1972) (vs group treated with 0 mg/kg).

demonstrated that SM-130686 enhanced pulsatile GH secretion in conscious male rats. These characteristics indicate that GHSs might lead to a more desirable treatment not only for GH deficiency but also for healthy elderly subjects.

GH plays a critical role in a variety of physiological processes, including longitudinal bone growth and body metabolism. With regards to body metabolism, it has been shown previously that GH possesses anabolic, lipolytic and anti-natriuretic actions, and thus affects body composition (Møller *et al.* 1995). The effects of GH on body composition in mature female rats have been investigated using a two-compartment model of fat mass and fat free mass (mainly composed of muscle, bone and water). Results showed that body weight gain after GH treatment was largely due to fat free mass accretion (Azain *et al.* 1995). It has also been reported that peptidic GHSs such as GHRP-6 (Bowers *et al.* 1984), G7039 (McDowell *et al.*



**Figure 7** Dose-response relationship of GH release from rat pituitary cells induced by ghrelin (○) or SM-130686 (●). Data are means  $\pm$  s.d. ( $n=3$ ).

1995) and NN703 (Hansen *et al.* 1999) significantly stimulate body weight gain in female rats after daily administration for more than 7 days. Peptidic GHSs have been known to stimulate food intake, possibly through direct action on the CNS (Locke *et al.* 1995, Okada *et al.* 1996), and thus the increase in body weight gain is partly due to fat mass gain.

Recently, an endogenous GHS ligand for the GHS receptor has been identified and designated as ghrelin (Kojima *et al.* 1999). Ghrelin increases body weight gain by stimulating fat mass gain without any change in fat free mass in mice (Tschöp *et al.* 2000). Thus, it may be that ghrelin itself enhances glucose catabolism and reduces fat utilization in mice and rats. Similar data were observed after administration of KP-102, a peptidic GHS, indicating a GHS/ghrelin receptor-mediated mechanism (Tschöp *et al.* 2000). In the present study, repetitive administration of SM-130686 significantly increased the fat free mass by almost the same amount as the gain in body weight. Further, we preliminarily confirmed that defatted dry mass (composed of body protein and minerals etc.) of rats given

**Table 2** Effect of SM-130686 on body composition in female rats. Values are means  $\pm$  s.d. of six rats per group

Parameter	SM-130686 (mg/kg per day)		
	0	6	20
Weight gain (g)	13.5 $\pm$ 2.3	15.1 $\pm$ 1.9	19.5 $\pm$ 2.1**
Fat free mass gain (g)	7.2 $\pm$ 6.3	8.9 $\pm$ 8.0	18.1 $\pm$ 7.5*
% body fat	18.0 $\pm$ 4.9	17.2 $\pm$ 4.2	14.5 $\pm$ 3.9
Plasma IGF-I (ng/ml)	729 $\pm$ 75	777 $\pm$ 39	842 $\pm$ 50**

\* $P<0.025$ , \*\* $P<0.005$ , using Williams test.

repetitive administration of vehicle, and 6 mg/kg and 20 mg/kg SM-130686 per day were 32.3 g, 32.3 g and 34.1 g respectively. These data suggest that repetitive administration of SM-130686 also increases body protein. The food intake of rats given 20 mg/kg SM-130686 per day (7.6 g/100 g body weight per day) was not significantly different from rats given vehicle (6.9 g/100 g body weight per day). Compared with ghrelin and other GHSs, the action of SM-130686 may be closer to GH itself and more advantageous for the treatment of GH deficiency patients without deteriorating body composition or inducing obesity.

The reason why SM-130686 acts differently from ghrelin still remains unknown. We have demonstrated that the GH-releasing activity of SM-130686 was less potent ( $52 \pm 12\%$ ) than ghrelin in rat pituitary cells, whereas SM-130686 has almost the same binding affinity as ghrelin (0.25 nM in Bednarek *et al.* 2000). These data indicate that SM-130686 might be a partial agonist for the GHS receptor, which may be one of the reasons for the difference between SM-130686 and ghrelin. Further studies will also be needed to unveil the mechanism of the action of SM-130686 in the endocrine system.

In summary, SM-130686 enhanced GH release from rat pituitary cells, possibly through a GHS receptor, and stimulated GH secretion both in anesthetized and conscious rats. Repetitive administration of SM-130686 significantly increased fat free mass gain in addition to body weight gain in normal female rats. Taken together, this indicates that repetitive SM-130686 treatment has an anabolic effect similar to repetitive GH treatment.

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