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Daily Low-Dose Administration of Growth Hormone Secretagogue Stimulates Pulsatile Growth Hormone Secretion and Elevates Plasma Insulin-like Growth Factor-1 Levels in Pigs

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Repeated administration of growth hormone secretagogues (GHSs) has proven to be a delicate matter owing to development of tolerance. The aim of the present study was to define conditions during which the responsiveness to the orally active NN703 was maintained over several days. Growing pigs were fitted with stomach and vascular catheters, permitting unstressed intragastric administrations and blood sampling. NN703 or vehicle was administered once daily. When NN703 was given at a dose of 18 mg/kg, there was a massive acute increase in plasma growth hormone (GH) levels, but this was only seen on the first day of administration. A dose of 1.8 mg/kg did not cause a significant acute increase in plasma GH concentrations, whereas stimulation of pulsatile GH release was sustained over a 4-d period. During the first 7 h following injection of vehicle, the area under the curve of plasma GH was 1211 ± 144 (μg/[L·7 h]), but increased to 1770 ± 269 and 1824 ± 198 (μg/[L·7 h]) on the first and fourth day of NN703 administration, respectively. Deconvolution analysis of the 7-h profiles revealed that the GH mass per burst as well as the GH burst amplitude were significantly ($p < 0.001$) increased during treatment with NN703, which led to an increase in pulsatile GH secretion rate ($p < 0.001$). Insulin-like growth factor-1 plasma concentrations increased steadily during NN703 administration ($p < 0.01$) and decreased after termination of treatment. The sustained increase in GH pulsatility observed with low-dose NN703 treatment suggests that development of tolerance to this GHS may be obviated by minimization of dose.

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Introduction

The last several years have seen the rapid development of orally active small molecules, growth hormone secretagogues (GHSs), which have the ability to stimulate the release of growth hormone (GH) from the pituitary gland *(1–5)*. Such progress was inspired by the early finding that small synthetic oligopeptides, the GH-releasing peptides (GHRPs) *(6)*, were potent GHSs that acted through pathways distinguisable from that of the endogenous GH-releasing hormone (GHRH) *(7,8)*. The relevance of this concept was greatly reinforced by the cloning and expression mapping of a specific target receptor for the GHSs *(9)*. The subsequent discovery of a novel ^{3}Ser-octanoylated 28 amino acid endogenous ligand, ghrelin *(10)*, for the GHS-receptor further extended the perspective of GHSs, not only in terms of GH release *(11,12)*, but also in the regulation of food intake and metabolism *(13)*.

Although the GHSs elicit GH release through distinct hypothalamo-pituitary receptor-effector pathways *(14)*, these agonists manifest important interactions with the GHRH system *(15)*. This was illustrated clinically by Bowers et al. *(16)*, who found that injection of a fixed dose of GHRH and increasing doses of GHRP-6 documented marked potentiation of GHRH action by otherwise minimally effective doses of GHRP-6 administered alone.

We reasoned that if GHSs could be used in small doses to stimulate the pulsatile GH release, long-term desensitization to the drug *(17)* might be avoided *(18,19)*. Initial studies with the orally available nonpeptidyl GHS NN703, presented in this article, showed that high doses rapidly lose effectiveness. To our knowledge, there is no published study directly addressing whether a low dose of GHS that does not evoke significant acute GH release is able to show a sustained stimulation of pulsatile GH release and increase

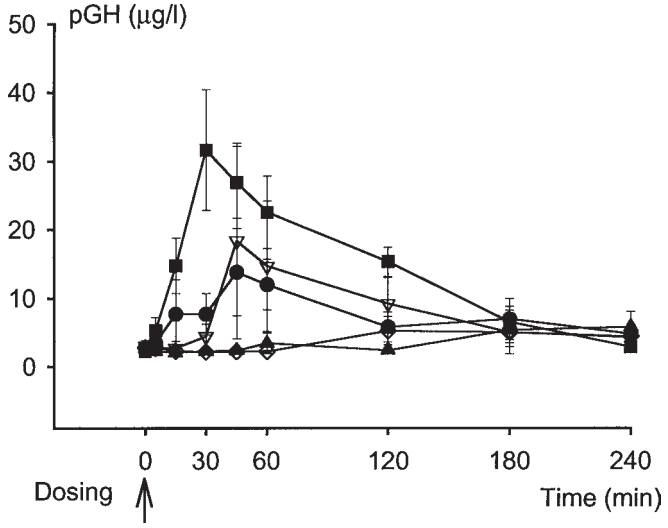

Fig. 1. Plasma GH levels in pigs after intragastric administration of NN703 at doses of (■-■) 18, (▽-▽) 6.0, (●-●) 3.0, (▲-▲) 1.8, and (◇-◇) 0.18 mg/kg body wt, respectively. Data represent mean levels of four to eight observations ± SE.

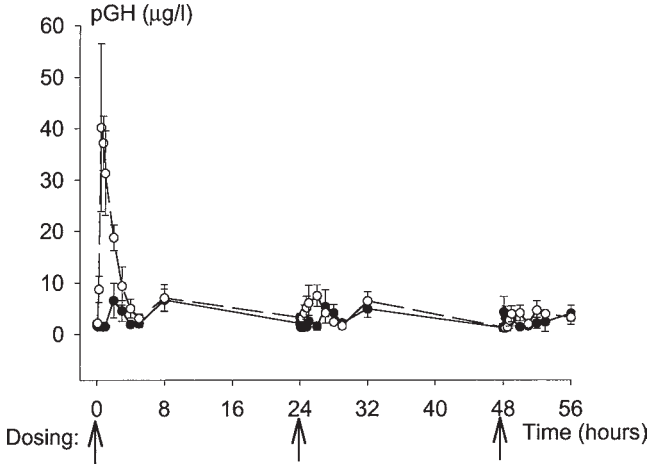

Fig. 2. Plasma GH levels in pigs after repeated intragastric dosing of vehicle (●-●) or NN703 at a dose of 18 mg/kg (○-○). Data represent mean levels of four observations ± SE.

insulin-like growth factor-1 (IGF-1) levels. Accordingly, the main aim of the current study was to explore this hypothesis.

Results

Dose Escalation Study

Intragastric administration of 3–18 mg/kg of NN703 caused a dose-dependent increase in circulating GH levels within 60 min (Fig. 1). At the highest dose, circulating GH levels peaked at approx 30 µg/L, which is two or three times higher than a spontaneous GH peak in this model. In doses of 0.18 or 1.8 mg/kg, no acute responses to NN703 were observed.

Repeated Dosing with High-Dose (18 mg/kg) NN703

The initial administration of NN703 stimulated a prompt GH release. During 8 h following this administration, plasma area under the curve (AUC) of GH increased from a baseline value of 1781 ± 460 to 5607 ± 777 µg/(L·8 h). However, following the third administration, the equivalent AUC decreased to 1665 ± 385 µg/(L·8 h) (Fig. 2).

Repeated Dosing with Low-Dose (1.8 mg/kg) NN703

Administration of a low dose of NN703 increased plasma GH concentrations minimally on initial administration (Fig. 3). Deconvolution analysis of GH profiles registered during 7 h after administrations revealed that the mass of GH secreted per burst as well as the GH secretory burst amplitude significantly ($p < 0.001$) increased in response to low-dose NN703, which led to an increase in pulsatile GH secretion rate ($p < 0.001$) (Table 1). Other parameters of basal and pulsatile GH secretion were unaffected. Plasma IGF-1 concentrations rose steadily over the 4 d of secretagogue administration ($p < 0.01$) (Fig. 4) and decreased after termination of treatment.

Discussion

An acutely effective high dose of NN703 failed to sustain stimulated GH output following three successive daily administrations. Such observations corroborate earlier findings with this and other GHSs in several species *(18–20)*. In vitro data *(17)* are consistent with desensitization of somatotrophs *(21)*. In addition, the wide distribution of neurons expressing the GHS receptor in the brain *(7,22)* and reported central nervous system responses in the rat to long-term exposure to GHS *(23)* point to multilevel desensitization including negative feedback by GH *(24,25)* and IGF-1 *(19)* as well as elevated somatostatinergic outflow *(26)*.

In a study by Chapman et al. *(27)*, GH-deficient adults were treated with two doses, 10 and 50 mg/d, of the orally active GHS, MK-677, for 4 d. Although both doses significantly increased mean serum GH concentrations and IGF-1 levels compared with baseline, there was surprisingly no significant further effect of the higher dose. These results illustrate that stimulation of GH secretion by GHS may become limited by desensitization and/or evolving negative feedback by an activated GH-IGF-1 axis. This notion led us to pursue a study in which a low dose of NN703 not evoking a massive acute release of GH was evaluated for its ability to stimulate persistently the pulsatile GH release and maintain elevated plasma IGF-1 concentrations. To this

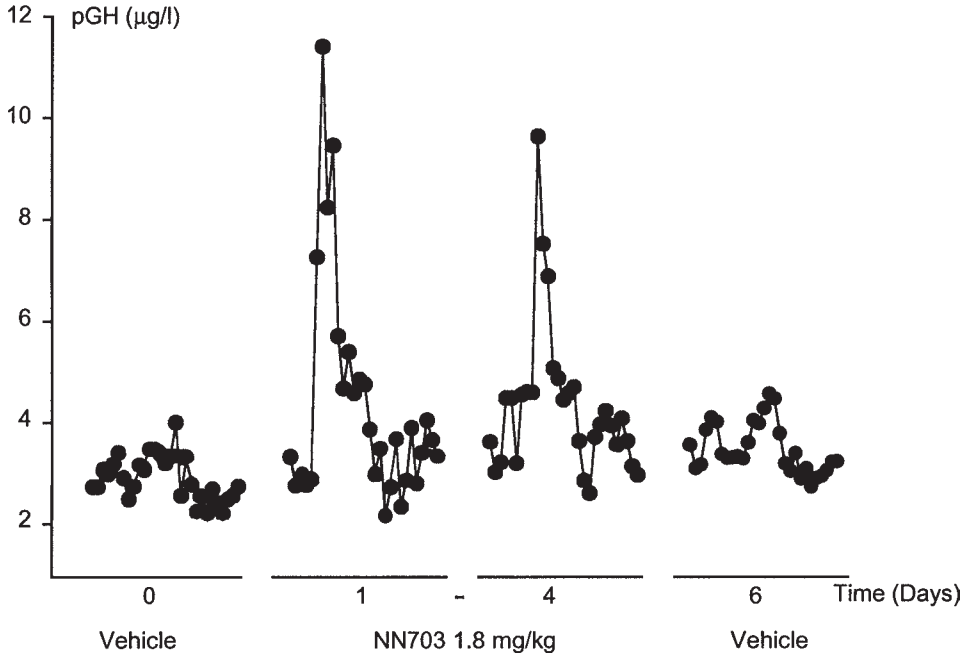

Fig. 3. Plasma GH levels in pigs after repeated intragastric dosing of vehicle on d 0 and 6 or NN703 on d 1–4 at a dose of 1.8 mg/kg. Data represent mean levels of nine observations.

Table 1
Deconvolution Analysis of GH Profiles Following Administration of Vehicle and Low-Dose (1.8 mg/kg) NN703

Variable	Period and treatment				*p* value of treatment vs vehicle
	Vehicle (d 0)	NN703 (d 1)	NN703 (d 4)	Vehicle (d 6)	
Basal GH secretion (µg/[L·min])	0.10 ± 0.03	0.08 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	<0.905
Half-life (min)	19.2 ± 2.5	19.5 ± 2.1	18.4 ± 1.0	21.6 ± 2.1	<0.462
No. of bursts (events/d)	5.0 ± 0.5	4.1 ± 0.6	4.4 ± 0.3	4.3 ± 0.6	<0.374
Half duration (min)	20.2 ± 4.9	15.7 ± 4.9	18.9 ± 4.7	19.5 ± 3.7	<0.339
Interval (min)	85.0 ± 8.6	120.8 ± 24.2	93.0 ± 7.8	108.2 ± 14.0	<0.324
Pulse secretion rate (µg/[L·7 h])	23.9 ± 7.4	45.3 ± 10.1[a]	31.7 ± 3.6[a]	14.0 ± 2.2	<0.001
Mass/burst (µg/L)	4.6 ± 1.2	13.2 ± 3.2[b]	7.3 ± 0.8[b]	3.5 ± 0.6	<0.001
GH AUC (µg/[L·7 h])	1211 ± 144	1770 ± 269	1824 ± 198	1428 ± 403	<0.001
GH mean level (µg/L)	2.9 ± 0.3	4.2 ± 0.6	4.4 ± 0.5	3.4 ± 1.0	<0.001
Amplitude (µg/[L·min])	0.30 ± 0.08	1.48 ± 0.48[c]	0.78 ± 0.30[c]	0.26 ± 0.11	<0.001

[a]NN703 d 1 vs d 4 ($p < 0.392$).
[b]NN703 d 1 vs d 4 ($p < 0.263$).
[c]NN703 d 1 vs d 4 ($p < 0.423$).

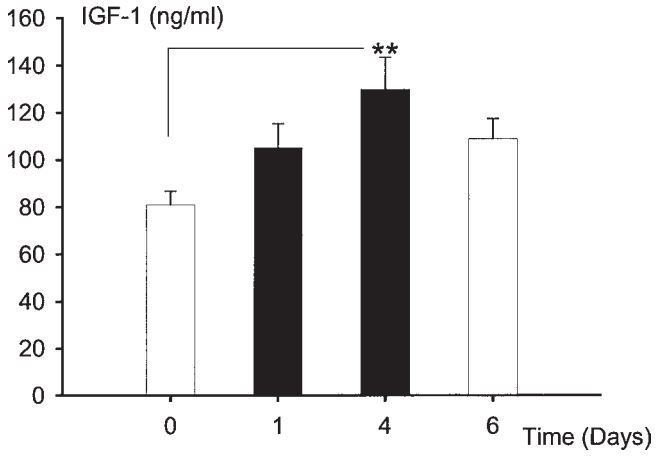

Fig. 4. Plasma IGF-1 levels in pigs after repeated intragastric dosing of vehicle on d 0 and 6 or NN703 on d 1–4 at a dose of 1.8 mg/kg. Data represent mean levels of seven observations ± SE. **, vehicle d0 vs. NN703 d4 ($p < 0.01$).

end, we examined GH responses to a low dose on the dose escalation scale where the acute stimulation of GH release had just disappeared. This stimulus level significantly increased integrated serum GH concentrations over 4 d compared with the pre- and postintervention baselines. Moreover, GH release remained pulsatile and NN703 was found to amplify selectively the mass of GH secreted per burst by increasing GH burst amplitude. This response mechanism resembles, to a large extent, that reported in the human *(28,29)*.

There was a nonsignificant tendency for the amplitude and the mass per burst to decline between the first and fourth day of exposure to NN703. Although integrated serum GH concentrations were almost identical on these 2 d and IGF-1 levels continued to increase on d 4, further studies will be required to determine the longer-term durability of this response. Interestingly, in a study of obese subjects *(30)* treated with MK-677, the GH response to the first injection was nearly five times higher than that recorded after 2 and 8 wk of treatment. Nevertheless, serum GH and IGF-1 remained significantly elevated compared with placebo throughout this period.

In summary, the sustained increase in GH pulsatility observed with low-dose NN703 treatment supports the notion that downregulation of in vivo responsiveness to this GHS may be obviated, at least partly, by minimization of dose. Whether this strategy preferentially limits loss of responsiveness at the hypothalamic level is presently unknown. However, the present dose deescalation strategy may have utility in evaluating and implementing longer-term administration of NN703 or other GHSs.

Materials and Methods

Animals

Danish Landrace pigs weighing about 40 kg were all fitted with stomach and vascular catheters, thus permitting unstressed gastric administrations and arterial blood sampling. After surgery, animals were allowed a 5 to 7-d period of recovery before studies began. The pigs received a standard diet in two meals given at 7:00 AM and 3:00 PM. Water was freely available.

Surgical Procedure

After an overnight fast, anesthesia was commenced with an im injection of a mixture of 1.7 mg/kg of Zolazepam (Zoletil Boeringer Ingelheim, Ingelheim am Rhein, Germany), 0.9 mg/kg of Xylazin (Rompun vet. Bayer, Leverkusen, Germany), 0.9 mg/kg of Ketamin (Ketaminol; Rosco, Taastrup, Denmark), 0.2 mg/kg of Methadon (Nycomed, Roskilde, Denmark), and 12 μg/kg of Atropin (A/S Gea, Copenhagen, Denmark) followed by an iv injection of 5 mg/kg of Propofol (Rapinovet; Abbott, Solna, Sweden). During surgery pigs were intubated and anesthesia was maintained by inhalation of a mixture of Isofluran (1.0–2.0%; Abbott) and oxygen (1.0–2.0 L/min). After introduction of anesthesia, the skin of the abdominal and neck regions was shaved and sterilized with 0.05% chlorhexidine in 70% ethanol and 0.4% iodine in 70% ethanol. In the right hypochondrium, a 40-cm transverse wound was opened, completely incising the abdominal muscles and peritoneum. The ventricle was punctured in the fundic region with a blunt diathermic needle before inserting a silicon cannula (3.1 × 1.6 mm, Silastic; Dow Corning, Midland, Michigan, USA). To keep the cannula in place, two silicone anchor cuffs were glued to the catheter with a distance of 2 cm. One of these cuffs was inserted in the gastric lumen and the other one was on the outside and was used to anchor the purse string suture closing the incision. The carotid artery was cannulated as described previously *(31)*.

Experimental Designs

Dose Escalation Study

In initial pilot studies five doses (n = 4–8) of NN703 (18.0, 6.0, 3.0, 1.8, and 0.18 mg/kg) were tested for acute GH response. Arterial blood samples were withdrawn at 0, 30 60, 120, 180, and 240 min after intragastric dosing. Data from these studies are presented in Fig. 1.

Repeated Dosing with High-Dose NN703

Four pigs received either vehicle or 18 mg/kg of NN703 in rotation according to a replicated 2 × 2 Latin square design. Treatment periods were 56 h and dosing was performed at 0, 24, and 48 h. Arterial blood samples were withdrawn at 0, 5 15, 30, 45, and 60 min after dosing, then every hour up to 8 h. Between treatment periods a 5- to 7-d washout period was allowed.

Repeated Dosing with Low-Dose NN703

Nine of 12 pigs went successfully through a sampling scheme starting with a saline control period on d 0. On d 1, 2, 3, and 4 the pigs received NN703 at a dose of 1.8 mg/kg body wt, and on d 6 another saline control period was run. NN703 or saline was administered once a day at 10:00 AM.

During both saline control periods, on the first and last day of NN703 treatment, venous blood samples were taken every 15 min during a 7-h period (10:00 AM to 6:00 PM) using a fully automated multichannel blood sampler (Accusampler; DiLab, Lund, Sweden).

Plasma Analyses

Plasma porcine GH *(32)* and IGF-1 *(33)* were analyzed as previously described. Because of technical errors, IGF-1 values in two of the pigs were not obtainable and results are thus based on seven animals.

Statistical Analyses

No statistical analyses were performed on data from the dose escalation and high-dose NN703 studies apart from calculation of means and standard errors. The GH profiles from the low-dose NN703 study were subjected to deconvolution analysis *(34)*. Statistical treatment of deconvolution measures was made on logarithmically transformed data to reduce heterogeneity of variance. The general effect of treatment was tested with analyses of variance for repeated measures where treatment and day of treatment and the interaction between these factors were considered main sources of variation. Calculations were executed with the MIXED procedure of the SAS program (version 8.0, SAS Institute, Cary, NC). The *t*-test for paired observations was used in limited preplanned cases. Data are presented as the mean ± SEM.

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