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Do Growth Hormone–Releasing Peptides Act as Ghrelin Secretagogues?

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NN703 is an orally active and selective growth hormone secretagogue (GHS) that was derived from growth hormone-releasing peptide-1(GHRP-1) via ipamorelin by a peptidomimetic approach and has now entered into phase II clinical trials. When the disposition in rats of NN703 and GHRP-6 was studied using whole-body autoradiography following administration of an iv dose of radiolabeled material, we found that a substantial amount of these secretagogues accumulate in the glandular part of the stomach. Because this is the site of synthesis and secretion of ghrelin, the endogenous GHS, we investigated the effect of resection of the gastrointestinal (GI) tract on growth hormone (GH) release induced by GHRP-6. This procedure significantly attenuated the GH secretion response by 60–70%. By contrast, the effect of GH-releasing hormone on GH release was not inhibited. The binding of GHRPs to the glandular part of the stomach and the blunted GH response to GHRP-6 following resection of the GI tract suggest a role for ghrelin as a mediator of part of the GH-releasing effect of GHRPs.

Key Word: NN703; ghrelin; growth hormone–releasing peptide; growth hormone secretagogue; drug disposition; oxyntic gland.

Introduction

Novel growth hormone secretagogues (GHSs) have been designed and developed by a peptidomimetic approach, using growth hormone–releasing peptide-1 (GHRP-1) as a template (**Fig. 1**) *(1,2).* The first compound from this series that progressed to clinical trials, ipamorelin, showed potency similar to GHRP-6 and proved to be a remarkably selective drug, that lacked effects on other hormones *(3).* However, the oral bioavailability (F_{po}) of ipamorelin was not satisfactory, and it was therefore decided to design a derivative with acceptable F_{po} in addition to high potency and selectivity. The result of this effort was NN703 (tabimorelin) *(4),* which has now entered into phase II clinical trials.

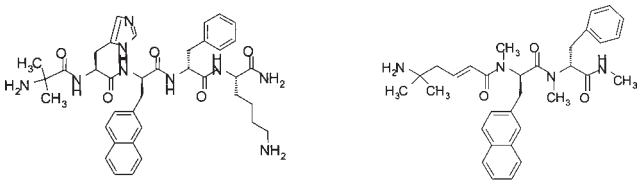

Fig. 1. Structural formulas of ipamorelin and NN703.

We studied the disposition in rats of NN703 and GHRP-6 using whole-body autoradiography (WBAR) following administration of an iv dose of radiolabeled material. We found that these secretagogues accumulate in the glandular part of the stomach where the endogenous GHS, ghrelin, is biosynthesized and released *(5).* This prompted us to investigate the effect of resection of the gastrointestinal (GI) tract on growth hormone (GH) release induced by GHRP-6.

Results

The disposition of either radioiodinated GHRP-6 or carbon-14 labeled NN703 10–15 min after an iv injection was studied by WBAR. Whereas the central nervous system was not significantly labeled by either compound, a substantial part of the radioactivity was recovered in the glandular part of the stomach (**Fig. 2 A,B**). To study whether the binding of GHRP-6 to the stomach is involved in eliciting the release of GH from the pituitary, a group of rats were submitted to resection of the GI tract and then challenged with GHRP-6. A significant reduction (60–70%) of the secretion of GH from 0 to 15 min was observed (**Fig. 3**). In a subsequent experiment groups of rats were gastrectomized as before and then challenged with either GHRP-6 or GH-releasing hormone (GHRH). A blunted GH-response to GHRP-6 was again observed, but, by contrast, the GH secretion following GHRH was significantly augmented (**Fig. 4**).

Discussion

GHRPs are thought to elicit GH secretion by a dual action at the hypothalamic and pituitary levels. The direct effect of

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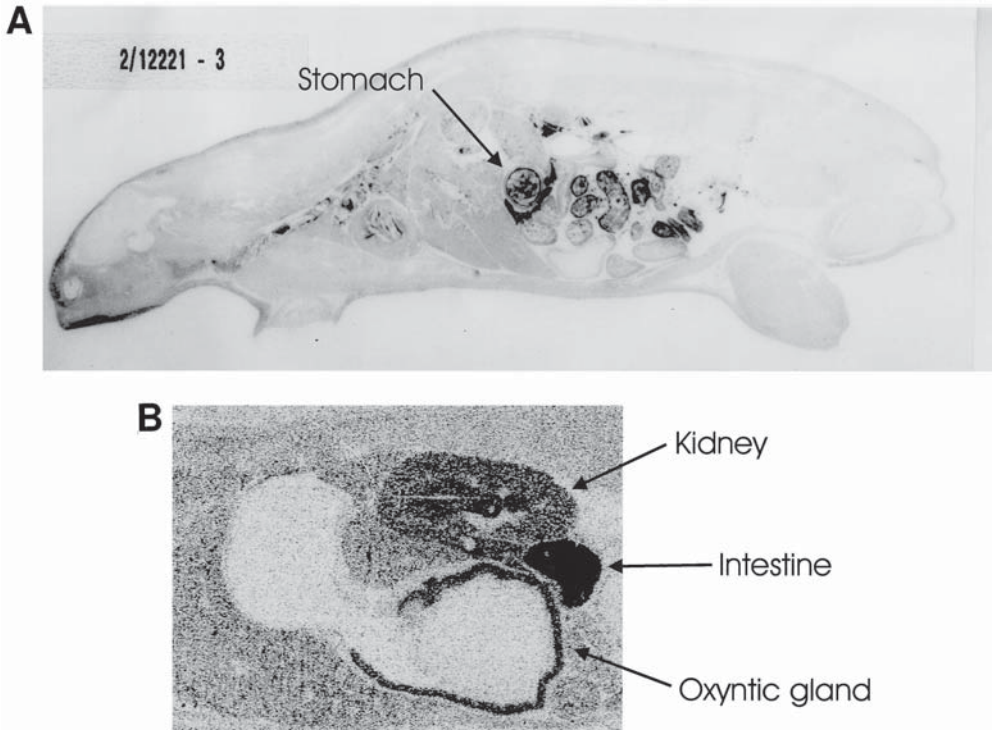

Fig. 2. Disposition of radiolabeled GHRP-6 (**A**) and NN703 (**B**) in rats at the peak of GH release as demonstrated by WBAR. (**a**). [^{125}I]His-Ala-GHRP-6 was injected 10 min prior to sacrifice. The arrow points to the gastric ventricle in which the glandular part is labeled. In addition, the pancreas and the intestinal lumen contain radiolabeled material. (**b**). [^{14}C]NN703 was injected 15 min prior to sacrifice. The magnification shows labeling as indicated.

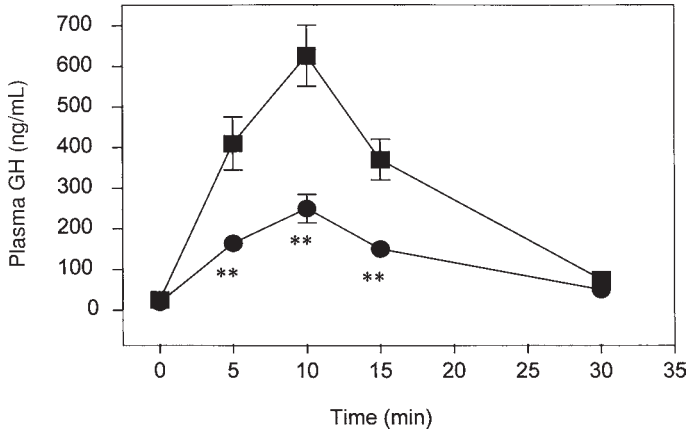

Fig. 3. GH release measured as plasma GH after iv injection of GHRP-6 (0.3 mg/kg) to normal (■) and GI tract–resected (●) pentobarbital-anesthetized rats. Values are means ± SD. Asterisks indicate statistical significance ($p < 0.05$, student's *t*-test).

GHRPs on GH release from somatotrophs is well established, because this is the primary screening assay by which most GHSs have been identified, including NN703. The effect at the hypothalamic level is more speculative and based on activation of indirect markers, such as c-fos expression or electrical activity following iv injection *(6)*, or presence of the GHS receptor *(7)*. To explain direct, central effects, penetration of the blood-brain barrier after peripheral administration of GHSs would seem mandatory, but this pharmacokinetic parameter has not been extensively described in the literature for most GHSs. By WBAR we were not able to demonstrate a brain target for GHRP-6 or NN703 at the peak of GH release (10–15 min). Surprisingly, however, we found an accumulation of both these secretagogues in the glandular part of the gastric ventricle. Because this is the organ responsible for the synthesis and secretion of the recently described endogenous GHS ghrelin *(5)*, we wondered whether binding of GHSs to the GI tract is involved in eliciting secretion of GH from the pituitary gland.

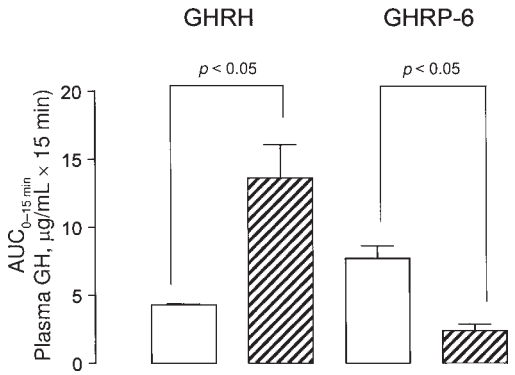

Fig. 4. GH secretion in response to GHRH (2µg/kg), and GHRP-6 (0.3 mg/kg), in intact (□) and GI tract-resected (▨) pentobarbital-anesthetized rats. Values are means ± SD. Statistical significance was evaluated by Student's *t*-test. AUC, area under the curve.

After resection of the whole GI tract, a profound decrease in the GH release in response to iv administration of GHRP-6 was observed. The residual GH release corresponded to 30% of the normal secretion, which might be accounted for by a direct effect of the secretagogue at the pituitary level. By contrast, GHRH-induced GH secretion was not inhibited by the resection. Actually a statistically significant increase in the GH response was observed. The reason for this phenomenon is unclear at present, but it might be related to a lower volume of distribution of GHRH following GI tract resection. However, it appears from the present data that resection of the GI tract selectively blunts the GH response to GHRP and not to GHRH.

It is tempting to speculate on the basis of the binding of GHRP-6 and NN703 to the glandular part of the stomach and the significant reduction in GH secretion following GI tract resection that ghrelin is involved in the pharmacologic action of GHRPs. The latter compounds might act as ghrelin secretagogues in addition to their direct effect at the pituitary level. Measurement of plasma ghrelin concentrations following systemic administration of GHRP should be conducted in order to verify this hypothesis.

Materials and Methods

Whole-Body Autoradiography

Sprague-Dawley rats were anesthetized with pentobarbital and dosed intravenously with radiolabeled GHS. In the first experiment, a male rat weighing 358 g was dosed with 40 µCi/468 µg of [^{125}I]His-Ala-GHRP-6, a fully bioactive analog of GHRP-6, and sacrificed 10 min later. In the second experiment, a male rat weighing 150 g was dosed with 1 mg/kg of [^{14}C]NN703, specific activity of 7.5 mCi/mmol, and sacrificed 15 min later. The rats were frozen at –80ºC and embedded in 2% carboxymethylcellulose. Longitudinal 25 µm sections were cut at five levels between the kidney and spine. After lyophilization for 48 h, the sections were placed on x-ray films for exposure at –20°C for an appropriate time.

Resection of GI Tract Followed by GHRP-6 and GHRH Stimulation

Male Sprague-Dawley rats (450–500 g) were anesthetized with pentobarbital sodium (45 mg/kg intraperitoneally) and placed on a heated (28ºC) table. Catheters were inserted into a carotid artery for blood pressure measurements and blood samplings and in a jugular vein for iv drug administration. The abdomen was opened by a midline incision and the GI tract, including the pancreas, was removed *in toto*. Initially a ligature was placed around the cardiac part of the stomach and from there and moving backward the whole intestinal tract was resected by carefully placing ligatures around all visible blood vessels. After surgery the animals were allowed to stabilize for 15 min, and animals with a mean blood pressure above 100 mmHg were used for the experiments. After collecting a control blood sample, GHRP-6 (300 µg/kg) or GHRH (2 µg/kg) was injected intravenously at time 0, and the GH-releasing profile was determined by an enzyme-linked immunosorbent assay of blood samples at times indicated.

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