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ORAL ACTIVITY OF THE GROWTH HORMONE RELEASING PEPTIDE HIS-D-TRP-ALA-TRP-D-PHE-LYS-NH_2 IN RATS, DOGS AND MONKEYS

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Summary

The purpose of this study was to evaluate the growth hormone (GH) releasing activity of orally administered His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 (GHRP-6, SK&F 110679) in rats, dogs and monkeys. Rats were administered GHRP-6 orally by gavage or parenterally through femoral artery catheters. Blood was collected before and after GHRP-6 administration for estimation of plasma GH and comparison of GH changes resulting from enteral and parenteral administration of the peptide. GHRP-6 was administered to dogs intravenously (i.v.) through cephalic vein catheters, intragastrically (i.g.) through esophagostomy tubes or intraduodenally (i.d.) through vascular access ports, and blood was collected before and after peptide administration for estimation of plasma GH. Cynomolgus monkeys were administered GHRP-6 i.g., and blood was collected from abdominal aorta for estimation of changes in plasma GH.

Enteral activity of GHRP-6 was observed in all 3 species tested. In rats, $ED_{50's}$ for enteral and parenteral administration of GHRP-6 were 4 mg/kg and 28 µg/kg, respectively. Thus in rats, enterally administered GHRP-6 was 0.7% as bioactive as the parenterally administered peptide. In dogs GHRP-6 was slightly less potent than in rats, with $ED_{50's}$ for i.g. and i.v. administration approximately 15 mg/kg and 125 µg/kg, respectively. However, enteral potency of GHRP-6 in dogs was 0.8% of parenteral potency, and thus, comparable to that in rats. Additionally, comparison of plasma GH levels following i.g. vs i.d. administration in dogs suggested greater activity by the i.d. route. Monkeys were the species most sensitive to enterally administered GHRP-6, with plasma GH increased in those receiving i.g. doses as low as 0.3 mg/kg and an ED_{50} of 0.75 mg/kg compared to 4 and 15 mg/kg in rats and dogs, respectively.

The results of this study demonstrate that GHRP-6 releases GH when administered directly into the gastrointestinal tract. Although enteral activity is approximately 1% of parenteral activity, GHRP-6 is potent, especially in primates which require relatively low doses to provoke GH release. These data suggest that orally active GHRP-6 may provide a practical therapeutic alternative to parenterally administered peptides such as GHRH, especially if enteral activity is enhanced with appropriate formulation.

Stable, orally active enkephalin analogs such as (D-Ala^2,MePhe^4,Met(O)^5-ol)-enkephalin (DAMME, FK 33-824; 1,2) stimulated growth hormone (GH) and prolactin release in animals and man (3,4), while lowering LH, FSH and cortisol levels (5). Unlike these general effects of DAMME, another met-enkephalin analog, Tyr-D-Trp-Gly-Phe-Met-NH_2 (D-Trp^2-MetEnk-NH_2) specifically released GH, without affecting any other pituitary hormone (6). Structural modification of this molecule led to the discovery of a potent GH-releasing peptide His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2 (GHRP-6; SK&F 110679), that retained the hypophysiotrophic specificity of its predecessor, D-Trp^2-MetEnk-NH_2 (7,8). GH-releasing activity of the

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new peptide was comparable by i.v., s.c., and i.p. routes in rats, monkeys, lambs, calves and chicks, indicating a lack of species specificity and resistance to activity loss when administered other than directly into the circulatory system (9). Recently, GHRP-6 was reported to be very active in humans, producing peaks of plasma GH approximately 70 ng/ml within 15 minutes of i.v. administration at a dose of 1ug/kg body weight (10).

Many conditions for which peptides are used therapeutically require their long-term administration. Polypeptides must often be administered parenterally since they are readily degraded by proteases in the gastrointestinal tract. Unfortunately, parenteral administration is least desirable in terms of patient comfort and compliance. Therefore, peptide administration has also been tested by vaginal, rectal, dermal, tracheal, nasal, buccal, and ocular routes (11,12). Despite its practical limitations, the oral route is generally preferred by physicians and patients.

In some cases, proteolytic degradation of orally administered peptides can be reduced by substituting d-amino acids for l-amino acids in the structural sequence. The resulting molecules are more resistant to attack by endopeptidases and in many cases retain physiological activity or even become superagonists (2). GHRP-6 contains d-amino acids at positions 2 and 5 and thus, may possess oral activity as do certain other enkephalin-derived peptides (2). Since GHRP-6 is relatively potent, producing high levels of serum GH in humans at doses of 1ug/kg and below (10), its potential for use by oral administration is good, even with poor bioavailability. Since oral activity would significantly enhance the practical utility of GHRP-6 as a therapeutic agent, the purpose of this study was to determine the GH-releasing activity of GHRP-6 administered directly into the gastrointestinal tract of rats, dogs and monkeys as an indication of its potential for oral use in humans.

Materials and Methods

Animals and facilities:

Rats: Male, Sprague-Dawley rats (2 - 4 months old) were purchased from Charles River Breeding Laboratories (CD/VAF; Cambridge, Massachusetts) and acclimated to local housing conditions for approximately 14 days prior to peptide administration. The rats were housed individually in suspended stainless-steel wire mesh cages equipped with an automatic watering system, and were provided water and Purina Certified Rodent Chow #5002 **ad libitum** except overnight prior to oral administration of GHRP-6, when food was withheld.

Dogs: Female mongrel dogs were purchased from Haycock Kennels (Perkasie, PA), acclimated to the laboratory conditions described above, given physical examinations and permanently identified with ear tattoos. Two groups of dogs were used: group 1 was 4-7 years old, while group 2 was 1-4 years old. Food (Purina Lab Canine Diet #5006) and tap water were available **ad libitum** except for 18-20 hours before administration of GHRP-6 when food was withheld.

Monkeys: Three male wild caught cynomolgus macaques (**Macaca fascicularis**; Hazelton Research Animals, Reston, VA and Alice, TX) were selected for use in this study based upon general health information obtained from ophthalmologic and physical examinations, electrocardiography, clinicopathologic evaluation and body weight. The monkeys were housed individually in stainless steel cages. Food (12 biscuits of Purina Monkey Chow #5045 and supplemental seasonal fruit) and tap water were available **ad libitum** except for approximately 16 hours before administration of GHRP-6, when food was withheld. The animals were 2.5-3 years old.

All animal rooms were maintained at $72^{o} \pm 4^{o}$F, at 50% ± 10% relative humidity, and with a photoperiod with 12 hour alternating phases of darkness and light. All other housing parameters and husbandry procedures met the recommendations set forth in the "Guide for the Care and Use of Laboratory Animals," (NIH Publication 85-23).

Compound and Administration:

GHRP-6 (SK&F 110679; His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2) produced by the Department of Synthetic Chemistry, SK&F R&D, is a white powder whose molecular weight is 873.03. Solutions for parenteral and enteral administration were prepared by dissolving the compound in sterile isotonic NaCl. The solutions were administered to rats by infusion for 15 seconds as 1ml/kg (intraarterially) or 5ml/kg (oral gavage); to dogs over 33 minutes via infusion pump (i.v.) or over 2 minutes via esophagostomy tube (i.g. or i.d.; dogs required slow i.v. infusion since they were more sensitive than rats to the hypotensive effects of parenterally administered peptide); to monkeys as a bolus of 1 ml/kg (intragastrically).

Surgery and Experimental Design:

Since the high amplitude spontaneous spikes of GH in the rat (15) could interfere with GHRP-6-stimulated GH release, systemic and oral activity of GHRP-6 was determined in rats anesthetized with pentobarbital, previously shown to suppress somatostatin release and thus blunt the ultradian rhythm of GH release (13, 14). Pentobarbital (35 mg/kg, i.p.) was administered to the rats to achieve a state of anesthesia sufficient to allow surgical placement of femoral arterial cannulae used for systemic administration of GHRP-6 and subsequent collection of blood samples for quantitation of plasma GH. Additional anesthesia was administered when vibrissae movement was observed, approximately every 30 minutes. Blood samples (200 ul) were drawn through femoral arterial cannulae before and after parenteral or enteral administration of a range of doses of GHRP-6.

Eight chronic dogs (group 1) were surgically prepared with esophagostomies for intragastric administration of GHRP-6. Four additional dogs (group 2) were surgically prepared with a subdermal Vascular Access Port (VAP, Norfolk Medical Products, Skokie, IL) with the attached cannula fixed directly into the duodenum 6-8 cm from the gastro-duodenal junction. The animals were allowed at least two weeks for recovery before receiving the peptide. Before and during the recovery period, the dogs were acclimated to standing in a sling used to restrain them during experimental periods up to 4 consecutive hours. All dogs received routine cleansing and aseptic preparation of the VAP injection site for the direct intraduodenal administration of fluids. Blood samples (1ml) were drawn from cephalic vein catheters at 5 to 15 minute intervals before, during and after administration of GHRP-6. A maximum intravenous reference dose of 0.5 mg/kg GHRP-6 was used to compare with i.g. and i.d. responses. All dogs received i.v. GHRP-6; therefore each dog served as its own control for comparing GH responses by i.g. or i.d. routes.

Cynomolgus monkeys were surgically prepared with abdominal aortic and gastric cannulae that were exteriorized and protected from damage within primate jackets (Alice King Chatham Medical Arts, Los Angeles, CA). After recovering from surgery, the monkeys were acclimated to experimental conditions which included handling, weighing and restraint in primate chairs for several hours each day. Each monkey received i.g. water vehicle and several GHRP-6 doses administered at two week intervals. During the intervals between experiments, the handling procedures were repeated every few days to insure that the animals remained acclimated. The monkeys were placed in restraining chairs 90 min before drug administration. Blood samples (800ul) were obtained through the arterial catheters at several times before and after administration of GHRP-6 or vehicle.

Blood Collection and GH Radioimmunoassay:

The heparinized blood samples were kept on ice until the end of each experiment, when they were centrifuged and the plasma stored at -20°C until assayed for growth hormone concentrations. Rat plasma GH was determined using a double antibody method with reagents (anti-rGH-S-5; rGH-RP-2; rGH-I-5) obtained from Dr. A. F. Parlow, Pituitary Hormones and Antisera Center, Torrence, CA. Intraassay and interassay coefficients of variation for the rat GH RIA were 3.7% and 9.3%, respectively. Reagents for the canine GH RIA also were obtained from Dr. Parlow (NIDDK-cGH-I-6; NIDDK-anti-cGH-S-5; NIDDK-cGH-RP-2), and the intraassay and interassay coefficients of variation were 2.4% and 7.7%, respectively. Monkey plasma GH concentrations were estimated with a hGH RIA kit (Diagnostic Products, Los Angeles, CA). The intraassay and interassay coefficients of variation using the kit reagents were 5% and 7%, respectively.

Data points are presented graphically as means ± S.E.M. for each experiment. Dose response curves were calculated from peak heights of plasma GH levels subsequent to GHRP-6 administration. ED_{50} values were derived from the dose-response curves.

Results

A time course of change in plasma GH concentrations following i.a. administration of GHRP-6 to pentobarbital anesthetized rats is shown in Fig. 1. Following administration of vehicle, plasma GH levels were stable with no evidence of the rhythmic ultradian changes reported for conscious rats (15). However, within 5 minutes of GHRP-6 administration, plasma GH rose above basal levels, reaching peak values within 10-15 minutes. The rise in plasma GH was dose-dependent over a range of 3-100 μg/kg. GH responses to 300 μg/kg were comparable in amplitude and duration to those resulting from 100 μg/kg (mean peak value, 1750 ng/ml). The ED_{50} for i.a. administered GHRP-6 calculated from a dose-response curve constructed from the peak height data (100μg/kg = 100%) was 28μg/kg (Fig. 1).

The data presented in Fig. 2 show that temporal changes in plasma GH concentrations for orally administered GHRP-6 in rats were similar to those resulting from i.a. administration. However, the most efficacious p.o. dose (10 mg/kg) produced a higher mean peak concentration of plasma GH than did the most efficacious i.a. dose (100 μg/kg). The dose response curve calculated from the oral data yielded an oral ED_{50} of approximately 4mg/kg (Figure 2). Thus, enterally administered GHRP-6 was only 0.7% as potent as the parenterally administered peptide, but was more efficacious.

The data presented in Fig. 3 compare changes in plasma GH resulting from i.v. and i.g. administration of GHRP-6 in dogs at doses of 0.5mg/kg and 5 or 25 mg/kg, respectively. In dogs GHRP-6 was slightly less potent than in rats, with ED_{50}'s for i.g. and i.v. (data not shown) administration approximately 15 mg/kg and 125 μg/kg, respectively. However, enteral activity of GHRP-6 in dogs was 0.8% of parenteral activity, or comparable to that observed in rats.

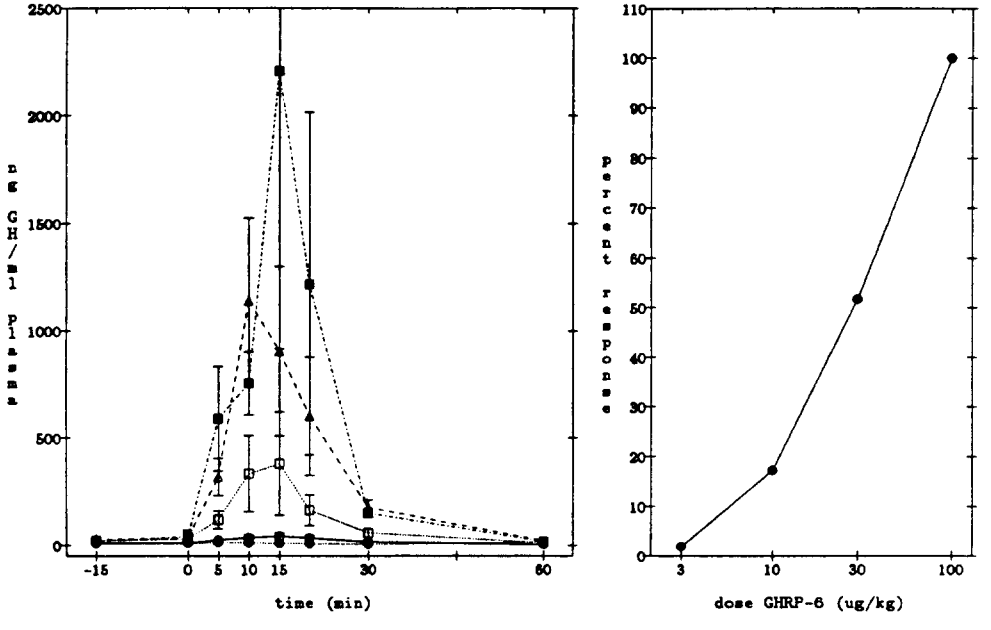

FIG. 1

GH release following intra-arterial administration of GHRP-6 to rats. Left: Time course of plasma rGH levels after various doses of GHRP-6 were administered to pentobarbital anesthetized male rats: (●) 0μg/kg, (○) 3μg/kg, (□) 10μg/kg, (△) 30μg/kg, (■) 100μg/kg. Shown are the mean and SEM of replicate determinations of GH content in samples from 3-6 rats per group. Right: Dose-response curve for i.a. GHRP-6-induced GH release, derived from the data shown in the left panel.

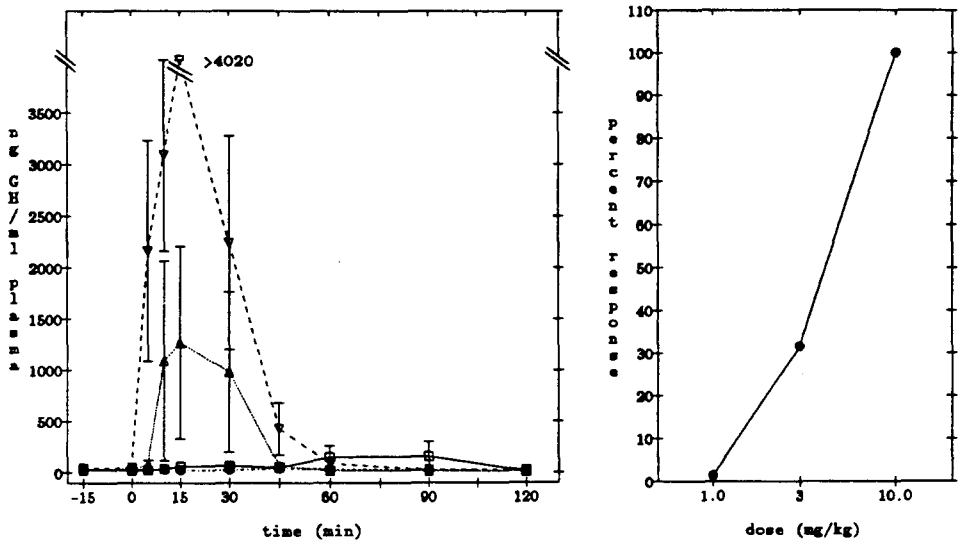

FIG. 2

GH release following oral administration of GHRP-6 to rats. Left: Time course of plasma rGH levels following oral administration of various doses of GHRP-6 to pentobarbital anesthetized male rats: (○) 0mg/kg, (□) 1mg/kg, (△) 3mg/kg, (▽) 10mg/kg. Shown are the mean and SEM of duplicate determinations of GH content from 2-3 rats per group. The upper limit of this assay system is 4020ng/ml; values above this point are expressed as >4020ng/ml. Right: Dose-response curve for GH release induced by oral administration of GHRP-6, derived from the data shown in the left panel.

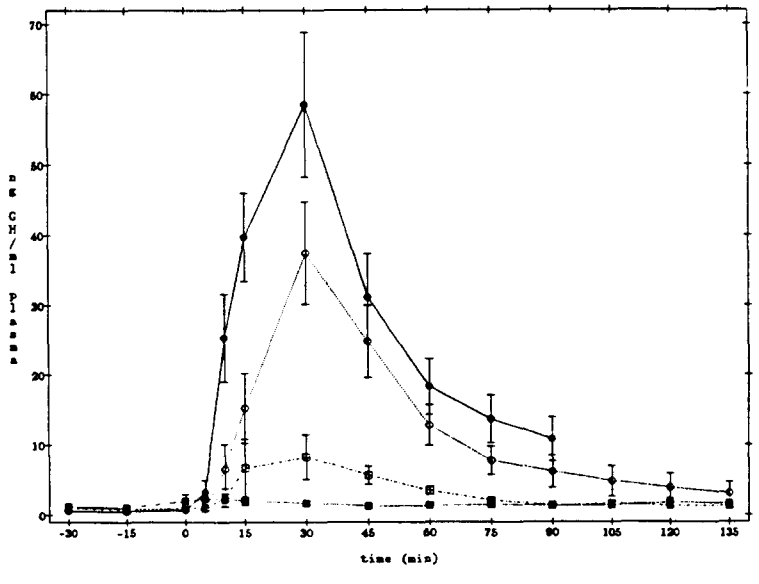

FIG. 3

Time course of plasma cGH levels following GHRP-6 administration to female dogs: (●) i.v. 0.5mg/kg, (○) i.g. 25mg/kg, (□) i.g. 5mg/kg, (■) i.g. saline vehicle. The graph shows the mean and SEM of the data from eight animals.

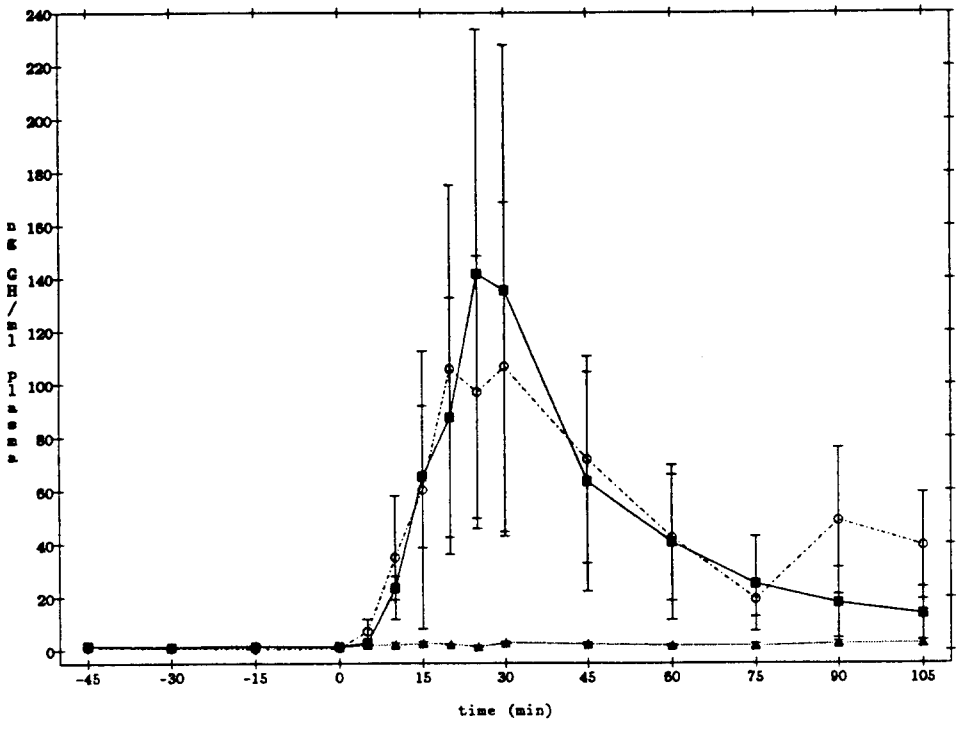

FIG. 4

Time course of plasma cGH levels following GHRP-6 administration to female dogs: (■) i.v. 0.5mg/kg, (○) i.d. 5mg/kg, (▲) i.d. saline vehicle. The graph shows the mean and SEM of the data from four animals.

Administration of 5mg/kg of GHRP-6 intraduodenally (i.d.) caused a greater elevation in plasma GH (Fig. 4) than that following i.g. dosing (Fig. 3). The increase in plasma GH in dogs administered 5 mg/kg GHRP-6 i.d. was 75% of that resulting from i.v. administration of 0.5 mg/kg. Five mg/kg administered i.g. increased plasma GH only 14% of the i.v. dose (0.5 mg/kg) suggesting that bioactivity of the orally administered peptide is increased if it is delivered directly to the duodenum.

Although monkeys were acclimated to the testing conditions, a stress-related increase in plasma GH occurred when they were placed in restraining chairs (Figure 5). However, 90 minutes after chairing, plasma GH had returned to baseline values. Thereafter, i.g. administration of water had no effect on plasma GH levels, while a dose-related increase in plasma GH followed i.g. administration of GHRP-6 (Fig. 5). Monkeys were the most sensitive of the 3 species tested to enterally-administered GHRP-6. Plasma GH rose in monkeys receiving as little as 0.3 mg/kg i.g. GHRP-6. The greater i.g. potency of GHRP-6 in monkeys also was evident in an ED_{50} of 0.75 mg/kg compared to 4 and 15 mg/kg in rats and dogs, respectively.

Discussion

The primary finding presented here is that His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2, a synthetic GH releasing peptide is orally active. Peptides with oral activity sufficient for practical use are atypical. In fact, Roemer et al. (2) reported that DAMME, a synthetic enkephalin analogue, "is one of the rare examples of a peptide showing pronounced biological activity after enteral administration". The relative biological activity of p.o. to i.v. FK 33-824 was 0.4%, or approximately one-half of that observed for GHRP-6 in this study. In addition, the ED_{50}'s for GHRP-6 biological activity in the three species tested were orders of magnitude lower than those for DAMME, underscoring the unusual attributes of GHRP-6 and its potential for practical use as an orally active GH-releasing peptide.

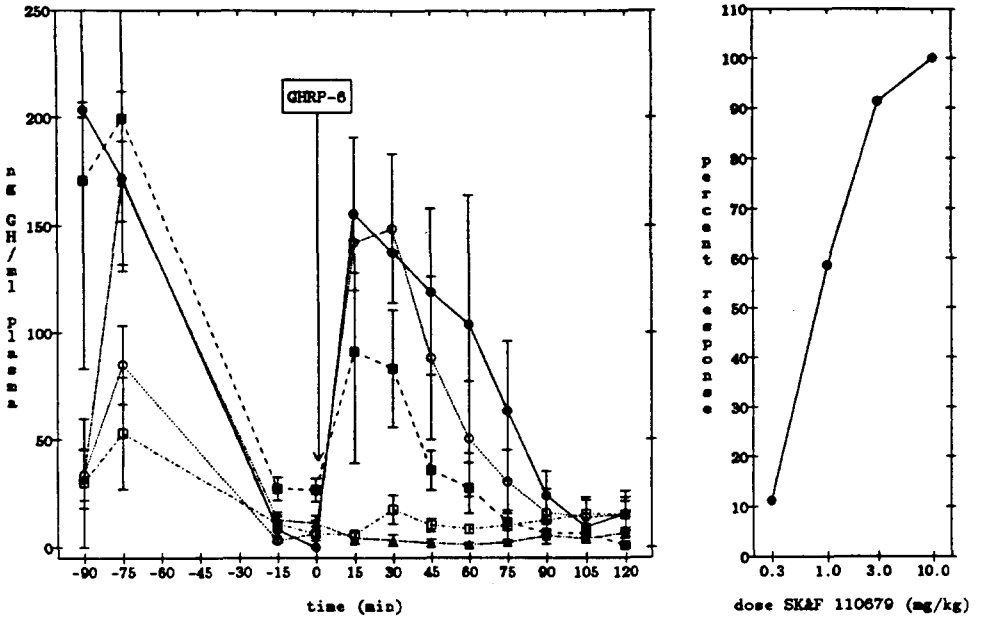

FIG. 5

GH release following gastric administration of GHRP-6 to male Cynomolgus monkeys. Left: Time course of plasma GH levels following administration of a range of doses of GHRP-6 via gastric cannulae: (▲) 0mg/kg, (□) 0.3mg/kg, (■) 1mg/kg, (○) 3mg/kg, (●) 10mg/kg. The values shown represent the means of duplicate determinations of plasma GH in samples from three juvenile Cynomolgus monkeys. The error bars represent the SEM. Right: Dose-response curve for the elevation in plasma GH levels following the intra-gastric administration of GHRP-6. The curve is derived from the peak heights shown in the left panel.

Investigation of complete dose-response relationships for i.g.and i.d. dosing in dogs was precluded by the limited availability of peptide. However, the results of our study in which a reference i.v. dose was given to each group of dogs indicate that much greater GH releasing activity follows i.d. than i.g. administration.

The ED_{50} for oral activity of GHRP-6 in monkeys was lower than in rats and dogs despite the fact that stress-related GH release occurred approximately 1 hour before peptide administration. These data suggest that primates are more sensitive than other species to the GH-releasing effects of the GHRP-6. This hypothesis is supported by on-going clinical trials in which GH release has been observed in young men administered oral doses <200 µg/kg GHRP-6 (Ilsen, unpublished data), and is consistent with the high potency of the i.v. administered peptide in man (10). It is presently unknown whether the differences in GHRP-6 activity represent species specific sensitivities, or species differences in absorption, distribution or biotransformation of the peptide.

Possible transformation of orally administered GHRP-6 to a more efficacious GH-releasing molecule in the rat may result from p.o. vs. i.v. administration of the hypophysiotrophic peptide. Alternatively, the slightly prolonged release of GH after i.g. administration of GHRP-6 might be explained by a prolonged duration of exposure resulting from absorption of the peptide. However, slow i.v. infusion of GHRP-6 in the present study in dogs did not produce significantly different temporal profiles of GH-release than did bolus administration in rats, suggesting that prolonged exposure to GHRP-6 does not necessarily prolong GH release. In any event, differential responses to p.o. and i.v. administration may have future therapeutic implications if it is determined that the area under the curve is more important than peak height in eliciting the biological effects of GH.

In conclusion, the results of this study demonstrate that GHRP-6 is orally active in 3 different species, albeit at approximately 1% of its i.v. potency. Although a similar ratio of activity by i.v. and p.o. routes of administration was previously observed for the peptide DAMME (2), biological activity by the oral route in that study required >100 mg/kg rather than concentrations of <1-15 mg/kg presently demonstrated for GHRP-6. This difference, in addition to the potential for greater potency of a formulation that releases the peptide in the duodenum, makes orally administered GHRP-6 an important practical alternative to GH and other GH-releasing peptides that require parenteral administration.

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