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Stimulation of the Growth Hormone (GH)-Insulin-Like Growth Factor I Axis by Daily Oral Administration of a GH Secretogogue (MK-677) in Healthy Elderly Subjects*

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ABSTRACT

Aging is associated with declining activity of the GH axis, possibly contributing to adverse body composition changes and increased incidence of cardiovascular disease. The stimulatory effects on the GH-insulin-like growth factor I (IGF-I) axis of orally administered MK-677, a GH-releasing peptide mimetic, were investigated. Thirty-two healthy subjects (15 women and 17 men, aged 64–81 yr) were enrolled in a randomized, double blind, placebo-controlled trial. They received placebo or 2, 10, or 25 mg MK-677, orally, once daily for 2 separate study periods of 14 and 28 days. At baseline and on day 14 of each study period, blood was collected every 20 min for 24 h to measure GH, PRL, and cortisol. Attributes of pulsatile GH release were assessed by 3 independent algorithms. MK-677 administration for 2 weeks increased GH concentrations in a dose-dependent manner, with 25 mg/day increasing mean 24-h GH concentration 97 ± 23% (mean ± SE; $P < 0.05$ *vs.* baseline). This increase was due to an enhancement of preexisting pulsatile GH secretion. GH pulse height and interpulse nadir concentrations increased significantly without significant changes in the number of pulses. With 25 mg/day MK-677 treatment, mean serum IGF-I concentrations increased into the normal range for young adults (141 ± 21 μg/L at baseline, 219 ± 21 μg/L at 2 weeks, and 265 ± 29 μg/L at 4 weeks; $P < 0.05$). MK-677 produced significant increases in fasting glucose (5.4 ± 0.3 to 6.8 ± 0.4 mmol/L at 4 weeks; $P < 0.01$ *vs.* baseline) and IGF-binding protein-3. Circulating cortisol concentrations did not change, and PRL concentrations increased 23%, but remained within the normal range. Once daily treatment of older people with oral MK-677 for up to 4 weeks enhanced pulsatile GH release, significantly increased serum GH and IGF-I concentrations, and, at a dose of 25 mg/day, restored serum IGF-I concentrations to those of young adults. (*J Clin Endocrinol Metab* **81:** 4249–4257, 1996)

PULSATILE secretion of GH by the anterior pituitary gland is controlled mainly by two hypothalamic peptides: somatostatin, which is inhibitory, and GH-releasing hormone (GHRH), which is stimulatory (1). In addition to stimulating linear growth before epiphyseal fusion, GH has metabolic effects that persist throughout life. These effects are exerted directly by GH and via its stimulation of insulin-like growth factor I (IGF-I) production (1). In physiological concentrations, GH is anabolic, stimulates muscle development and strength, stimulates loss of fat tissue (particularly from central abdominal sites), and increases bone density (2). GH also exerts cardioprotective effects on blood lipid concentrations, explaining why adult GH deficiency may predispose to premature atherosclerosis and the resultant increased mortality from cardiovascular disease (2, 3).

Normal human aging is associated with declining serum concentrations of GH and IGF-I (4–7). Although normal aging is not typically associated with profound GH deficiency as occurs in patients with pituitary disease (8, 9), mean GH concentrations in people over 60 yr of age are, on the average, about one third to one half those in young adults (4, 9–11). This reduction may contribute to the decreases in muscle and bone mass and the increases in adipose tissue that accompany normal aging (12). These changes have been partially reversed by GH administration for 6 months to otherwise healthy older men and women (13, 14). The disadvantages of GH therapy are the high cost, the need for parenteral administration, and side-effects, including fluid retention, carpal tunnel syndrome, and glucose intolerance (2, 12–14). We speculate that such side-effects may result from prolonged nonphysiological elevations of circulating GH concentrations after daily sc GH injections (15). If this is the case, enhancement of endogenous pulsatile GH secretion by an

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orally administered secretagogue may be a more desirable therapeutic strategy than parenteral GH.

GH-releasing peptide (GHRP) is a synthetic hexapeptide that was developed specifically to stimulate growth. It stimulates pulsatile GH secretion in humans, probably by actions on both the hypothalamus and pituitary, through a novel receptor (16–18). The natural ligand, whose effect it mimics, has yet to be discovered. Compounds have been developed that mimic the stimulatory actions of GHRP on GH release and do not interact with muscarinic or nicotinic cholinergic receptors (19, 20). These compounds are more potent secretagogues than GHRH in older adults (21). We report the effects of oral administration for 2–4 weeks of the spiropiperidine MK-677 (20) to healthy older adults. This compound was selected because of its high oral bioavailability and its long duration of action.

Experimental Subjects

The study was approved by the human investigational review boards of the participating centers (University of Virginia, University of Chicago, and Clinical Studies, Florida). Each subject gave written informed consent before enrollment in the study.

Thirty-two subjects, 15 women and 17 men, aged 64–81 yr (mean ± SD, 70.2 ± 3.8), were studied in 2 panels. All were healthy nonsmokers with a body mass index (BMI) between 19.9–30.2 kg/m^2 (mean ± SD, 24.6 ± 2.3). The mean age and BMI did not differ between panels or treatment groups. This age group was chosen because it constitutes a significant portion of elderly subjects who would be candidates for such therapy. Because spontaneous GH secretion is inversely related to BMI (6), it was important to include the wide range of BMIs found in this population. The only medications allowed were stable doses of thyroid hormone replacement, less than 1000 mg acetaminophen/day, and up to 1 aspirin tablet/day. Exclusion criteria included a history of diabetes mellitus; significant cardiac, vascular, or other disease; other hormone treatments (estrogen, testosterone, *etc.*); unusual or extreme dietary habits; or consumption of more than 6 cups of caffeinated beverages/day. All subjects had unremarkable clinical histories and physical examinations; normal urinalyses, electrocardiograms, and chest x-rays; and normal biochemical indexes of renal, hepatic, hematological, and thyroid function. Glycated hemoglobin and serum concentrations of PRL, FSH, and LH (women) and testosterone (men) were in the age-adjusted normal ranges.

Materials and Methods

Study design (Fig. 1)

Subjects were entered into a randomized, double blind, placebo-controlled trial, in which they received once daily the oral study drug (MK-677 or placebo) for each of two 14-day study periods (periods I and II) separated by a 14- to 21-day washout period. At the end of period II, subjects received the study drug for an additional 2-week extension period for collection of IGF-I and safety data. Subjects were studied in one of two panels. In panel A, the study drug was given once a day in the evening (between 2200–2300 h). In panel B, subjects received the study drug in both the morning (between 0700–0900 h) and the evening (between 2200–2300 h); to blind the treatment time of the active drug, at least one of these two treatments per day was placebo.

Period I. Subjects were admitted to the Clinical Research Center (CRC) the day before the study, to acclimate to the unit. Regular CRC diets were consumed during all admissions. Alcohol consumption was not permitted. An iv cannula for blood sampling was inserted into an arm vein by 0700 h on study day 1. Starting at 0800 h on study day 1, blood samples were collected every 20 min for 24 h for measurement of GH, cortisol, and PRL, and a 24-h urine collection was started for measurement of urinary free cortisol. At 0900 h on the next day (study day 2), 1 h after completion of sampling, subjects received the first dose of the study drug; from study days 3–15, subjects took the study drug between

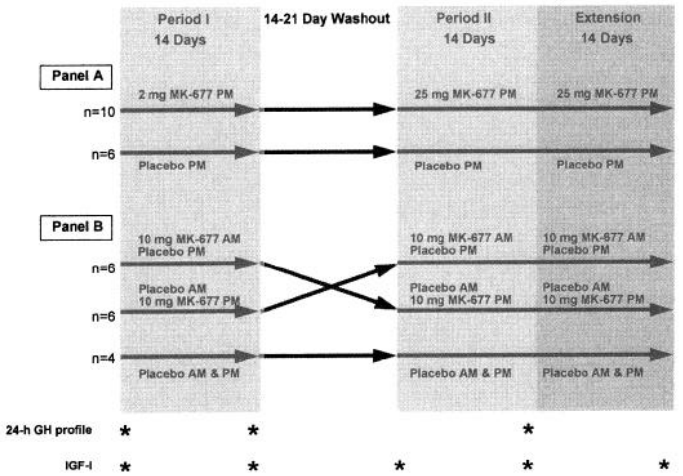

FIG. 1. Schematic of the study design. In panel A, all study drug was given once a day in the evening (between 2200–2300 h). In panel B, subjects received study drug in both the morning (between 0700–0900 h) and evening (between 2200–2300 h); to blind treatment time of active drug, at least one of these two treatments per day was placebo.

2200–2300 h (and also between 0700–0900 h in panel B). Compliance was monitored by drug log and Smart-Cap (APREX Corp., Fremont, CA), a top for the medication container that contains a sensor and monitoring device to record each time the container is opened. Subjects were instructed to consume no more than six cups of caffeinated beverages and two alcoholic drinks per day during this period, but had no other dietary limitations. They were instructed to avoid strenuous exercise, but encouraged to continue modest exercise as part of their daily routine.

Subjects were readmitted to the CRC in the evening of study day 14 and underwent repeat 24-h blood and urine collections beginning at 0800 h on study day 15, as on study day 2. The last dose of treatment drug was taken in the evening of study day 15.

Period II. After a washout period of 14–21 days, subjects returned to the CRC, took the first dose of the study drug at 0900 h, were observed for 4 h, and then were discharged. The remainder of the period was the same as period I, with repeat admission and sampling after 2 weeks. Subjects in panels A and B continued to take study drug for an additional 2-week extension period (*i.e.* a total of 28 continuous days of drug administration) and were then seen for assessment of possible side-effects and collection of fasting urine and blood samples.

Analytical methods

Assays. Serum GH concentrations were measured in duplicate by chemiluminescence assay (Nichols Institute Diagnostics, San Juan Capistrano, CA), modified as previously described (22). The sensitivity of the assay was 0.002 μg/L, and the measured GH concentrations in all samples were above this detection limit. The intraassay coefficients of variation were 5.4% at 0.04 μg/L, 4.8% at 0.4 μg/L, 5.7% at 3.4 μg/L, and 9.9% at 8.4 μg/L. The interassay coefficients of variation were 5.6% at 0.04 μg/L and 7.9% at 8.4 μg/L. All 24-h GH profiles from each subject were run in one assay. Cortisol was measured by fluorescence polarization immunoassay (Abbott TDx, Abbott Laboratories, Abbott Park, IL). Values below the lowest standard were reported as less than 83 nmol/L (<3 μg/dL). PRL was measured in a chemiluminescence immunoassay (ACS 180, Ciba Corning Diagnostics Corp., Medfield, MA) with a sensitivity of 0.3 μg/L. Cortisol, GH, and PRL assays were performed at the University of Virginia Medicine Clinical and CRC Core Laboratories. All other assays, including routine serum chemistry, hematology, screening hormone levels, and urinary free cortisol, were performed by Endocrine Sciences (Calabasas Hills, CA). Serum IGF-I was measured by RIA after acid-ethanol extraction, with an assay sensitivity of 10 μg/L. The expected values are 202–453 μg/L (mean, 330) for 21–25 yr, 52–372 μg/L (mean, 161) for 60–69 yr, and 63–223 μg/L (mean, 153) for 70–79 yr.

Analysis of pulsatile GH release. GH concentration profiles were analyzed by three methods: 1) the Cluster peak detection algorithm, version 6.0 (23); the threshold parameters used (test peak = 1, test nadir = 1, *t* statistic = 1) had a sensitivity of 75% for detection of GH concentration pulses and a positive predictive accuracy of 93% in a validation study employing computer simulations of GH pulse series at 20-min intervals; 2) a multiple parameter deconvolution technique; pulsatile and basal GH secretion rates and the half-life of GH disappearance were simultaneously resolved (6, 24) (sensitivity, 85%; positive predictive accuracy, 96% in the validation study above); and 3) a modification of the Ultra algorithm; the threshold for significance of a pulse was 2 times the intraassay coefficient of variation in the relevant concentration range (25). The amount of GH secreted per pulse was estimated using a deconvolution method (5). To estimate 24-h GH production rates, the GH volume of distribution was assumed to be 7% of body weight.

Statistical methods. The results of evening administration of 10 mg MK-677 to 12 subjects in panel B were combined and compared to evening doses administered in panel A. In addition, the results of evening *vs.* morning administration of 10 mg MK-677 in panel B were compared. The results from the 10 subjects who received placebo in all three periods in panels A and B were also combined. The 2 and 4 week treatment results for the placebo-treated subjects were those for treatment period II.

Data collected after 2 and 4 weeks of drug administration were expressed as a percentage of the pretreatment baseline level for statistical analysis. Baseline GH values were those at the beginning of period I. All other baseline values were those at the beginning of the treatment period under analysis. For data collected after 2 weeks of treatment, ANOVA, excluding 2 mg data, provided a pooled estimate of the intersubject SD of percent changes. This pooled estimate was used to compute *t* tests to evaluate the significance of changes from pretreatment baseline and also the significance of changes in response to MK-677 in relation to those observed in the placebo group. When statistical evaluation suggested that the distribution of percent change values was not normal and/or the variability of these values was not homogeneous across treatment groups, the data were expressed as log-transformed fold change [*i.e.* log(post/pre)] for statistical analysis. In those cases, geometric means and appropriately back-transformed SEs are provided to summarize response in the various treatment groups. The response to 25 mg MK-677 after 4 weeks of therapy was similarly evaluated. The response to 10 mg MK-677 at 4 weeks was not evaluated because only six subjects received morning and evening dosing for 4 weeks. The significance of differences in response to morning and evening administration of 10 mg MK-677 was evaluated with statistical methods for the analysis of a two-period cross-over study. Two-sample *t* tests were used to compare the response to 10 mg MK-677 administered in the morning with the response to placebo.

Results

Compliance

Compliance data were available for all subjects; in subjects randomized to active drug, overall compliance was more than 99%. Five of 22 subjects receiving active drug missed 1 dose, and 1 subject missed 2 doses.

Side-effects

Treatment with MK-677 was generally well tolerated. There were no serious clinical or laboratory adverse experiences. There were three reports in panel A of mild abdominal pain and five reports of mild appetite increase in panels A and B (all in drug-treated subjects). Body weight did not change significantly with MK-677 treatment.

Effect of evening MK-677 treatment

GH. Oral administration of MK-677 produced dose-dependent increases in mean 24-h GH concentrations (Fig. 2),

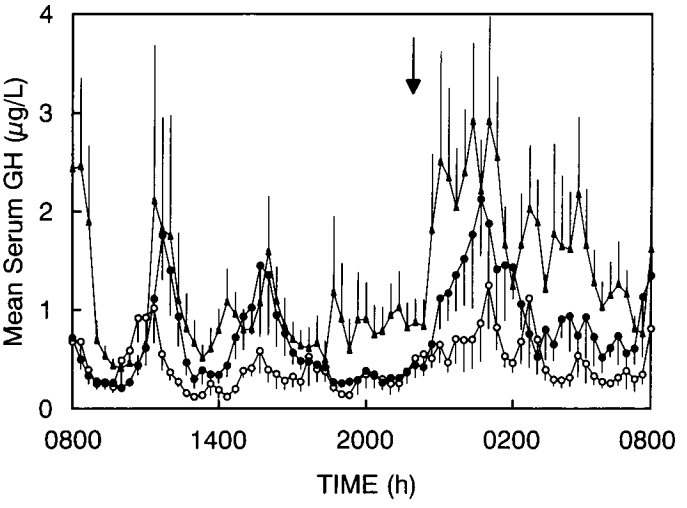

FIG. 2. Mean (±se) serum GH concentrations (micrograms per L) in older subjects after 2 weeks of treatment with placebo (○; n = 10), 10 mg/day MK-677 (●; n = 12), and 25 mg/day MK-677 (▲; n = 10). Evening treatment time (between 2200–2300 h) is indicated by an *arrow*.

which were statistically significant for treatment with 10 and 25 mg, but not 2 mg MK-677 (Table 1). After 2 weeks of placebo administration, there was no significant change in mean 24-h GH concentrations (8 ± 16% greater than at baseline; P = NS), whereas the increases were 57 ± 13% and 97 ± 23% (range, 30–330%) with 10 and 25 mg MK-677, respectively ($P < 0.05$ *vs.* baseline for both doses; Fig. 3, *upper panel*).

Examination of individual 24-h GH concentration profiles (Fig. 4) suggested that MK-677 treatment enhanced pulsatile GH release rather than a sustained increase in serum GH concentrations. This interpretation was supported by the results of Cluster analysis (Table 1), which revealed that treatment with 10 and 25 mg MK-677 resulted in statistically significant increases in the height of GH pulses and interpeak nadir GH concentrations, without any change in the number of GH pulses. Similar results were obtained with the Ultra peak detection algorithm (data not shown). Multiple parameter deconvolution analysis (6, 24) indicated that the increases in serum GH concentrations resulted from increased GH secretion without changes in GH clearance rates. Treatment with 25 mg MK-677 for 14 days resulted in a 1.7-fold increase in the amount of GH secreted per 24 h compared to baseline (277 ± 32 *vs.* 164 ± 27 μg; P = 0.017) without a significant change in GH disappearance half-life (21.0 ± 1.7 *vs.* 20.2 ± 0.8 min). The increase in GH secretion was accounted for by a 1.6-fold increase in the mass of GH secreted per pulse (3.3 ± 0.4 *vs.* 2.1 ± 0.3 μg/L distribution volume; P = 0.045) without a significant change in the number of GH secretory pulses per 24 h (13.1 ± 0.5 *vs.* 11.9 ± 0.8) or the contribution of basal secretion to total 24-h production (20 ± 3.7% *vs.* 17.6 ± 4%). The second, independent deconvolution technique (5) estimated the increase in 24-h GH production rates with 25 mg MK-677 to be slightly larger (364 ± 20 *vs.* 178 ± 8 μg/24 h; P = 0.011) due to shorter estimates of the GH half-life (16.7 ± 0.8 min).

IGF-I. Administration of MK-677 also resulted in dose-dependent increases in serum IGF-I concentrations (Table 1 and

TABLE 1. Analysis of 24-h GH concentration profiles and serum IGF-I concentrations at baseline and after 2 weeks of treatment with oral MK-677

MK-677 dose	24-h Mean GH conc. (μg/L)[a]		Peak no./24 h		Peak ht. (μg/L)[a]		Interpeak nadir (μg/L)[a]		IGF-I (μg/L)[a]	
	Baseline	2 weeks	Baseline	2 weeks	Baseline	2 weeks	Baseline	2 weeks	Baseline	2 weeks
Placebo (n = 10)	0.39 ± 0.07	0.42 ± 0.08	10.4 ± 0.7	10.8 ± 0.8	0.78 ± 0.2	0.79 ± 0.2	0.16 ± 0.03	0.17 ± 0.04	124 ± 15	123 ± 13
2 mg (n = 10)[b]	0.58 ± 0.08	0.61 ± 0.11	10.2 ± 0.8	10.6 ± 0.5	1.12 ± 0.2	1.10 ± 0.2	0.18 ± 0.04	0.18 ± 0.05	142 ± 20	155 ± 24
10 mg PM (n = 12)	0.43 ± 0.07	0.68 ± 0.09[c,d]	11.0 ± 0.7	11.3 ± 0.4	0.89 ± 0.2	1.26 ± 0.2[c]	0.12 ± 0.02	0.23 ± 0.03[c,d]	123 ± 13	164 ± 15[c,d]
10 mg AM (n = 12)	0.43 ± 0.07	0.80 ± 0.10[c,d]	11.0 ± 0.7	12.2 ± 0.6	0.89 ± 0.2	1.48 ± 0.2[c,d]	0.12 ± 0.02	0.32 ± 0.04[c,d,e]	112 ± 12	182 ± 15[c,d,e]
25 mg (n = 10)[b]	0.58 ± 0.08	1.14 ± 0.18[c,d]	10.2 ± 0.8	10.7 ± 0.6	1.12 ± 0.2	2.03 ± 0.3[c,d]	0.18 ± 0.04	0.53 ± 0.13[c,d]	141 ± 21	219 ± 32[c,d]
All subjects (n = 32)	0.46 ± 0.04		10.5 ± 0.4		0.92 ± 0.1		0.15 ± 0.01		128 ± 9	

Attributes of pulsatile GH release were assessed by the Cluster algorithm. The 10 mg AM row represents morning (AM) treatment (10 mg MK-677); all other doses were administered in the evening (PM).

[a] Values are expressed as geometric mean ± geometric SE.
[b] The same subjects received 2 and 25 mg in different treatment periods.
[c] Statistically significant change from pretreatment baseline ($P < 0.05$).
[d] The percent change from baseline is statistically different from that in the placebo group ($P < 0.05$).
[e] Statistically significant difference between response to 10 mg PM and that to 10 mg AM ($P < 0.05$).

FIG. 3. Percent changes from baseline (geometric mean ± geometric SE) of 24-h mean GH concentrations (micrograms per L) after 2 weeks of treatment (*upper panel*) and of serum IGF-I concentrations (micrograms per L) after 2 and 4 weeks of treatment (*lower panel*) with placebo (n = 10) and once daily oral evening (PM) MK-677 doses of 2 mg (n = 10), 10 mg (n = 12), and 25 mg (n = 10). *, Significant change from baseline ($P \leq 0.05$).

Fig. 3, *lower panel*). The highest dose (25 mg/day) increased IGF-I concentrations 55 ± 10% (−1.5–155%) at 2 weeks and 88 ± 16% (33–202%) at 4 weeks. Serum IGF-I concentrations increased in all subjects treated with 25 mg/day. In 8 of the 10 subjects, IGF-I concentrations were within the normal range for adults 21–25 yr old after 4 weeks of treatment compared to 2 of 10 subjects at baseline (Fig. 5). The mean IGF-I concentration of these 10 subjects increased into the young adult normal range after both 2 and 4 weeks.

Comparison of evening vs. morning treatment with 10 mg/day MK-677

GH. The 24-h mean GH profiles after evening and morning administration of placebo or 10 mg/day MK-677 are shown in Fig. 6. The 24-h mean GH percent increase from baseline was significant after both morning and evening oral admin-

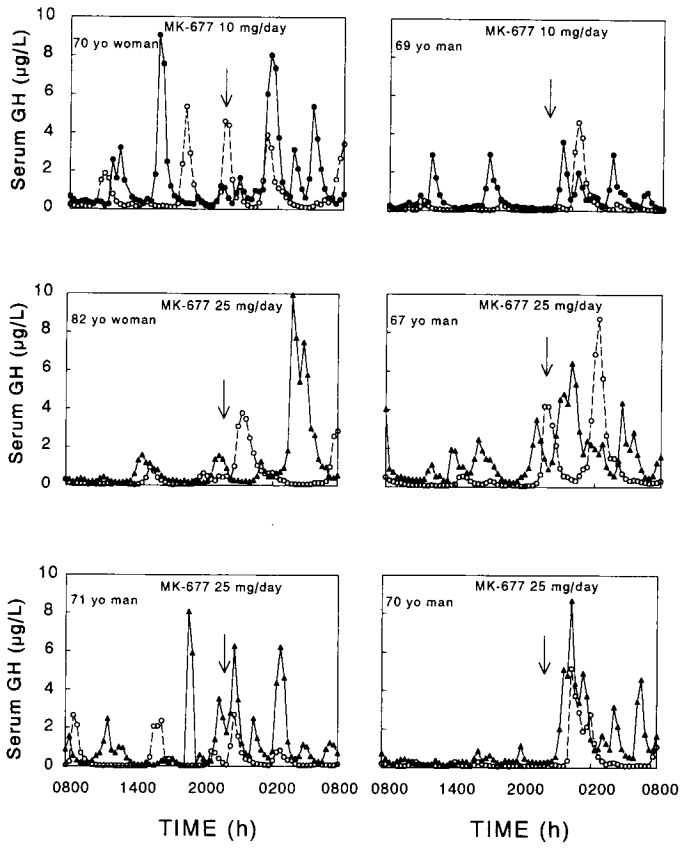

FIG. 4. Twenty-four-hour GH concentration profiles of six older subjects at baseline (○) and on day 14 of treatment with oral MK-677, administered once daily in the evening between 2200–2300 h. Blood samples were collected every 20 min. *Two upper panels*, 10 mg/day MK-677 (●); *four lower panels*, 25 mg/day MK-677 (▲).

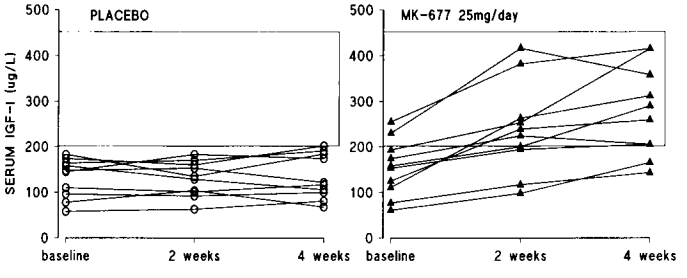

FIG. 5. Serum IGF-I concentrations (micrograms per L) of individual subjects at baseline and after 2 and 4 weeks of treatment with daily evening oral placebo (○; n = 10; *left panel*) or 25 mg MK-677 (●; n = 10; *right panel*). The *shaded zone* represents the assay normal range for adults 21–25 yr old (202–453 μg/L).

istration of 10 mg MK-677 for 2 weeks (Fig. 7, *top panel*). Although 24-h mean GH and GH peak height were numerically higher ($P = 0.09$ and $P = 0.11$, respectively), only the interpeak nadir GH concentrations were significantly different ($P = 0.01$; Table 1).

IGF-I. The IGF-I response after 2 weeks of 10 mg MK-677 morning treatment was significantly greater than that after evening treatment (164 ± 15 *vs.* 182 ± 15; $P < 0.05$; Table 1 and Fig. 7, *lower panel*).

Although there was a trend toward a greater response to the drug in older subjects, the response to MK-677 in individual subjects could not be predicted from any baseline measurement. For example, in the 10 subjects treated with 25 mg/day MK-677, no significant correlations ($P > 0.1$) were detected between the percent changes in either the mean 24-h mean GH concentration or the serum IGF-I concentration after 2 weeks of treatment and any of the following baseline measurements: mean 24-h GH concentration, IGF-I concentration, age, BMI, or age-BMI product.

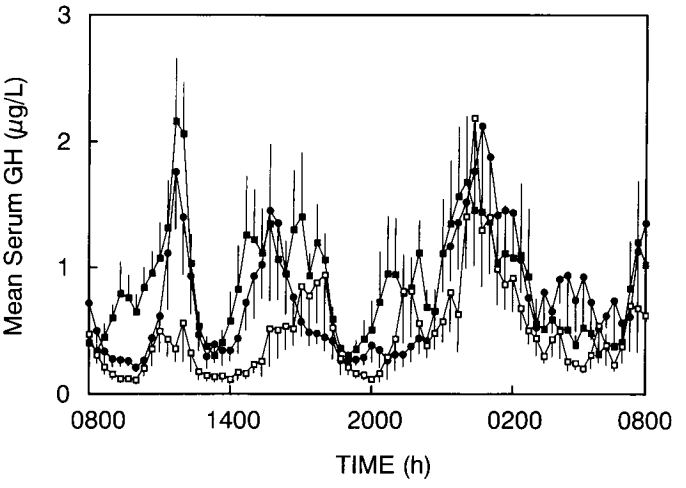

FIG. 6. Mean (±SE) serum GH concentrations (micrograms per L) in 12 older subjects at baseline (□) and after 2 weeks of daily oral treatment with 10 mg/day MK-677 administered in the morning (■) and 10 mg/day MK-677 administered in the evening (●). Morning doses were administered between 0700–0900 h, and evening doses were given between 2200–2300 h.

Other hormone and glucose results

Results of treatment with 2 mg MK-677 are not shown, as there were no associated significant changes in any parameter. Serum cortisol concentrations were determined every 20 min for 24 h in all panel A subjects. Mean serum cortisol was not significantly different from baseline after 25 mg MK-677 for 14 days (mean ± SE, 226.2 ± 5.5 *vs.* 231.8 ± 5.5 nmol/L; $P = 0.31$), and the ultradian pattern of serum cortisol concentrations was preserved (Fig. 8). Similarly, 24-h urinary free cortisol measurements were not significantly different (data not shown).

The mean serum PRL concentration increased 24% after the administration of 25 mg MK-677 (mean ± SE, 7 ± 0.5 to 8.6 ± 0.7 μg/L; $P \leq 0.01$ *vs.* baseline). No gender-associated differences in this response were detected. Serum T_3 and TSH were not significantly affected by MK-677 treatment. T_4 concentrations were significantly lower than baseline after 2 weeks of treatment with both placebo and 25 mg MK-677; the changes in the MK-677-treated group were not significantly different from those in the control group.

The effects of evening treatment with 10 and 25 mg MK-677 on IGF-II; IGF-binding protein-1 (IGFBP-1), -2, and -3; fasting glucose; and fasting insulin concentrations are shown in Table 2. MK-677 treatment was associated with statistically significant increases in fasting concentrations of IGF-II, IGF-BP-3, glucose, and insulin and decreases in IGFBP-1 and -2.

Pretreatment fasting blood glucose concentrations were below 8 mmol/L (144 mg/dL) in all subjects. MK-677 treat-

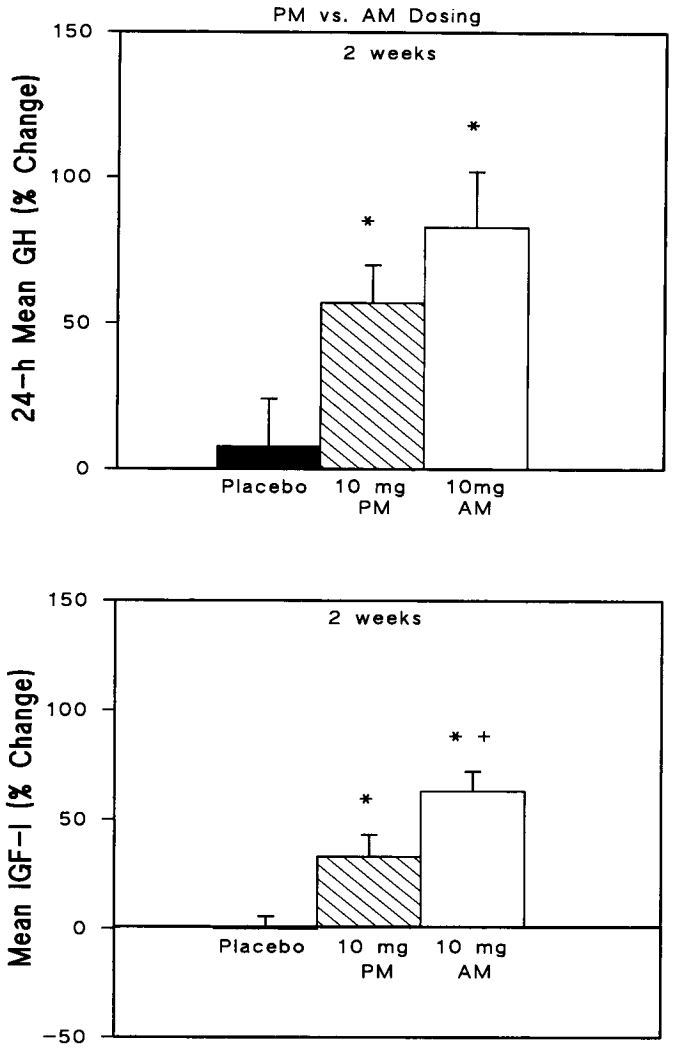

FIG. 7. Percent changes from baseline (geometric mean ± geometric SE) of 24-h mean GH concentrations (micrograms per L; *upper panel*) and serum IGF-I concentrations (micrograms per L; *lower panel*) after daily oral administration of 10 mg/day MK-677 for 2 weeks in the evening (PM) *vs.* morning (AM) administration of the same dose (n = 12). *, $P < 0.05$ *vs.* placebo (n = 10); +, $P < 0.05$, AM *vs.* PM dosing.

ment was associated with statistically significant dose-dependent increases in fasting blood glucose concentrations. After the administration of 25 mg MK-677, glucose concentrations had increased 25.3 ± 6.6% and 26.9 ± 6.8% above baseline by 2 and 4 weeks, respectively. Three of the 10 subjects who received 25 mg/day and 1 of the 12 who received 10 mg/day had increases in fasting glucose concentrations to above 8 mmol/L at either 2 or 4 weeks compared to none of the placebo-treated subjects. The highest fasting blood glucose level measured was 9.7 mmol/L in a subject who had received 10 mg MK-677 for 2 weeks in the evening. The change in glucose after administration of 25 mg MK-677 correlated with BMI (r = 0.77; $P < 0.01$).

Discussion

Once daily oral administration of MK-677 to older adults increased serum GH concentrations in a dose-dependent

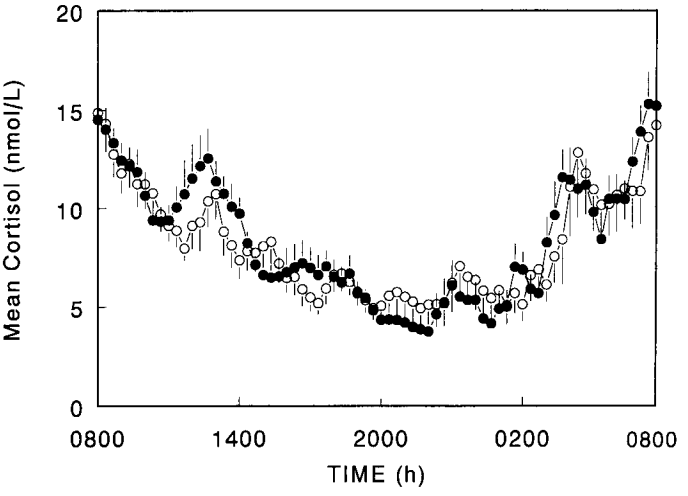

FIG. 8. Mean (±SE) serum cortisol concentrations of 10 older subjects at baseline (○) and on day 14 of treatment with oral 25 mg/day MK-677 (●). Blood samples were collected every 20 min. Multiply by 0.0362 to convert from Systeme International units (nanomoles per L) to micrograms per dL.

manner by enhancing pulsatile GH secretion. Serum IGF-I concentrations increased into the normal range for young adults in 8 of 10 subjects after 4 weeks of treatment with 25 mg/day MK-677, with a mean increase of 88%. Administration of MK-677 in the morning resulted in greater increases in serum IGF-I compared to the effects of evening treatment. This is the first study to demonstrate that serum GH and IGF-I concentrations can be increased in older adults by chronic administration of an oral GH secretagogue.

MK-677 is a spiropiperidine that mimics the actions of GHRP-6 (19, 20). Both MK-677 and GHRP-6 bind to the same unique non-GHRH, nonsomatostatin receptors in the pituitary to stimulate GH secretion via protein kinase C- and calcium-dependent mechanisms (17–20, 26–29). In addition to direct effects on the pituitary, MK-677 and GHRP-6 probably act on the hypothalamus (17). GHRP-6 has greatly reduced GH-releasing efficacy when administered to subjects with hypothalamic-pituitary disconnection (30, 31) and has a synergistic stimulatory effect on GH secretion when coadministered with GHRH (32). This suggests that it acts as a functional somatostatin antagonist and/or releases another, as yet unidentified, hypothalamic releasing factor (17). Both GHRP-6 and the GHRP-mimetic secretagogues stimulate the hypothalamic arcuate nucleus neurons in which GHRH is synthesized (33, 34). In sheep, hexarelin, a GHRP-6 analog, stimulates hypothalamic secretion of GHRH (35). In older adults, the GH response to GHRP-6 and GHRP-mimetic secretagogues is greater than that to GHRH (21, 36).

The net effect of these actions is that both GHRP-6 (16) and MK-677 enhance the preexisting pulsatile pattern of GH secretion. The primary effect of aging on GH secretion is to decrease the amplitude of GH pulses (5, 6). Oral administration of MK-677 partially reversed these effects of aging by increasing both the height of the GH pulses and the nadir GH concentrations between pulses. These higher serum GH concentrations resulted from an increased mass of GH secreted per pulse, with no significant change in the number of GH secretory pulses or the half-life of GH clearance. Similar

TABLE 2. Effect of oral evening treatment with placebo (PBO; n = 10), 10 mg/day MK-677 (n = 12), and 25 mg/day MK-677 (n = 10) for 2 and 4 weeks on physiological indicators of GH activity in olders adults

Dose/day	Baseline	2 weeks			4 weeks		
		Conc.	P^a	P^b	Conc.	P^a	P^b
IGF-II (mg/L)[c]							
PBO	425 ± 50	382 ± 39			405 ± 39		
10 mg	406 ± 28	419 ± 54					
25 mg	411 ± 26	452 ± 35			492 ± 31	<0.01	<0.05
IGFBP-1 (mg/L)[c]							
PBO	15.4 ± 2.0	14.7 ± 3.0			21.3 ± 3.6	<0.05	
10 mg	19.3 ± 3.7	15.3 ± 3.5					
25 mg	16.2 ± 4.2	16.5 ± 4.0			10.3 ± 2.6	<0.05	<0.01
IGFBP-2 (mg/L)[c]							
PBO[d]	655 ± 121	543 ± 108	<0.01		637 ± 105		
10 mg[d]	488 ± 67	392 ± 69	<0.01				
25 mg	593 ± 97	413 ± 66	<0.01		419 ± 79	<0.01	<0.01
IGFBP-3 (mg/L)[c]							
PBO	2.98 ± 0.3	2.58 ± 0.2			2.8 ± 0.3		
10 mg	2.55 ± 0.3	2.76 ± 0.4		<0.05			
25 mg	2.86 ± 0.2	3.26 ± 0.2	<0.05	<0.01	3.8 ± 0.2	<0.01	<0.01
Fasting glucose (mmol/L)[c,e]							
PBO[f]	5.2 ± 0.3	5.3 ± 0.3			5.3 ± 0.2		
10 mg	5.4 ± 0.2	6.3 ± 0.4	<0.01	<0.05			
25 mg	5.4 ± 0.3	6.7 ± 0.3	<0.01	<0.01	6.8 ± 0.4	<0.01	<0.01
Fasting insulin (mU/L)[g]							
PBO	8.3 ± 1.1	7.7 ± 1.4			8.2 ± 1.3		
10 mg	6.1 ± 1.8	8.7 ± 2.1					
25 mg	10.6 ± 0.9	12.9 ± 1.5			16.2 ± 2.5	<0.01	<0.05

[a] P values to assess the significance of changes from baseline.
[b] P values to assess the significance of the comparison with PBO.
[c] Values expressed as the mean ± SE.
[d] n = 8, n = 11 for PBO and 10 mg, respectively, due to insufficient blood sample for assay.
[e] Multiply by 18.018 to convert to mg/dL.
[f] n = 9 due to insufficient blood sample for assay.
[g] Values expressed as the geometric mean ± geometric SE.

effects in older adults were observed with a continuous iv infusion of a related compound, L-692,429 (37). The increased interpeak GH concentrations may be the result of increased basal (nonpulsatile) secretion or the larger GH secretory pulses (*i.e.* with larger pulses, the GH concentrations do not decline to baseline before the onset of the next pulse). Nevertheless, it is clear that the majority of GH secretion remained pulsatile during MK-677 treatment. Whereas basal secretion accounts for 38–69% of 24-h GH production in acromegaly (38), less than 20% of GH secretion was attributable to basal secretion during MK-677 treatment. As the metabolic actions of GH are modulated by the pattern of GH exposure to tissues (39), we speculate that enhancement of pulsatile GH release may produce fewer side-effects and greater benefits than the sustained increase in GH concentrations produced by daily sc GH administration (15), but this remains to be determined.

The greater stimulation of GH release by morning than evening MK-677 administration was an unexpected finding, although a similar conclusion had been suggested by two earlier studies. However, both of those studies unfortunately were limited by the use of less sensitive GH assays (16, 40). Somatostatin release is thought to be stimulated by food ingestion and decreased during sleep, resulting in greater GH secretion rates at night than during the day in fed subjects (1). Thus, our results support the hypothesis that MK-677 functionally antagonizes somatostatin action.

The effects of MK-677 are relatively specific to the GH-IGF-I axis. Blood concentrations of cortisol and thyroid hormones were not significantly affected by the drug. Mean serum PRL levels increased approximately 24% after daily treatment with 25 mg MK-677; this increase is statistically, although probably not clinically, significant. Consistent with the known effects of GH (2, 15, 41), circulating concentrations of IGF-II and IGFBP-3 increased, IGFBP-1 and -2 levels decreased, and fasting blood glucose and insulin concentrations increased. The changes in glucose were correlated with BMI, suggesting that the GH stimulatory effects of MK-677 may result in impaired glucose tolerance in individuals with predisposing risk factors. It is not known whether these effects on carbohydrate metabolism will persist with longer term administration of MK-677. If so, the usefulness of this drug could be limited. In GH-deficient subjects, GH replacement therapy results in insulin resistance at 6 weeks, but this effect is diminished at 26 weeks, by which time significant decreases in body fat had occurred (42). Thus, if enhanced GH secretion produced by chronic MK-677 treatment results in loss of body fat, then it is possible that insulin sensitivity will improve over time.

If prolonged treatment with oral GH secretagogues results in favorable effects on body composition, functional capacity, and serum lipids, then such secretagogues may have a therapeutic role in a variety of conditions where the pituitary is intact but GH secretion is reduced. Candidates for treatment could include older adults with musculoskeletal impairment and patients with catabolic conditions for which

short term GH therapy has been shown to have beneficial effects, including human immunodeficiency virus/acquired immunodeficiency syndrome wasting (43), burns (44), chronic obstructive pulmonary disease (45), and those requiring parenteral nutrition after major surgery (46). The majority of children with GH deficiency and short stature have normal or near-normal GH release in response to GHRH (47), indicating a dominant hypothalamic rather than pituitary cause of their GH deficiency. As chronic treatment with parenteral GHRH significantly increases growth velocity in these children (48), oral GH secretagogues may be efficacious in this setting as well. Consistent with this possibility, we recently found that oral administration of MK-677 increases circulating IGF-I concentrations and enhances pulsatile GH release in selected GH-deficient adults who were treated with GH during childhood (49).

In summary, once daily oral treatment of healthy older adults with the GH secretagogue MK-677 for up to 4 weeks enhanced pulsatile GH release and produced dose-responsive increases in circulating concentrations of GH and IGF-I. At a dose of 25 mg/day, MK-677 restored serum IGF-I concentrations in most older subjects to levels seen in young adults. Administration of 10 mg/day MK-677 in the morning increased IGF-I to a greater extent than treatment in the evening. Further studies are needed to establish the long term safety of this drug, particularly its effect on glucose and insulin levels, and to determine the effect of long term administration of such compounds in a variety of conditions associated with GH deficiency. Our findings suggest that oral GH secretagogues may provide significant therapeutic advantages over administration of GH in these conditions.

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