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Kisspeptin-54 Stimulates the Hypothalamic-Pituitary Gonadal Axis in Human Males

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Context: Mutation of the G protein-coupled receptor 54 is associated with a failure of reproductive function. The endogenous neuropeptide agonist for G protein-coupled receptor 54, kisspeptin, potently stimulates the hypothalamic-pituitary-gonadal axis in rodents and primates.

Objective: The present study was designed to determine the effects of elevating circulating kisspeptin levels on LH, FSH, and testosterone in male volunteers.

Design: This was a double-blind, placebo-controlled, crossover study.

Setting: This was a hospital-based study.

Participants: Male volunteers (n = 6) were recruited.

Interventions: Each volunteer received a 90-min iv infusion of kisspeptin-54 (4 pmol/kg·min) and a control infusion of saline (0.9%) in random order.

Main Outcome Measure: Plasma LH, FSH, and testosterone concentrations were measured.

Results: Kisspeptin-54 infusion significantly increased plasma LH, FSH, and testosterone concentrations compared with saline infusion (mean 90-min LH: kisspeptin, 10.8 ± 1.5 *vs.* saline, 4.2 ± 0.5 U/liter, $P < 0.001$; mean 90-min FSH: kisspeptin, 3.9 ± 0.7 *vs.* saline, 3.2 ± 0.6 U/liter, $P < 0.001$; mean 180-min testosterone: kisspeptin, 24.9 ± 1.7 *vs.* saline, 21.7 ± 2.2 nmol/liter, $P < 0.001$). The plasma half-life of kisspeptin-54 was calculated to be 27.6 ± 1.1 min. The mean metabolic clearance rate was 3.2 ± 0.2 ml/kg·min, and the volume of distribution was 128.9 ± 12.5 ml/kg.

Conclusion: Elevation of plasma concentrations of kisspeptin in human males significantly increases circulating LH, FSH, and testosterone levels. Kisspeptin infusion provides a novel mechanism for hypothalamic-pituitary-gonadal axis manipulation in disorders of the reproductive system. **(*J Clin Endocrinol Metab* 90: 6609–6615, 2005)**

KISSPEPTIN IS A 54-amino acid peptide, encoded by the *KiSS-1* gene (1–4), which activates the previously orphan G protein-coupled receptor GPR54 (4). Recent evidence suggests the kisspeptin/GPR54 system is a key regulator of reproduction. GPR54-deficient ($GPR54^{-/-}$) mice have abnormal sexual development, have low circulating gonadotropin concentrations, and are infertile (5, 6). GPR54 mutations have been shown to cause hypogonadotrophic hypogonadism in humans (5, 7, 8).

Endogenous forms of kisspeptin 54, 14, and 13 amino acids in length have been isolated from human placenta. The common C-terminal decapeptide shared by these forms, kisspeptin-10, is the minimum sequence necessary for receptor activation (2–4) and is secreted by cultured human trophoblasts (9). All kisspeptin fragments, including kisspeptin-10, have a similar affinity and efficacy *in vitro* at the GPR54 (4).

Central or peripheral administration of kisspeptin stimulates the hypothalamic-pituitary-gonadal (HPG) axis in animal models. Intracerebroventricular injection of kisspeptin-10 or kisspeptin-54 potently increases circulating concentrations of LH and FSH in both male and female prepubertal and adult rodents (10–14). Intracerebroventricular kisspeptin-10 has been shown to potently stimulate LH release in agonadal juvenile male monkeys (15). Peripheral administration of kisspeptin-10 also stimulates plasma LH and FSH in prepubertal and adult rats and LH in agonadal juvenile male monkeys (12, 13, 15, 16). In addition, icv or ip kisspeptin-10 increases circulating testosterone in adult male rats (12). Chronic central kisspeptin administration induces precocious puberty in female rats (17) and restores pubertal activation in a rat model of undernutrition (18). The blockade of local kisspeptin action in the preoptic area of the hypothalamus with a specific monoclonal antibody to rat kisspeptin completely abolishes the proestrous LH surge and inhibits estrous cyclicity in female rats (19).

The stimulatory effects of kisspeptin on the HPG axis appear to be mediated via the hypothalamic GnRH system. The central and peripheral effects of kisspeptin on LH and FSH are blocked by GnRH antagonists (10, 16, 20). Peripheral kisspeptin-54 or central kisspeptin-52 induce c-*fos* immunoreactivity (IR) in the majority of GnRH neurons in the rat hypothalamus (16, 20). Kisspeptin-10 stimulates the release of GnRH from *in vitro* hypothalamic explants (12) and increases the GnRH concentration in cerebrospinal fluid in

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Abbreviations: AcN, Acetonitrile; FPLC, fast protein liquid chromatography; GPR54, G protein-coupled receptor 54; HPG, hypothalamic-pituitary-gonadal; IR, immunoreactivity; MCR, metabolic clearance rate; RFRP, RF amide-related peptide; TFA, trifluoroacetic acid; VD, volume of distribution.

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sheep (21), suggesting that the action of kisspeptin on the HPG axis is mediated via GnRH release. Hypothalamic *KiSS-1* expression is regulated by circulating sex steroids in rodents and primates, suggesting that kisspeptin is involved in the HPG negative feedback cycle (11, 15, 20, 22, 23).

The effects of kisspeptin-54 administration to human subjects have not been investigated to date. The present study was designed to determine the effects of iv administration of kisspeptin-54 in normal male volunteers on plasma LH, FSH, testosterone, and inhibin B levels and to determine the pharmacokinetics of kisspeptin-IR.

Subjects and Methods

Kisspeptin-54

Human kisspeptin-54 was synthesized by the Advanced Biotechnology Centre (Imperial College, London, UK). The product was purified to homogeneity by reverse-phase HPLC to give more than 95% purity. Electrospray mass spectroscopy of the kisspeptin-54 (comprising one major peak with an average molecular weight of 5848) (supplemental Fig. 1, published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org/) and amino acid analysis were used to confirm the identity of the peptide. The stability of kisspeptin-54 was analyzed from the infusate during and after infusions. This resulted in one major peak on fast protein liquid chromatography (FPLC) (method described below) with a recovery more than 85% of the expected concentration. The Limulus Amoebocyte Lysate assay test (Associates of Cape Cod, Liverpool, UK) for pyrogen was negative, and the peptide was sterile on culture (Microbiology Department, Hammersmith Hospital, London, UK). Toxicology of the kisspeptin-54 was tested in C57BL/6 mice. Mice were given an ip injection of kisspeptin-54 (2 nmol/mouse) or saline (n = 10 per group) and killed 48 h later, and tissues (heart, lung, stomach, pancreas, small bowel, large bowel, liver spleen, and kidney) were immediately removed. Histological examination (Prof. G. Stamp, Histopathology Department, Imperial College) confirmed no abnormalities. Bioactivity of the peptide was confirmed by ip administration of kisspeptin-54 (2 nmol) to C57BL/6 mice. This resulted in a significant 3-fold increase in LH release at 20 min after injection compared with saline-injected mice (data not shown). Kisspeptin-54 was used for all studies, because current evidence suggests that it is more efficacious than shorter kisspeptin fragments *in vivo* (12, 16).

Subjects

Eleven healthy male subjects participated in study 1 and six in study 2. Mean age was 27.4 ± 1.2 (mean ± SEM) yr, and body mass index was 24.4 ± 0.6 kg/m^2. Subjects gave written informed consent, and ethical approval was obtained from the Hammersmith and Queen Charlotte's & Chelsea Hospitals Research Ethics Committee (no. 04/Q0406/151). Studies were performed in accordance with the Declaration of Helsinki. Subjects were taking no medication and had no allergies or abnormalities on physical examination and electrocardiogram. They had no evidence of abnormal renal or liver function, and baseline hemoglobin, glucose, LH, FSH, testosterone, and prolactin levels were normal. Subjects refrained from alcohol, strenuous exercise, and sexual activity for 24 h before each infusion.

Protocol

To establish a dose response for the effects of iv infusion of kisspeptin-54 on LH, FSH, and testosterone in male volunteers. This study was blinded to the subjects but not the investigators. Each volunteer received a 90-min infusion of kisspeptin-54. On the morning of each study, a cannula was inserted into a large forearm vein in both arms: one for collection of blood, and the second for infusion of kisspeptin-54. The subjects remained supine throughout the study. All subjects were given a standard breakfast (24) on arrival, to minimize any changes in gonadotrophins due to differences in nutritional status. Kisspeptin-54 was dissolved in saline containing gelofusine (5% vol/vol) (B. Braun Medical, Sheffield, UK) to minimize peptide adsorption to the infusion system (25) and was infused over 90 min. During the first 30 min of infusion, the volunteers were infused with 0.125, 0.25, 0.5, 1, 2, 4, 8, 12, 18, 27, or 40 pmol/kg·min. The infusion rate for each volunteer was then halved for the remaining 60 min of each infusion. This dosing regimen was designed to achieve a steady-state concentration of serum kisspeptin during the infusion period (26). Each dose was administered to three subjects. Blood was sampled on arrival (t = −30) and then at t = 0, after which the infusion was started. Blood was then sampled at t = 10, 20, 30, 40, 50, 60, 70, 80, 90, 92, 95, 100, 105, 110, 120, 150, 180, 210, and 240 min (Fig. 1). Blood was collected into lithium-heparin tubes (LIP, Cambridge, UK) containing 5000 kallikrein inhibitor units (0.2 ml) aprotinin (Trasylol, Bayer, Newbury, UK) and stored on ice. After centrifugation, plasma was immediately separated and stored at −20 C until measurement of LH, FSH, and kisspeptin-IR at all time points and testosterone and SHBG at t = −30, 0, 30, 60, 90, 120, 150, 180, 210, and 240. Blood pressure and pulse were measured every 15 min for the first 180 min and every 30 min thereafter. Subjects were asked whether they felt any nausea or other side effects every 30 min. Samples of kisspeptin-54 infusate were collected before and after termination of the infusion, and the kisspeptin-IR measured by RIA to verify the infusion rate.

To establish the time course of the effects of iv infusion of kisspeptin-54 in male volunteers on LH, FSH, and testosterone. This was a double-blind, placebo-controlled, crossover study. All subjects were given a standard breakfast on arrival and lunch (24) at 240 min. Each subject (n = 6) received an iv infusion of kisspeptin-54 and a control infusion of saline (0.9%) at least 3 d apart, in random order. The dose of kisspeptin-54 used was 4 pmol/kg·min (infusion rate of 0.3 ml/min) for the first 30 min, followed by half this dose (2 pmol/kg·min) and rate (0.15 ml/min) for the remaining 60 min of the infusion. This dose of kisspeptin-54 was chosen because it was the lowest infusion dose that maximally increased LH levels in male volunteers in study 1. Rates of saline infusion were the same as for kisspeptin-54. The protocol for this study was identical to that in study 1 except that blood was also sampled at the additional time points t = 300, 360, 420, 480, and 1440 min. Plasma kisspeptin-IR, LH, FSH, and testosterone were measured for all additional time points.

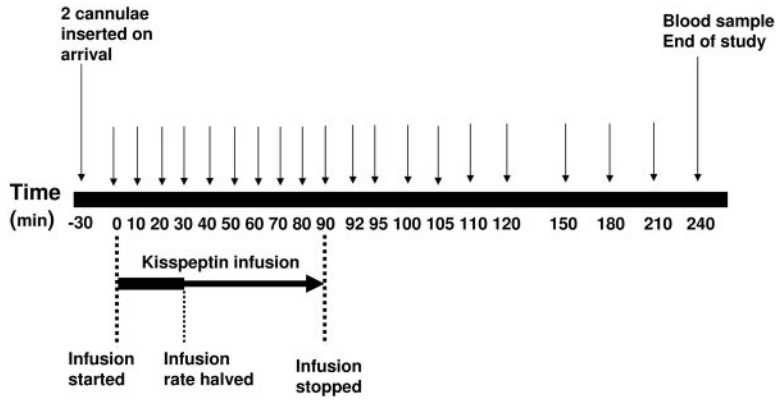

FIG. 1. Diagram showing the kisspeptin-54 infusion protocol.

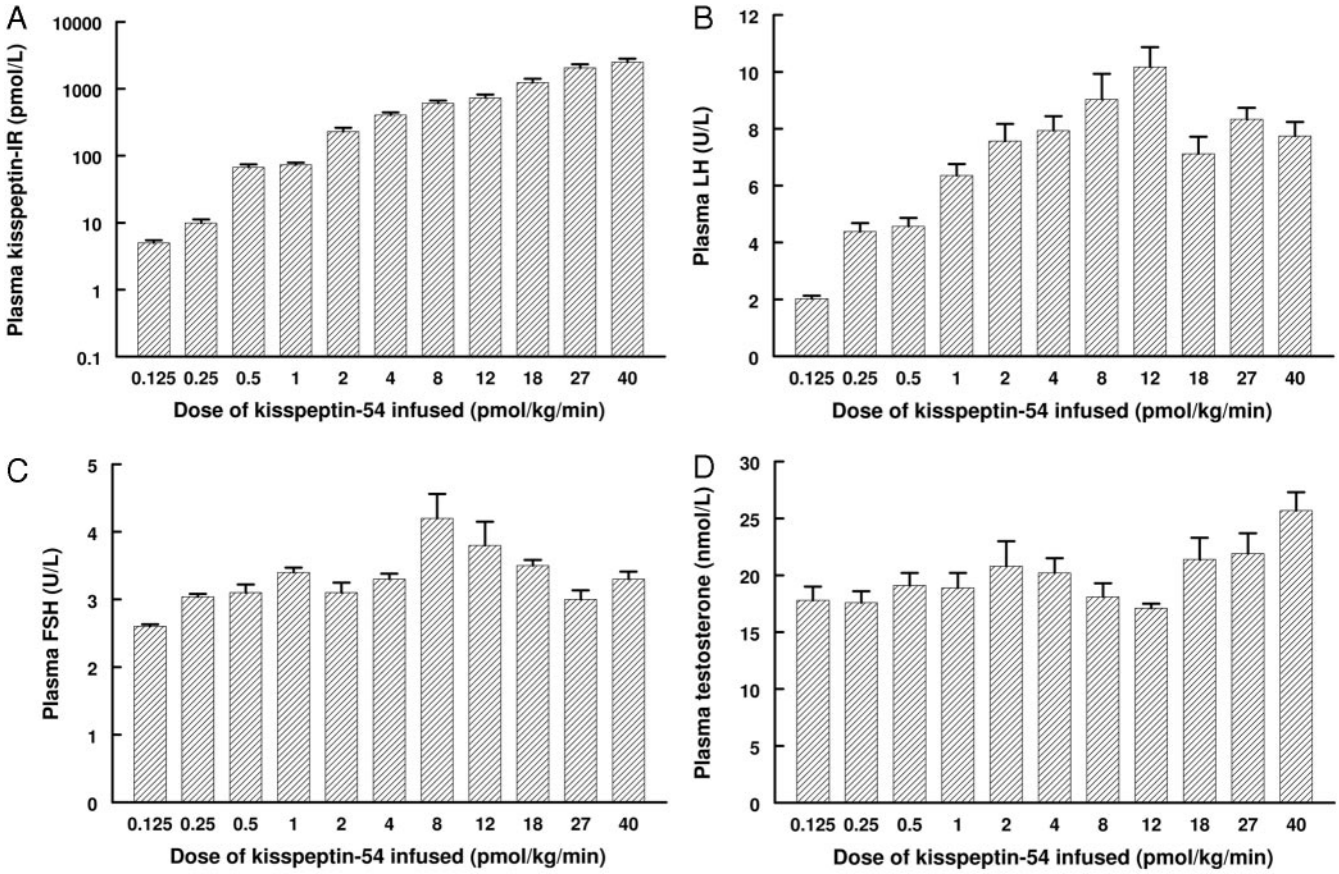

FIG. 2. Effects of kisspeptin-54 infusion in male volunteers on mean over time of plasma kisspeptin-IR (A), LH (B), FSH (C), and testosterone (D) at kisspeptin-54 infusion doses of 0.125, 0.25, 0.5, 1, 2, 4, 8, 12, 18, 27, and 40 pmol/kg·min. Mean over time plasma values were calculated as the mean plasma value at t = −30, 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90 min. Three male volunteers were infused with each dose of kisspeptin-54.

Plasma inhibin B was also measured at t = 0, 30, 60, 90, 120, 150, 180, 210, and 240. Subjects were also asked to complete a visual analog score [adapted from Both *et al.* (27)] to determine their degree of sexual arousal rated on a linear 7-point scale, from "not sexually aroused at all" to "very strongly sexually aroused" at time t = 0, 30, 60, 90, 120, 150, 180, 210, 240, 300, 360, 420, and 480 min.

Calculations

The decay curve of kisspeptin-IR from kisspeptin-54 infusions in study 2 was converted to natural logarithms and plotted against time for each subject. The resulting straight-line plot was used to derive the half-time of disappearance ($t_{1/2}$) for infused kisspeptin-54 for each subject. The metabolic clearance rate (MCR) of kisspeptin-IR was calculated for each volunteer from the steady-state concentration (taken as the mean plateau kisspeptin-IR at t = 40, 50, 60, 70, 80, and 90 min) and the measured infusion rate at which this concentration was stable, where MCR = infusion rate/CSS, where CSS is the steady-state concentration. The apparent volume of distribution (VD) was calculated from the $t_{1/2}$ and MCR using the formula $VD = MCR \times t_{1/2} \times 1.44$ (24). Mean ± SEM values for $t_{1/2}$, MCR, and VD were calculated from the results of the six kisspeptin-54 infusions in study 2.

Analytical methods

LH, FSH, testosterone, and inhibin B. LH, FSH, and total testosterone were measured using the automated Chemiluminescent Microparticle Immunoassays (ARCHITECT LH, ARCHITECT FSH, and ARCHITECT testosterone; Abbott Laboratories, Abbott Park, IL). SHBG was measured using a solid-phase two-site chemiluminescent immunometric assay (DPC Immulite; Euro/DPC, Llanberis, UK). Inhibin B was measured by ELISA (Diagnostic Systems Laboratories, Oxford, UK). The normal ranges for males are as follows: LH, 4–14 U/liter; FSH, 1.5–8 U/liter; testosterone, 10–28 nmol/liter; SHBG, 20–40 nmol/liter; and inhibin B, less than 400 pg/ml. The intraassay and interassay coefficients of variation were 2.5 and 3.6% for the LH assay, 2.9 and 3.7% for the FSH assay, 4.5 and 4.1% for the total testosterone assay, 6.5 and 8.7% for the SHBG assay, and 4.9 and 6.7% for the inhibin B assay, respectively.

Kisspeptin RIA. Antibody GQ2 was raised in a sheep immunized with synthetic human kisspeptin-54 (Bachem UK, Merseyside, UK) conjugated to BSA by glutaraldehyde and used at a final dilution of 1: 3,500,000. The antibody cross reacted 100% with human kisspeptin-54, kisspeptin-14, and kisspeptin-10 and less than 0.01% with any other related human RF amide peptide, including prolactin releasing peptide, RF amide-related peptide (RFRP1), RFRP2, RFRP 3, neuropeptide FF, and neuropeptide AF. The ^{125}I-kisspeptin-54 label was prepared using the iodogen method and purified by reverse-phase HPLC on a C18 column (Waters, Milford, MA) over a 15–45% 90-min gradient of acetonitrile (AcN)/water/0.1% trifluoroacetic acid (TFA). The specific activity of kisspeptin label was 56 Bq/fmol. The assay was performed in duplicate using dilutions of neat plasma in 0.7 ml of 0.06 M phosphate buffer (pH 7.2) containing 0.3% BSA and incubated for 3 d at 4 C. Free and antibody-bound label were then separated by charcoal adsorption. The assay detected changes of 2 pmol/liter of plasma kisspeptin with a 95% confidence limit. The intraassay and interassay coefficients of variation were 8.3 and 10.2%, respectively.

Analysis of kisspeptin-IR in human plasma. Kisspeptin-IR was analyzed in human plasma from volunteers (n = 3) in study 2 during their kisspeptin infusion day at t = 60 min (plateau of kisspeptin infusion) and at t = 110 min (20 min after the kisspeptin-54 infusion had been stopped). Peptide

was extracted from plasma using Sep-Pak C18 cartridges (Waters, Hertfordshire, UK) as described previously (28). Briefly, Sep-Pak C18 cartridges were activated using 10 ml of 100% methanol and then 20 ml water. A 2-ml volume of plasma was mixed with 2 ml of 0.1 M HCl and loaded onto the cartridge. The cartridge was then washed with 10 ml of 4% acetic acid (vol/vol). The Sep-Pak bound sample was eluted in 1.5 ml methanol, and this eluant was dried in a Savant vacuum centrifuge and reconstituted in water plus 0.05% TFA (vol/vol) for FPLC. Peptide extracts from plasma were dissolved in 0.6 ml distilled water plus TFA 0.05% (vol/vol). Of this volume, 0.5 ml was fractionated by FPLC on a high-resolution reverse-phase (Pep RPC 1 ml high resolution) C18 column (Pharmacia, Uppsala, Sweden) as described previously (28). The column was eluted with a 10–40% gradient of AcN/water 0.05% (vol/vol) TFA over 40 min, and fractions were collected at 1-min intervals. The kisspeptin-IR in all fractions was determined by RIA. The remaining 0.1 ml was used to calculate the percentage recovery. Recovery was calculated as kisspeptin-IR recovered from each sample compared with kisspeptin-IR loaded onto the FPLC column and was expressed as a percentage.

Statistical analysis

All results are presented as mean ± SEM. In study 1, linear regression analysis was used to calculate the correlation between infusion rates of kisspeptin-54 and mean plateau plasma kisspeptin-IR.

For study 2, LH, FSH, testosterone, inhibin B, and visual analog scores on the kisspeptin-54 infusion day were compared with those on the saline infusion day using a two-way repeated-measure ANOVA. In all cases, $P < 0.05$ was considered significant.

Results

No nausea or other side effects were reported by any subject during any of the infusions. Kisspeptin-54 had no effect on blood pressure or pulse rate at any of the doses infused (data not shown).

Study

Dose response for the effects of iv infusion of kisspeptin-54 on LH, FSH, and testosterone in male volunteers. Infusions of kisspeptin-54 to male volunteers resulted in a rise in plasma kisspeptin-IR to a plateau level by t = 40 min, and kisspeptin-IR remained constant until t = 90 min (data not shown). Increasing infusion rates of kisspeptin-54 in male volunteers resulted in a dose-dependent increase in mean plasma kisspeptin-IR over time (calculated as the mean plasma kisspeptin-IR at t = −30, 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90 min) (Fig. 2A) and correlated with mean plateau plasma kisspeptin-IR (calculated as the mean plasma kisspeptin-IR at t = 40, 50, 60, 70, 80, and 90 min; $r^2 = 0.97$; $P < 0.001$) (Fig. 3).

Infusion of kisspeptin-54 in male volunteers at an infusion rate above 0.25 pmol/kg·min resulted in an increase in mean LH over time (calculated as the mean plasma LH at t = −30, 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90 min) (Fig. 2B). There was a dose-dependent increase in mean LH over time from an infusion rate of 0.25 pmol·kg^{-1}·min^{-1} up to 12 pmol/kg·min. Although administration of kisspeptin-54 at infusion rates higher than 12 pmol/kg·min did not result in a higher mean LH concentration, the LH rise appeared to be sustained for a longer period (data not shown). The LH at t = 90 min (end of the infusion) is plotted against the mean plasma plateau kisspeptin-IR achieved for each volunteer in Fig. 4. This shows that a plasma kisspeptin-IR above 300 pmol/liter results in a near maximal LH release. The aim of the kisspeptin-54 infusion in study 2 was to achieve a plasma kisspeptin-IR of approximately 300 pmol/liter. An infusion rate of 4 pmol/kg·min was therefore used in study 2 using the data from study 1 as a guide.

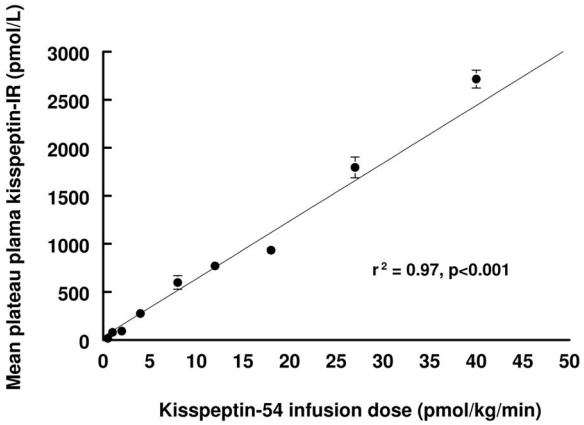

FIG. 3. Graph showing mean plasma plateau kisspeptin-IR achieved in male volunteers during 90-min iv kisspeptin-54 infusions. Plateau plasma kisspeptin-IR was achieved in subjects 40 min into the infusion and remained constant until the end of the infusion at 90 min. The mean plasma plateau kisspeptin-IR was calculated for each infusion from the mean of the plasma kisspeptin-IR achieved in male volunteers during the infusion at t = 40, 50, 60, 70, 80, and 90 min.

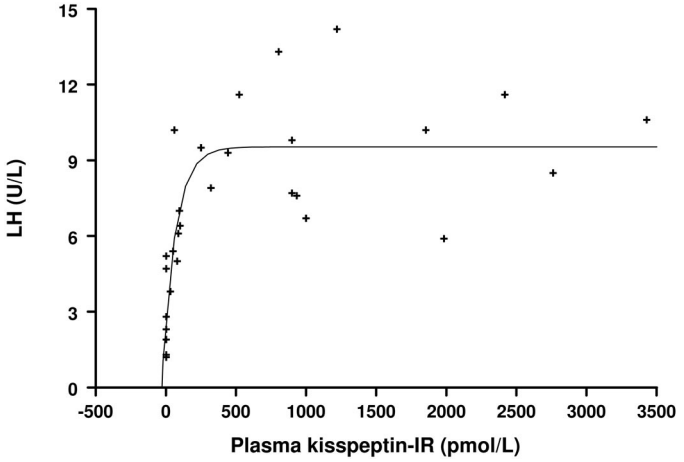

FIG. 4. Graph showing plasma LH levels at t = 90 min *vs.* mean plateau plasma kisspeptin-IR achieved during the infusion for each male volunteer.

Infusion of kisspeptin-54 to male volunteers increased mean FSH over time (calculated as the mean plasma FSH at t = −30, 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90 min) at a kisspeptin-54 infusion rate above 0.25 pmol/kg·min (Fig. 2C). There was also a trend toward an increase in mean testosterone over time (calculated as the mean plasma testosterone at t = −30, 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90 min) (Fig. 2D). However, these effects were not dose dependent. SHBG levels did not significantly change within an individual during any of the infusions (data not shown).

Time course of the effects of iv infusion of kisspeptin-54 in male volunteers on LH, FSH, and testosterone. Plasma kisspeptin-IR was undetectable on saline infusion days. Infusion of kisspeptin-54 (4 pmol/kg·min) to male volunteers resulted in a rise in plasma kisspeptin-IR to a mean plateau level of 303 ±

10 pmol/liter (Fig. 5A). After termination of the infusion, kisspeptin-IR returned to baseline by t = 300 min. Kisspeptin-54 infusion resulted in a significant increase in LH, FSH, and testosterone release compared with saline infusion (mean 90-min LH: kisspeptin, 10.8 ± 1.5 *vs.* saline, 4.2 ± 0.5 U/liter, $P < 0.001$; mean 90-min FSH: kisspeptin, 3.9 ± 0.7 *vs.* saline, 3.2 ± 0.6 U/liter, $P < 0.001$; mean 180-min testosterone: kisspeptin, 24.9 ± 1.7 *vs.* saline, 21.7 ± 2.2 nmol/liter, $P < 0.001$) (Fig. 5, B, C, and E). SHBG levels did not significantly change within an individual during any of the infusions (data not shown).

Infusion of kisspeptin-54 in male volunteers had no effect

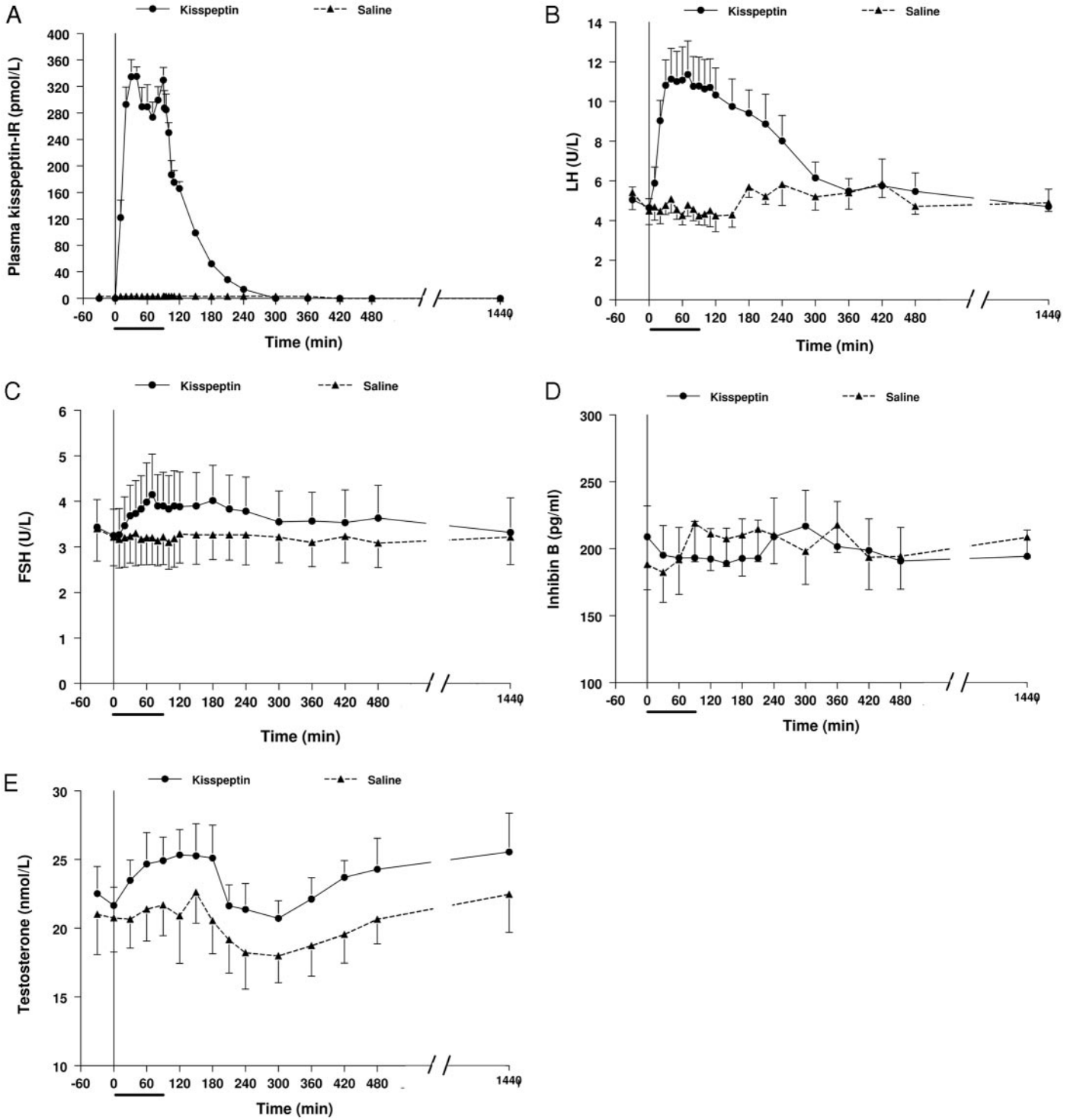

FIG. 5. Effects of kisspeptin-54 (4 pmol/kg·min) or saline infusion in male volunteers (n = 6) on mean plasma kisspeptin-IR (A), LH (B), FSH (C), inhibin B (D), and testosterone (E). Each volunteer received both kisspeptin-54 and saline infusions and thus acted as their own control.

on inhibin B release (Fig. 5D) or sexual arousal scores (mean arousal score: kisspeptin, 1.1 ± 0.1 *vs.* saline, 1.5 ± 0.2; $P = 0.3$).

Reverse-phase FPLC

Reverse-phase FPLC was used to further analyze kisspeptin-IR extracted from plasma by Sep-Pak cartridge. All columns had a recovery greater than 60%. In each plasma extract, the kisspeptin RIA detected a single immunoreactive peak corresponding to synthetic kisspeptin-54. A representative profile is shown in Fig. 6.

Pharmacokinetics

Plasma kisspeptin-IR was below the detection limit of the assay before infusion of kisspeptin-54 (<2 pmol/liter). After achievement of a plateau level of kisspeptin-IR, the course of kisspeptin-IR disappearance followed first-order kinetics. The plasma half-life of kisspeptin-IR was calculated to be 27.6 ± 1.1 min. The mean MCR was 3.2 ± 0.2 m/kg·min, and the VD was 128.9 ± 12.5 ml/kg.

Discussion

The kisspeptin/GPR54 system has been shown recently to be critical to normal reproductive development in rodents and man (5–8). Kisspeptin potently stimulates the HPG axis in rodents and primates (10–16), but there are no studies demonstrating the effects of kisspeptin administration in humans. Our data demonstrates that systemic administration of kisspeptin-54 to male volunteers results in a significant increase in plasma LH, FSH, and testosterone. In our dose finding study, plasma kisspeptin-IR achieved in male volunteers ranged from undetectable (<2 pmol/liter) to levels of a similar magnitude to the dramatically elevated kisspeptin-IR observed in human pregnancy (~3000 pmol/liter) (29). No side effects were observed at any dose. Administration of 4 pmol/kg·min of kisspeptin-54 resulted in a mean plateau plasma kisspeptin-IR of approximately 300 pmol/liter, which resulted in a mean peak LH of 11.4 ± 1.7 U/liter. Higher levels of kisspeptin-IR appeared to result in a more sustained effect on peak LH but did not further increase the magnitude of the LH peak. Similar to GnRH infusion in humans, increasing plasma kisspeptin-IR increased FSH and showed a trend to increasing testosterone in male volunteers, but these effects did not appear to be dose dependent (30). This is consistent with previous studies in rodents that demonstrate that kisspeptin appears to have a more potent effect on LH release than FSH release (12, 14).

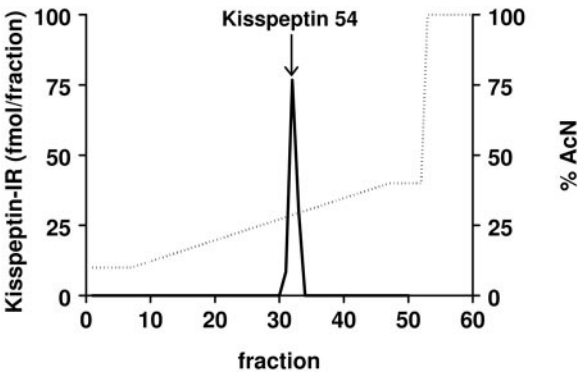

FIG. 6. Representative elution profile of kisspeptin-IR extracted from 2 ml plasma by Sep-Pak cartridge and fractionated by reverse-phase FPLC. *Dotted line* represents percentage AcN. Kisspeptin 54, Elution position of synthetic kisspeptin-54 indicated by an *arrow*.

Study 2 was a double-blind randomized controlled trial, with each subject receiving saline or kisspeptin on separate days. On the saline infusion day, plasma kisspeptin-IR was below the detection limit of our assay (<2 pmol/liter). This is consistent with a previous report showing that basal plasma kisspeptin-IR in males is 1.3 ± 0.1 pmol/liter (29). The infusion rate of kisspeptin-54 for this study was chosen to achieve a mean plateau kisspeptin-IR of approximately 300 pmol/liter, the lowest plasma kisspeptin-IR shown in study 1 to maximally increase LH. There was a significant rise in LH, FSH, and testosterone but no increase in inhibin B levels. Inhibin B is the major circulating inhibin in man and inhibits the secretion of FSH via a negative feedback mechanism (30). Although the FSH levels were increased by kisspeptin-54 infusion, the magnitude or duration of this rise may have been insufficient to alter inhibin B levels. In addition, inhibin B levels are also affected by factors other than FSH, including spermatogenesis. The pharmacokinetics of circulating kisspeptin-IR was assessed. The MCR of kisspeptin-IR was similar in magnitude to the normal glomerular filtration rate. The VD was a little over double the total body water volume and suggests that kisspeptin-IR is not extensively tissue bound. No endogenous kisspeptin-IR was detectable in plasma by RIA before the onset of infusions. In study 1, plasma kisspeptin-IR achieved correlated strongly with the dose of kisspeptin-54 infused, suggesting that the pharmacokinetics of kisspeptin-IR do not alter across the dose range studied.

Our results demonstrate that systemic administration of kisspeptin-54 can acutely increase circulating levels of LH, FSH, and testosterone release in human males. This is in keeping with previous studies in rodents and primates suggesting that the kisspeptin system may operate similarly in mammals (12, 14–16). It has been shown that chronic central administration of kisspeptin-54 can induce precocious puberty in female rats (17) and restore pubertal activation in undernourished rats (18). The kisspeptin system may have a therapeutic value in man, for example, in stimulating puberty. In humans, circulating kisspeptin-IR is 7000-fold higher than basal levels during the third trimester of pregnancy, but the role of plasma kisspeptin-IR in pregnancy is still unclear (29). It is possible that these high circulating concentrations chronically stimulate GnRH release, leading to a down-regulation of the HPG axis, as is seen with synthetic GnRH agonists (31). In summary, we have demonstrated that systemic administration of kisspeptin-54 to male volunteers results in a significant increase in circulating LH, FSH, and testosterone. Additional work is required to assess the therapeutic possibilities of kisspeptin.

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