Desensitization of gonadotropin responses to kisspeptin in the female rat: analyses of LH and FSH secretion at different developmental and metabolic states

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Roa J, Vigo E, García-Galiano D, Castellano JM, Navarro VM, Pineda R, Diéguez C, Aguilar E, Pinilla L, Tena-Sempere M. Desensitization of gonadotropin responses to kisspeptin in the female rat: analyses of LH and FSH secretion at different developmental and metabolic states. Am J Physiol Endocrinol Metab 294: E1088–E1096, 2008. First published April 15, 2008; doi:10.1152/ajpendo.90240.2008.—Kisspeptins have emerged as potent elicitors of gonadotropin secretion and, therefore, putative targets for pharmacological intervention. In this context, desensitization of gonadotropin responses to continuous administration of kisspeptins has begun to be characterized, but information so far available is mostly restricted to LH responses in males, whereas the similar phenomenon in females, of obvious therapeutic interest, remains virtually unexplored. We report herein LH and FSH responses to continuous intracerebral administration of kisspeptin in female rats at different developmental and metabolic states. Infusion of kisspeptin-10 to adult female rats induced a transient elevation in serum LH concentrations, followed by a precipitous drop and normalization of LH levels thereafter. Elevation of LH after kisspeptin infusion was prolonged in underfed animals; a phenomenon mimicked by leptin administration. Conversely, FSH levels were persistently heightened along continuous kisspeptin infusion, but duration of this response was shortened by undernutrition. In pubertal females, LH and FSH levels remained elevated at the end of a 7-day infusion of kisspeptin; responses whose magnitude was augmented by subnutrition but not mimicked by leptin. In all settings, terminal gonadotropin-releasing hormone responses were fully preserved, suggesting that eventual desensitization must occur upstream from the pituitary. In summary, our current data document the pharmacological consequences of continuous administration of kisspeptin to female rats, with remarkable differences being detected between LH and FSH responses, in different developmental and metabolic states. These observations of potential pharmacological interest might help also to delineate the physiological roles of kisspeptins in the dynamic regulation of gonadotropin secretion in the female.

KiSS-1; G protein-coupled receptor 54; gonadotropin-releasing hormone; constant infusion; fasting; leptin; puberty; luteinizing hormone; follicle-stimulating hormone

THE SECRETION OF PITUITARY GONADOTROPINS LH AND FSH IS dictated by the pulsatile release of the hypothalamic decapetide gonadotropin-releasing hormone (GnRH), which is ultimately driven by the complex interaction of a plethora of excitatory and inhibitory signals of central and peripheral origin that are thought to integrate at the level of GnRH neurons (15, 16). Very recently, our knowledge of the neuroendocrine networks controlling GnRH secretion has been substantially enlarged by the identification of the essential roles of kisspeptins, products of the KiSS-1 gene, and their receptor, G protein-coupled receptor 54 (GPR54), in the regulation of key facets of reproductive function in different mammalian species (33, 36). Thus genetic inactivation of GPR54 and KiSS-1 has been reported to cause sexual immaturity and hypogonadotropic hypogonadism (8, 10, 19, 39). In addition, hypothalamic KiSS-1 neurons have been shown to be essential elements for pubertal activation of the gonadotropic axis, through a complex maturational process that is likely to involve increased kisspeptin tone, enhanced GPR54 signaling efficiency, and the rise of the number of their appositions to GnRH neurons at the time of puberty (4, 7, 14, 26, 45). Moreover, the KiSS-1/GPR54 system has been implicated in the dynamic regulation of gonadotropin secretion in adulthood, playing key roles in conveying both positive and negative feedback effects of sex steroids and the metabolic control of the gonadotropic axis and its modulation by endogenous rhythms and environmental cues (5, 26, 35, 36, 42–44). The ultimate mechanism whereby kisspeptins elicit gonadotropin secretion primarily involves a direct excitatory action at the level of GnRH neurons, as demonstrated by a number of pharmacological and physiological data in rodents and sheep (33, 36).

Concerning gonadotropin secretion, the pharmacological effects of kisspeptins, mostly of kisspeptin-10 and kisspeptin-54 (metastin), have been thoroughly analyzed in a number of mammalian species after administration of the peptides via different routes and at a wide range of doses for both LH and FSH (11, 13, 22, 27, 28, 32, 40, 47, 48). These analyses collectively point out that kisspeptins are likely the most potent elicitors of GnRH/gonadotropin secretion known so far (36). This feature, together with their pivotal roles in the control of the gonadotropic axis, makes them suitable targets for pharmacological intervention of the reproductive function. Of note, activation of GPR54 signaling by kisspeptins as a means to stimulate gonadotropin secretion seems to have optimal physiological characteristics (i.e., secretion of the endogenous GnRH releasable pool) vs. stimulation of the gonadotropic axis...
by pharmacological boluses of GnRH, which is prone to desensitization after continuous or repeated administration (1, 48). Notwithstanding, the available information demonstrates that the stimulatory effects of kisspeptin on LH secretion may be desensitized after protocols of continuous administration (34, 38, 46): a phenomenon of potential therapeutic interest, especially considering the conspicuous lack of GPR54 antagonists.

Notably, preliminary characterization of desensitization to the effects of kisspeptins has been solely conducted in the male, either the juvenile and adult monkey or the adult male rat (34, 38, 46). Moreover, the above analyses have only considered LH responses, whereas potential changes in FSH secretion after chronic kisspeptin administration remain totally unexplored. Considering the specific, somewhat differential, features of LH and FSH secretion in general (18), and their responses to kisspeptin in particular (27, 28), and taking into account the obvious therapeutic interest of optimization of amenable protocols of pharmacological intervention of the female gonadotropic axis, the present work was undertaken to provide an integral analysis of gonadotropin (LH and FSH) responses to continuous intracerebral infusion of kisspeptin-10 in the female rat. Given that substantial changes in the functionality of the female gonadotropic axis are detected along sexual maturation and under different metabolic/nutritional conditions, our experiments were comparatively conducted in peripubertal and adult cyclic females, either fed ad libitum or after chronic subnutrition. In the latter, considering the proven roles of leptin in signaling the state of energy stores to the centers governing the gonadotropic axis (3, 12) and its particular role in the modulation of kisspeptin signaling (6, 41), the effects of continuous infusion of kisspeptin-10 were compared with those of leptin, selectively in underfed animals.

MATERIALS AND METHODS

Animals and drugs. Wistar female rats bred in the vivarium of the University of Córdoba were used. The animals were maintained under constant conditions of light (14 h of light, from 7:00) and temperature (22°C) with free access to pelleted food and tap water unless otherwise stated. Experimental procedures were approved by the Córdoba University Ethical Committee for Animal Experimentation and were conducted in accordance with the European Union normative for care and use of experimental animals. The animals were humanely killed by decapitation at the end of the experimental settings when trunk blood samples and sex organs (uterus and/or ovaries) were collected. Rat/mouse KISS-1 (110-119)-NH2, the rodent analog of the human C-terminal KISS-1 decapetide KISS-1 (112-121)-NH2, was obtained from Phoenix Pharmaceuticals (Belmont, CA). This peptide fragment, which has been previously shown to maximally bind and activate GPR54 in transfected CHO cells, will be referred hereafter as kisspeptin-10 or kisspeptin. The decapetide GnRH was purchased from Sigma Chemical (St. Louis, MO), while recombinant leptin was obtained from ProSpec-Tany TechnoGene (Rehovot, Israel).

Experimental design. To provide an integral analysis of potential desensitization events after continuous exposure to kisspeptin in the female, experiments monitoring the circulating levels of both gonadotropins were conducted in intact female rats at different stages of postnatal development (pubertal vs. cyclic adult) and under different metabolic states (fed ad libitum vs. subnutrition). As a general protocol for kisspeptin administration, groups of female rats (n = 10–12) were submitted to continuous intracerebral infusion of effective doses of 7.5 nmol (10 μg/day) of kisspeptin-10 for 7 days following previously published procedures (6). The animals were implanted with osmotic minipumps (1 μl/h delivery rate: Alzet mini-osmotic pump model #2001, DURECT, Cupertino, CA, USA) containing vehicle (physiological saline) or kisspeptin-10 at a final concentration 7.5 nmol/24 μl. The osmotic pumps were placed intradermally between the scapulas and connected to intracerebroventricular cannulas to allow central delivery of the peptides. Correct positioning of minipumps and intracerebral cannulas was confirmed postmortem.

In experiment 1, the effects of continuous intracerebroventricular infusion of kisspeptin-10 on LH and FSH secretion were tested in cyclic female rats along the 7-day period of chronic administration of the peptide. Adult virgin female rats, weighing 257.7 ± 9.9 g at the beginning of the experiment, were monitored for estrous cyclicity by daily vaginal cytology; only rats with at least two consecutive regular 4-day estrous cycles were used for subsequent pharmacological studies. As a general procedure, blood samples were taken by jugular veni-puncture while the animals were under light ether anesthesia, before pump implantation (day 0), and on a daily basis (at 9:00–10:00) after the beginning of infusion. To screen for potential desensitization events at the GnRH receptor level, on the last day of kisspeptin administration (day 7) all animals were subjected to a terminal GnRH provocative test, involving intraperitoneal administration of a single bolus of GnRH (1 μg/rat) and blood sampling before (0 min) and at 15 min after administration of the decapetide.

With the use of a similar design, in experiment 2 the effects of continuous intracerebral administration of kisspeptin-10 on gonadotropin secretion were evaluated in adult female rats under negative energy balance conditions. Regularly cycling adult virgin female rats, weighing 267.5 ± 4.5 g, were checked for regular cyclicity as described in experiment 1 and submitted to a standard protocol of chronic subnutrition, consisting in a 50% reduction in daily calorie intake along the study period. Underfeeding was started at diestrus, and the animals were subsequently monitored for estrous cyclicity by daily vaginal cytology. After four complete estrous cycles (16 days) when all females showed persistent vaginal diestrus due to sustained food restriction, the rats were implanted with osmotic minipumps containing either physiological saline or kisspeptin-10, as described above. Additional groups of underfed females were implanted with minipumps containing effective doses of leptin (1 nmol/day), according to previous references (6). In all groups, blood samples were taken at selected time points after pump implantation. To minimize the effect of the sampling procedure on underfed animals, serum samples were obtained only at days 1, 3, 5, and 7 after the beginning of kisspeptin infusion. As described for fed animals, the experiment was terminated by a GnRH provocative test, conducted as described above on the last day of kisspeptin/leptin administration.

In experiment 3, the effects of continuous intracerebral administration of kisspeptin-10 in terms of on LH and FSH secretion were monitored in peripubertal female rats following an experimental procedure grossly similar to that of experiment 1; osmotic minipumps were implanted on day 30 and constant infusion of kisspeptin-10 (7.5 nmol/day) proceeded for 7 days until day 37. However, to minimize the effect of repeated blood sampling on puberty onset, only terminal blood samples were taken from vehicle- and kisspeptin-infused animals. In all experimental animals, body weights and vaginal opening were daily monitored. On the latter, detailed inspection was conducted in each animal to determine the date of complete canalization of the vagina. In addition, as done in adult animals, terminal GnRH provocative tests were conducted in all animals.

Finally, in experiment 4, terminal LH and FSH responses to continuous intracerebroventricular administration of kisspeptin-10 were analyzed in peripubertal female rats subjected to chronic undernutrition during the prepubertal period. To this end, a protocol of 30% restriction in daily food intake (vs. age-matched control females fed ad libitum) was initiated on day 23 postpartum, following previously published procedures (5). Chronic intracerebral infusion of kisspeptin-10 was conducted between days 30 and 37 postpartum, as de-
Digestive and Kidney Diseases National Hormone and Peptide Institutes of Health (A. F. Parlow, National Institute of Diabetes and Metabolism). Double-antibody method and RIA kits supplied by the National Institutes of Health. Hormone concentrations were assayed in selected experimental groups, using commercial kits from Linco Research (St. Charles, MI), following the instructions of the manufacturer. The sensitivity of the assay was 0.05 ng/tube and the intra-assay coefficient of variation was <5%. Accuracy of hormone determinations was confirmed by assessment of rat serum samples of known hormone concentrations used as external controls.

Presentation of data and statistics. Hormonal determinations were conducted in duplicate, with a minimal total number of 10 samples per group. Hormonal data are means ± SE. Results were analyzed for statistically significant differences using single or repeated ANOVA followed by Student-Newman-Keuls multiple range test (SigmaStat 2.0, Jandel, San Rafael, CA). *P < 0.05 was considered significant.

RESULTS

Effects of continuous kisspeptin infusion on LH and FSH secretion in adult female rats. In an attempt to characterize the consequences of continuous administration of kisspeptin on the circulating levels of both gonadotropins in the female, a standard protocol of intracerebral infusion of kisspeptin-10 for 7 days was implemented in adult, regularly cyclic rats, starting on the day of diestrus-1. Of note, protocols of central administration were implemented in an attempt to achieve high effective doses of kisspeptin at the hypothalamus. In keeping with previous results of acute or repeated injection of the peptide to adult males (5, 47), final body weights remained unaltered in female rats after 7-day treatment with kisspeptin-10 (258.4 ± 9.4 vs. 258.6 ± 6.6 g in vehicle-infused controls), and daily food intake was not modified (data not shown). In addition, chronic infusion of kisspeptin resulted in a marginal increase in uterus weight at the end of the treatment (172.9 ± 12.3 vs. 155.3 ± 5.8 mg/100 g body wt in vehicle-treated rats), which did not reach statistical significance.

Regular (daily) blood sampling of kisspeptin-infused females demonstrated that serum LH levels were significantly enhanced 24 h (day 1) after initiation of chronic treatments remained elevated up to 48 h (day 2) after continuous kisspeptin administration and significantly dropped thereafter, with circulating LH concentrations below control values on day 3 and normalized levels from day 4 of infusion onwards (Fig. 1A). Yet, despite the loss of LH stimulation after >2 days of chronic

Fig. 1. Pattern of LH secretion in adult, cyclic female rats after continuous intracerebroventricular infusion of kisspeptin-10 (Kp-10). A and B: hormonal data from adult females fed ad libitum are shown. Daily (9:00–10:00) levels of LH on the day of diestrus-1 (day 0) and during the course of the 7-day period of Kp-10 administration are presented in A, whereas in B, LH responses to terminal GnRH provocative tests are shown. In the latter, the animals were infused with either vehicle (Veh) or Kp-10 for 7 days and injected or not with GnRH on day 7. C and D: hormonal data from adult females subjected to a protocol of 50% reduction in daily calorie intake are presented. C: serum LH levels on days 1, 3, 5, and 7 after initiation of vehicle or Kp-10 infusion to underfed females are plotted; LH data from pair-fed animals infused with leptin (Lep) are also presented. D: LH responses to terminal GnRH tests in adult underfed female rats, chronically treated with vehicle, kisspeptin, or leptin are shown. **P < 0.01 vs. corresponding values in vehicle-infused groups (ANOVA followed by Student-Newman-Keuls multiple range test).
administration, terminal GnRH provocative tests showed that kisspeptin-infused female rats retain their ability to maximally respond to GnRH in terms of LH secretion; responses whose magnitude was similar to that of vehicle-treated animals (Fig. 1B).

Chronic subnutrition (consisting in 50% reduction in daily calorie intake for 4 complete estrous cycles) resulted in a significant lowering of body weight (226.9 ± 4.2 g, on the day of implantation of osmotic minipumps). Sustained subnutrition in vehicle-infused animals resulted in further lowering of body weight at the end of the 7-day treatment period (206.5 ± 10.5 g). Constant infusion of kisspeptin-10 to underfed females did not significantly modify final body (208.5 ± 9.6 g) or uterus weights (90.0 ± 7.4 vs. 105.0 ± 7.6 mg/100 g body wt in vehicle-treated rats), while it significantly increased ovarian weights (45.0 ± 1.9 vs. 37.5 ± 2.2 mg/100 g body wt in vehicle-treated rats; \(P < 0.01\)) and circulating LH levels. In detail, our analyses involving repeated blood sampling and hormone determinations on days 1, 3, 5, and 7 after the beginning of kisspeptin infusion revealed that, in contrast to fed rats, LH levels were persistently elevated in food-deprived rats up to day 5 after the beginning of chronic infusion of kisspeptin, while on day 7 no significant differences were found between vehicle- and kisspeptin-treated animals (Fig. 1C). Yet, as was the case in females fed ad libitum, GnRH stimulation in underfed rats at the end of the treatment period with kisspeptin-10 resulted in robust LH responses, similar in magnitude to those observed in vehicle-treated animals (Fig. 1D).

Given the profound effect of food restriction on body weight (total reduction >22% at the end of the study period), the effects of infusion of kisspeptin-10 described above were compared with those induced by chronic central administration of the adipocyte-derived hormone leptin to underfed animals. Indeed, our protocol of persistent undernutrition caused a state of marked hypo-leptinemia (0.285 ± 0.073 vs. 1.94 ± 0.266 ng/ml in control fed rats; \(P < 0.01\)); leptin being recognized as a putative stimulator of KISS-1 gene expression at the hypothalamus (6, 41). In our setting, constant intracerebroventricular infusion of leptin failed to significantly reduce final body weights, probably because of the imposed 50% reduction in daily calorie intake (196.5 ± 3.0 g), while it significantly increased uterus weights (155.9 ± 11.6 vs. 105.0 ± 7.6 mg/100 g body wt in vehicle-treated rats; \(P < 0.01\)). In addition, leptin infusion evoked a profile of LH responses similar to that of kisspeptin-10, with persistently elevated LH levels up to day 5 and loss of stimulation thereafter (day 7; Fig. 1C). Likewise, terminal provocative tests demonstrated persistent LH responses to GnRH in leptin-infused animals (Fig. 1D).

In addition to LH secretion, FSH responses were monitored in the different experimental groups indicated above. In cyclic females fed ad libitum, daily blood sampling at 9:00–10:00 showed the dynamic fluctuation of FSH levels across the cycle, with low levels at diestrus and proestrus and peaks of secretion on the morning of the corresponding estrus (i.e., the secondary surge of FSH, on days 3 and 7 of the experiment, as checked by vaginal cytology). Contrary to LH, chronic infusion of kisspeptin-10 resulted in a persistent elevation of FSH levels throughout the study period. Indeed, at the end of the kisspeptin infusion (corresponding to estrus), serum FSH concentrations were significantly higher than the endogenously elevated levels of the secondary surge of FSH (Fig. 2A). Likewise,
terminal provocative tests demonstrated that FSH responses to exogenous GnRH were not only conserved but even enhanced after chronic administration of kisspeptin (Fig. 2B).

Analysis of FSH responses to kisspeptin was also conducted in underfed animals and compared with those evoked by leptin administration. In contrast to animals fed ad libitum, the duration of persistent FSH stimulation after continuous treatment with kisspeptin-10 was considerably short lived: significant elevation of FSH levels over basal values was only detected on day 1, whereas thereafter FSH concentrations remained similar to those of vehicle-infused animals. Chronic leptin administration to female rats at undernutrition fully mimicked the pattern of FSH response to continuous kisspeptin, with significant elevation of FSH levels on day 1 and subsequent loss of stimulation (Fig. 2C). Nonetheless, terminal provocative tests demonstrated that the rapid loss of the FSH-releasing effect of continuous infusion of kisspeptin or leptin in underfed females is not due to desensitization at the GnRH receptor level, as acute FSH responses to GnRH were preserved in those groups (Fig. 2D).

Effects of continuous kisspeptin infusion on LH and FSH secretion in pubertal female rats. Considering the significant changes in the functionality of the gonadotropic axis that take place in the female during postnatal maturation, the above responses in cyclic adult rats were compared with those detected in peripubertal females. A protocol of constant intracerebroventricular infusion of kisspeptin, similar to that of adult animals, was implemented between days 30 and 37 postpartum. As it was the case in adult rats, continuous infusion of kisspeptin-10 failed to modify final body weights (117.9 ± 2.5 vs. 119.6 ± 2.0 g in vehicle-infused controls) or daily food intake (data not shown) in pubertal females. In this setting, chronic infusion of kisspeptin did not change uterus weights (103.5 ± 22.6 vs. 122.3 ± 20.9 mg/100 g body wt in vehicle-treated rats) at the end of the 7-day treatment period, nor did it significantly alter the age of occurrence of vaginal opening (Fig. 3A).

To minimize the effect of regular (daily) blood sampling on pubertal development of immature animals, hormonal analyses were conducted solely at the end of the period of chronic infusion of kisspeptin-10. In contrast to data in adult females, these analyses revealed that serum LH levels were significantly elevated even 7 days after initiation of continuous kisspeptin administration. Likewise, terminal tests demonstrated fully preserved LH responses to an effective bolus of GnRH (Fig. 3B). Similar profiles were observed for FSH secretion: modestly, but significantly, elevated FSH levels were detected at day 7 after kisspeptin-10 infusion, which coincided with totally conserved terminal FSH responses to GnRH (Fig. 3B).

A standard protocol of chronic subnutrition (consisting in 30% reduction in daily calorie intake starting at day 23 postpartum) was used to explore the effect of a negative energy balance on the pattern of LH and FSH responses to continuous infusion of kisspeptin. Such a procedure resulted in a significant lowering of body weights at the end of the study period (day 37: 78.5 ± 0.8 vs. 119.6 ± 2.0 g in controls fed ad libitum), overtly decreased circulating leptin levels (0.52 ± 0.04 vs. 1.48 ± 0.19 ng/ml in fed animals; P < 0.01), and fully prevented vaginal opening (Fig. 4A). Constant infusion of kisspeptin-10 to underfed pubertal females did not modify final body weights (117.9 ± 2.5 vs. 119.6 ± 2.0 g in vehicle-infused controls) or daily food intake (data not shown) in pubertal females. In this setting, chronic infusion of kisspeptin did not change uterus weights (103.5 ± 22.6 vs. 122.3 ± 20.9 mg/100 g body wt in vehicle-treated rats) at the end of the 7-day treatment period, nor did it significantly alter the age of occurrence of vaginal opening (Fig. 3A).

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body weights (77.0 ± 1.1 g) but significantly increased uterus weights (92.0 ± 24.5 vs. 51.7 ± 3.0 mg/100 g body wt in vehicle-treated rats; \( P = 0.05 \)) and restored vaginal opening in >62% of the animals at the end of the 7-day treatment period (Fig. 4A). Likewise, terminal serum LH levels were significantly elevated in underfed females chronically infused with kisspeptin-10 (Fig. 4B); an increase whose magnitude was higher than that detected in pair-aged females fed ad libitum. A similar profile was observed for FSH responses, with persistently elevated levels (higher than those of control fed rats) even after 7 days of continuous administration of kisspeptin-10. Furthermore, gonadotropin responses to GnRH stimulation were fully preserved in the case of LH and even enhanced in the case of FSH in kisspeptin-infused females (Fig. 4B).

Finally, as was done for adult animals, the effects of kisspeptin infusion were compared with those induced by chronic central administration of leptin to underfed pubertal female rats. Constant intracerebroventricular infusion of leptin tended to decrease final body weight despite the imposed calorie restriction (73.6 ± 2.6 vs. 78.5 ± 0.8 g in pair-aged females fed ad libitum; \( P = 0.061 \)) and marginally, but not significantly, increased uterus weights (77.0 ± 12.0 mg/100 g body wt). However, in contrast to kisspeptin treatment, our protocol of continuous administration of leptin did not evoke a persistent elevation of serum LH and FSH levels on day 7 of treatment, while it was able to rescue vaginal opening only in 25% of animals (Fig. 4, A and B). Terminal provocative tests demonstrated persistent LH and FSH responses to GnRH in leptin-infused animals (Fig. 4D). Yet, the net magnitude of gonadotropin responses to GnRH was significantly lower in this group than in kisspeptin-infused rats.

DISCUSSION

Pharmacological manipulation of the female gonadotropic axis holds considerable therapeutic interest in a wide range of pathological conditions, including puberty disorders, endocrine-related tumors, endometriosis, and ovarian insufficiency (23). While GnRH analogs, with either agonistic or antagonistic activities, are used routinely in those pathologies, optimization of more physiological, amenable protocols of hormonal intervention on the reproductive system remains of great interest. In this context, the recent identification of kisspeptins as very potent stimulators of gonadotropin secretion in a number of mammalian species, including the human, has raised the interesting possibility that the products of KiSS-1 gene and their putative receptor GPR54 might be suitable targets for therapeutic intervention of the gonadotropic axis (36). Thus kisspeptins have been shown to potently elicit LH and FSH secretion even at very low doses and after systemic administration of kisspeptins (36). Indeed, as kisspeptins primarily act by inducing the secretion of the releasable pool of endogenous GnRH, such a procedure seems more physiological and accordingly less prone to rapid desensitization than the pharmacological activation of the axis by an exogenous bolus of GnRH (48). Nonetheless, evidence is mounting that continuous exposure to kisspeptins is also able to induce desensitization to their potent gonadotropin-releasing effects (34, 38, 46). This
possibility is of potential therapeutic interest, given the lack of pharmacological antagonists of GPR54. Yet, the evidence so far available on this phenomenon appears limited to the analyses of LH responses only in the male (34, 38, 46). Indeed, to our knowledge, protocols of constant infusion of kisspeptin to females have been only reported in the sheep, but these involved solely very short infusion periods (<8 h) with sustained gonadotropin responses being observed in those settings (2).

To evaluate the potential occurrence of desensitization of gonadotropin responses to kisspeptin in the female, protocols of central (intracerebroventricular) infusion of kisspeptin were implemented as a means to achieve high effective concentrations at the hypothalamus with moderate doses of kisspeptin. Our data in models of constant intracerebroventricular infusion to adult cyclic female rats demonstrate that, as in adult male rats and monkeys, the stimulatory effects of kisspeptin-10 on LH secretion are extinguished after a rather short period of chronic exposure to the peptide, which in our experiments was >48 h. The duration of such a window of persistent stimulation of LH secretion appears to differ between males and females, as previous studies in male rats and monkeys indicate that, despite continuous exposure to kisspeptin and the initial heightening of its circulating levels, LH concentrations were normalized within 24 h of initiation of infusion (34, 38, 46). Of note, however, those previous analyses involved systemic, rather than central, administration of higher doses of kisspeptins (kisspeptin-54 or kisspeptin-10), which hampers direct comparison of results across studies. In any event, the loss of LH stimulation after chronic administration of kisspeptin-10 in adult female rats cannot be attributed to degradation of the peptide or premature exhaustion of the pumps, as indirectly evidenced by persistent FSH responses in the very same animals (see Fig. 2A). Likewise, the conserved LH responses to GnRH in chronically infused females demonstrate that such a desensitization to the stimulatory effects of kisspeptin in terms of LH secretion must take place upstream from the GnRH receptor in keeping with data in the male monkey (38).

One of the most salient observations of our study is that, despite rapidly extinguished LH responses, chronic infusion of kisspeptin-10 did not obliterate, but rather persistently augmented, FSH secretion in the female rat. Indeed, at the end of the 7-day infusion period, circulating levels of FSH in kisspeptin-treated animals were significantly higher than in vehicle-infused controls, despite the occurrence of the secondary surge of FSH in the latter. In addition, FSH responses to exogenous GnRH were not only preserved but were even enhanced after chronic infusion of kisspeptin-10. These data are the first to disclose that in the female rat conditions of persistently elevated kisspeptin tone might lead to clear-cut dissociation of gonadotropin levels, with selective stimulation of FSH secretion only. From a mechanistic standpoint, the fact that the loss of LH stimulation after continuous kisspeptin exposure was accompanied by persistent elevation of FSH levels casts doubts on a simple desensitization of GPR54 as unique causative factor for the former phenomenon. Alternatively, it is tempting to speculate that chronically elevated kisspeptin input results in changes in the pattern of GnRH release in terms of frequency and pulse amplitude, which might preferentially drive FSH secretion (i.e., low frequency pulses) (9, 21). Admittedly, however, acute (single) administration of kisspeptin seems unable to significantly alter the patterns of pulsatile release of GnRH, as very recently reported in female rats (17). Yet, the effects of constant infusion, rather than acute injection, have not been so far explored, and thorough analyses of early and late changes in LH and FSH pulsatility after chronic administration of kisspeptin might shed light on the underlying mechanisms. In addition, we cannot exclude on the basis of our current data that additional, peripheral events, such as changes in circulating inhibin, might be partially involved in the observed dissociation of FSH and LH responses in cyclic females. Nonetheless, the possibility that the above phenomenon (differential gonadotropin responses to prolonged exposure to kisspeptin) might contribute to some physiological and pathological conditions of dissociated LH and FSH secretion (24, 25, 31, 37) merits further investigation.

Metabolic stress is known to have a deep effect in the functionality of the female reproductive axis, with persistent conditions of negative energy balance being associated to variable degrees of hypogonadotropic hypogonadism and infertility (12). Notably, we (5, 6, 20) have previously documented that energy insufficiency is also coupled to decreased hypothalamic expression of KiSS-1. In our study, chronic undernutrition in adult female rats markedly altered the patterns of gonadotropin responses to continuous infusion of kisspeptin-10. Thus, while the period of persistent stimulation of LH secretion was significantly protracted by undernutrition (from 48 h in fed animals to >5 days in underfed females), the time window for FSH stimulation was considerably shortened, with significant elevation of FSH levels being observed in underfed females only at day 1 after initiation of kisspeptin infusion. Such a switch in LH and FSH responses strongly suggests that adverse metabolic conditions alter the pattern of GnRH secretion in response to kisspeptin and, together with the preserved responses to exogenous GnRH, further emphasize the concept that extinction of gonadotropin responses after continuous exposure to kisspeptin is primarily related to functional changes in the profile of GnRH secretion rather than with the mere downregulation of GPR54 and/or GnRH receptors in their corresponding target cells.

From a neuroendocrine perspective, puberty in the female is defined by the progressive heightening of the neurosecretory activity of the GnRH pulse generator that ultimately results in the full activation of the reproductive axis and the instauration of a cyclic pattern of gonadotropin secretion (30). Recently, KiSS-1 neurons in the forebrain have been implicated in the awakening of the GnRH system at puberty through a complex, multifaceted mechanism that involves not only an increase in the endogenous KiSS-1 tone but also an enhancement in the responsiveness to kisspeptin stimulation (4, 14, 26). Our data from models of chronic infusion of kisspeptin-10 in pubertal females suggest that at puberty the GnRH/gonadotropin system of the female is less sensitive to the loss of LH responsiveness to persistently elevated kisspeptin levels than in adulthood. Indeed, while in adult cyclic females stimulation of LH secretion was no longer observed after 48 h of chronic infusion of kisspeptin-10, in pubertal animals LH concentrations were persistently elevated even 7 days after constant kisspeptin administration. This state of resistance to desensitization might be mechanistically relevant to explain why the persistent increase in endogenous KiSS-1 expression at puberty (26, 40) is not linked to the lowering of circulating levels of gonadotropins at this critical stage of sexual maturation. Yet, it is to be
noted that, despite the observed elevation of LH and FSH levels, infusion of kisspeptin-10 between days 30 and 37, i.e., coincident with the peak of hypothalamic expression of KiSS-1 (26), failed to significantly advance the age of vaginal opening (see Fig. 3A). This observation suggests that further enhancement of kisspeptin tone at this period does not result in acceleration of puberty onset. This is in contrast with the precocity of pubertal maturation induced by intermittent central administration of kisspeptin at an earlier developmental window (between days 26 and 31 postpartum), when lower expression of KiSS-1 mRNA is detected at the hypothalamus (29).

Leptin is well recognized as a pivotal metabolic regulator of puberty onset and gonadotropin secretion, with a predominant permissive/stimulatory function at the hypothalamus (3). Very recently, a role for leptin as a positive modulator of KiSS-1 expression at the hypothalamus has emerged in rodents (6, 41), thus suggesting that such a leptin-kisspeptin pathway participates in signaling the state of the energy reserves of the organism onto GnRH neurons (33, 36). In our experiments, continuous central infusion of leptin to adult female rats at undernutrition evoked a pattern of gonadotropin stimulation strikingly similar to that induced by chronic treatment with kisspeptin, indirectly supporting that the mechanism whereby leptin modulates the gonadotropic axis in the adult female involves activation of the hypothalamic KiSS-1 system. Conversely, leptin infusion to underfed female rats at puberty was apparently less effective than continuous kisspeptin administration, as shown by the lower rate of vaginal opening and the lack of stimulation of LH and FSH secretion at the end of leptin treatment. These observations suggest that our protocol of leptin administration (1 nmol/day between days 30 and 37) was not sufficient to evoke a full activation of the KiSS-1 system in the hypothalamus at puberty, in keeping with the contention that leptin is a permissive metabolic factor, rather than the triggering signal, for puberty onset (12). Admittedly, however, protoclated protocols of leptin infusion (e.g., starting at weaning) have been reported to advance puberty onset in food-restricted rats (49), suggesting that supraphysiological leptin stimulation might be sufficient to trigger puberty in rodents.

In conclusion, we present herein an integral study of the patterns of gonadotropin secretion after continuous administration of kisspeptin-10 in the female rat. By the combined analysis of LH and FSH secretory responses at different developmental and metabolic states of the female gonadotropic axis, we document the complexity of the process of desensitization to kisspeptin effects in terms of gonadotropin secretion, which involves differential LH and FSH responses at puberty and adulthood that are under the influence of metabolic cues. Obviously, as protocols of central infusion of kisspeptin were used in our study, direct translation of these data into procedures for pharmacological intervention of the gonadotropic axis, which would certainly need systemic administration, must be done with caution. Nonetheless, as proof-of-principle, our results might assist to better define therapeutic procedures based on the use of GPR54 analogs. In addition, our results may contribute also to provide a deeper insight into the mechanisms whereby kisspeptin signaling may participate in the differential regulation of LH and FSH secretion in certain physiological or pathological conditions in the female.

**REFERENCES**


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