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Leptin effects on pulsatile gonadotropin releasing hormone secretion from the adult rat hypothalamus and interaction with cocaine and amphetamine regulated transcript peptide and neuropeptide Y

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Abstract

Leptin may act as a negative feedback signal to the hypothalamic control of appetite through suppression of neuropeptide Y (NPY) secretion and stimulation of cocaine and amphetamine regulated transcript (CART). We aimed at studying the effects of leptin, CART and NPY on the hypothalamic control of the pituitary–gonadal system. Pulsatile gonadotropin-releasing hormone (GnRH) secretion was studied in vitro using retrochiasmatic hypothalamic explants from adult rats. In the female, GnRH pulse amplitude was significantly increased by leptin (10^{-7} M) and CART (10^{-6} M) irrespective of the estrus cycle phase while no such effects were seen in the male. The GnRH interpulse interval was not affected in both sexes. Passive immunoneutralization against CART caused a reduction in GnRH pulse amplitude in the female. A slight but significant increase in GnRH pulse amplitude was caused by NPY (10^{-7} M) in the female. However, GnRH pulse amplitude was not affected by a Y5-receptor antagonist (10^{-6} M) while the interpulse interval was significantly increased as shown previously in the male. The increase in GnRH pulse amplitude caused by leptin was totally prevented by coincubation with an anti-CART antiserum whereas it was not affected by coincubation with the NPY Y5-receptor antagonist (10^{-7} M). In conclusion, leptin and NPY show separate permissive effects on GnRH secretion in the adult rat hypothalamus. In both sexes, NPY is prominently involved in the control of the frequency of pulsatile GnRH secretion through the Y5 receptor subtype. Leptin causes a female-specific facilitatory effect on GnRH pulse amplitude which is mediated by CART and which occurs irrespective of the estrus cycle phase.

Keywords: NPY-receptor subtype; Estrus cycle; Pulsatility

1. Introduction

Alteration in energy balance may influence reproductive function in many species. Food restriction can alter adult reproductive function [1,2] and delay the timing of pubertal onset [3,4] by suppression of luteinizing hormone (LH) secretion. Many studies showed that leptin, a key regulator of food intake and energy balance, may play a regulatory role in the hypothalamic–pituitary–gonadal axis. In female ob/ob mice, leptin administration increased basal LH levels and restored fertility [5–7]. Other experiments have demonstrated that intracerebroventricular (i.c.v.) injection of leptin antiserum led to a decrease in LH pulsatility and an impairment of reproductive function [8].

Neuropeptide (NPY) and, more recently, the cocaine and amphetamine regulated transcript (CART) were shown to be involved in the mechanisms of leptin action on food intake. The hypothalamic transcripts of NPY, a potent stimulator of food intake, were increased by food restric-

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tion [9] and reduced by i.c.v. administration of leptin [10,11]. Leptin administration to obese (ob/ob) mice stimulated the mRNA expression of CART, a hypothalamic inhibitor of food intake [12]. Therefore, it appeared interesting to study whether NPY and CART could be involved in leptin effects on the hypothalamic–pituitary–gonadal system.

Discrepant stimulatory and inhibitory effects of NPY on sexual maturation and reproduction were observed depending on species, steroidal environment [13], site of NPY administration in the brain [14] and chronic [15–17] versus acute pattern of infusion [18]. CART effects on the reproductive axis were unknown till we reported very recently a study of pulsatile GnRH secretion from hypothalamic explants of prepubertal male and female rats [19]. The frequency of GnRH pulsatility was stimulated by leptin. CART was likely to mediate such an effect. Using the same paradigm, NPY was found to accelerate GnRH pulsatility through the Y5-receptor subtype which appeared to be not involved in mediation of leptin effects [20]. Here, we used hypothalamic explants from adult male rats and female rats at different phases of the estrus cycle. We aimed at studying the effects of leptin and the possible mediating role of NPY and CART in the regulation of frequency and amplitude of pulsatile GnRH secretion.

2. Materials and methods

2.1. Animals

Male and female Wistar rats were used. They were housed in temperature and light-controlled conditions (22°C, lights on between 07:00 and 19:00) with water and standard rat pellet ad libitum. The protocols were approved by the University Committee on animal research.

2.2. Hypothalamic explants incubation

The animals were sacrificed by decapitation between 10:00 and 11:00 for all experiments except during the estrous cycle when some experiments were started in the afternoon around 16:00. The retrochiasmatic hypothalamus was rapidly dissected after decapitation and transfered into a static incubator as described previously [21,22]. The studied retrochiasmatic explants did contain GnRH axons and terminals but virtually no GnRH cell bodies [23]. In each experiments, 12–15 explants were studied individually for 4–6 h, through collection and renewal of the incubation medium (0.5 ml) every 7.5 min. The samples were frozen until the specific GnRH radioimmunoassay was performed.

2.3. GnRH RIA

GnRH was measured in the collected fractions using a highly sensitive RIA [21,22]. The values below the limit of detection (5 pg/7.5 min) were assigned that value. The GnRH antiserum was generously gifted by Dr. Y.F. Chen and V.D. Ramirez (Urbana, IL, USA) [24]. This antiserum was highly specific for GnRH without significant cross reactivity of any of the peptides used in the experiments (leptin, NPY and CART).

2.4. Study protocols

Using hypothalamic explants from adult (50-day-old) male and female rats, the frequency and amplitude of pulsatile GnRH secretion was studied without (control) or in the presence of different compounds: mouse recombinant leptin (R&D system, Abingdon, UK), Porcine NPY (Ferring, Copenhagen, Denmark), $CART_{(52-102)}$ (Novo Nordisk, Bagsvaerd, Denmark) and an anti-CART rabbit antiserum (Novo Nordisk) were used at a 1:1000 dilution. A NPY-Y5 receptor antagonist, *trans*-naphtalene-1-sulfonic acid-4-{(4-(3-dimethylamino-propylamino)-quinazolin-2-ylamino)-methyl}-cyclohexylmethyl}-amide [25] was synthesized in the Medicinal Chemistry Department of Ferring Research (Chilworth, UK).

The frequency and amplitude of spontaneous pulsatile GnRH secretion and the effects of leptin (10^{-7} M) were studied using explants from 50-day-old female rats which were obtained in the morning or in the afternoon of each phase of the estrus cycle. Vaginal smears were performed at the time of experiments to determine the estrus cycle phase. A similar study was performed using explants from male rats aged 50 days. Using explants of 50-day-old female rats which were obtained at 10:00, the effects of CART (10^{-6} M) were studied. In this protocol, each explant was used as its own control since it was incubated for 3 h without CART (control) and for the subsequent 3-h period, with 10^{-6} M of CART. Following the same protocol, the effects of an anti-CART antiserum used alone or together with leptin (10^{-7} M) were studied.

Using explants from adult female rats, the effects of NPY (10^{-7} M) and of the NPY-Y5 receptor antagonist (10^{-6} M) on the frequency and the amplitude of pulsatile GnRH secretion were studied. Each explant was used as its own control.

2.5. Statistical analysis

The occurrence of significant pulses of GnRH secretion was determined using the Pulsar program as described previously [26]. The mean ($\pm$S.D.) interpulse interval and pulse amplitude were calculated. Comparisons were performed using the unpaired, two-tailed Student's *t*-test, with $P < 0.05$ as the threshold for significance of differences.

3. Results

3.1. Pulsatile GnRH secretion at different phases of the estrus cycle

Using hypothalamic explants of adult female rats obtained in the morning as well as in the afternoon (Table 1), the mean GnRH interpulse interval was around 40 min and did not change throughout the estrus cycle. Using explants obtained in the morning, the mean GnRH pulse amplitude did not change significantly throughout the cycle. Using explants obtained in the afternoon, when the preovulatory LH surge is known to occur on proestrus, a significantly increased pulse amplitude was seen on proestrus compared to the other phases. Since no differences were observed between estrus, metestrus and diestrus in these and the following experiments, the data obtained during these three phases were pooled in the subsequent analysis.

3.2. Effects of leptin on frequency and amplitude of pulsatile GnRH secretion

Using hypothalamic explants obtained from adult female rats in the morning, the interpulse interval was not affected by leptin (10^{-7} M) while the GnRH pulse amplitude was significantly increased, irrespective of the cycle phase (Table 2). Using explants obtained in the afternoon, the GnRH interpulse interval was not affected by leptin. The GnRH pulse amplitude was significantly increased by leptin (10^{-7} M) irrespective of the estrus cycle phases (Fig. 1). The total GnRH secretion was significantly increased by leptin except in the afternoon of proestrus when GnRH secretion was already increased in control conditions. Since leptin effects were seen unequivocally in the morning at all phases of the cycle, the subsequent experiments were carried out using explants obtained in the morning, irrespective of the cycle phase.

Using hypothalamic explants from adult male rats, leptin affected neither the GnRH interpulse interval (39.5±3.4 vs. 40.3±3.7 min, leptin vs. control) nor the GnRH pulse amplitude (10.8±1.0 vs. 11.3±1.3 pg/7.5 min, leptin vs. control).

3.3. Effect of CART-peptide and NPY on frequency and amplitude of pulsatile GnRH secretion

As shown in Fig. 1, using explants from adult female rats, the GnRH pulse amplitude was significantly increased by 10^{-6} M of CART (13.6±4.5 vs. 9.5±2.2 pg/7.5 min, CART vs. control) while the interpulse interval was not affected (data not shown). A less important but significant increase in GnRH pulse amplitude was caused by 10^{-7} M of NPY (12.2±1.7 vs. 10.6±0.9 pg/7.5 min, NPY vs. control) while the interpulse interval was not affected (40.9±3.8 vs. 39.6±3.4 min).

As shown in Fig. 2, the anti-CART antiserum significantly reduced GnRH pulse amplitude (9.2±1.7 vs. 11.8±1.6 pg/7.5 min, anti-CART vs. control) while the

Table 1
Mean interpulse interval and pulse amplitude of GnRH secretion from hypothalamic explants obtained in the morning or in the afternoon at different phases of the estrus cycle[a]

Cycle phase		Proestrus	Estrus	Metestrus	Diestrus
Interpulse interval (min)	Morning	41.2±3.9	45.0±8.2	41.4±3.8	41.2±3.9
	Afternoon	39.7±3.6	40.3±3.9	40.2±3.9	38.9±3.1
Pulse amplitude (pg/7.5 min)	Morning	9.6±2.6	7.6±4.5	6.3±4.1	6.5±4.6
	Afternoon	11.1±0.8*	7.5±0.5	7.9±0.7	7.1± 0.6

[a] Data are mean±S.D. with a mean of four explants studied in each group.
*, $P < 0.05$ versus the other cycle phases.

Table 2
Effects of leptin on frequency and amplitude of pulsatile GnRH secretion using hypothalamic explants of adult female rats obtained in the morning or in the afternoon at the proestrus phase compared with the other phases of the cycle[a]

	Interpulse interval (min)		Amplitude (pg/7.5 min)		Total secretion (pg/3 h)	
	Control	Leptin	Control	Leptin	Control	Leptin
Morning						
Proestrus	41.2±3.9	39.7±3.5	9.6±2.6	18.3±6.2*	204.3±24.1	285.6±39.6*
Other phases	42.0±4.9	41.5±4.2	6.5±4.2	12.6±6.0*	178.4±27.4	237.1±55.6*
Afternoon						
Proestrus	40.5±3.9	41.2±3.9	11.1±0.8	13.1±1.2*	223.8±22.0	233.0±25.7
Other phases	39.7±3.5	40.1±3.6	7.5±0.7	12.0±1.2*	171.5±11.9	242.4±19.2*

[a] Leptin was used at a 10^{-7} M concentration. The data are mean±S.D., a mean of four explants being studied at each phase.
*, $P < 0.05$ versus control.

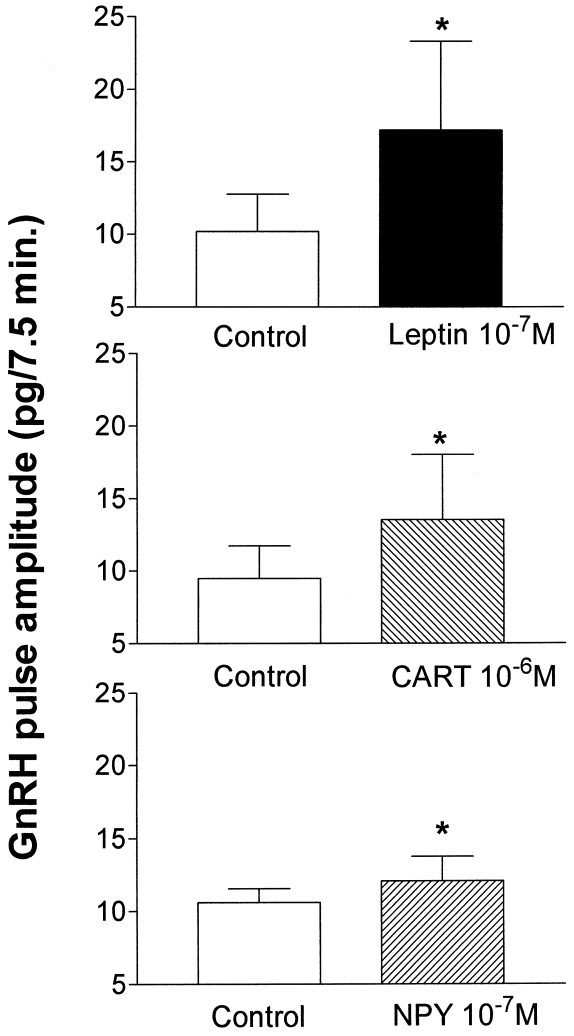

Fig. 1. Effect of leptin, CART and NPY on the amplitude of pulsatile GnRH secretion from hypothalamic explants of normally cycling adult female rats. These data are mean±S.D. with a mean of ten explants studied in each groups. *, $P < 0.05$ versus control.

interpulse interval was not affected (38.8±3.0 vs. 40.0±3.7 min). In contrast, GnRH pulse amplitude was not affected by the Y5 receptor antagonist (10.2±0.7 vs. 10.0±0.8 pg/7.5 min, Y5 receptor antagonist vs. control) while the interpulse interval was significantly increased (52.0±3.6 vs. 39.8±3.6 min).

3.4. Effect of anti-CART antiserum and NPY Y5-receptor antagonist on leptin-induced stimulation of GnRH secretion

Using explants of adult female rats, the increase in GnRH pulse amplitude caused by leptin (10^{-7} M) was totally prevented by coincubation with an anti-CART antiserum whereas it was not affected by coincubation with the NPY Y5-receptor antagonist (Fig. 3).

4. Discussion

The preovulatory LH surge was known to take place during the afternoon of proestrus [27–29]. In this study, using explants obtained in the afternoon of the different cycle phases, we showed that the frequency of GnRH pulsatility did not change whereas the amplitude showed about a two fold increase on the afternoon of proestrus. Several in vivo studies showed that GnRH secretion in pituitary stalk plasma was markedly increased at the time of the preovulatory LH surge [30–32]. It was interesting to observe that this process was associated with an increased GnRH pulse amplitude in vitro whereas we showed that changes in pulse frequency without changes in amplitude characterized the period preceding onset of puberty [33].

An interplay between various inhibitory and excitatory neurotransmitters may occur on proestrus, leading to the preovulatory GnRH discharge [34]. Leptin might be involved since it was shown to stimulate GnRH secretion from the median eminence and arcuate nucleus [35,36]. Using explants from the prepubertal female rat, we found that the frequency of GnRH pulsatility was accelerated by leptin in a dose-dependent manner [20]. Intracerebroventricular administration of leptin antiserum to adult female rats resulted in an impairment of reproductive function with persistent anestrus [8]. Here, using hypothalamic explants from adult female rats, leptin was found to increase GnRH pulse amplitude whatever the estrus cycle phase and the time (morning or afternoon) when the explant was obtained. These data indicated an overall facilitatory role of leptin on the amplitude of GnRH secretion instead of a specific contribution to the occurrence of the preovulatory LH surge. In agreement with our in vitro data, it was shown in vivo that i.c.v. administration of a leptin antiserum caused reduction of LH pulse amplitude without a change in frequency [8].

Since the increase in GnRH pulse amplitude caused by leptin in the female was not seen in the male, it is possible that this effect is dependent on sex steroids. Such a concept is consistent with the facilitatory effects of estrogens on leptin receptor gene expression [37]. However, because leptin had stimulatory effects in the prepubertal hypothalamus from rats of both sexes, we may hypothesize that testosterone might account for disappearance of leptin effects in the male instead of estrogen for persistent effects in the female.

Leptin could influence GnRH secretion by acting transsynaptically rather than by a direct effect [38]. Indeed, little coexpression of ob-receptor and GnRH was found in the rat at the protein or mRNA levels [39]. CART might be a hypothalamic mediator of leptin effects on GnRH neurons. Using explants from 15-day-old rats, our previous studies showed that CART could be involved in mediating leptin stimulatory effects on the frequency of GnRH pulsatility [19]. Using hypothalamic explants from Zucker rats homozygous for the leptin receptor mutation, we

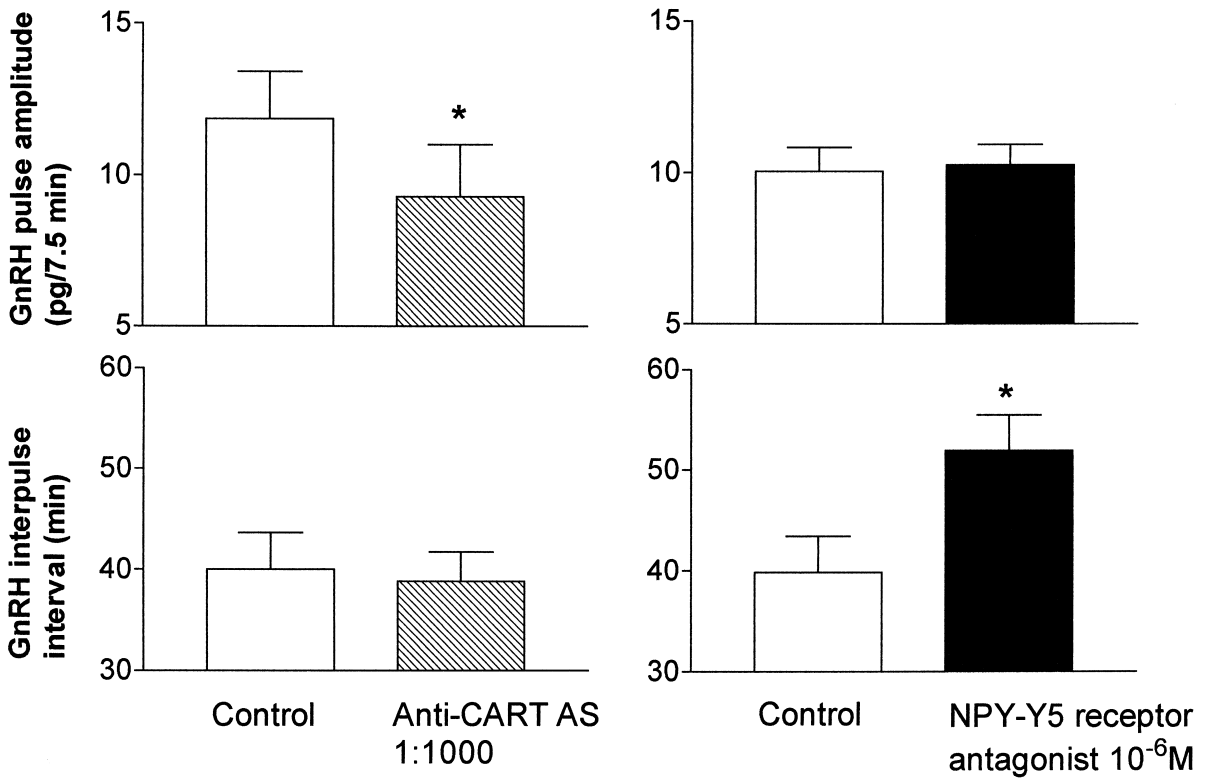

Fig. 2. Effect of an anti-CART antiserum and a NPY Y5-receptor antagonist on the frequency and amplitude of pulsatile GnRH secretion from hypothalamic explants of normally cycling adult female rats. These data are means±S.D. with a mean of five explants studied in each group. *, $P < 0.05$ versus control.

found that GnRH pulse frequency was not affected by leptin but significantly accelerated by the CART-peptide. In the present study, using explants from adult female rats, the GnRH pulse amplitude was similarly affected by CART and leptin, further suggesting the mediating role of CART. A role for the endogenous CART-peptide is supported by the decreased GnRH pulse amplitude after CART immunoneutralization. The antiserum could totally prevent leptin effects on GnRH pulse amplitude at 50 days whereas, used at the same concentration, the antiserum could only partially overcome leptin effects on GnRH pulse frequency at 15 days. Thus, though CART appeared to be involved in mediating leptin effects at different stages throughout life, the absolute activity or the importance of CART relative to other mediators may change with age.

Neuropeptide Y, a potent stimulator of food intake, was shown to affect the reproductive axis in a number of species including rats, though discrepant stimulatory and inhibitory effects were observed [13–18]. In the rat, the involvement of NPY in the preovulatory GnRH and LH surges was supported by the concomitant increase of NPY gene expression in the arcuate nucleus [40] and NPY tissue content in the median eminence [41]. In addition, NPY stimulation of GnRH release was maximal under conditions leading to GnRH surges [42,43]. The pituitary responsiveness to pulsatile GnRH stimulation was enhanced by NPY in proestrus rats [44]. Thus, the contribution of NPY to the preovulatory LH surge could involve both a direct action on GnRH secretion [13,45] and a potentiation of the pituitary response to GnRH [43,46]. The actions of NPY are mediated through distinct receptor subtypes showing different affinity for NPY agonists which have been cloned [47]. The Y5-receptor [48,49] and Y1-receptor [50] were shown to be involved in NPY-induced food intake. Our previous data obtained with different NPY agonists were consistent with the involvement of the Y5-receptor subtype in the stimulatory effect on the frequency of GnRH pulsatility in the prepubertal hypothalamus [20]. In the present study, the contribution of NPY to the preovulatory increase in GnRH pulse amplitude appeared to be equivocal since exogenous NPY had only modest effect and prevention of endogenous NPY effects through a Y5 receptor antagonist did not alter GnRH pulse amplitude. We did however not separate NPY effects in relation to the cycle phase. Such experiments as well as studies using antagonists for different receptor subtypes (Y1, Y4) possibly involved in the adult female [51] are warranted. It is noteworthy that the Y5 receptor antagonist revealed an endogenous facilitatory NPY effect on the frequency of pulsatile GnRH secretion in the adult female hypothalamus which was similar to that recently

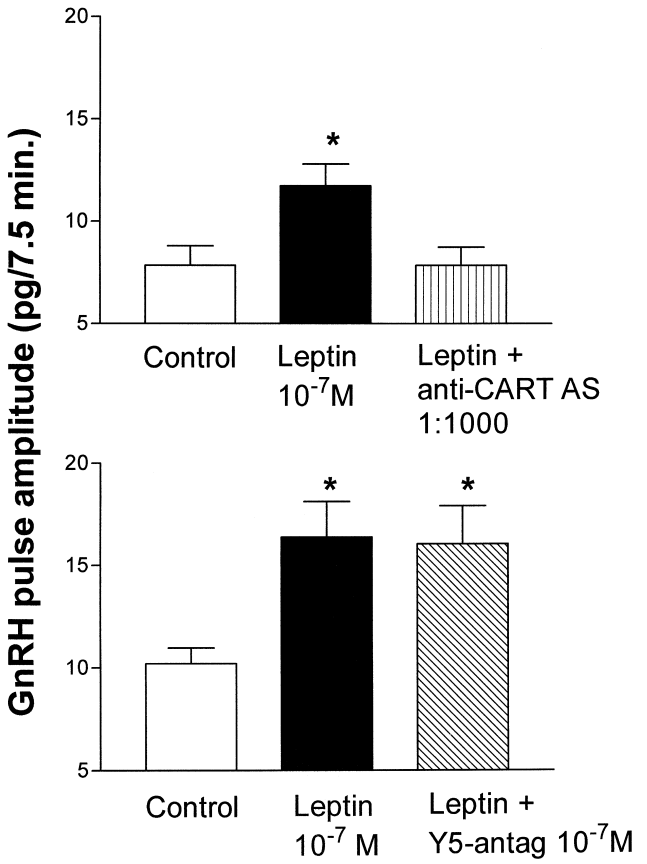

Fig. 3. Effect of leptin on the amplitude of pulsatile GnRH secretion and effect of an anti-CART antiserum (upper panel) and of a NPY Y5-receptor antagonist (lower panel) on leptin-induced stimulation of GnRH secretion from explants of the adult female rats hypothalamus. These data are mean±S.D. with a mean of six explants studied in each group. *, $P < 0.05$ versus control.

reported in the adult male [20]. Such an NPY effect which is not sexually dimorphic and different from the female-specific leptin stimulation of GnRH pulse amplitude, added to the dissociation between NPY and leptin effects. This dissociation was critically observed using explants from prepubertal rats where NPY could accelerate the frequency of pulsatile GnRH secretion through a mechanism not involving the leptin–CART pathway [20]. Here, a similar dissociation was found in the adult female hypothalamus.

In conclusion, using a hypothalamic explant model, we demonstrated an increase in amplitude of pulsatile GnRH secretion in the afternoon of proestrus. Both leptin and NPY showed a permissive effect on the amplitude of GnRH secretion in the adult female hypothalamus with distinct mechanisms for the two peptides. Endogenous NPY was found to control the frequency of pulsatile GnRH secretion through the Y5 receptor subtype in the adult hypothalamus of both sexes. In contrast, leptin showed a female-specific facilitatory effect on GnRH pulse amplitude, irrespective of the estrus cycle phase. CART was confirmed to be involved in the leptin effect on pulsatile GnRH secretion in the adult female as it was shown earlier in the prepubertal hypothalamus in both sexes.

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References

[1] Badger TM, Lynch EA, Fox PH. Effects of fasting on luteinizing hormone dynamics in the male rat. J Nutr 1985;115:788–97.
[2] Cagampang FRA, Maeda K-I, Yokoyama A, Ôta K. Effect of food deprivation on the pulsatile LH release in the cycling and ovariectomized female rat. Horm Metab Res 1990;22:269–72.
[3] Foster DL, Olster DH. Effect of restricted nutrition on puberty in the lamb: patterns of tonic luteinizing hormone (LH) secretion and competency of the LH surge system. Endocrinology 1985;116:375–81.
[4] Kennedy GC, Mitra J. Body weight and food intake as initiating factors for puberty in the rat. J Physiol 1963;166:408–18.
[5] Barash IA, Cheung CC, Weigle DS, Ren H, Kabigting EB, Kuijper JL, Clifton DK, Steiner RA. Leptin is a metabolic signal to the reproductive system. Endocrinology 1996;137:3144–7.
[6] Chehab FF, Lim ME, Lu R. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. Nat Genet 1996;12:318–20.
[7] Mounzih K, Lu R, Chehab FF. Leptin treatment rescues the sterility of genetically obese ob/ob males. Endocrinology 1997;138:1190–3.
[8] Carro E, Pinilla L, Seoane LM, Considine RV, Aguilar E, Casanueva FF, Dieguez C. Influence of endogenous leptin tone on the estrous cycle and luteinizing hormone pulsatility in female rats. Neuroendocrinology 1997;66:375–7.
[9] Dryden S, Frankish HM, Wang Q, William G. Neuropeptide Y and energy balance: one way ahead for the treatment of obesity? Eur J Clin Invest 1994;246:293–308.
[10] Cusin I, Rohner-Jeanrenaud F, Stricker-Krongrad A, Jeanrenaud B. The weight reducing effects of an intracerebroventricular injection of leptin in genetically obese fa/fa rats: reduced sensitivity compared with lean animals. Diabetes 1996;45:1446–50.
[11] Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG. Identification of targets of leptin action in rat hypothalamus. J Clin Invest 1996;98:1101–6.
[12] Kristensen P, Judge ME, Thim L, Ribel U, Christjansen KN, Wulff BS, Clausen JT, Jensen PB, Madsen OD, Vrang N, Larsen PJ, Hastrup S. Hypothalamic CART is a new anorectic peptide regulated by leptin. Nature 1998;393:72–6.
[13] Khorram O, Pau KYF, Spies HG. Bimodal effects of neuropeptide Y

on hypothalamic release of gonadotrophin hormone in conscious rabbits. Neuroendocrinology 1987;45:290–7.
[14] Pau KYF, Berria M, Hess DL, Spies HG. Hypothalamic site-dependent effects of neuropeptide Y on gonadotropin-releasing hormone secretion in rhesus macaques. J Neuroendocrinol 1995;7:63–7.
[15] Cruaz NM, Pierroz DD, Rohner-Jeanrenaud F, Sizonenko PC, Aubert ML. Evidence that neuropeptide Y could represent a neuroendocrine inhibitor of sexual maturation in unfavorable metabolic conditions in the rat. Endocrinology 1993;133:1891–4.
[16] Pierroz DD, Gruaz NM, d'Allèves V, Aubert ML. Chronic administration of neuropeptide Y into the lateral ventricle starting at 30 days of life delays sexual maturation in the female rat. Neuroendocrinology 1995;61:293–300.
[17] Pierroz DD, Catzeflis C, Aebi AC, Rivier JE, Aubert ML. Chronic administration of Neuropeptide Y into the lateral ventricle inhibits both the pituitary-testicular axis and growth hormone and insulin-like growth factor 1 secretion in intact adult male rats. Endocrinology 1996;137:3–12.
[18] Minami S, Sarkar DK. Central administration of neuropeptide Y induces precocious puberty in female rats. Neuroendocrinology 1992;56:930–4.
[19] Lebrethon MC, vandersmissen E, gérard A, Parent AS, Bourguignon JP. Cocaine-and amphetamine-regulated transcript (CART)-peptide mediation of leptin stimulatory effect on the rat gonadotrophin-releasing hormone pulse generator in vitro. J Neuroendocrinol 2000;12:383–5.
[20] Lebrethon MC, vandersmissen E, Gérard A, Parent AS, Junien JL, Bourguignon JP. In vitro stimulation of the prepubertal rat gonadotrophin-releasing hormone pulse generator by leptin and neuropeptide Y through distinct mechanisms. Endocrinology 2000;141:1464–9.
[21] Bourguignon JP, Gérard A, Franchimont P. Direct activation of GnRH secretion through different receptors to neuroexitatory amino acids. Neuroendocrinology 1989;49:402–8.
[22] Bourguignon JP, Gérard A, Mathieu J, Simons J, Franchimont P. Pulsatile release of Gonadotrophin-releasing hormone from hypothalamic explants is restrained by blockade of N-methyl-D,L-aspartate receptors. Endocrinology 1989;125:1090–6.
[23] Purnelle G, Gérard A, Czajkowski V, Bourguignon JP. Pulsatile secretion of gonadotrophin-releasing hormone by rat hypothalamic explants without cell bodies of GnRH neurons. Neuroendocrinology 1997;66:305–12.
[24] Hartter DE, Ramirez VD. Responsiveness of immature versus adult male rat hypothalami to dibutyryl cyclic AMP-and Forskolin-induced LHRH release in vitro. Neuroendocrinology 1985;40:476–82.
[25] Ruëger H., Schmidlin T., Rigollier P., Yamaguchi Y., Tintelnot-Blomley M., Schilling W., Criscione L., Mah R. Receptor antagonists. Basel, Switzerland:Novartis A.G., 1997: Patent W.O. 97/20823, example 53, p. 91.
[26] Bourguignon JP, Gérard A, Debougnoux G, Rose JR, Franchimont P. Pulsatile release of gonadotrophin-releasing hormone (GnRH) from the rat hypothalamus in vitro: calcium and glucose dependency and inhibition by superactive GnRH analogs. Endocrinology 1987;121:993–9.
[27] Blake CA. A detailed characterization of the proestrus luteinizing hormone surge. Endocrinology 1976;98:445–50.
[28] Allen LG, Kalra SP. Evidence that a decrease in opioid tone may evoke preovulatory luteinizing hormone release in the rat. Endocrinology 1986;118:2375–81.
[29] Allen LG, Hahn E, Caton D, Kalra SP. Evidence that a decrease in opioid tone on proestrus changes the episodic pattern of LH secretion: implications in the preovulatory LH hypersecretion. Endocrinology 1988;122:1004–13.
[30] Sarkar DK, Chiappa SA, Fink G. Gonadotropin-releasing hormone surge in proestrus rats. Nature 1976;264:461–3.
[31] Ching M. Correlative surges of LHRH, LH and FSH in pituitary stalk plasma and systemic plasma of rat during proestrus: effects of anesthetics. Neuroendocrinology 1982;34:279–85.
[32] Levine JE, Ramirez VD. In vivo release of luteinizing hormone-releasing hormone estimated with push–pull cannulae from the mediobasal hypothalami of ovariectomized, steroid-primed rats. Endocrinology 1980;107:1782–90.
[33] Bourguignon JP, Gérard A, Alvarez Gonzalez ML, Franchimont P. The neuroendocrine mechanism of onset of puberty: sequential reduction in activity of inhibitory and facilitatory N-methyl-D-aspartate receptors. J Clin Invest 1992;90:1736–44.
[34] Kalra SP, Mandatory neuropeptide-steroid signaling for the preovulatory luteinizing hormone-releasing hormone discharge, Endocr Rev 1993;14:507-538.
[35] Yu WH, Kimura M, Walczewska A, Karanth S, Mc Cann SM. Role of leptin in hypothalamic-pituitary function. Proc Natl Acad Sci USA 1997;94:1023–8.
[36] Mizuno M, Kasuya E, Mogi K, Teresawa E. Leptin stimulates in vivo LHRH release in the stalk-median eminence in adult ovariectomized female rhesus monkeys. In: Program of the 28th Annual Meeting of the Society for Neuroscience, Los Angeles, CA, 1998, Abstract 244 -14.
[37] Bennet PA, Lindell K, Karlsson C, Robinson ICAF, Carlsson LMS, Carlsson B. Differential expression and regulation of leptin receptor isoforms in the rat brain: effects of fasting and oestrogen. Neuroendocrinology 1998;67:29–36.
[38] Cunningham MJ, Clifton DK, Steiner RA. Leptin's actions on the reproductive axis: perspectives and mechanisms. Biol Reprod 1999;60:216–22.
[39] Hakansson ML, Brown H, Ghilardi N, Skoda RC, Meister B. Leptin receptor immunoreactivity in chemically defined target neurons of the hypothalamus. J Neurosci 1998;18:559–72.
[40] Bauer-Dantoin AC, Urban JH, Levine JE. Neuropeptide Y gene expression in the arcuate nucleus is increased during preovulatory luteinizing hormone surges. Endocrinology 1992;131:2953–8.
[41] Sahu A, Jacobson W, Crowley WR, Kalra SP. Dynamic changes in neuropeptide Y concentrations in the median eminence in association with preovulatory luteinizing hormone release in the rat. J Neuroendocrinol 1989;1:83–7.
[42] Woller MJ, Teresawa E. Estradiol enhances the action of neuropeptide Y on in vivo luteinizing hormone-releasing hormone release in the ovariectomized rhesus monkey. Neuroendocrinology 1992;56:921–5.
[43] Sabatino FD, Collins P, Mc Donald JK. Neuropeptide Y stimulation of luteinizing hormone-releasing hormone secretion from the median eminence in vitro by estrogen-dependent and extracellular Ca^{2+}-independent mechanisms. Endocrinology 1989;124:2089–98.
[44] Bauer-Dantoin AC, Mc Donald JK, Levine JE. Neuropeptide Y potentiates luteinizing hormone (LH)-releasing hormone-stimulated LH surges in pentobarbital-blocked proestrus rats. Endocrinology 1991;129:402–8.
[45] Crowley WR, Kalra SP. Neuropeptide Y stimulates the release of luteinizing hormone-releasing hormone from medial basal hypothalamus in vitro: modulation by ovarian hormones. Neuroendocrinology 1987;46:97–103.
[46] Crowley WR, Hassid A, Kalra SP. Neuropeptide Y enhances the release of luteinizing hormone induced by luteinizing hormone-releasing hormone. Endocrinology 1987;120:941–5.
[47] Bloqvist AG, Herzog H. Y receptor subtype: how many more? Trends Neurosci 1997;20:294–8.
[48] Tang-Christensen M, Kristensen P, Stidsen CE, Brand CL, Larsen PJ. Central administration of Y5 receptor antisense decreases spontaneous food intake and attenuates feeding in response to exogenous neuropeptide Y. J Endocrinol 1998;159:307–12.
[49] Criscione L, Rigollier P, Batzl-Hartmann C, Rüeger H, Stricker-Krongrad A, Wyss P, Brunner L, Whitebread S, Yamaguchi Y, Gerald C, Heurich RO, Walker MW, Chiesi M, Schilling W, Hofbauer KG, Levens N. Food intake in free-feeding and energy-deprived lean rats

is mediated by the neuropeptide Y5 receptor. J Clin Invest 1998;102:2136–45.
[50] Kalra SP, Dube MG, Pu S, Xu B, Horvath TL, Kalra PS. Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. Endocrine Rev 1999;20:68–100.
[51] Jain MR, Pu S, Kalra PS, Kalra SP. Evidence that stimulation of two modalities of pituitary luteinizing hormone release in ovarian steroid-primed ovariectomized rats may involve neuropeptide Y Y1 and Y4 receptors. Endocrinology 1999;140:5171–7.