Corticotropin-releasing factor receptor subtypes mediating nutritional suppression of estrous behavior in Syrian hamsters

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Seymour, Patricia L., Samantha L. Dettloff, Juli E. Jones, and George N. Wade. Corticotropin-releasing factor receptor subtypes mediating nutritional suppression of estrous behavior in Syrian hamsters. Am J Physiol Regul Integr Comp Physiol 289: R418–R423, 2005. First published April 14, 2005; doi:10.1152/ajpregu.00168.2005.—Caloric deprivation inhibits reproduction, including copulatory behaviors, in female mammals. Decreases in metabolic fuel availability are detected in the hindbrain, and this information is relayed to the forebrain circuits controlling estrous behavior by neuropeptide Y (NPY) projections. In the forebrain, the nutritional inhibition of estrous behavior appears to be mediated by corticotropin-releasing factor (CRF) or urocortin-signaling systems. Intracerebroventricular (ICV) infusion of the CRF antagonist, astressin, prevents the suppression of lordosis by food deprivation and by NPY treatment in Syrian hamsters. These experiments sought to determine which CRF receptor type(s) is involved. ICV infusion of the CRF receptor subtype CRF R2-selective agonists urocortin 2 and 3 (UCN2, UCN3) inhibited sexual receptivity in hormone-primed, ovariectomized hamsters. Furthermore, the CRF R2-selective antagonist, astressin 2B, prevented the inhibition of estrous behavior by UCN2 and by NPY, consistent with a role for CRF R2. On the other hand, astressin 2B did not prevent the inhibition of behavior induced by 48-h food deprivation or ICV administration of CRF, a mixed CRF R1 and CRF R2 agonist, suggesting that activation of CRF R1 signaling is sufficient to inhibit sexual receptivity in hamsters. Although administration of CRF R1-selective antagonists (NBI-27914 and CP-154,526) failed to reverse the inhibition of receptivity by CRF treatment, we could not confirm their biological effectiveness in hamsters. The most parsimonious interpretation of these findings is that, although NPY inhibits estrous behavior via downstream CRF R2 signaling, food deprivation may exert its inhibition via both CRF R1 and CRF R2 and that redundant neuropeptide systems may be involved.

FOR FEMALE MAMMALS, REPRODUCTIVE endeavors are energetically expensive. As a result, fertility and sexual behaviors are often suppressed in the face of decreased energy availability (7, 39, 45). Numerous situations that disrupt energy balance also affect central corticotropin-releasing factor (CRF) signaling, and these coincident processes may be functionally related (4, 33). It is well established that CRF signaling inhibits reproductive physiology (34, 35) in multiple species and copulatory behaviors (18, 41–43) in rats and Syrian hamsters (but not in musk shrews (38)). CRF receptor signaling mediates the inhibition of estrous behavior in response to food deprivation in Syrian hamsters (18).

Previous work in Syrian hamsters suggests that metabolic fuel availability is detected in the hindbrain and that this information is relayed to the forebrain synthetically (24, 27, 45), acting on or in conjunction with CRF-receptor-expressing pathways to inhibit estrous behavior (18). Hindbrain neuropeptide Y (NPY) neurons may convey the nutritional information to the forebrain because astressin, a nonspecific CRF antagonist, prevents the suppression of estrous behavior by both food deprivation and intracerebroventricular (ICV) NPY administration in Syrian hamsters (18). Because this work was done with ligands that bind to both of the receptor subtypes (CRF R1 and CRF R2), it does not address which CRF-related ligand or receptor type acts in this capacity physiologically (18).

At present, four CRF receptor ligands have been identified in mammalian nervous system. CRF and urocortin 1 (UCN1) activate both CRF R1 and CRF R2, whereas urocortins 2 and 3 (UCN2, UCN3) activate only CRF R2. Each of the receptor subtypes is known to mediate distinct homeostatic and metabolic functions (2, 10, 31). Generally speaking, CRF R1 is thought to be the major regulator of hypothalamic-pituitary-adrenal activity and is also implicated in the etiology of anxiety and depression (1, 3). In contrast, work with knockout mice and pharmacological manipulations implicates CRF R2 in the control of energy balance and recovery from stress (13, 17, 21, 46), although there is some controversy regarding the latter (1, 28). It is not known which receptor subtype(s) or endogenous ligand(s) mediates the suppression of reproduction in metabolic cues or in response to the presence of NPY.

To shed some light on the CRF receptor type(s) mediating the effects of negative energy balance on female copulatory behavior, we examined the effects of selective agonists and antagonists on estrous behavior in ad libitum-fed and nutritionally challenged Syrian hamsters.

MATERIALS AND METHODS

Animals and surgery. Female Syrian hamsters (Mesocricetus auratus) weighing 80–100 g were purchased from Charles River Breeding Laboratories and fed PMI laboratory rodent diet (no. 5001) ad libitum except where otherwise noted. Animals were singly housed in stainless-steel, wire-bottom cages in a room maintained at 20 ± 2°C with a 14:10-h light-dark cycle (lights on at 0700). Animals were anesthetized with pentobarbital sodium (Nembutal; 80 mg/kg) and supplemented when necessary with methoxyflurane (Metofane), bilaterally ovarioctomized, and implanted with unilateral cannula aimed at the lateral ventricle: 1.1 mm anterior to bregma, 1.7 mm lateral to the midline, and 2.2 mm ventral to the dura. Three stainless steel screws were affixed to the skull and fastened with α-methylcyanoacrylate (Krazyglue). The cannula was fixed in place with dental cement (Duralon). A dummy cannula was inserted into the guide cannula to prevent blockage and was cleaned routinely. To determine proper cannula placement, each animal was administered 100 ng of
angiotensin ICV in 5 μl of artificial cerebrospinal fluid and was observed for 2 min following the infusion. When immediate drinking was observed, the animal was considered to have a ventricular cannula placement and included in the experiments. The University of Massachusetts Institutional Animal Care and Use Committee approved all procedures.

Behavior testing. Hamsters were injected with 2.5 μg of estradiol benzoate in sesame oil 48 h before testing, followed by 500 μg of progesterone dissolved in 5% benzyl alcohol and 15% benzyl benzoate in sesame oil 6 h before testing. Animals were tested before infusion as well as 30 min after peptide infusion. Time of testing took place between 1200 and 1500. Other than in the food deprivation experiment, animals that did not display lordosis for 80 s or more on the pretest were eliminated from the study for that week. During testing for lordosis, females were placed in a Plexiglas arena (30 × 36 × 30 cm) and allowed to habituate for 5 min. A male was then placed in the arena, and the female’s flanks and anogenital region were stroked continuously with a soft artist’s paintbrush (30). The number of seconds the female spent in the lordosis position was recorded during the 3-min testing period. Males were not allowed to mount or intromit. At least 1 wk elapsed between behavioral tests. Food intake was recorded in all experiments. Food was removed from the cages 2 h before measurement to synchronize meals. After tests for estrous behavior were completed, animals were returned to their home cages, and food intake measurements were taken at 1, 2, and 24 h postinfusion. None of the treatments affected food intake, and the data are not reported.

Drug administration. ICV infusions were made in a volume of 5–15 μl. For all infusions, we used a KD Scientific infusion pump and Hamilton syringes. In all infusion experiments, drug infusion occurred 30 min before behavioral testing. UCN2 (1.0–2.0 nmol; American Peptide), UCN3 (0.2–2.0 nmol; American Peptide), and NBI-27914 (4.2–21.0 nmol; Toceir) were dissolved in DMSO and distilled H2O. CRF (0.2 nmol; Phoenix Peptide), NPY (0.48 nmol; Phoenix Peptide), astressin (4.2 nmol; American Peptide), and astressin 2B (4.2 nmol; a gift of Jean Rivier) were dissolved in distilled H2O. CP-154,526 was administered via intraperitoneal (1 mg/animal; dissolved in 5% emulphor, 5% DMSO, and 90% distilled H2O) injection 30 min before infusion of CRF. Drug doses were chosen on the basis of previously published work in hamsters (corticotropin-releasing hormone, NPY, UCN2, UCN3, astressin, and astressin 2B), relative affinities for CRF receptors, or else published work in rats (NBI-27914 and CP-154,526).

Cortisol radioimmunoassay. Animals were decapitated within 1 min of handling. Trunk blood was allowed to clot for 24 h at 4°C and was separated by centrifugation at 3,000 g for 30 min. Serum was aspirated and frozen at −20°C until time of radioimmunoassay. Serum cortisol was determined with the cortisol Coat-a-Count kit from Diagnostic Products.

Statistical analyses. Animals were placed in treatment groups based on their pretest lordosis scores to ensure that each group had a similar mean lordosis duration on the pretest. Changes in lordosis duration from the pretest to the posttest were calculated for each treatment group and compared with controls using one-way ANOVAs. Post hoc analysis with Fisher’s least significant difference test was performed for significant ANOVAs. Results were considered significant at \( P \leq 0.05 \).

**RESULTS**

Selective CRFR2 activation by UCN3 and UCN2. UCN2 and UCN3 are selective endogenous agonists of CRFR2 (22). Furthermore, UCN3 cells project to CRFR2-rich areas in rats, suggesting that it may be a principal endogenous ligand for CRFR2 (17, 23). To test the ability of CRFR2 activation to suppress lordosis, we first infused UCN3 ICV in steroid-primed hamsters. Lordosis duration was significantly depressed when either 1 or 2 nmol was administered (\( P < 0.02 \), Fig. 1). Although not known to synapse in close proximity to neurons containing either CRF receptor, UCN2 has a higher affinity for CRFR2 than does UCN3, and it is also highly selective for CRFR2 (17). ICV infusions of 0.2 and 2.0 nmol UCN2 significantly reduced lordosis duration (\( P < 0.001 \), Fig. 2).

**CRFR2 antagonism by astressin 2B.** The nonselective CRF antagonist astressin prevents the inhibition of estrous behavior in Syrian hamsters when coinfused with CRF or NPY (18). Astressin 2B is a selective antagonist for CRFR2 (36). To examine the necessity of CRFR2 activation in the suppression of estrous behavior, we coinfused astressin 2B (4.2 nmol) along with CRF (0.2 nmol). The lordosis-inhibiting action of CRF was not attenuated by coadministration of astressin 2B. On the other hand, astressin (4.2 nmol) did block the effects of CRF (\( P < 0.01 \), Fig. 3), and astressin 2B (4.2 nmol) was effective in preventing the suppression of lordosis duration when coadministered with UCN2 (0.2 nmol) (\( P < 0.05 \), Fig. 4).

**NPY antagonism by astressin 2B.** The suppressive effects of NPY on lordosis can be prevented by a nonselective blockade of CRF signaling using astressin. In light of this finding, it has been suggested that NPY exerts its effects on lordosis via
CRF-related circuitry (18, 45). To characterize the CRF subtype that mediates this effect, we coinfused NPY with astressin 2B. If NPY neurotransmission is reliant on CRFR2-expressing pathways, then the suppression of lordosis induced by NPY should be attenuated or prevented by coadministration with astressin 2B. Indeed, both astressin and astressin 2B (4.2 nmol, each) prevented the inhibition of lordosis by NPY (0.48 nmol) ($P < 0.05$, Fig. 5).

**CRF and CRFR1 antagonism.** Given that astressin 2B did not block the effects of CRF on lordosis, it is possible that CRFR1 activation alone is capable of suppressing estrous behavior. We did not have access to any CRFR1-selective agonists, but information can be gleaned from CRFR1 blockade in the presence of nonselective CRF agonists. When administered intraperitoneally 30 min before ICV administration of CRF (0.2 nmol), CP-154,526 (1 mg), a small-molecule CRFR1 antagonist (40), did not block the suppression of lordosis duration (data not shown). Likewise, NBI-27914, another small-molecule antagonist of CRFR1 (15), failed to attenuate the lordosis-inhibiting effects of CRF (0.2 nmol) when given ICV at doses between 4.2 and 21 nmol (data not shown). However, these results are inconclusive because neither peptide’s efficacy could be confirmed. In our hands, neither compound blocked the rise in plasma cortisol induced by ICV CRF infusion, even when given in heroic doses (data not shown).

**Food deprivation and CRF antagonism.** Removal of all food at the time of the estradiol benzoate injection 48 h before testing significantly suppresses lordosis duration in ovariectomized, steroid-primed Syrian hamsters, and this suppression was reversed by ICV administration of astressin 30 min before behavioral testing (18). Given that astressin 2B blocks the suppressive effects of NPY on estrous behavior, it is possible that blockade of the CRFR2 would be sufficient to attenuate the suppression of lordosis induced by food deprivation. However, this was not the case; astressin (4.2 nmol), but not astressin 2B (4.2 nmol), significantly increased lordosis duration in food-deprived hamsters ($P < 0.05$, Fig. 6).

**DISCUSSION**

Activation of CRFR2 is sufficient to inhibit sexual receptivity in Syrian hamsters because ICV infusion of UCN3 or UCN2 decreases lordosis duration (Figs. 1 and 2, Table 1). Several neural sites that participate in the control of estrous behavior, including the medial preoptic area, lateral septum, and ventromedial hypothalamus (29), express significant levels of CRFR2 and receive inputs of CRF and/or the urocortins in...
rats (23, 44). Thus there exists a neuroanatomical substrate that could account for the effects of CRF2 activation.

However, our data indicate that activation of CRF1 alone is also capable of attenuating lordosis because astressin, but not astressin 2B, prevented the inhibition of estrous behavior by CRF (Fig. 3, Table 1) and food deprivation (Fig. 6, Table 1). This dose of astressin 2B is behaviorally effective in Syrian hamsters, as indicated by the fact that it blocked the effects of UCN2 on lordosis duration (Fig. 4). Although activation of either receptor type appears to be capable of inhibiting estrous behavior, these data do not speak to the identity of the CRF receptor type(s) mediating endogenous suppression of sexual receptivity during nutritional challenges. At this time, the most parsimonious conclusion is that both receptor types are involved because antagonism of both receptor types is required to reverse the effects of food deprivation (Fig. 6).

Blockade of CRF2 was sufficient to prevent the suppression of lordosis by NPY (Fig. 5; Table 1), and NPY plays a significant role in controlling energy balance and reproduction (20, 45). Central administration of NPY causes a profound decrease in estrous behavior as well as hyperphagia in rats and hamsters (8, 9, 18). Furthermore, NPY is an important regulator of luteinizing hormone (LH) secretion (20, 32). NPY knockout mice fail to exhibit a decrease in circulating LH levels after food deprivation (14), and immunotoxic lesions of NPY-containing neurons that project from the hindbrain to the forebrain prevent the suppression of ovulation by 2-deoxy-D-glucose, an inhibitor of glucose oxidation in rats (16). Upregulation of NPY neurons in times of negative energy balance is well documented in rats (12); in hamsters, there is an increase in NPY mRNA in response to food deprivation as well (19, 25).

NPY neurons synapse in close proximity to CRF cell bodies in the paraventricular nucleus, suggesting a functional relationship (37). Therefore, there is reason to believe that NPY acts upstream of and possibly directly on CRF neurons in the paraventricular nucleus. In conjunction with previous behavioral work (18), these data provide strong support for the contention that CRF is a downstream mediator of NPY effects on lordosis, and the present data demonstrate that this effect is mediated via CRF2 activity (Fig. 5). Although the role of CRF2 activity in times of negative energy balance requires further investigation, we can postulate that situations known to increase NPY neurotransmission will likely induce activity in downstream CRF2-expressing neurons.

In theory, any of the four endogenous ligands for CRF2 could mediate the suppression of estrous behavior by NPY. However, not all are equally likely. In rats, neither CRF nor UCN1 shows a colocalization with CRF2 expression, but UCN3 does (5, 6, 23). The distribution of UCN2 is not well established, and the extent of its colocalization with CRF2 is unclear. In addition, ICV administration of CRF fails to induce c-Fos expression in regions of known CRF2 expression; rather, it induces c-Fos immunoreactivity in areas rich in CRF1 mRNA in rats (5). Thus one can make a strong case for a role of UCN3 in suppression of estrous behavior by NPY. The jury is still out on UCN2, and CRF and UCN1 appear to be unlikely.

It would be a mistake to conclude that NPY acts solely via CRF2 signaling to mediate nutritional suppression of female sexual behavior. As noted above, antagonism of CRF2 alone is insufficient to prevent the decrease in lordosis duration caused by food deprivation. If NPY acts solely by CRF2-associated processes, then neurotransmitters in addition to NPY are likely to be involved. Furthermore, although food deprivation increases NPY expression in hamster brain, not all metabolic challenges that inhibit sexual receptivity do so (19). On the other hand, inactivation of NPY systems is sufficient to prevent the effects of nutritional manipulations on LH secretion and estrous cyclicity (14, 16). However, mechanisms mediating nutritional control of ovulation and estrous behavior differ in some instances. For example, inhibition of glucose metabolism alone is sufficient to inhibit LH secretion in sheep and rats, whereas inhibition of both glucose and fatty oxidation is required to suppress estrous behavior in Syrian hamsters (11, 26). Thus it is possible that redundant systems actually inhibit sexual receptivity in the face of undernutrition.

Previously, we had suggested that neurons responsive to CRF or one of the urocortins may represent the sole “final common pathway” responsible for suppression of estrous behavior in food-deprived animals (18). However, although our group (18) consistently observed increases in lordosis duration in weakly receptive, food-deprived hamsters infused with astressin, this phenomenon depends on the degree of inhibition. That is, we have subsequently found that food-deprived animals that show no lordosis whatsoever in the pretests do not exhibit any increase in lordosis duration following astressin infusion, (Seymour, Jones, and Wade, unpublished observations). This phenomenon is robust, and we have observed it repeatedly. It is conceivable that in some animals food deprivation elicits sufficient release of CRF or one of the urocortins that it cannot be adequately reversed by the doses of astressin we are able to infuse (limited by solubility and volume). Perhaps a more likely hypothesis is that there are redundant neurotransmitter/neuropeptide systems that act in parallel with CRF/urocortin mechanisms. This would not be at all unexpected, given the redundancy observed in the physiological controls of appetite.

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