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Neuropeptide Y (NPY) neurons have been implicated in the regulation of reproductive hormone secretions in a variety of physiological contexts. There is considerable evidence, for example, that a preovulatory NPY surge facilitates preovulatory release of gonadotropin-releasing hormone (GnRH) in proestrous rats [1, 2], and to potentiate GnRH-induced luteinizing hormone (LH) secretion from pituitary gonadotropes [3–7]. These effects appear to be mediated by NPY Y1 receptors expressed in hypothalamic [8, 9] and pituitary [6] cells respectively. Other studies have demonstrated that intracerebroventricular NPY applications can induce changes in basal LH secretion in a steroid dependent manner. NPY is stimulatory in the presence of gonadal steroids [8] and inhibitory in their absence, e.g. in gonadectomized animals. Inhibitory actions appear to be mediated by NPY Y5 receptors [10] and/or Y2 receptors [11]. It has also been proposed that NPY exerts inhibitory actions on the reproductive axis prior to the onset of puberty, and that these may wane as the pubertal acceleration of GnRH pulsatility proceeds [12].

Given the multiplicity of effects of exogenous NPY, the physiological importance of endogenous NPY actions have been difficult to establish. Immunoneutralization of NPY in the brain [13] or in the peripheral circulation [1] has been shown to block or attenuate gonadotropin surges in rats, supporting an obligatory role for the peptide in the generation of preovulatory GnRH and LH surges. Infusion of NPY antibodies into the hypothalamus of monkeys has been found to interrupt GnRH pulsatility [14], implicating synaptic NPY release in the facilitation of basal GnRH pulse generation. Recent studies have also made ooMTofF@ToorespmplCiTix.tK@F@T1ix.tkcFKTrecoMTofF@TubtypxprMmrF@+9GMTakk@F3TgjseffeMmrF@tois+

N.Y., USA) and dried overnight. Tissue pellets were resuspended in 1 ml of PBS (pH 7.4) (each sample) for later GnRH RIA.

The LH and follicular-stimulating hormone (FSH) RIAs were performed using reagents provided by the National Institute of Diabetes and Digestive and Kidney Diseases, including the LH RP-3 and FSH RP-2 reference preparations. The intraassay coefficients of variation for the LH and FSH assays were 8.9 and 5.5%, respectively. Progesterone was assayed with the Immuchem Prog ^{125}I kit (ICN Pharmaceuticals, Costa Mesa, Calif., USA), and E_2 was assayed using the KE2D1 kit (Diagnostic Products Corp., Los Angeles, Calif., USA).

Ovarian Histology

Ovaries were removed at autopsy and fixed overnight in a 4% paraformaldehyde solution. The tissues were then embedded in paraffin, cut into 20- m sections on a sliding microtome, and stained with hematoxylin/eosin for subsequent light microscope analysis. The number of corpora lutea present in representative sections throughout the ovaries was tabulated for each of the genotypes.

Basal Hormone Levels and Responses to Ovariectomy

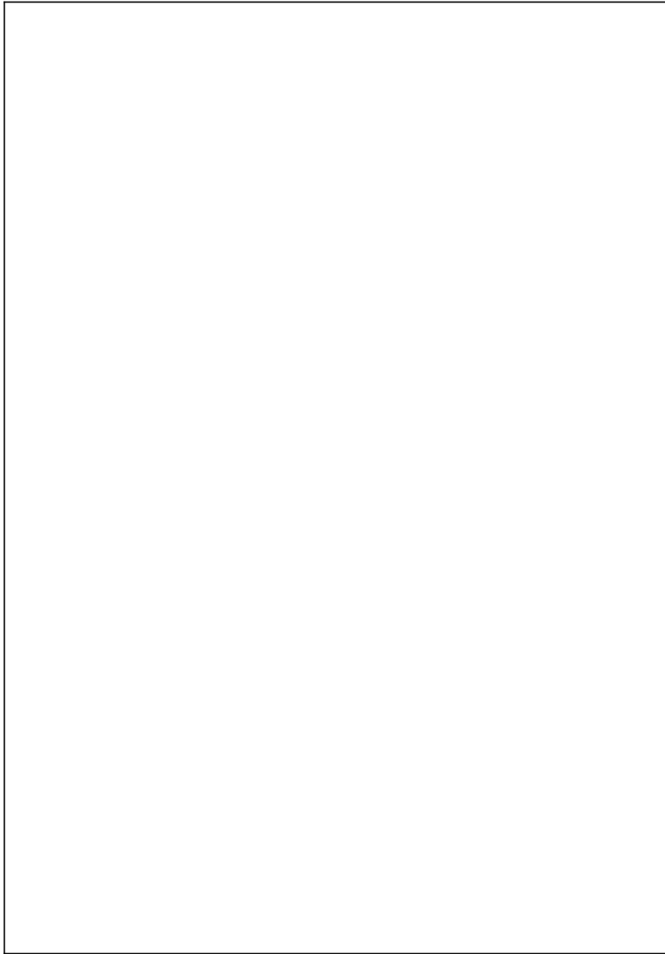
Estrous cycles were monitored in female WT and NPY-KO mice by daily inspection of vaginal cytology as described by Bingel and Schwartz [20]. Mice were selected for serum sample collection after they had exhibited at least two consecutive estrous cycles. For basal level hormone measurements, WT (n = 11) and NPY-deficient mice (n = 12) were sacrificed between 12.00 and 13.00 h of metestrus. The LH, FSH, estrogen and progesterone levels in the sera, and hypothalamic GnRH content, were measured by respective hormone RIAs. Additional groups of WT and NPY-KO mice were anesthetized via methoxyflurane inhalation and bilaterally ovariectomized (OVX). On day 5 or 10 after OVX surgery, these WT and NPY-KO mice (n = 5 for each group) were anesthetized and blood samples obtained via cardiac puncture between 12.00 and 13.00 h. Serum LH, FSH and hypothalamic GnRH were measured by RIAs.

Pheromone-Induced LH Surges

Female mice were individually housed in cages. After exposure to male urine-soaked mouse bedding, WT and NPY-KO mice exhibiting proestrous-like vaginal cytology were anesthetized and killed between 18.30 and 19.00 h via cardiac puncture and exsanguination. Only blood samples from animals showing natural uterus ballooning were used [20]. Serum samples from these animals were stored at –70°C for subsequent LH and FSH RIA. The time of sacrifice was chosen on the basis of previous experiments [21] which showed that the ascending phase of the LH surge in intact, pheromonally stimulated mice occurs between 17.00 and 18.00 h, and that the LH peak occurs at approximately 19.00 h; the same study demonstrated that LH levels return to baseline in these animals by 21.00–22.00 h. In preliminary experiments, we similarly found that LH levels are at or near the minimum level of detectability at 17.00 and 21.00 h of proestrus in both the WT and NPY-KO genotypes (data not shown).

E_2/E_2B-Induced LH Surges

Mice of both genotypes were submitted to OVX and subsets of both animal groups were given estrogen treatments. OVX was performed on the mice at 09.00 h under methoxyflurane anesthesia, and 1-cm Silastic brand capsules (0.04 inches internal diameter, 0.085 inches outer diameter) containing 1.5 g of E_2 mixed into silicone type A medical adhesive were placed subcutaneously (s.c.) under one flank. Controls received empty capsule implants. At 09.00 h on day 6 following surgery and E_2 capsule implantation, each mouse received a s.c. injection of 1 g of E_2B in sesame oil. Between 18.30 and 19.00 h on day 7, mice were sacrificed via cardiac puncture and exsanguination. Control groups received either control capsule (without E_2) implantation and vehicle s.c. injection, control capsule implantation and E_2B injection, or E_2



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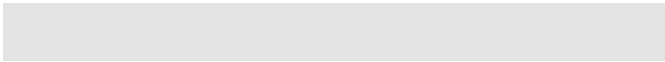

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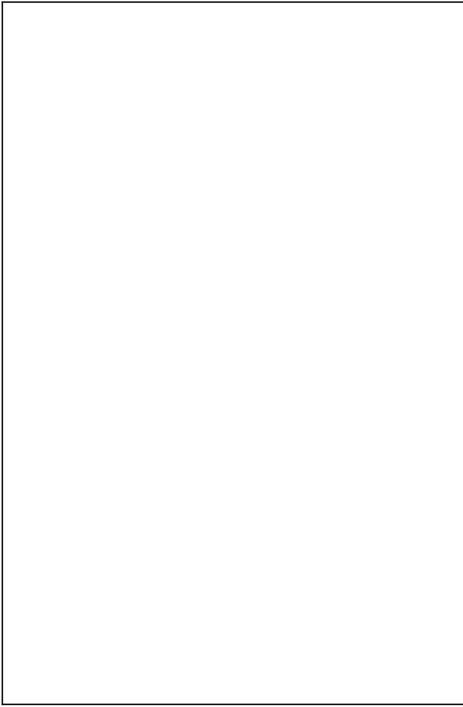

F . 3. Male pheromone-induced preovulatory LH surges and the

LH surges (7.33 B 0.97 ng/ml; n = 10), reached levels that were significantly increased in comparison to both the untreated OVX WT controls (n = 10; p = 0.0009) and the OVX WT animals treated with an E_2-filled capsule alone (n = 11; p = 0.0003) (fig. 4a). By contrast, NPY-KO mice receiving both the E_2 capsule and s.c. E_2B injection exhibited LH levels that only minimally exceeded those in

reveal that NPY-KO mice do exhibit a substantial and specific deficit in their reproductive hormonal secretions; they release preovulatory and estrogen-induced LH surges that are greatly diminished in amplitude compared to those of their WT counterparts.

There is considerable support for the idea that NPY exerts actions at both the hypothalamic and pituitary levels to facilitate release of GnRH and LH surges, respectively. The peptide stimulates GnRH release in vitro and in vivo in a steroid-dependent manner [27, 28], and likewise facilitates GnRH-stimulated LH secretion in vitro and in vivo, especially under conditions which normally lead to LH surges. That these actions are important in the generation of LH surges derives support from numerous observations, i.e. that NPY mRNA levels [29], NPY tissue content in median eminence [2], NPY release in median eminence [30] and NPY levels in the portal vasculature [1] are all acutely increased in association with the initiation of GnRH and LH surges. Moreover, intracerebral administration of NPY antiserum blocks the release of LH surges [31], as does passive immunoneutralization in the peripheral circulation [1].

Given the foregoing body of evidence, we hypothesized that ablation of the NPY gene would be accompanied by a major diminution of the LH surge. Our observations in both pheromone-stimulated and estrogen-primed NPY-KO mice have revealed that such an attenuation is indeed characteristic of the NPY-deficient animal. Our findings in the NPY-KO mice thus confirm the role of NPY for normal amplification of preovulatory LH surges. The extent of the attenuation that we observed in these mice, moreover, is comparable to the degree to which an NPY Y1 receptor antagonist blunts the LH surge in proestrous rats (i.e. 65%) [6]. Our results are therefore also consistent with the assumption that activation of the Y1 receptor subtype may account for NPY facilitation of the surge, a hypothesis which remains to be tested directly by comparing amplitudes of LH surges between strains of mice bearing different deletions of specific NPY receptor subtype genes.

Despite the substantial attenuation of LH surge amplitude, NPY-KO mice did not exhibit any significant reduction in corpora lutea number, pregnancy rate or litter size. It thus appears that ovulation can occur normally in these mice in response to LH surges of amplitudes reduced up to 35% with respect to those observed in WT mice. This phenomenon has long been known in female rats, in which as little as 15% of the spontaneous LH surge is apparently sufficient to trigger ovulations [32]. The reasons for such suprathreshold LH surge under normal conditions are not clear, but may reflect the special characteristics of rodents housed under relatively neutral laboratory conditions. Relatively little environmental stress, as well as ad libitum feeding, may confer a maximally fertile state to the normal laboratory rodent by providing unlimited metabolic energy for reg unlim-

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