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GONADAL STEROIDS AND NEUROSECRETION: FACILITATORY INFLUENCE ON LHRH AND NEUROPEPTIDE Y

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Summary—Luteinizing hormone releasing hormone (LHRH) is regarded as the primary hypothalamic signal that controls reproduction in the rat. Neuropeptide Y, a recently isolated hypothalamic peptide, appears to regulate LHRH secretion. Our studies show that gonadal steroids act in multiple ways to enhance the neurosecretory functions of each of these neuronal systems and, in addition, they promote excitation by NPY of LHRH release from the hypothalamic nerve terminals.

INTRODUCTION

The concept that gonadal steroids may regulate gonadotropin secretion was enunciated more than 50 yr ago [1, 2]. It has been generally understood that the tight reciprocal relationship between the circulating levels of gonadal steroids and hypothalamic luteinizing hormone releasing hormone (LHRH) secretion restrains LH release to low basal levels in rats of both sexes. Additionally, in female rats, this closed feedback loop is interrupted on every 4th or 5th day of the estrous cycle by the stimulatory feedback effects of rising titers of estradiol which, in conjunction with progesterone, induce a massive discharge of preovulatory gonadotropins [3, 4]. Evidence suggests that the central feedback action of steroids is mediated by hypothalamic neurons that preferentially concentrate gonadal steroids [5, 6]. It appears that a subset of steroid concentrating neurons in the preoptic area (POA) and the medial basal hypothalamus (MBH) may locally communicate with the components of the neural circuitry involved in the regulation of LH secretion [7–10].

What is the precise nature of influence exerted by gonadal steroids on LHRH secretion? Major advances in techniques to measure LHRH levels in tissues and body fluids by radioimmunoassay have provided a degree of accuracy that allows the analysis of the effects of gonadal steroids on hypothalamic LHRH release *in vitro* and *in vivo* and to relate them to the dynamic shifts in tissue levels that precede or occur concurrently [4, 9, 10]. Our studies over the past decade have enabled us to formulate a working hypothesis on the mode of gonadal steroid action in regulating the secretory function of LHRH neurons [11–13]. As represented in Fig. 1, our results suggest that gonadal steroids, in general, exert a facilitatory influence on the neurosecretory activity of LHRH neurons [13–15]. Since this view appears contrary to the widely believed notion derived from the concept of gonadotropin regulation by the negative feedback action of steroids, it is our attention to review the experimental findings that led to formulation of our hypothesis and the ongoing studies that emphasize the complex nature of the central action of gonadal steroids.

Recently, we proposed that pituitary LH secretion may be regulated by a distinct neural circuitry, the components of which communicate by diverse chemical signals [4, 9, 10]. A variety of neurochemical signals in the hypothalamus that include biogenic amines and neuropeptides, have the potential to play an important role in the regulation of LH release [4, 9, 10]. It is striking that a number of neuropeptides in the rat hypothalamus are localized in the close vicinity of the LHRH neuronal network [16–21]. This morphological proximity is suggestive of a local interaction between LHRH and neuropeptides in the moment-to-moment control of LH secretion (Fig. 1; Refs. [10, 22]. Because of our longstanding interest in delineating the central physiological effects of gonadal steroids, we recently undertook to systematically investigate the effects of gonadal steroids on the activity of two peptidergic neural systems implicated in the regulation of LH secretion, viz. secretion of opioid peptides and neuropeptide Y (NPY) [4, 9, 10]. In this communication, we have emphasized that gonadal steroids facilitate communications between NPY and LHRH neurons locally in the hypothalamus and that this interaction may have a physiological relevance in the control of gonadotropin secretion (Fig. 1).

Gonadal steroids and LHRH

A number of studies show that in the absence of gonadal secretion, as that prevails after castration, LHRH content of the MBH decreases markedly and this depletion occurs exclusively in the median eminence (ME) region [4, 7–9, 23]. There are two views to account for this castration-induced

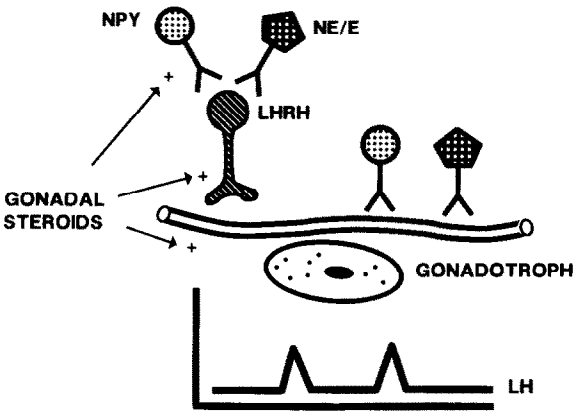

Fig. 1. A schematic representation of the facilitatory effects (+) of gonadal steroids on LHRH, NPY and catecholaminergic (norepinephrine, NE; epinephrine, E) systems in the hypothalamus. It is possible that NPY and NE/E may be released either in the vicinity of the LHRH network in the hypothalamus, and/or like LHRH, they may be discharged into the hypophysial portal system to act at the level of pituitary gonadotrops. Refer to text for details on the facilitatory effects of gonadal steroids on LHRH and NPY systems and Refs. [4, 9, 10, 47] for catecholaminergic-LHRH interactions.

depletion in the ME LHRH levels. It is proposed that in the absence of negative feedback effects of gonadal steroids in castrated rats, the rate of LHRH secretion may exceed the rate of replenishment in the perikarya in the POA-MBH and in the ME nerve terminals. Further, it is implied that this shift in the balance in favor of release over replenishment results in a progressive decrease in LHRH stores until a new dynamic relationship between these two neurosecretory events is established. The strongest evidence to support this view is the demonstration of accelerated episodic LH discharge in association with the progressive biphasic fall in the MBH LHRH stores [7–9]. Implicit in these correlative studies is the assumption that castration-induced LH hypersecretion is a function of accelerated LHRH release into the hypophysial portal system. Evidence gathered first from *in vitro* studies [4, 10, 24, 25] and later corroborated to a large extent by *in vivo* studies [26] has disputed this assumption. In fact, direct analysis of LHRH secretion patterns show that there may be a decrease in LHRH secretion from the hypothalami of castrated rats. Further, *in vitro* LHRH release in response to a variety of secretogogues, such as naloxone, K^+ and Cu^+, was reduced from the hypothalami of castrated rats [24, 25, 27]. This dimunition in LHRH release correlated with the diminished hypothalamic LHRH levels in castrated rats [7, 8, 24, 25, 27]. This strongly suggested that the postulated pulse generator *in vivo* may, likewise, evoke smaller amounts of LHRH/pulse from the hypothalami of castrated than that from intact rats.

Alternatively, it was proposed that gonadal steroids may promote LHRH biosynthesis and secretion in intact rats over the rate that prevails in castrated rats [15, 28, 29]. Several lines of evidence favor this hypothesis of a "trophic" influence of gonadal steroids on the neurosecretory function of LHRH neurons. These are enumerated as follows:

(1) Gonadal steroid replacement can reinstate hypothalamic LHRH levels in castrated rats and the extended time-course of action required to elicit this response suggested that the effects of testosterone, estradiol 17β and dihydrotestosterone [7, 8, 23, 28–30] on this neurosecretory process may be exerted independently on the effect on LHRH release.

(2) Gonadal steroid-induced hypothalamic LHRH increase can be evoked even by those extremely small concentrations of testosterone [29] or estradiol 17β [28] that fail to suppress LH release and the facilitatory action is restricted to the ME region in the MBH [7, 8, 23, 30, 31]. Thus, it is possible that under the influence of steroids new LHRH may become available for storage and/or release in the ME nerve terminals in conjunction with unabated LH secretion.

(3) Castration resulted in a progressive decrease in GnRH Associated Peptide (GAP), a fragment normally elaborated during the processing of the LHRH precursor, with a time course similar to that for the depletion of hypothalamic LHRH levels [32, 33]. This evidence is in line with our thesis that there may be a diminution in the rate of LHRH production in castrated rats [15, 28, 29].

(4) Progesterone treatment acutely raises ME LHRH levels without affecting LH release in a manner normally seen prior to preovulatory LH secretion on proestrus [13, 34–38].

(5) The evidence to date from the *in vitro* [4, 24, 25] and *in vivo* [26, 38] studies show a decrease in the rate of LHRH release from the hypothalami of gonadectomized rats as compared to intact rats.

(6) More recently, we have studied LHRH release *in vitro* from the hypothalami of rats in different phases of the estrous cycle and concurrently com-

pared it with the LHRH release response from the hypothalami of ovariectomized rats [40]. Our studies show that circulating estradiol 17β, and possibly progesterone, concentrations during the estrous cycle [38, 41], in general, permit higher LHRH release as compared to that seen from the hypothalami of ovariectomized rats. We found that LHRH output from the MBH of ovariectomized rats was lowest and it was comparable to that seen from the rats in estrus, a phase characterized by little ovarian steroid secretion. A significant augmentation in LHRH release *in vitro* occurred from the hypothalami of rats on diestrus I, when estrogen and progesterone titers are significantly higher. Additionally, when estrogen alone rose as on the morning of proestrus, the hypothalami of these rats also released high amounts of LHRH. Thus, a positive correlation exists between LHRH release *in vitro* and the rising titers of ovarian steroids during the estrous cycle. Seemingly, the circulating concentrations of gonadal steroids in male and female rats maintain LHRH release at a high level and in the event of a loss of this steroid support, LHRH secretion recedes to a new low level.

Therefore, cumulatively these findings are consistent with our proposal that gonadal steroids not only augment the appearance of new LHRH, but they may promote release as well with different thresholds for modulating the two neurosecretory responses (Fig. 1; Refs. [28, 29]). Our studies show that the "trophic" influence on LHRH appearance in the ME nerve terminals, perhaps a result of increased formation of new precursor LHRH protein in the perikarya and/or accelerated assembly of the biologically active decapeptide in the ME nerve terminals, may require extremely small concentrations of steroids which exert no appreciable effect on LH release [10, 14, 15, 28, 29].

Gonadal steroids and NPY

Neuropeptide Y-containing neurons are widely distributed in the rat brain. The highest levels of NPY are found in the hypothalamus, wherein immunocytochemical mapping studies show a cluster of NPY immunoreactive perikarya in the arcuate nucleus, periventricular nucleus and septum and a dense network of fibers emanating from these cells project into the ME and paraventricular nuclei; a somewhat sparse NPY fiber distribution was seen in other hypothalamic regions including the preoptic area and the ventral medial hypothalamus—regions long known to be innervated by LHRH and steroid concentrating neurons [5, 6, 16–19, 21, 42]. This overlapping topography of the three systems within the hypothalamus and the reported co-existence of NPY and adrenergic transmitters in some neurons [20, 43], led us first to examine the effects of NPY on LH release and later to assess the interaction, if any, between the gonadal steroid milieu and the neurosecretory function of hypothalamic NPY neurons.

Intracerebroventricular administration of NPY in unprimed, ovariectomized rats inhibited LH release [44] and continuous central infusions decreased the amplitude and frequency of LH episodes [45]. LH secretion after continuous NPY infusion decreased rapidly to a low basal range normally attained after ovarian steroid treatment in ovariectomized rats. However, pretreatment of ovariectomized rats with estrogen and progesterone completely reversed the NPY-induced LH response. In steroid-primed ovariectomized rats [44], intraventricular injections of NPY stimulated LH release in a reliable dose-related fashion. The stimulatory effects of NPY on LH release were also observed in intact male rats [46]. The unexpected facilitation of LH release by NPY in the presence of gonadal steroids prompted us to further explore the nature of the interaction between NPY and LHRH neurons that may normally occur in the hypothalami of intact rats [47]. Although NPY was found to be completely ineffective in eliciting *in vitro* LHRH release from the MBH of ovariectomized rats, pretreatment of ovariectomized rats with estrogen facilitated stimulation of LHRH release by NPY. Further, in physiological ranges (10^{-9}–10^{-6} M), NPY evoked a dose-related stimulation of LHRH from the MBH of ovariectomized rats primed with estrogen and progesterone. In fact, this concurrent estrogen, progesterone pretreatment elicited the highest stimulation of LHRH release in response to 10^{-6} M NPY. In additional studies, we noted that NPY also potentiated the K^+-induced LHRH release from the hypothalamus. Thus, it appears likely that NPY may be intimately involved in the release of LHRH into the hypophysial portal circulation and this participation may be dependent upon the hypothalamic microenvironment created under the influence of gonadal steroids. Taken together, our observations are in accord with the proposal that the NPY system may be an intrahypothalamic neural circuit that exerts an excitatory influence on LHRH release in intact rats (Fig. 1). We speculate that these excitatory NPY effects may be mediated by axo-axonic contacts between NPY and LHRH neurons and the interaction between them is gonadal steroid-dependent. The precise mechanism and site(s) whereby gonadal steroids act to modulate the effects of NPY on LHRH release remain to be investigated. Nevertheless, these observations are in line with the emerging notion that the gonadal steroidal milieu may also facilitate the reception of neuropeptides at their target sites in the hypothalamus [47].

Prompted by our previous studies which implied that gonadal steroids can affect some key aspects of LHRH neurosecretion, we have assessed the effects of gonadal steroids on hypothalamic NPY concentrations *in vivo* and release *in vitro*. The effect of progesterone on the ME NPY levels prior to and

during the LH surge in estrogen-primed ovariectomized rats was studied [48]. Progesterone treatment rapidly increased NPY stores in the ME at the time when LH levels were basal; thereafter, as the LH surge began and reached peak levels, NPY concentrations in the ME progressively decreased. Interestingly, LHRH levels in the same ME samples displayed an identical dynamic pattern, an initial rise followed by a progressive fall as the rate of LH secretion was accelerated. It seems that progesterone can stimulate the accumulation not only of LHRH, but also of neuropeptides, such as NPY that have the potential to regulate LHRH release. Since we have shown that NPY stimulates LHRH release from the MBH [47], we speculate that at some time point after the initial accumulation in the ME nerve terminals, NPY is released in the vicinity of LHRH neurons leading, in turn, to LHRH secretion followed by a precipitous rise in LH release.

Additional evidence suggests that in male rats androgens may exert a facilitatory effect on NPY release [49]. We noted that K^+ readily stimulated the *in vitro* release of NPY from the MBH of male rats but the magnitude of response appeared to depend upon the presence of testis. A 15-min depolarizing pulse of K^+ stimulated a significantly higher NPY release from the MBH of intact than from castrated rats. In summary, these observations reveal that gonadal steroids may act in multiple ways to modulate the function of the NPY neuronal system (Fig. 1). Our data show that gonadal steroids facilitate two neural events, the appearance and release of NPY from the ME nerve terminals and the postsynaptic NPY effects subsequent to release.

CONCLUDING REMARKS

The realization that neuropeptides other than LHRH may play a regulatory role in the secretion of gonadotropins has led us to explore the effects of gonadal steroids on neuropeptides in the rat brain. Inceasing evidence favors the view that gonadal steroids exert a facilitatory effect on the LHRH neuronal system in a complex manner. Moreover, other components of the neural circuitry that control LH secretion may also be subject to independent modulation by gonadal steroids at multiple levels. Our studies show that gonadal steroids may activate the production and release, and facilitate the postsynaptic expression of NPY. We speculate that gonadal steroids may act in the brain in three likely ways (Fig. 2). It is possible that neurosecretory neurons (NS), such as LHRH or NPY, may concentrate steroids wherein they may directly affect neurosecretion (Fig. 2A), alternatively, steroid concentrating (SC) neurons may modulate the function of NS neurons either by direct synaptic communications (Fig. 2B) or indirectly via other unidentified neutral link(s) that may be transposed between the two systems (Fig. 2C). In the case of LHRH neurons, the first possibility seems unlikely as we proposed earlier [7, 8] and later corroborated by Shivers *et al.*[50]. Although the first possibility has not yet been completely ruled out for NPY neurons, a restricted distribution pattern of NPY perikarya in the hypothalamus favors the latter two functional relationships. Overall, gonadal steroids may be viewed as humoral signals that exert beneficial effects on those neuropeptidergic systems which control not only the pituitary gonadotropin

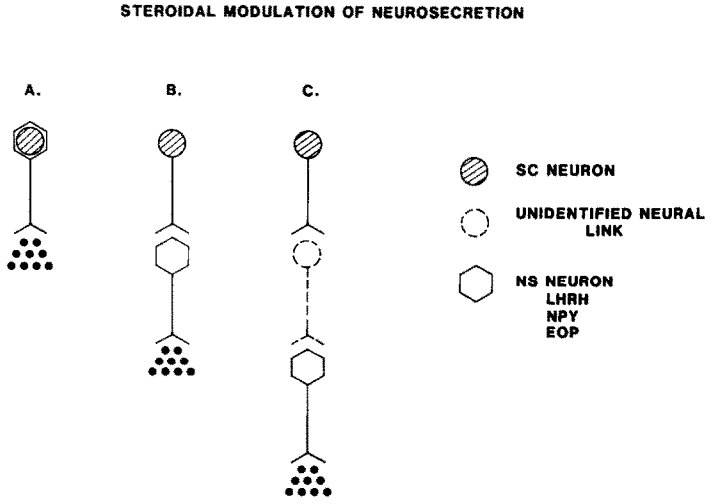

Fig. 2. A schematic representation of the three ways (A, B, C) gonadal steroids may facilitate the secretion of regulatory peptides shown on the bottom right. For details see text. SC—steroid concentrating neuron; NS—neurosecretory neuron, LHRH—luteinizing hormone releasing hormone; NPY—neuropeptide Y; EOP—endogenous opioid peptide.

secretion, but also appetite and sexual behaviors [23, 51, 52].

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