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Gender Specificity in the Neural Regulation of the Response to Stress

New Leads from Classical Paradigms

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Abstract

Pronounced gender-related differences are observable in the regulation of the limbic-hypothalamic-pituitary-adrenal (LHPA) activity under basal and stress-related conditions, and by circulating glucocorticoid levels. This article reviews recent studies that have unequivocally demonstrated that these differences emerge from the organizational effects of gonadal steroids during early brain development. Although largely masked by the dominating role of glucocorticoids in maintaining feedback thresholds, gonadal steroids continue to exert gender-specific activational effects on the LHPA axis through adulthood. The importance of these modulatory effects of gonadal steroids may be reflected in gender differences in the incidence of psychopathologies that are accompanied by symptoms of LHPA dysregulation. One goal of this review is to highlight the need for further investigations into the (still elusive) cellular and molecular mechanisms underlying the activational effects of sex steroids, which may provide leads for neuroprotective hormone replacement strategies.

Index Entries: Sex steroids; stress; pituitary-adrenal axis; glucocorticoid feedback; brain development; menopause; neuroprotection.

The Regulation of the Neuroendocrine Response to Stress: Simple and Elusive

When asked to describe a neuroendocrine regulatory circuit, most endocrinologists would probably refer to the one that governs the secretory response to stress. Indeed, at first look, there is barely anything more clear and straightforward than the events elicited in the endocrine system by a stressful challenge. The secretory response is elicited by well-known triggers in the central nervous system (CNS), whose output, together with the ancilliary contributions of circulation-borne factors, con-

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verge at the pituitary compartment, the ultimate secretory product is a steroid hormone with an immense efficacy that induces a host of biological effects through virtually ubiquitous receptors, which also control the drive of the neuroendocrine cascade at different levels and modalities through feedback mechanisms (Munck et al., 1984; Sapolsky, 1992; Chrousos and Gold, 1992). Thus, it seems that not much can be said beyond the textbook wisdom about the function and principles of regulation of the limbic-hypothalamo-pituitary-adrenal (LHPA) axis (Fig. 1). Nature has implemented a disarming teleology in the organization and regulation of this cascade that has a tremendous importance for survival in, and adaptation to, the permanently changing environment. Regardless of its origin and perception modality, virtually every threat for homeostasis elicits a secretory response in the LHPA axis that mobilizes a host of physiological systems with the ultimate goal of meeting the requirements of enhanced energy expenditure, securing the functional capacity, and decreasing the vulnerability of systems of primary importance to life.

Confusion emerges when one comes to the point of why such an important and, obviously, robust system may deteriorate by means of its own secretory products in a mode that is probably best described as an "obliteration of the most sensitive control mechanisms." First, a "time bomb" is laid down in the duality of requirements concerning the quality of the endocrine response to stress: it must mobilize the organism's reserves for a struggle with an endangerment of unpredictable duration, while being able to rapidly "reset" in order to maintain responsiveness to coincident and subsequent challenges (Johnson et al., 1992). In other words, the LHPA axis is faced with the apparently conflicting necessity of sustaining a challenge of unpredictable intensity and duration, and adequately deciphering the significance of intercurrent homeostatic threats. Second, the responsiveness of the end-point of the LHPA cascade, i.e., the adrenal cortex, is burdened by certain "primitivity." Its secretory products are synthesized and released immediately upon stimulation, with no possibility of storage and fractionated secretion, i.e., in view of the enormous functional reserve of the adrenocortical system, there is always a possibility that the secretory response exceeds the demands of the threatening situation. Finally, the most sensitive target for the regulatory action of glucocorticoids on the LHPA axis, the hippocampus, serves multiple functions that extend beyond the control of the endocrine response to stress. Its high sensitivity to glucocorticoids, however, renders the hippocampus highly vulnerable to prolonged exposure to elevated glucocorticoid levels (Sapolsky et al., 1986; Jacobson and Sapolsky, 1991). Thus, persistent adrenocortical hypersecretion can not only alter the sensitivity of the main regulatory component of the LHPA axis, but can also interfere with its role in processing cognitive information and gating stress perception (Gold et al., 1988).

The view that the ability to rapidly terminate the endocrine response to stress is a critical measure of adequate regulation of the LHPA axis evolved into a major tenet of neuroendocrine physiology, accordingly, aberrations in this respect became increasingly implicated in the pathogenesis of a host of disorders that are associated with symptoms of both abnormal pituitary-adrenal secretion and cognitive-emotional impairment, e.g., major depression, anxiety, anorexia nervosa, and Alzheimer's disease (McEwen, 1987; Sapolsky and Plotsky, 1990; Holsboer et al., 1992). Interestingly, a major evolution also occured in our thinking about the pathophysiological significance of disturbed pituitary-adrenal function in these disorders. Whereas signs of basal hypercorticism and impaired responsiveness to stress and glucocorticoid administration were initially considered as mere epiphenomena that provided certain diagnostic cues, currently, dysregulation of the LHPA axis is causally implicated in the progression of already established cognitive-emotional impairment and pathological changes in the CNS found in these disorders (Holsboer, 1995). However, although there is little doubt about the functional dam-

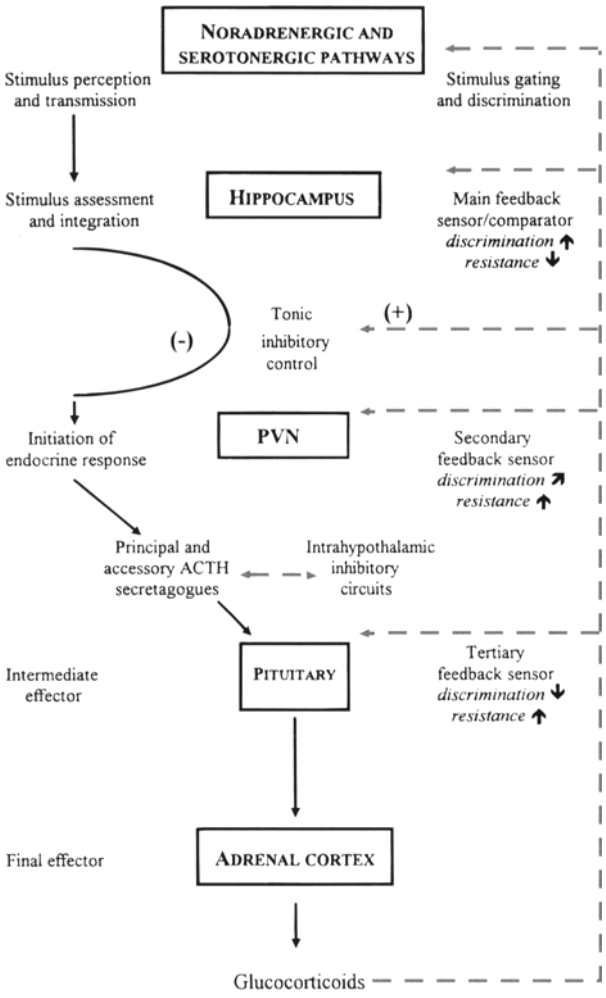

Fig. 1. Schematic representation of "feedforward" (solid black lines) and "feedback" (dashed gray lines) loops involved in the regulation of the LHPA axis. Exteroceptive and interoceptive stimuli converge on structures of the CNS to increase drive on pituitary (ACTH) and adrenocortical (glucocorticoid) secretion. PVN = paraventricular nucleus of the hypothalamus, wherein corticotropin-releasing hormone (CRH) and arginine-vasopressin (AVP) producing neurons are located. The terms discrimination and resistance characterize sensitivity to glucocorticoid feedback and vulnerability by corticosteroids, respectively.

age inflicted on the CNS by persistently elevated adrenocortical activity, gender differences in the susceptibility to this pathogenic factor have been considered only sporadically. Interest in this respect is justified by indications that several disorders that are associated with symptoms of impaired LHPA regulation (e.g., major depression, anorexia nervosa) show clear signs of gender prevalence (Weismann et al., 1993; Kessler et al., 1993). In addition, the concept that individual vulnerability to chronic stress and/or elevated glucocorticoids may be determined by sex-specific variables emerges from observations that several structural and functional aspects of the mammalian CNS are characterized by sexual dimorphism (Madeira and Lieberman, 1995; Guillamón and Segovia, 1996); gonadal hormones exert powerful neurotropic effects in the developing and mature brain (McEwen, 1988; McEwen et al., 1991); substantial similarities and common targets exist with regard to the cellular and molecular mechanisms of action of gonadal and adrenal steroids (Meyer et al., 1989; Carson-Jurica et al., 1990; Wahli and Martinez, 1991; Zilliacus et al., 1995), and receptors for gonadal and adrenal steroids are frequently colocalized in brain structures involved in LHPA regulation (Simerly et al., 1990; Aronsson et al., 1988; Hagihara et al., 1992) (Fig. 2). Thus, a major objective of the studies summarized below was to examine the role of gonadal steroids for the sex-specific organization and regulation of the neuroendocrine response to stress, as well as to scrutinize the importance of gonadal secretions for the emergence of differences in the susceptibility of neuroendocrine regulatory circuits to the pathological effects of chronically elevated glucocorticoid levels.

Gender, Sex Steroids, and Endocrine Response to Stress: What, How, and Why?

Since the first reports on sex differences in the magnitude of the secretory response to stress in rats, several studies addressed various aspects of this issue. The principal findings of those investigations can be summarized as follows: the female rat displays a stronger pituitary-adrenal secretory response to stress that

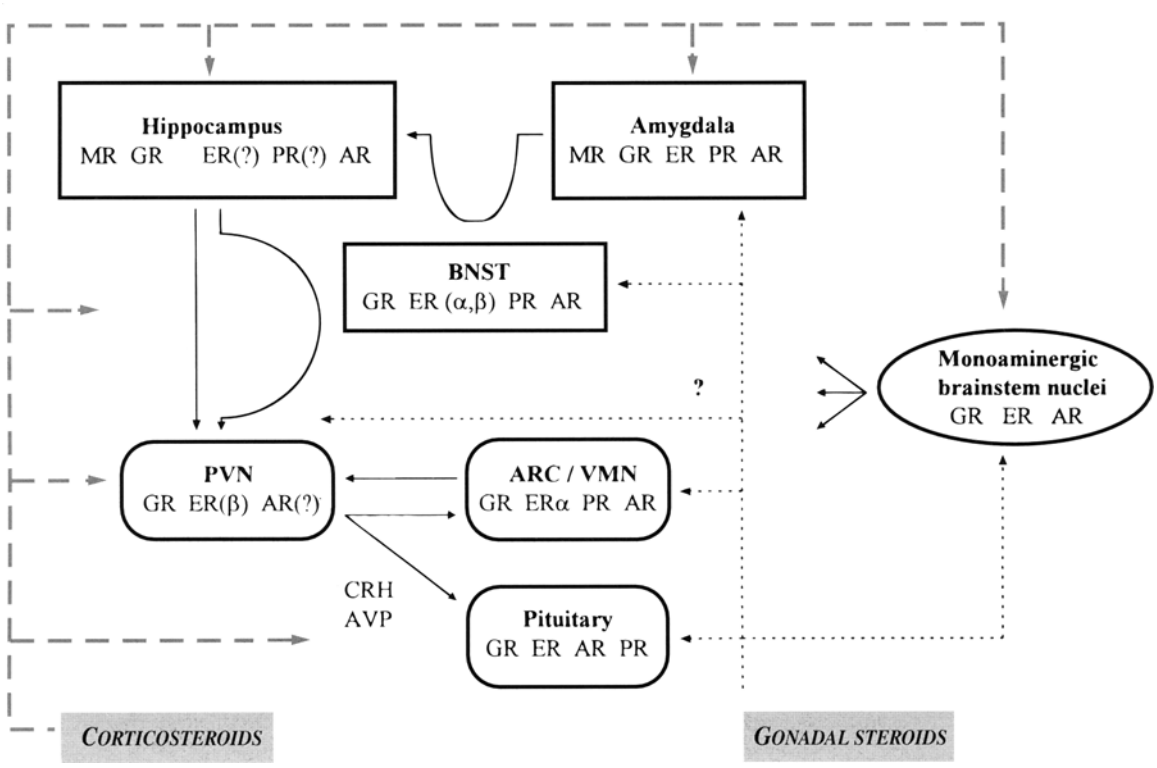

Fig. 2. Colocalization of corticosteroid and gonadal steroid receptors in brain areas relevant to the regulation of the LHPA axis, based on receptor binding, immunocytochemical and *in situ* hybridization studies (refs. Madeira and Lieberman, 1995; Simerly et al., 1990; Aronsson et al., 1988; Haghara et al., 1992; Rainbow et al., 1982; Pelletier et al., 1988; Shughrue et al,. 1996; Lauber et al., 1991). Solid arrows depict established anatomical connections, dashed arrows denote sites of regulatory actions of adrenocortical (gray) and gonadal (black) steroids. Abbreviations: BNST = bed nucleus of the stria terminalis; PVN = hypothalamic paraventricular nucleus; ARC/VMN = hypothalamic arcuate and ventromedial nuclei; MR = mineralocorticoid receptor; GR = glucocorticoid receptor; ER = estrogen receptor; PR = progestin receptor; AR = androgen receptor; CRH = corticotropin-releasing hormone; AVP = arginine-vasopressin.

largely depends on the presence of intact gonads (gender differences tend to disappear following gonadectomy) (Kitay, 1961, 1963; Critchlow et al., 1963; Le Mevel et al., 1979; Kant et al., 1983); in the female rat, the endocrine responsiveness to stress varies during the ovarian cycle (Buckungham et al., 1978; Viau and Meaney, 1991), and several components of the regulatory circuits that govern the LHPA activity and responsiveness are either sexually dimorphic or subject to the influence of gonadal secretions (refs. Turner and Weawer, 1985; Peiffer et al., 1991; Ahima et al., 1992; Burgess and Handa, 1992; Carey et al., 1995; *see* also Fig. 2). Several studies have examined the role of individual gonadal steroids for the emergence of these gender differences. Whereas observations of glucocorticoid-like effects of androgens on stress-induced pituitary-adrenal secretions in males suggest that these gonadal steroids may act as accessory restrainers of the LHPA activity in the male (Bingaman et al., 1994; Handa et al., 1994), estrogens stimulate the biosynthesis of the several hypothalamic activators of the LHPA axis, such as corticotropin-releasing hormone (CRH) (Bohler et al., 1990; Vamvakopoulos and Chrousos, 1993), arginine-vasopressin and oxytocin (Greer et al., 1986; De Vries et al., 1986; Adan and Burbach, 1992). However, for several reasons, it is difficult to clearly define the contribution of individual gonadal steroids to

gender-specific characteristics of LHPA regulation. First, sex steroids act in the brain in a dual fashion that is best described by the terms *organizing* and *activating* (Phoenix et al., 1959): Physiological fluctuations in gonadal secretions in sexually mature females elicit phasic responses (activating effects) in neural substrates that have already experienced irreversible morphological and biochemical hard wiring through sex steroids during early brain development (i.e., sex hormone-dependent organization). Second, there are considerable male-female differences with regard to qualitative, quantitative, and temporal characteristics of gonadal secretions. Third, regulation of the LHPA axis is dominated by glucocorticoids and, thus, subtle modulatory effects of sex steroids may not be readily appreciated.

These considerations largely determined our approach to further examination of gender differences in the regulation of the endocrine response to stress. On the one hand, there was a necessity to define whether gonadal secretions interact with glucocorticoid-mediated regulation of the LHPA axis, on the other, it was intriguing to examine whether sex differences in LHPA regulation emerge from the organizing actions of gonadal steroids in the brain. In most studies, the activating and organizing effects of sex steroids on neural circuits that account for stress-induced stimulation and glucocorticoid-mediated physiological reset or dysregulation of LHPA activity were monitored by measuring changes in the gene expression of hypothalamic CRH and hippocampal corticosteroid receptors, together with determinations of pituitary-adrenal secretions. These parameters have been shown to adequately illustrate the functional state of neural mechanisms of regulation of the LHPA axis (Chrousos and Gold, 1992; Johnson et al., 1992; Jacobson and Sapolsky, 1991; Gold et al., 1988; Holsboer et al., 1992), although, even beyond the scope of this review, the importance of the pituitary for the control of its secretory output deserves closer attention.

When examining these parameters under resting conditions, we made the surprising observation that, despite higher basal (nocturnal) secretion of corticosterone, diestrous females displayed lower CRH mRNA levels in the hypothalamic paraventricular nucleus (PVN) (Patchev et al., 1995). In accordance with earlier observations (Bohler et al., 1990; Vamvakopoulos and Chrousos, 1993), CRH-encoding transcripts in the PVN were increased by estrogen administration in females, thus, confirming the view that phasic estrogen-mediated increase in CRH gene expression might underlie the high responsiveness to stress seen in proestrous rats (Patchev et al., 1995). On the other hand, when compared with males, diestrous female rats showed significantly higher densities of transcripts encoding type I (MR) and type II (GR) corticosteroid receptors in the hippocampus (Almeida et al., 1997; Patchev and Almeida, 1996). Whereas gonadectomy failed to influence the sex differences in MR transcription, GR mRNA levels were decreased in females by both ovariectomy and short-term estradiol replacement (Patchev and Almeida, 1996). In view of the fact that CRH gene expression in the PVN is under the tonic control of extrahypothalamic structures, a major implication of these findings is that, under basal conditions, the female hippocampus may exert a stronger restraint on hypothalamic CRH synthesis. During proestrus, CRH gene transcription may temporarily escape from this tonic suppression, because of either estrogen-mediated stimulation or/and transient decrease in hippocampal GR density. Further, it seems that the presence of gonadal secretions is required for the maintenance of female-specific higher levels of GR mRNA in the hippocampus, and that in the male, hypothalamic CRH and hippocampal corticosteroid receptors are largely refractory to both gonadectomy and estrogen administration.

The assumption that glucocorticoid-mediated feedback operates at a lower discrimination threshold in the female rat was confirmed by examining the ability of various doses of the synthetic glucocorticoid dexamethasone (DEX) to suppress the nocturnal rise in pituitary-adrenal secretions (Almeida et al., 1997). As

shown in Table 1, several glucocorticoid-dependent parameters, such as ACTH and corticosterone levels, CRH-encoding transcripts and thymus mass, responded earlier and/or more dramatically to DEX in the diestrous female than in the intact male. The observations that gonadectomy generally abolished these gender differences and decreased hippocampal GR mRNA levels in females indicate that the sensitivity to glucocorticoids might represent a primary mechanism through which sex steroids modulate the activity of the LHPA axis.

However, since these findings emerged from adrenal-intact animals, it was important to address the question whether gonadal steroids might influence the parameters of interest in the absence of endogenous glucocorticoid control. The expectation that gonadal steroids may alter the responsiveness of the LHPA axis proved to be valid in that progesterone treatment, especially when preceded by estrogen priming, significantly increased the gene transcription of MR in primary hippocampal cell cultures as well as in the hippocampus in vivo (Castrén et al., 1995). The results of a detailed study (Patchev and Almeida, 1996), in which adrenalectomized (ADX) and/or gonadectomized (GDX) male and female rats were supplemented with different gonadal hormone regimens, are summarized in Tables 2A and B. Measurements of CRH-, MR- and GR-encoding mRNAs in the PVN and hippocampus showed that gonadal-steroid deprivation fails to affect these parameters in the presence of intact adrenals; administration of estrogens and androgens alters the gene expression of hippocampal corticosteroid receptors in a glucocorticoid-like fashion, and gonadal steroids fail to influence adrenalectomy-elevated gene transcription of CRH. Taken together, these observations strongly indicated the hippocampus as a site of gonadal and adrenal steroid interactions, at least as far as LHPA regulation is concerned; further the results suggest that hypothalamic CRH is primarily controlled by glucocorticoids. The possibility that sex steroids might act on the PVN indirectly, e.g., by modifying the hippocampal control pathway should not be excluded, however, it seems that in this respect also, glucocorticoids are the more powerful and, probably indispensable, regulatory factor.

For a long time, the former view was supported by findings that showed only moderate expression of estrogen receptors (ER) in the PVN itself (Simerly et al., 1990; Rainbow et al., 1982; Pelletier et al., 1988). Thus, transsynaptic effects originating in other brain structures that project to the PVN, or occurring secondary to alterations at higher (extrahypothalamic) levels of control of the LHPA axis, served as plausible explanations for sex steroid-induced changes in the synthesis of hypothalamic factors (e.g., CRH, vasopressin, oxytocin) related to the neuroendocrine response to stress. An exciting alternative emerged from a report (Shugrue et al., 1996) that documented a strong presence of the recently cloned ERβ (Kuiper et al., 1996) in the PVN and estrogen-sensitive limbic structures (such as the BNST). The circumstance that ERβ was barely present in the hypothalamic ventromedial nucleus, a site characterized by a high abundance of the classic isoform ERα, suggests that a duality of receptor mechanisms might underlie the differential neurotropic actions of estrogens: the ERα-isoform is apparently responsible for estrogen effects related to neuroendocrine control of reproductive function and sexual behavior, whereas ERβ might be the mediator of estrogen influences on neural targets unrelated to reproduction. Mapping of ERβ in extrahypothalamic sites subserving the LHPA axis, especially the hippocampus, will contribute significantly to resolving the enigma of estrogen modulation of the response to stress.

The LHPA Axis Under Glucocorticoid Pressure: What Do Sex Steroids Do?

Whereas glucocorticoids may play a leading role in LHPA regulation, it is nevertheless interesting to examine the interactive effects of gonadal and adrenal steroids in this process.

Table 1
Minimal Effective Doses of Dexamethasone (in μg/kg Body Wt) Required for a Significant Suppression of Nocturnal Pituitary-Adrenal Secretions, CRH Gene Expression in the PVN, and Thymus Involution in Intact and Gonadectomized Adult Male and Female Rats

Parameter	Intact males	Castrated males	Intact females	Ovariectomized females
Serum corticosterone	100	20	20	20[b]
Plasma ACTH	100	100	40	40
CRH mRNA	100	100[a]	40	200
Thymus weight	200	40	20	20

[a,b] Significantly greater or lower response magnitude, respectively, as compared to responses seen in gonad-intact rats of the same sex that have received the same dose of dexamethasone (based on Almeida et al., 1997). Dexamethasone was injected 6 h before testing.

Coadministration of individual gonadal steroids and high doses of corticosterone in adrenalectomized and gonadectomized rats revealed that glucocorticoid effects on regulatory components of the LHPA axis are reinforced or attenuated by sex steroids in a gender-specific fashion (Patchev and Almeida, 1996): The androgen dihydrotestosterone (DHT) potentiated glucocorticoid-induced decreases in CRH and MR gene expression in males; estradiol augmented the suppressive effect of corticosterone on GR transcripts in the female hippocampus; and progesterone, with or without estrogen priming, counteracted glucocorticoid effects on CRH and GR gene transcription in the female, but not male, hippocampus (Table 2C). In accordance with earlier findings (Bingaman et al., 1994; Almeida et al., 1997, Handa et al., 1994; Patchev et al., 1995), these observations indicate that androgens and estrogens may gender-specifically "assist" glucocorticoids in their influence on the key regulators of LHPA activity, however, further investigations are required to elucidate the significance of the observed effects of progesterone. It seems warranted to consider progesterone as a female-specific "buffer" against excessive effects of glucocorticoids, and there is ample evidence for the interference of progesterone with glucocorticoid-mediated signaling at common molecular targets (Castrén et al., 1995; Svec, 1977 von der Ahe, 1985; Strahle et al., 1989). However, the basis for these gender-specific effects of progesterone remains elusive at present. Earlier reports on male-female differences in the density of progesterone receptors in certain brain structures (Brown et al., 1987; Lauber et al., 1991) are a good starting point. In the context of the sequential pattern of sex-steroid secretion during the ovarian cycle, a major step forward would be to provide convincing support for the hypothesis that induction of progesterone receptors by estrogens could be the proper cause for the gender-specific attenuation of glucocorticoid effects by progesterone. Verification of the latter assumption would be of particular value with regard to menopause-related pathophysiological mechanisms of increased vulnerability of the brain to stress and glucocorticoids. The clinical significance of this issue has been outlined by Schmidt and Rubinow (1991) and, more recently, by Young (1995). Although the available evidence is only partial (Patchev and Almeida, 1996; Castrén et al., 1995), we nevertheless suggest that decreased estrogen secretion in menopausal subjects means that the female CNS may become deprived of a major protector against the detrimental effects of high glucocorticoid levels. Simply stated, we assume that the female is less vulnerable to glucocorticoids so long as sufficient estrogen secretion is provided in order to periodically promote in-

Table 2
The Modulatory (Activational) Role of Individual Gonadal Steroids on the Gene Expression of CRH in the Hypothalamic PVN and Corticosteroid Receptors in the Hippocampus of Male and Female Rats

	Males			Females		
Treatment	CRH	MR	GR	CRH	MR	GR
Panel A						
ADX	⇧	⇧	⇧	⇧	⇧	⇧
GDX						↓
ADX + GDX	⇵	⇧	⇵	⇵	⇧	⇵
ADX + GDX + CORT	↓	↓	↓	↓		↓
Panel B						
ADX + GDX + E_2			↓		↓	
+ P	↓					
+ E_2 + P		Not tested				↓
+ DHT		↓	↓		↓	
Panel C						
ADX + GDX + CORT + E_2						↓
+ CORT + P				⇧		⇧
+ CORT + E_2 + P		Not tested		⇧		⇧
+ CORT + DHT	↓	↓				

Panel A: effects of partial (adrenalectomy, ADX; gonadectomy, GDX) or complete steroid hormone deprivation (ADX+GDX), and chronic supplementation with high doses of corticosterone (CORT).

Panel B: effects of supplementation with individual gonadal steroids in ADX+GDX animals.

Panel C: effects of gonadal steroid treatment in rats bearing CORT implants. Arrows denote directions of changes as compared to the initial experimental condition (e. g., intact animals in Panel A, ADX+GDX in Panel B, and ADX+GDX with CORT-implants in Panel C (based on Patchev and Almeida, 1996).

creases in progesterone receptors. If this assumption proves true, it will certainly encourage a re-evaluation of the benefits of hormone replacement therapy (HRT) in the menopause, including its utility in protection against stress and hypercortisolism. The male brain apparently loses its responsiveness to several aspects of estrogen action during early development (Don Carlos et al., 1995; Brown et al., 1996), perhaps including induction of progesterone receptors. Therefore, the male has to get along without the added insurance provided by progesterone agains the deleterious effects of excessive glucocorticoid exposure. However, views on the therapeutic use of estrogens in men may experience a dramatic change with the introduction of tissue-selective estrogen derivatives that are devoid of classic side effects in reproductive organs and secondary sex characteristics, yet selectively influence the CNS (Oettel et al., 1995).

Gender-Specific Organization of Mechanisms That Control the LHPA Axis

Sex-steroid-dependent organization of neuroendocrine regulatory circuits accounts for

male-female differences in the secretory pattern of pituitary gonadotropins and expression of gender-specific sexual behavior. The pioneering studies of Phoenix et al. (1959), Barraclough (1967) and Gorski (1971) convincingly demonstrated that morpho-functional determination of the brain gender under natural conditions results from a brief exposure to sex-specific gonadal steroids during a critical period of perinatal development. Briefly, the concept of sex-hormone-dependent brain organization asserts that a "female-by-default" neural control of gonadal function and sexual behavior becomes "defeminized" through exposure to temporarily enhanced levels of testosterone during early brain development. In the male rat, this process apparently occurs within the final days of gestation; however, in this species, the infant brain remains susceptible to altered sex-steroid levels for the first 2 wk of postnatal life. This knowledge provided the cornerstone of a paradigm that became a classic in experimental neuroendocrinology: Neonatal orchidectomy of male rats, or exposure of female rats to inappropriate (male-like) testosterone levels, irreversibly changes the patterns of gonadotropin secretion and sexual behavior to such that they resemble those of the opposite (genetic) sex (Fig. 3). Ample evidence was subsequently provided in support of the view that androgen-induced defeminization depends on the aromatization of testosterone to estrogens, the latter acting through estrogen receptors (Christensen and Gorski, 1978; MacLusky and Naftolin, 1981; Patchev et al., 1996).

In view of the fact that this paradigm has been sucessfully used for decades to investigate different aspects of hormone-dependent control of sexual behavior and its associated neurochemical mechanisms, we exploited this approach in a series of studies to examine whether the foundations of gender differences in the neural control of the endocrine response to stress are laid down during sex-steroid-dependent brain differentiation. Based on the experience gained from investigations of the activating effects of gonadal steroids on LHPA regulation, we developed a battery of tests for screening gender-specific characteristics of basal and stress-induced pituitary-adrenal secretions in rats that had been exposed to an altered sex-steroid milieu during early postnatal development. The parameters of interest included amplitude of the nocturnal increase in adrenocortical secretion; amplitude of the stress-induced secretory response; efficacy of a threshold dose of dexamethasone in attenuating stress-induced corticosterone secretion; and transcription of genes encoding CRH in the PVN and corticosteroid receptors in the hippocampus.

The results of those studies (Patchev et al., 1995; Patchev et al., 1996) indicated that modifications of the gonadal steroid environment during early development are associated with significant changes in the sex-specific characteristics of the LHPA axis: neonatal orchidectomy produced female-like increases in nocturnal and stress-induced corticosterone secretion, increased sensitivity of the LHPA axis to the suppressive effect of dexamethasone, lower CRH mRNA levels in the PVN, and an increased number of MR- and GR-encoding transcripts in the hippocampus. Conversely, neonatal estrogenization of female pups resulted (in addition to obliteration of ovarian cyclicity) in several male-like changes in LHPA regulation, as expressed by modest increases in nocturnal and stress-related secretory activity, diminished sensitivity to exogenous glucocorticoids, higher steady-state levels of CRH mRNA in the hypothalamus, and a decreased density of MR and GR transcripts in the hippocampus. In addition, neonatal estrogenization was associated with a male-like refractoriness of CRH and GR gene transcription to acute estrogen treatment in adulthood. Taken together, these findings provide convincing evidence for the view that sex hormone-dependent brain organization extends beyond the regulation of gonadal secretion and sexual behavior to encompass the neuroendocrine circuitry that controls the pituitary-adrenal axis.

A further attempt to precisely identify the targets of sex- steroid action within the hypo-

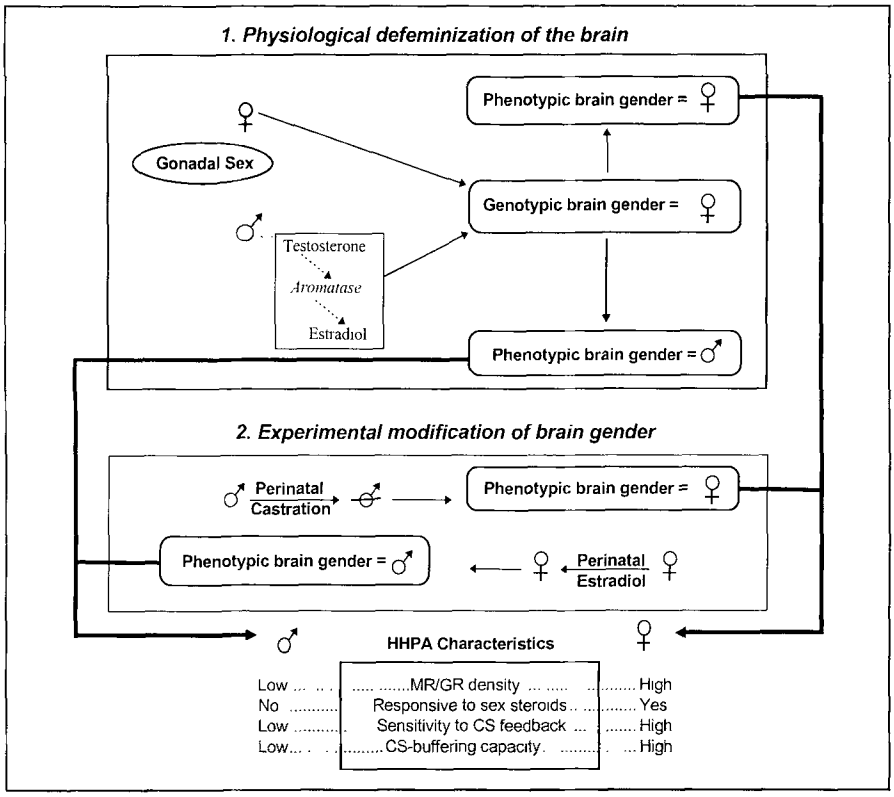

Fig. 3. Natural processes leading to the development of a "male-type" brain (upper half), and experimental manipulations (lower half) which, if made during a critical window of time during development (the first 10 postnatal days in the rat), lead to the maintenance of a phenotypically "female," or the development of a phenotypically "male," brain.

thalamic compartment of the LHPA axis was made by using phenotypically-defined cultures of paraventricular neurons originating from animals of different postnatal ages (Hellbach et al., 1993). These studies revealed that CRH- and AVP-producing neurons emerging from juvenile rats aged 17–20 d (i.e., beyond the critical period of hormone-dependent brain differentiation) display male-female differences in the quality of their responsiveness to glucocorticoids, whereas cultures originating from young (5–10 d-old) neonatally estrogenized female pups showed a male-like pattern in the responsiveness of CRH release to dexamethasone. Together with the observations that GR densities in postnatal PVN neurons display gender differences resembling those found in the hippocampus of intact males and females, and that GR in neuronal cultures prepared from hypothalami of neonatally-estrogenized female rats show male-like binding characteristics (Hellbach et al., 1995), these data suggest that the sensors of glucocorticoid feedback, i.e., corticosteroid receptors in the brain, are major targets of the action of gonadal steroids in shaping the sex-specific regulation of the LHPA axis.

The studies described above clearly indicate that gonadal steroids profoundly influence the regulation of the neuroendocrine response to stress in terms of both organization of the neuronal circuits during early development and operation under the conditions of fluctuating sex-hormone levels throughout life. However, the already mentioned intricacy of LHPA regulation makes it difficult to provide a clear state-

ment of where and how gonadal steroids interact with the primary mechanisms that control the magnitude and duration of the stress response in males and females. Evidence from those studies suggests that sex differences in the secretory response to stress may be engrained in gender-specifically organized mechanisms that ultimately influence glucocorticoid feedback on the stress-induced secretory cascade. Thus, the facts that density and/or sensitivity of hippocampal and hypothalamic corticosteroid receptors can be programmed by gonadal hormones, and that sex steroids can interact with the cellular and molecular mechanisms of signal transmission by glucocorticoids (Meyer et al., 1989; Carson-Jurica et al., 1990; Wahli and Martinez, 1991; Zilliacus, et al., 1995), points to brain corticosteroid receptors as a potential target of sex-hormone action. However, several other factors merit attention in future studies. For example, the role of CBG as a sex-specific gate for the amount of glucocorticoid encountered by the brain remains to be further examined. Indeed, CBG levels display clear gender differences that are also affected by changes in circulating gonadal steroid concentrations; however, Gala and Westphal (1965) reported that there are no significant sex differences in free (i.e., brain-penetrating) glucocorticoid concentrations. In addition, our findings that dexamethasone, a glucocorticoid that does not bind to CBG, is more effective in restraining LHPA activation in female than in male rats (Almeida et al., 1997) suggest that gender differences in the responsiveness to glucocorticoids are rather a matter of responsiveness to, than of corticosteroid availability. Further, the contribution of the pituitary to gender differences in the responsiveness to stress is poorly known, a situation that also applies with regard to sex differences in the responsiveness and secretory capacity of the adrenal cortex (Fonzo et al., 1967). Finally, apart from the mentioned colocalization of gonadal- and adrenal-steroid-hormone receptors in brain structures directly involved in LHPA regulation, sex-steroid receptors are strongly expressed in several brain sites (e.g., amygdala, lower brain stem nuclei) that project to the centers that integrate and control the endocrine response to stress (hippocampus and PVN) (Simerly et al., 1990). Whether sex steroids, upon binding to those sites, can modify the perception, transmission, and assessment of intero- and exteroceptive stressful stimuli requires more careful examination. Interactions between different classes of steroid hormones and their receptors at the molecular level represent a further area of research that could elucidate the role of sex steroids in the control of the neuroendocrine response to stress. Competition for similar response elements (Wahli and Martinez, 1991; Zilliacus et al., 1995) or common transcription factors (Meyer et al., 1989), formation of heterodimers involving different classes of steroid receptors (Trapp and Holsboer, 1996), and mutual influences on gene transcription (Meyer et al., 1989; Patchev and Almeida, 1996; Castrén et al., 1995) exemplify only a few of the possibilities of cross-talk between gonadal and adrenal steroids in neuroendocrine regulation.

Conclusions

Until now, the influence of gonadal hormones on the characteristics of the response to stress has been only partially elucidated. Nevertheless, it seems to be firmly established that sex steroids act on two major components that elicit and control the stress response: hypothalamic CRH amd hippocampal corticosteroid receptors. The principal message from these studies (albeit limitated to the rat) may be summarized as follows:

1. Sex differences in the endocrine response to stress persist in the presence of physiological amounts of gonadal secretions and dwindle upon removal of the gonads.
2. Female rats display large diurnal oscillations in pituitary-adrenal secretions and can mount a stronger secretory response to stress; however, glucocorticoid-mediated restraint of ACTH and corticosterone fluctuations is obvi-

ously more efficient than that in males. Higher densities of hippocampal and hypothalamic corticosteroid receptors probably form the basis for the more sensitive feedback control in the female, the operating thresholds of which can be modified by circulating estrogen levels during the ovarian cycle.

3. Gonadal steroids may interact with glucocorticoids in controlling the LHPA axis in sex-specific potentiating and buffering fashions; the capacity of progesterone to attenuate the effects of high doses of glucocorticoids in the female brain (although progesterone itself proves efficient in dampening the secretory response to stress in a mode resembling glucocorticoids) opens new avenues for examining the significance of altered ovarian secretions in the menopause on the responsiveness to stressful stimuli and the resistance of LHPA regulation to chronic stress and elevated glucocorticoid levels.
4. The neural circuits involved in control of the LHPA axis are maleable by altered gonadal hormone levels during early brain development. Again, sensitivity of the hippocampus and PVN to glucocorticoids appears to be the parameter that is primarily influenced in a sex-specific manner by gonadal steroids.
5. Gonadal steroids alone can barely compete with glucocorticoids for the leading role in controlling the LHPA axis; however, they undoubtedly modify the efficacy of glucocorticoids in the brain either directly (by acting as transcription factors) or transsynaptically.

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