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MINIREVIEW
Pathologic Effect of Estradiol on the Hypothalamus

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ABSTRACT

Estradiol provides physiological signals to the brain throughout life that are indispensable for the development and regulation of reproductive function. In addition to its multiple physiological actions, we have shown that estradiol is also selectively cytotoxic to β-endorphin neurons in the hypothalamic arcuate nucleus. The mechanism underlying this neurotoxic action appears to involve the conversion of estradiol to catechol estrogen and subsequent oxidation to *o*-semiquinone free radicals. The estradiol-induced loss of β-endorphin neurons engenders a compensatory increment in μ opioid binding in the medial preoptic area rendering this region supersensitive to residual β-endorphin or to other endogenous opioids. The consequent persistent opioid inhibition results in a cascade of neuroendocrine deficits that are ultimately expressed as a chronically attenuated plasma LH pattern to which the ovaries respond by becoming anovulatory and polycystic. This neurotoxic action of estradiol may contribute to a number of reproductive disorders in humans and in animals in which aberrant hypothalamic function is a major component.

ESTRADIOL-INDUCED HYPOTHALAMIC PATHOLOGY

Gonadal steroids in general, and estradiol (E_2) in particular, act upon the hypothalamus throughout life. Steroids contribute to the sexual differentiation, development, and maturation of the hypothalamus and provide physiological signals to the hypothalamus and pituitary that are essential for the regulation of the ovulatory cycle. These normal physiological interactions between E_2 and the hypothalamus have been extensively characterized. There is, however, another dimension to the role of estradiol that is less well appreciated. Estradiol exerts a specific pathologic effect on the hypothalamus that may contribute to a variety of female reproductive disorders with a significant hypothalamic component. We first discovered this pathologic action using a single high dose of estradiol valerate (EV) [1]. Subsequently we found that this pathology actually develops in response to physiological levels of estradiol [2, 3]. Indeed, to some extent, the hypothalamic damage occurs with time in untreated animals as a consequence of normal physiological exposure to E_2 [2, 4].

A single large injection of estradiol valerate (EV) given to an adult female normally cycling rat produces (within 4 wk) chronic anovulation, persistent vaginal cornification, and polycystic ovaries [1, 5–7]. This single EV treatment also initiates a progressive multifocal lesion throughout the hypothalamic arcuate nucleus [1, 2]. Other hypothalamic nuclei and limbic structures, such as the amygdala, are unaffected. Within lesion foci, electron microscopy demonstrates the presence of degenerating neuronal elements (often identified as dendrites) as well as reactive microglial cells and an unusual variety of reactive astrocytes, characterized by numerous, pleomorphic dense inclusions [1]. If the ovaries are removed prior to EV injection, the lesion does not occur [2], indicating that the pathology evolves in response to uninterrupted and unopposed exposure to physiological concentrations of E_2 produced by the ovaries as a result of the initial EV insult to the neuroendocrine axis. Chronic exposure to physiologic concentrations of E_2 by means of silastic implants in ovariectomized rats also generates the arcuate pathology [3]. The lesion does not occur in response to androgens or progestins [3, 8]. Moreover, 5α-reduced androgen impedes the pathologic action of E_2 on the arcuate nucleus [3].

Two classes of neuroendocrine defects are associated with this arcuate pathology. The first is a blocked gonadotropin surge mechanism. Since gonadotropin surges can be reinitiated by hemiovariectomy [9, 10], this deficit appears to be a reversible consequence of the unchanging steroidal milieu engendered by the noncycling, polycystic ovaries. The second aberration is an intractable ovary-independent impairment in the hypothalamic circuitry regulating tonic LH release, resulting in a chronically suppressed plasma LH pattern. This defect is reflected in retarded depletion of hypothalamic GnRH [11] and in a corresponding deficit in pituitary GnRH receptor induction following bilateral ovariectomy [12]. It also accounts for the inability of hemiovariectomy to restore normal basal plasma LH levels despite the reinitiation of cyclic LH surges. Although these surges are probably also defective, they are sufficient, at least, to produce corpora lutea [9].

Neuronal Circuitry Affected by Estradiol Toxicity

Recently it has been possible to correlate the intractable ovary-independent hypothalamic dysfunction with the EV-induced lesion in the arcuate nucleus. The arcuate nucleus

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contains neurons that project to the medial preoptic area (MPOA), the region in which most GnRH cells are located. Many of the afferents from the arcuate nucleus have been shown to contribute to the regulation of LH by directly or indirectly influencing the GnRH neuronal system [13]. Some of these inputs, such as those that release neuropeptide Y [14, 15] or neurotensin [16, 17], are excitatory, whereas others such as β-endorphin-containing inputs [15, 18] are inhibitory. Remarkably, although these neuronal populations are densely intermingled in the arcuate nucleus, the pathologic action of estradiol appears selective for the β-endorphin neurons.

Within 8 wk of EV treatment, the hypothalamic concentration of β-endorphin and the number of β-endorphin immunoreactive neurons in the arcuate nucleus are diminished by about 60% [19]. In contrast, EV treatment has no effect on the numbers of arcuate neurons immunoreactive for neurotensin, somatostatin, or tyrosine hydroxylase. Likewise, EV treatment does not appear to affect the hypothalamic concentrations of Met-enkephalin or of neuropeptide Y [19].

Two lines of indirect evidence indicate that β-endorphin neurons are probably destroyed, as opposed to inhibited or otherwise compromised, by the EV treatment. Cell counts performed on Nissl-stained material via unbiased stereological methods reveal a total loss of neurons in the arcuate nucleus in the EV-treated rats; this loss corresponds to the EV-induced decrement in β-endorphin neurons estimated via quantitative immunocytochemistry [19]. Secondly, quantitative analysis of cell shape in the remaining β-endorphin neurons in EV-treated rats indicates numerous distorted immunoreactive perikarya and dendritic processes, suggesting that many of the surviving cells are in the process of degenerating [19].

Beta-endorphin is a powerful inhibitor of LH release. The selective loss of a significant number of β-endorphin afferents to the MPOA, therefore, should result in enhanced pituitary LH secretion. Paradoxically, the opposite is true. The EV-induced destruction of β-endorphin neurons coincides with a marked suppression of the plasma LH pattern [20, 21]. One explanation for this incongruity involves a singular response of β-endorphin target cells in the MPOA to the loss of β-endorphin afferents.

Long before estradiol was found to selectively destroy β-endorphin cells, opioid (naloxone) binding in the anterior hypothalamus of EV-treated rats [22] and of E_2-implanted rats [23] had been shown to be significantly greater than in normal controls. Radioautographic studies using specific opioid ligands demonstrated that this increment was due to a selective increase in μ opioid binding and that it was confined to the region of the MPOA densely populated with GnRH neurons [24]. This suggested that β-endorphin target elements in the MPOA, possibly the GnRH cells themselves, exhibit a compensatory up-regulation of μ binding sites in response to the partial deafferentation. This would render these cells supersensitive to residual β-endorphin or to other endogenous μ ligands such as Met-enkephalin. The resultant chronic opioid inhibition would then account for the suppressed plasma LH pattern characterizing the EV-treated rat [20]. The interpretation that the increment in μ binding in the MPOA is a compensatory response to deafferentation is supported by observations in monosodium glutamate-lesioned rats [25]. Treatment of neonates with monosodium glutamate results in a selective injury to the arcuate nucleus with a marked loss of β-endorphin neurons. Animals thus lesioned exhibited significantly more μ opioid binding in the MPOA than nonlesioned controls. Moreover, they showed a strong inverse correlation between the extent of the loss in hypothalamic β-endorphin and the increment in μ binding.

The identity of the opioid target structures in the MPOA that express additional μ opioid receptors in response to the loss of β-endorphin afferents is not yet known. A likely candidate would be the GnRH neurons themselves. β-Endorphin axons synapse directly on GnRH neurons [26, 27] and may account for as much as 10% of the total synaptic input of these cells [27]. There is, however, evidence indicating that opioid suppression of GnRH involves presynaptic inhibition of facilitatory alpha adrenergic inputs to GnRH neurons [28, 29]. Although this evidence is not unequivocal [30], there is a real possibility that the ascending noradrenergic system may be directly involved in events occurring in the MPOA of the EV-treated rat.

In contrast to the putative hypersensitivity to opioids hypothesized above, there is evidence from one study that ovariectomized rats exposed to chronic supraphysiologic levels of estradiol for 17 days acquire a marked insensitivity to naloxone [31]. Because this occurs only after 17 days, it may reflect an early stage at which β-endorphin neurons are just beginning to degenerate. The resultant incipient loss of β-endorphin afferents to target neurons in the MPOA may not yet initiate compensatory up-regulation of μ opioid receptors. Thus the opioid target neurons may simply be deprived of ligand. Alternatively, the naloxone insensitivity seen in this study may result from physiological alterations that are not directly related to β-endorphin cell loss.

In addition to the loss of β-endorphin cells, other changes occur within the arcuate nucleus following EV treatment. There is a significant, albeit transient, reduction in the number of axosomatic and axodendritic shaft synapses [32]. This alteration is accompanied by a decrease in the concentration of small intramembrane particles in membranes of dendritic shafts and perikarya [33]. Although the import of these modifications is not clear, they undoubtedly reflect some sort of synaptic remodeling. Whether this is secondary to the loss of β-endorphin cells or is itself caused by the estradiol exposure is unknown.

Pituitary Deficits

The low mean plasma LH concentration in the EV-treated rat is not much greater than the GnRH-independent nadir

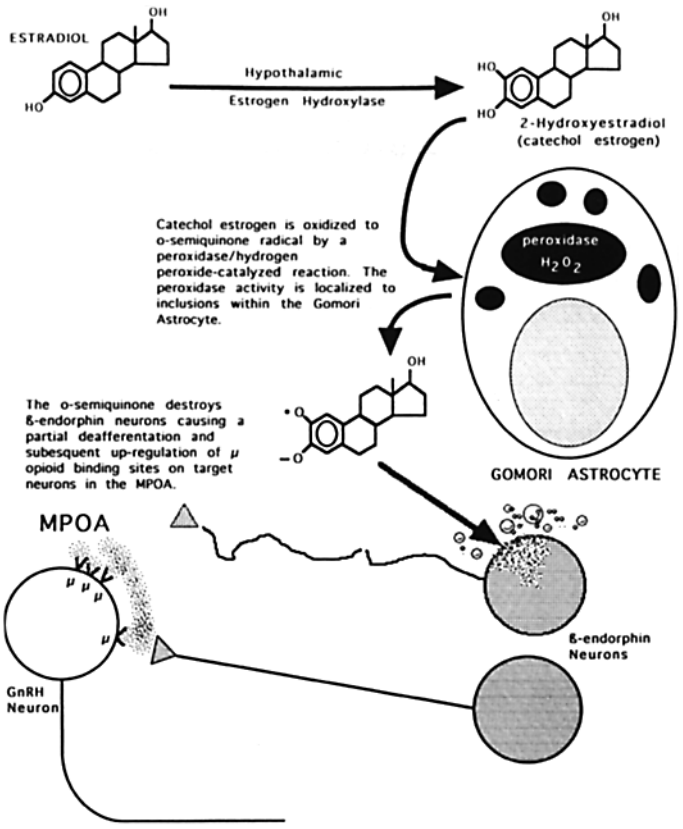

FIG. 1. Pathologic action of E_2 on the hypothalamus. This diagram summarizes our interpretations of the data reviewed in this article and represents a hypothetical model explaining the pathologic effect of E_2 on the hypothalamo-pituitary axis.

[34]. The pituitary LH content, LH responses to GnRH, and LH replenishment following GnRH-stimulated release are severely attenuated [5, 6, 11]. Since all of these LH parameters are, in large measure, regulated by GnRH [35–38], this cluster of deficits is consistent with a long-standing aberrant GnRH signal engendered by the arcuate lesion. Moreover, these deficits persist 28 days following ovariectomy; this is consistent with a physical lesion at the hypothalamic level that ultimately affects the GnRH system. It is also not surprising that the same parameters for FSH either are minimally affected or are normal, since the regulation of LH is far more dependent on GnRH than is that of FSH [42, 43]. The accumulation of hypothalamic and subsequent pituitary defects in the EV-treated rat ultimately translates into an abnormal attenuated plasma LH pattern. The plasma LH pattern in animals in estrus is characterized by large-amplitude LH pulses interspersed among more frequent low-amplitude pulses [20]. Following EV treatment there is a progressive diminution in the number and amplitude of the large pulses such that by Day 16, they disappear altogether [21]. Conversely, there is an equivalent increase in the frequency of small-amplitude pulses, such that the total pulse frequency remains constant. In contrast, the pulsatile FSH pattern is indistinguishable from that characterizing normal estrus. This accords with observations that pituitary FSH function in the EV-treated rat is relatively unimpaired [6, 11].

Coincident with the striking deficits in pituitary LH content, release, and replenishment is a marked diminution in LHβ mRNA. LH/FSHα and FSHβ mRNAs are unaffected. The expression of gonadotropin subunit mRNAs is directly, although differentially, related to the stimulatory action of GnRH [39–41]. It is not surprising, therefore, that the steady state concentration of LHβ mRNA in the EV-lesioned rat is about 30% that of normally cycling controls (unpublished). Although ovariectomy in the EV-treated rats was seen to induce a significant increase in LHβ mRNA, the content was only one-half of that seen in ovariectomized controls.

Two lines of evidence link the cluster of pituitary deficits to sustained opioid inhibition of the GnRH system. The first is the nature of the alterations in the endogenous episodic plasma LH pattern in the EV-treated rat. In the rat, β-endorphin acts to reduce LH secretion by primarily decreasing the amplitude of GnRH pulses [44]. It most often does not modify other parameters such as pulse frequency or duration. Thus, the EV-induced diminution in mean LH pulse amplitude with no change in pulse frequency is consistent with opioid inhibition of the GnRH system [44]. Moreover, it has been shown that specific activation of μ opioid receptors in the MPOA diminishes the amplitude of plasma LH pulses but has no effect on pulse frequency [45]. The low-amplitude, normal-frequency, LH pattern characterizing the EV-treated rat, therefore, is precisely what would be predicted to result from the observed increase in μ binding in the MPOA [24].

Another aspect of pituitary activity that is highly dependent on the pattern of exposure to GnRH is the pituitary concentration of GnRH receptors [46, 47]. In the EV-treated rat, the pituitary concentration of GnRH receptors is chronically low [12]. Although ovariectomy in normal rats results in a marked increase in GnRH receptors, it does not improve the receptor concentration in the EV-treated animals. Opiatergic blockade, however, reinitiates cyclicity, restores normal ovarian morphology, and increases the pituitary concentration of GnRH receptors in EV-treated rats [48], suggesting that the low receptor content in the EV-treated rat reflects chronic opioid suppression of the endogenous GnRH pattern.

Mechanism of Estradiol Neurotoxicity

Until recently, it has not been at all clear how and why estrogen is selectively pathogenic to the arcuate nucleus. Since estradiol target cells in the arcuate nucleus, such as those expressing tyrosine hydroxylase or Met-enkephalin, are spared by the lesion, it is unlikely that the neuronal damage is directly mediated by a conventional E_2-receptor interaction. Moreover, other hypothalamic nuclei, rich in E_2 receptors and/or afferents from E_2-receptive neurons, do not exhibit this pathologic response to E_2 [1, 2]. However,

recent studies by H. Schipper suggest the following hypothesis [49]. A unique population of periventricular astrocytes containing dense Gomori-positive inclusions are concentrated in the arcuate nucleus. The astrocytic inclusions exhibit a nonenzymatic peroxidase activity and therefore stain intensely with diaminobenzidine (DAB) [50, 51]. EV treatment greatly increases the number and size of these inclusions [1, 2]. This peroxidase activity, by catalyzing the formation of highly cytotoxic free radicals, could mediate the local pathologic effect of E_2 on the arcuate nucleus.

The hypothalamus contains the enzymes (estrogen2/4-hydroxylase) necessary for converting estradiol to 2- or 4-hydroxyestradiol (catechol estrogen, OHE_2) [52]. OHE_2 thus generated can be transformed to reactive *o*-semiquinone radicals by a peroxidase/H_2O_2-catalyzed reaction [53]. Indeed, the participation of cultured peroxidase-positive astrocytes in the oxidation of catechol estrogens to their respective *o*-semiquinones has recently been demonstrated through electron spin resonance spectroscopy [54].

We suspect that a reaction of this sort is mediated by the peroxidase-positive astrocytes in the arcuate nucleus of the EV-treated rat. It has been shown that 5α-reduced androgen, a class of steroids shown to inhibit hydroxylase activity [55], blocks estradiol induction of the arcuate lesion [3]. We have also demonstrated that antioxidants such as vitamin E [56], and 21-amino steroids (unpublished results) block the EV-induced loss of hypothalamic β-endorphin and prevent the loss of cyclicity and the formation of polycystic ovaries.

Significance of the Estradiol-Induced Neuropathology

The pathologic action of estradiol on the hypothalamus and the subsequent neuroendocrine aberrations have thus far been studied largely in the EV-treated rat. There are, however, diverse anovulatory conditions in a range of species that share important features with the EV-treated model, suggesting cross-species sensitivity of the hypothalamus to the pathologic effects of estradiol. Estradiol-treated mice, for example, exhibit reproductive and pituitary deficits similar to those characterizing the EV-treated rat [57–59]. Moreover, the EV-induced anovulatory state shares features with the process of normal aging in the rodent. The lesion in the arcuate nucleus [2, 4], as well as reductions in hypothalamic β-endorphin concentrations [60], proopiomelanocortin mRNA [61, 62], and loss of β-endorphin neurons [63], have all been associated with reproductive aging in the female. In addition to these decreases in β-endorphin parameters, aging rodents show decline in LH secretion [62, 64] as well as hypothalamic supersensitivity to endogenous opioid inhibition [65].

Pathologic processes similar to those observed in the EV-treated rat may contribute to a variety of veterinary and human reproductive disorders. Irreversible anovulatory acyclicity characterized by multicystic ovaries and accompanied by hypothalamic pathology also occurs in sheep and cattle as a consequence of ingestion of forage crops containing nonsteroidal estrogenic compounds [66–69]. In humans, excessive endogenous opioid tone has been implicated in the attenuation of plasma LH characterizing hypothalamic amenorrhea [70, 71] and anorexia nervosa [72]. Excessive endogenous opioid function has also been implicated in the fatigue and depression associated with premenstrual syndrome, symptoms thought to reflect diminished noradrenergic transmission engendered by inappropriate opioid inhibition [73]. The possibility that the pathology described above could contribute or predispose to these or similar dysfunctions is very real, as the human hypothalamus is fully capable of converting E_2 to 2-OHE_2 [74, 75]. Moreover, the unique Gomori glia that are hypothesized to mediate the pathologic action of OHE_2 in the rat have also been identified in the human hypothalamus [76].

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