Biomedical **Reviews** 1997; 7: 67-74

ISSN 1310-392X

INTERACTION OF GONADAL STEROIDS AND GROWTH FACTORS IN BRAIN SEX DIFFERENTIATION

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SUMMARY

• *Sex hormones have developmental trophic actions on neurons and glial cells and activational effects in the adult brain. It has been proposed that sex steroids may interact with peptide trophic factors to induce part of their biological effects in the nervous system. The first evidence of such an interaction was provided by Toran-Allerand et al (Brain Res 1980; 184: 517-524), showing that in explant cultures of fetal rodent hypolhalamus, estrogen and insulin have synergistic effects on nei/rite growth, an effect probably mediated by insulin-like growth factor-1 receptors. Recent data indicate that estrogen and insulin-like growth factor-! signaling pathways interact on hypothalamic neurons to regulate survival and differentiation and that sex steroids interact with a variety of different trophic signals in vivo to regulate neuroendocrine events. These findings suggest that trophic factors may be involved in the genesis of sex differences in the developing brain and in the maintenance of a sexually differentiated brain function in the adult. (BioinedRev 1997; 7: 67-74)*

INTRODUCTION

• Hormonal steroids modulate behavior, brain physiology and neuroendocrine function by exerting a variety of different effects on specific neuronal populations. The mechanism of action of hormonal steroids in the nervous system, as in other tissues, involves the activation of specific nuclear receptors that act as transcription factors for specific genes (1,2). In addition, steroid hormones may modulate neuronal excitability by having rapid non-genomic actions (3). Neural activity is also modulated by sex steroid metabolites produced by nerve cells and by local steroids that are synthesized *de novo* in the brain from cholesterol (4). In this review we examine evidence generated in studies on rodents indicating that sex steroids may act as trophic factors for neural cells and as promoters of neural differentiation and plasticity. This trophic and plastic effect of sex steroids may be mediated in part by interaction with other trophic molecules of peptide nature. In particular, complex interactions of one such molecule, insulin-like growth factor-I (IGF-I), and estradiol have been documented recently in hypothalamic neurons.

Rccived for publication 5 April 1997 and accepted 3 June 1997.
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SEX HORMONES AND CELL COMMUNICATION IN BRAIN

• Due to the complex pattern of cell interactions in the development of the central nervous system (CNS), it is difficult to determine whether the effect of sex steroids on a given neural cell type is directly exerted on that cell or mediated by other neurons or glial cells. Since many neurons express receptors for gonadal steroids and since gonadal hormones and their metabolites may have a variety of direct rapid effects on neuronal membranes, it is presumable that steroids will directly affect many different neurons throughout the brain. However, indi-

reel effects are probably very important as well. Acting on their target neurons, for example, gonadal hormones may induce the release of neurotransmitters and trophic factors that could affect the development and function of other neurons. Furthermore, by promoting neuronal survival and differentiation of their target cells, gonadal hormones could also affect the survival and differentiation of the neurons that contact these cells.

The action of sex hormones in the brain involves not only neuron-to-neuron communication since glial cells are also affected by sex hormones and their metabolites (5). Glial cells are a source of growth factors for neurons, promoting neuronal survival and differentiation. Several laboratories have shown that glial cells in culture express receptors for sex steroids (6, 7), suggesting that at least in early stages of brain development glial cells may be a direct target of these hormones (6, 7). Furthermore, it has been reported that hypothalamic glia *in situ* expresses estrogen receptors, based on immunohistochemical evidence (8). Sex hormones and neurosteroids affect myelination by acting on oligodendrocytes and Schwann cells (9,10) and modulate the response of nerve tissue to injury by acting on microglia and astroglia (5, 1 1). In addition to being a target for sex steroids, glial cells are also involved in their metabolism (12) and in the synthesis of neurosteroids (10, 13-15) and participate in the organizational and activational effects of sex steroids on synapse formation (16, 17) and synaptic plasticity (18,19).

GONADAL HORMONES ACT AS NEUROTROPHIC FACTORS

• It is classically recognized that sex steroids exert organizational effects during the critical period for brain sexual differentiation and activational effects in the adult brain (20). During the CNS development, gonadal steroids determine the number of neurons in several brain areas. One of the best studied examples is the sexually dimorphic nucleus of the preoptic area in rodents, which is larger in volume in males than in females (21, 22). This difference in nuclear size is at least partially due to the action of gonadal steroids during the critical period, promoting the survival of a specific population of neurons (23). Another example of a sexually dimorphic structure in the CNS of rodents is the spinal nucleus of the bulbocavernosus (24). Androgens prevent normal cell death of moloneurons of this nucleus (25). These studies suggest that sex steroids may act as neurotrophic factors, promoting the survival of specific neurons or regulating apoptosis (26).

Neurotrophic effects of sex steroids have been characterized *in vitro.* Estradiol promotes the survival of cultured neurons from the amygdala, the hypothalamus and the spinal cord (26a-29). The effect of estradiol on the survival of cultured hypothalamic neurons is mimicked by testosterone, but not by the non-aromatizable androgen dihydrotestosterone (28). The effect appears to be mediated in part through the estrogen receptor since it is saturable and can be blocked by estrogen receptor antagonists, such as tamoxifen and ICI 182,780, and by the inhibition of estrogen receptor synthesis in the cultures by using an antisense oligonucleotide against estrogen receptor mRNA (28, 29).

In addition to the effects on cell number, sex steroids also affect neuronal differentiation in several brain areas, modulating the growth of axons, the number and branching of dendrites and the number of dendritic spines. Estradiol modulates, for instance, the growth of neurites in hypothalamic neurons in explant cultures (30,31) and monolayer cultures (29,32, 33), while in the spinal nucleus of the bulbocavernosus, androgens regulate dendritic outgrowth during the neonatal period and dendritic retraction during the pubertal period (34, 35). Gonadal steroid-dependent sex differences in neuronal processes have been detected in other brain structures, including the preoptic area (36) and hippocampus (37). These effects of gonadal hormones on the differentiation of neuronal processes are mediated, at least in part, by the modulation of cytoskeletal proteins (29, 32, 38).

Hormonal modifications in the growth of neuronal processes may result in changes in the pattern of neuronal connectivity. It is well established that during the critical period gonadal steroids influence the formation of synaptic contacts among neurons, resulting in specific sex differences in neuronal connectivity. Furthermore, the effect of sexrhormoncs on synapses is not restricted to the developmental period. Synapses are plastic structures that may be modified in the adult brain in response to changing physiological conditions, including modifications in hormone levels (18, 39). Gonadal hormones influence synapse formation in areas of the CNS involved in the control of reproductive behavior, such as the ventromedial hypothalamic nucleus, lateral septum and the amygdala and in areas involved in the control of the release of pituitary hormones, such as the hypothalamic arcuate nucleus and the preoptic area. In addition, gonadal hormones may affect neuronal connectivity in cognitive areas, such as the hippocampal formation and the cerebral cortex (18,37, 39-41).

INTERACTION OF SEX STEROIDS AND TROPHIC FACTORS

• The complexity of gonadal steroid action on the brain is not only a consequence of the complexity of cellular interactions in the neural tissue. Interactions between the signaling cascades of membrane receptors for peptidic factors and the steroid hormone receptors should also be taken into consideration. One of the best characterized neurotrophic factors, and the first one discovered, is nerve growth factor (NGF). In some brain areas and during specific developmental stages estradiol and NGF may affect the same target neurons. Genes encoding

NGP and the low-affinity receptor for NGP (p75) are expressed in subpopulations of neurons of hypothalamic neuroendocrine areas (42). Furthermore, it has been shown thatestrogen receptors colocalize with p75 in cholinergic neurons of the basal forebrain of developing rodents (43). Estradiol may therefore exert part of its trophic effects modulating the action of NGF. This is further supported by the fact that estradiol is able to modulate the levels of NGF receptors in PC 12 cells, in cerebral cortical cultures and in dorsal root ganglion neurons of adult female rats (44-46). Moreover, putative estrogen responsive elements have been identified in the NGP gene and in the genes for the NGF tyrosinc kinase (trkA) and low-affinity receptors (46). By regulating trkA or p75 levels, estradiol may affect programmed neumnaldcath (26,47).

A putative estrogen receptor element has been identified also in the gene encoding brain-derived neurotrophic factor (4 8). The receptor trkB for this ncurotrophin is expressed in neurons from hypothalamic neuroendocrine areas, as well as trkC, which is thercceptorforneurotrophin-3(42).

In addition to neurotrophins, other trophic factors may also be relevant in regard to the action of sex steroids in the brain. These include basic fibroblast growth factor (bPGF) and transforming growth factors-ex (TGF-a) and -p (TGF-p), which appear to he involved in the maturation of the neuroendocrine hypothalamus and/or in the regulation of luteinizing hormone-releasing hormone (LHRH) neurons (49-52). Sex steroids may interact with some of these factors to exert their effects on neurons and glial cells. Also, estrogen modulates the expression of TGF-(% in (he hypothalamus (50). This factor is produced by hypothalamic glial cells and appears to regulate LHRH neurons (49, 50, 53). Gene expression of TGF-(% increases in glial cells at the time of puberty in regions of the hypothalamus involved in LHRH controll (50).

Another factor that may be involved in the effects of gonadal hormones on brain is IGF-I. This factor is highly expressed by glia and neurons of the developing brain (54,55) and has prominent trophic actions, stimulating the survival and differentiation of specific neural cell populations, including hypothalamic neurons in culture (28. 56). IGF-1 may also act as a hormonal signal and may be involved in the feed-back regulation of growth hormone by affecting the synthesis or release of growth hormone-releasing hormone or somatostatin by hypothalamic neurons (57,58). IGF-1 may also affect the reproductive axis by mo(.lulatingthcsecretionol'LHRH(59-6l). ..

INTERACTION OF ESTRADIOL AND INSULIN-LIKE GROWTH FACTOR'1

In several tissues and cell types estrogen uprcgulates 1GP-1 gene expression (62-68) and modulates IGF-1 action by affecting the levels of IGP-I receptors (63, 69) and IGF-binding proteins (70,73) which appear to modulate the availability of IGF-I to target cells. Likewise, IGF-I may regulate steroid hormone action by stimulating the synthesis of steroid hormones (74-76) and steroid hormone receptors (77-79). Furthermore, IGF-I, as other growth factors, may activate the estrogen receptor in different cell lines, including neuroblastoma cells (80-85). This activation of the estrogen receptor occurs in the absence of the hormone. It is mediated by IGF-I receptor membrane-associated signaling pathways (82,85) and involves the activation function 2 domain of the estrogen receptor in neuroblastoma cells (85) and the activation function 1 domain in other cell lines (81,82).

The first evidence of an interaction between IGP-I and estrogen on neural cells was obtained in explant cultures of fetal rodent hypothalamus where estrogen and insulin have synergistic effects on neurite growth, an effect that is probably mediated through IGP-I receptors (86). Furthermore, estrogen modulates IGF-I receptors and binding proteins in monolayer hypothalamic cultures (87) and estrogen effects on hypothalamic neuronal survival and neurite growth are mediated by IGF-I (29). Hypothalamic cultures exposed to estradiol or IGF-I show a significant increase in neuronal survival and in the growth of neuronal processes. Inhibition of IGP-I synthesis in the cultures with an antisensc oligonucleotide to mRNA'^'" results in a significant decrease in the stimulatory effects of 17B-estradiol on the number of neurons and the extension of neuronal processes (29). This indicates that IGF-I is necessary for the manifestation of the hormonal effect, suggesting that estradiol may induce neuronal survival and differentiation by activation ofIGF-I signaling cascades. Also, the effect of IGF-I on the survival and differentiation of hypothalamic neurons depends on the estrogen receptor (29). Both the pure estrogen receptor antagonist ICI 182,780 or an antisense oligode-oxynucleotide to the estrogen receptor mRNA block the effects of IGF-I. This indicates that estrogen receptors are necessary for the action of IGF-I on hypothalamic survival and neuritic growth, suggesting that IGP-I may activate, either directly or indirectly, estrogen receptors.

Interactions of sex steroids and IGF-I are also detected in the brain m j;Y«. IGF-I levels increase in the arcuate nucleus and median eminence of male and female rats at the time of puberty, with males reaching higher levels than females (88). This sex difference is abolished by perinatal administration of testosterone to females. Furthermore, in females there is an abrupt increase in IGF-I levels between the morning and the afternoon of the first proestrus. Henceforth, IGP-I levels fluctuate in the arcuate nucleus and median eminence according to the different stages of the estrus cycle. IGF-I levels are high in the afternoon of proestrus, after the peak of estrogen in plasma, remain increased on the morning of the following day and then decrease to basal conditions by the morning of metestrus (88). In addi-

lion, IGP-I levels decrease by ovariectomy and increase in a dose-dependent manner when ovariectomized rats are injected with 17B-estradiol, an effect that is blocked by the simultaneous administration of progesterone (88). These changes, linked to fluctuations of gonadotrophins, suggest that the modulation by sex steroids of the levels of IGF-I in the mediobasal hypothalamus may be related to LHRH regulation. Moreover, these changes do not reflect modifications in the local synthesis of IGP-T (61,88; see also 89), being probably related to differences in the accumulation of IGP-I from extracerebral sources (61).

CONCLUSION

M Effects of sex hormones on different neuronal populations result in the development of local sex differences in neuronal number and connectivity. Part of these actions of sex hormones may be exerted by the modulation of trophic factor signaling among brain cells. Direct evidence of an interaction of sex hormones and trophic factors on neuronal survival and differentiation has been obtained in the hypothalamus. Furthermore, a variety of trophic factors interact with gonadal hormones in the brain to regulate neuroendocrine events. Recent findings in hypothalamic cultures suggest that the survival and differentiation of neurons expressing hormone receptors and the resulting sexual differentiation of brain circuitry and function may depend on the coordinated play of hormonal and neurotrophic signals acting on neurons and glial cells.

ACKNOWLEDGEMENTS

M This work was supported by Pundacidn Ramdn Areces, DGICYT. NATO (CRG930387), NIH (HD13587), and Pundacidn Endocrinologfa y Nutricidn.

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