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SEX HORMONES AS GROWTH PROMOTING FACTORS FOR THE ENDOCRINE HYPOTHALAMUS

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SUMMARY

Evidence is accumulating that the role of gonadal steroids in establishing adult schedules of gonadotropin release relates to the effects of these hormones on neuronal growth. Androgens or estrogens applied to organ cultures of neonatal hypothalamus greatly increase neuritic growth. Facilitation by estrogen of synaptogenesis in the hypophysiotropic area of neonatal rats has also been demonstrated. Early in this critical period of brain development the hypophysiotropic area in the rat is markedly undeveloped and susceptible to the growth promoting influences of gonadal steroids. Steroids hormones could promote the growth of afferents to the receptive hypophysiotropic areas. These afferents could then dominate the receptive space on hypophysiotropic neurons, while in the absence of steroid, all afferents would compete equally for synaptic space. Thus steroid hormones may function in establishing sex specificity by stimulating growth which results in sex specific neuronal circuitry.

INTRODUCTION

This first paper of a Symposium on hormones and growth describes studies on brain growth and differentiation to highlight the interdisciplinary approach to problems which involve basic and clinical considerations. The scope of the field includes the period of fetal intrapartum and post-natal development and has been approached from studies on humans and lower species. Presently, it appears that many early developmental events are carried over into the post-natal period and that these events, therefore, should not be considered only as isolated occurrences; e.g. offspring with small brains at birth retain subnormal growth patterns and show impaired mental performance in later life [1], and intra-partum asphyxia results in long-term damage. At present it is unknown whether the latter is due to neuronal death resulting from the intra-partum accident or the secondary effects on the subsequently growing and remodelling brain. Furthermore, antepartum events may set the stage (organization) for the effects of, and responses to later exposure to agents such as steroid hormones (functional–anatomic events); evidence is accumulating that remodelling or plasticity may be a normal part of post-natal life within specific regions of the central nervous system (CNS).

We will review these matters in the framework of the effects of sex steroid hormones on CNS neuronal growth, circuitry remodelling, and disease. Although there are a number of other species studied, certain aspects of the developing rodent neuroendocrine brain will be considered as model systems. Because of the brevity required, it will not be possible to incorporate much of what is known regarding the effects of non-steroidal hormones on neuronal growth. However, it is clear that there are many non-steroidal endocrine interactions involved in brain differentiation and development [2, 3].

SEX STEROIDS AND NEUROENDOCRINE DEVELOPMENT

Much of what is known about the role of sex steroids in brain differentiation and development has been derived largely from observations on the hypothalamic neuronal systems that regulate gonadotrophin secretion in the adult. In 1952, Harris and Jacobsohn[4] showed that pituitaries transplanted from the sellas of adult male rats to those of adult females released LH in the cyclic manner characteristic of normal females. This proved that Holweg and Junkmann were correct in suggesting that the mechanisms governing sex specific patterns of gonadotrophin secretion were encoded within the brain rather than within the pituitary gland [5]. There is now a large body of evidence suggesting that sex specificity wth respect to gonadotrophin secretion depends upon sex specific circuitry within the brain which, in the adult, drives the cyclic schedule of gonadotrophin release characteristic of a female or the tonic secretion characteristic of the male, and is determined by the presence of the testes and by the availability of their gonadal steroids to the brain during a critical period of neuronal development [6]. This was confirmed by the observation that neonatal female rats treated with either testosterone or estrogen within the first ten days of life (days 21 to 31 post-conception) developed acyclic patterns of LH secretion as adults, while male rats gonadectomized within the first few post-partum days established regular cyclic patterns of LH release as adults [7].

It was clear, therefore, that some parameters of neuronal differentiation or development are under the direct influence of gonadal steroids during early neonatal development. The narrow, fixed perinatal time intervals in which neonatal reversal of adult sexual function can be effected indicate that there is a well defined critical period in which gonadal steroids exert

a determining influence on neuronal development within the neuroendocrine brain. The duration and temporal location of this critical period exhibits considerable species variability. However, within any species it appears to occupy a fixed constant interval in the developmental continuum [7].

The hypothalamus is a specific region of the brain that is subject to the influences of sex steroids during the critical period, and subsequent hypothalamic development determines sex specific patterns of gonadotrophin secretion in the adult. All of the above functions in the adult result from this brain differentiation plus the presence of normally functioning gonads, i.e. without a cycling ovary, there no cycling of gonadotrophins. Implants of crystalline testosterone or estradiol micro-pellets into the female neonatal rat brain were effective in producing acyclic patterns of LH secretion in the adults only when the site of the implant was the hypothalamus [7, 8, 9]. Although there remains some controversy as to the specific intrahypothalamic locus that is responsive to implanted steroid, it is clear that gonadal steroids presented to the hypothalamus during a critical period of development direct subsequent differentiation of the gonadotrophin controlling neuronal system. The medial preoptic area (MPOA) and the medial basal hypothalamus (MBH) are populated by neurons that exhibit heavy labelling in autoradiographs following administration of tritiated estrogen or androgen [10, 11, 12].

In the rat, evidence exists to indicate that a major portion of the brain differentiating effect of the testes is due to *in situ* conversion of androgens to estrogens [13]. Further, the presence of the aromatization system has been demonstrated in the hypothalamus of perinatal rats and second trimester human fetuses [14].

The gonadotrophin regulating system of the hypothalamus is, therefore, a system of choice in investigating hormonal influences on neuronal differentiation and development. Advantages in studying this system include its relative anatomical and functional simplicity and the clear differences in the male and female patterns of gonadotrophin secretion that are unequivocally diagnostic of sexual differentiation in the rodent [7].

CLOSER INSPECTION OF NEUROENDOCRINE BRAIN DEVELOPMENT

Although developmental studies of the neuroendocrine hypothalamus are, as yet, sparse, there is nonetheless some recent information on the role of sex steroids in fashioning the developing hypophysiotropic circuitry. In 1973, Raisman and Field[15] showed that the medial preoptic area of the adult rat is sexually dimorphic with respect to its nonstrial synaptic connections. There are significantly more synapses of non-amygdalar origin on dendritic spines in the MPOA of the female than the male. Moreover, gonadectomy of neonatal males results in cyclic patterns of LH secretion in the adult, and typical female synaptology in the MPOA of the adult. Treatment of neonatal females with estradiol results in male type MPOA synaptology and in acyclicity in the adult.

It would appear then, that the availability of gonadal steroids during the critical period determines patterns of axonal connections in the MPOA. This is particularly significant since the MPOA probably regulates LH secretion by way of axonal connections with LH releasing factor (LRF) neurosecretory cells in the anterior and medial basal hypothalamus [16–22]. There is also evidence suggesting the MPOA itself harbours LRF neurosecretory cells [24]. In any event, it is clear that the MPOA is an essential structure to the gonadotropin regulating system of the hypothalamus.

The possibility that sex steroids effect synaptogenesis in the developing hypothalamus is further suggested by Matsumoto and Arai[23]. They found that treatment of female rats with estradiol benzoate during the first thirty days of life resulted in twice the number of axodendritic synapses in the arcuate nucleus compared to oil treated controls. The origin and physiologic significance of these synaptic connections is not yet known. There is strong evidence that the arcuate nucleus contains LRF elaborating tuberoinfundibular cells and is a major component of the gonadotrophin regulating system [24]. The steroid treatment did not seem to alter the rate of formation of axosomatic synapses in the arcuate nucleus which may suggest that the synaptogenic effect of estradiol may be specific to particular axon systems, although much work remains before this finding can be properly interpreted.

The role of sex steroids in hypothalamic neuronal development has recently been investigated by Toran-Allerand in explanted neonatal mouse hypothalamic tissue [25]. She found that the addition of estradiol or testosterone to hypothalamic explants *in vitro* resulted in accelerated and intense proliferation of neuronal processes as compared to tissue at the same hypothalamic level cultured in control media. Hypothalamic sections cultured in media mixed with antiserum to estrogen showed severe retardation and delay in neurite formation as well as large numbers of undifferentiated neuroblasts (personal communication). Whether the effects of gonadal steroid in this explant system are truly synaptogenic remains to be determined by electron microscopy. Preliminary observations at the light microscopic level of explants stained with a modified silver impregnation method do, however, indicate a profusion of axon terminals in tissue treated with steroid.

Thus, although the effects of gonadal steroids on developing brains may be manifold, it appears that they selectively facilitate neuronal growth and synaptogenesis in the hypophysiotrophic hypothalamus. Ray Walsh of our laboratory has examined the fine structure of the arcuate nucleus in the neonatal male

and female rat and found that the early neonatal arcuate nucleus is morphologically undeveloped. Definitive synapses are rare and the neuronal perikarya are, for the most part, cytologically undifferentiated, as are most of the glial cells. Growth cones are a prominent feature of the nucleus at this stage. The paucity of synapses and the abundance of growth cones indicates that the circuitry of the nucleus during the first few post-natal days is at a very early stage of development and that functional connections that determine adult patterns of neuronal activity have yet to be established. The synaptic organization of the arcuate nucleus could, therefore, be subject to modifying influences of gonadal steroids during the early post-natal period. A similar state of morphologic immaturity was observed in the MPOA of the rat during the early post-natal period [26].

ORGANIZATIONAL IMPLICATIONS IN THE DEVELOPING RAT

If indeed sex steroids facilitate neuronal development in the neuroendocrine hypothalamus, the question arises as to how this effect could result in functional sexual differentiation. Although, at present, this is a matter of pure speculation, it may be reasonable to suppose that developing target neurons within a hypothalamus exposed to sex steroid during the critical period would grow and branch more rapidly than neurons that are indifferent to the steroid. They could then establish synaptic connections on recipient neurons before the other unaffected axonal systems and would therefore monopolize the synaptic space of these recipient cells. The other afferent connections developing more slowly would arrive at their destination at a time at which much of the available receptive dendritic surface is already occupied by steroid activated connections. Thus, the electrophysiological behaviour of the recipient neurons would be largely dominated by the steroid facilitated afferents. In a hypothalamus in the absence of sex steroid, competition of afferent axons for synaptic space would be more balanced and thus the behaviour of recipient neurons would be determined by afferents which could have little influence in steroid exposed brains.

Inevitably, the foregoing hypothesis is an over-simplification. Karyometric changes occur in a number of hypothalamic nuclei under a variety of experimental and developmental conditions, and these alterations probably reflect an action of hormone on some organizational aspect of metabolism which may or may not be related to synaptogenesis [27–29]. Neonatal manipulations of the steroid milieu apparently alter the rate at which cerebellar neurons incorporate radio-labelled amino acids in the adult, a phenomenon which has little to do with reproductive endocrine function and may be unrelated to neuronal growth [30]. In addition, axonal development continues throughout adult life in the posterior ventromedial nucleus (VM), part of the neuroendocrine hypothalamus, so that the organizational effects of gonadal steroids must be distinguished from later functional–anatomic effects.

Nevertheless, although much remains to be clarified, it seems that the development of sex specific hypothalamic circuitry may provide an excellent model for the study of the organizational influences of gonadal steroid on the developing nervous system.

Studies are presently underway in our laboratory to determine the differential rates of development of a number of morphological parameters in male and female rats. Also under study is the effect of exogenous sex steroids on the rate of synaptogenesis in the arcuate nucleus and MPOA of the neonatal rat.

FUNCTIONAL–ANATOMIC IMPLICATIONS IN THE ADULT RAT

The formation of new neuronal circuits in the neuroendocrine hypothalamus is not limited to the neonatal period of development. Mark Van Houten of our laboratory has frequently observed growth cones in the posterior region of VM (PVM) in adult rats. These are cytologically identical to those commonly seen in developing prenatal or neonatal nervous tissue. In addition, the PVM exhibits reactive astrocytes and microglial cells in association with synaptic terminals in various stages of degeneration. All of these observations were made in normal adult male rats and strongly suggest that, at least in the PVM, synapses are eliminated and renewed on a constant basis. This would mean that neuronal circuits within the PVM are essentially plastic and that circuit remodelling is a continuous process in the adult. To what extent this process is related to hormones, steroid or otherwise, is unknown. It should be emphasized, however, that the VM is probably a source of LRF neurosecretory cells [31–33] and is undoubtedly a target for gonadal steroids in the adult [12]. Furthermore, neurons in VM have been implicated as targets for sex steroids in the neonate on the basis of karyometric changes in adult VM in response to neonatal castration or androgen treatment [29]. The possible role of sex steroids in supporting or facilitating neuronal plasticity in the adult rat is currently under investigation in our laboratory. It may be that this phenomenon occurs independently of any hormone influences. Although similar indications of circuit remodelling were observed by Sotelo and Palay[34] in Deiters nucleus of the rat—a region which is most likely indifferent to most, if not all, hormones—functional–anatomic influences of gonadal steroid well into adult life must be carefully considered.

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DISCUSSIONS

Arai. I was very much impressed by your interesting paper, Dr. Naftolin. I would like to know from the quantitative viewpoint which is more important, aromatization of the androgens in the brain or binding of the steroids with the brain tissue in exerting the effect of androgens to a central nervous system.

Naftolin. Thank you for your interesting comment. The question is what is quantitatively more important aromatization or binding of steroids. At this time the evidence favours aromatization as a primary step in rat brain differentiation. This allows androgens because they are not bound by these proteins to enter the cell as androgens and then within the cell be converted to estrogenic active products. This does not say that androgens as dihydrotestosterone or testosterone itself have no effect but allows for the effect of estrogens by filtering out the unwanted estrogens and allowing for *in situ* metabolism.

Grumbach. Dr Naftolin, could you briefly respond to the question of binding in the human fetus where human α-feto protein does not appear to be an important binder?

Naftolin. To my understanding it is not clear what binding proteins are present in the human fetus during this particular period of gestation or what properties they exhibit. We believe that in the rat, circulating estrogen binding proteins (such as α-feto protein) are protective against undesirable effects of generally available estrogens from the dam and fetus.

Posner. Dr. Naftolin, you have dealt only briefly with the role of aromatisation in determining the central effect of steroids. Perhaps you could briefly review why you think that the conversion to estrogen is the important regulating event and why there may not be direct effects of the androgen itself.

Naftolin. In the case of organisation of the nervous system, the candidates made by the testis include testosterone and estrogen. In this context, the evidence is that whatever testosterone does, estrogen does better by a potency to 10–1000 fold. Moreover, others have shown that intermediate compounds such as 19-hydroxy-testosterone are of intermediate potency in causing brain differentiation, while ring A reduced compounds such as dihydrotestosterone have no or little effect. You can block the effect of testosterone by pretreatment with anti-estrogens such as MER-25. In addition to the evidence presented in my talk, picogrammes of estrogens have been shown to cause brain differentiation when injected into critical brain areas, and recently Dr Parivigi, in our laboratory has shown that catechal estrogens can cause brain differentiation as do the primary estrogens. Our finding of aromatization in the neonatal rats hypothalamus seems to confirm to the proposal that aromatization is a necessary part of rodent brain differentiation.