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Sexual Dimorphism in the Developmental Regulation of Brain Aromatase

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Steroid sex hormones have an organizational role in gender-specific brain development. Aromatase (cytochrome $P450_{AR}$), converting testosterone (T) to estradiol-17β (E_2) is a key enzyme in brain development and the regulation of aromatase determines the availability of E_2 effective for neural differentiation. Gender differences in brain development and behaviour are likely to be influenced by E_2 acting during sensitive periods. This differentiating action has been demonstrated in rodent and avian species, but also probably occurs in primates including humans. In rodents, E_2 is formed in various hypothalamic areas of the brain during fetal and postnatal development. The question considered here is whether hypothalamic aromatase activity is gender-specific during sensitive phases of behavioural and brain development, and when these sensitive phases occur. *In vitro* preoptic and limbic aromatase activity has been measured in two strains of wild mice, genetically selected for behavioural aggression based on attack latency, and in the BALB/c mouse. Short attack latency males show a different developmental pattern of aromatase activity in hypothalamus and amygdala to long attack latency males. Using primary brain cell cultures of the BALB/c mouse, sex differences in hypothalamic aromatase activity during both early embryonic and later perinatal development can be demonstrated, with higher E_2 formation in males. The sex dimorphisms are brain region specific, since no differences between male and female are detectable in cultured cortical cells. Immunoreactive staining with a polyclonal aromatase antibody identifies a neuronal rather than an astroglial localization of the enzyme. T increases fetal brain aromatase activity and numbers of aromatase-immunoreactive hypothalamic neuronal cell bodies. T appears to influence the growth of hypothalamic neurons containing aromatase. Differentiation of sexually dimorphic brain mechanisms may involve maturation of a gender-specific network of estrogen-forming neurons which are steroid-sensitive in early development.

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INTRODUCTION

The way in which transient effects of steroids on brain development interact with the changing environment of the fetus and neonate is still not understood. However, sexual differentiation of the mammalian brain is known to occur during steroid-sensitive phases in the perinatal period and to be affected particularly by exposure to estrogens [1, 2]. The organizational effects of estrogen result in male-specific neuronal morphology [3], control of reproductive behaviour and patterns of gonadotrophin secretion. Estrogens are also crucial for synaptogenesis and dendrite formation [4]. The current view of steroid action during brain ontogeny suggests that a sexually differentiated phenotype develops as a consequence of a single steroidal message from the gonads acting on an undifferentiated bipotential substrate in the brain. This hypothesis may be an over-simplification in that it is difficult to envisage one hormonal signal being responsible for all the disparate changes occurring in the brain during sexual differentiation [5]. However, theoretically two steroid-dependent processes are involved in male mammalian behavioural development, masculinization and defeminization. These processes appear to occur at different stages in perinatal development. Permanent "organizing" effects of steroids on the brain can also

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be distinguished from "activating" effects required for adult behaviour [6]. Evidence of a role for estrogen has come from mutant testicular feminized male (tfm) rats and mice lacking androgen receptors; males are defeminized by postnatal estrogen dispite an insensitivity to androgen [7].

Estrogen formation by the aromatization of androgen in the brain is now well understood. The conversion is catalyzed by an enzymatic complex consisting of cytochrome $P450_{arom}$ and NADPH-cytochrome $P450$-reductase. Aromatase activity is detectable during brain development mainly in limbic structures and in the hypothalamus, which are both implicated in the control of reproductive functions such as behaviour and neuroendocrine control. The aromatase gene has been cloned and sequenced in a number of species [8]. Understanding the aromatase enzyme complex, which is a member of the $P450$ superfamily, is likely to provide a key to how early gene expression of an enzyme regulates formation of a steroid critical for sexual differentiation of the brain. However, there are important species differences which have to be taken into account in understanding brain development and the steroid metabolizing enzymes involved. In mammals, interpretation of the role of steroidogenic enzymes in the developing embryonic and perinatal brain is complicated by the feto–placental unit which partly controls availability of steroids in the fetal brain. It is, however, possible to apply concepts derived from other species such as birds in which the embryonic brain aromatase system is more open to investigation than the developing mammalian aromatase system. The first part of this review briefly discusses concepts derived from experimental work on the adult avian brain aromatase system which is closely linked to behavioural mechanisms, and the second part applies these concepts to studies of the fetal and neonatal mammalian brain.

COMPARATIVE STUDIES OF BRAIN AROMATASE REGULATION

Studies of the avian brain have revealed the complexity of steroid metabolism in the hypothalamus [9]. An array of metabolites, both biologically active and inactive, are formed in the brain areas associated subsequently with adult behaviour. These developing enzyme systems cannot be studied realistically in isolation, because various pathways of androgen metabolism exist in the same brain areas, and there may be interchange of metabolites between brain neurones involved in the control of behaviour or the neuroendocrine system. Radioisotope studies, notably of the avian (dove) brain, have led to four hypotheses on the role of androgen-metabolizing enzymes in behaviour. First, these brain enzymes activate androgens to effective forms in neurones involved in behavioural and neuroendocrine control. Second, androgens are converted to biologically inactive forms, these inactivating enzymes influence testosterone levels available to target cells during sensitive periods of early development when hormones exert their effects. Third, steroid metabolites are formed by competing pathways which modulate enzymatic production of the effective steroid. Fourth, environmental factors such as social stimuli and stress, known to affect brain development, influence metabolic pathways that form both active and inactive metabolites from androgen. Regulation of the production of estrogens from androgens within brain cells is the best studied example. In the ring dove, kinetic studies of aromatase activity showed differences in K_m between brain areas and between brain and ovary in the adult. It is not clear what modulates aromatase gene expression or whether mRNAs for aromatase in different estrogen target sites are derived from different gene transcripts. Adult preoptic aromatase activity can be increased markedly by androgen action. Exogenous testosterone increases the V_{max} of the preoptic enzyme 5-fold in the dove (without changing the K_m of the enzyme). This effect, therefore, involves induced aromatase gene expression and/or changes in the catalytic efficiency. Formation and action of estrogen via its receptor may occur in the same brain areas, and possibly in the same cells. Aromatase and estrogen receptors are co-distributed in the dove brain, but the degree of cellular co-localization has not been established yet. This is true for other species which have been studied [10]. Castration of the male ring dove drastically reduces aromatase activity, but does not affect the number of estrogen receptor cells in the preoptic area detected by immunocytochemistry [11]. The effects of androgen on brain aromatase activity have been demonstrated in other avian species (for review see [10]) suggesting that steroid regulation of brain androgen metabolism is a widespread phenomenon. Rapid changes in environmental stimuli derived from socio–sexual interaction have short-term effects on brain estrogen formation in the male dove. Visual stimuli from courtship interactions rapidly increase brain aromatase activity [12], an experimentally induced increase which parallels that of the normal male reproductive cycle. Therefore, socio–sexual stimuli have a crucial influence on testosterone metabolism in the brain. Photoperiod also influences estrogen formation in the brain. In addition to regulation mediated through changes in gene expression, endogenous brain-derived inhibitors of the aromatase (e.g. $5\alpha/\beta$-reduced androstanes) may influence estrogen formation. Other pathways of androgen metabolism also regulate brain aromatase activity by reduction of the substrate testosterone, and, therefore, the amount of estrogen formed.

Can the idea derived from research on the adult avian brain, that steroids and environmental stimuli modulate the aromatase system, be applied to brain development? Prenatal sex differences have been found in preoptic aromatase activity levels in the rat [13, 14] and ferret [15, 16]. However, in the prenatal rat [17]

and fetal ferret [18] treatment of pregnant females with testosterone, 5α-dihydrotestosterone or flutamide, a potent androgen receptor antagonist, does not influence fetal brain aromatase activity. Research on the mammalian brain suggests, therefore, that steroids do not directly modify developing brain aromatase activity before birth. However, the effects of fetal exposure to hormones via maternal treatment are difficult to interpret in developing mammals, because the metabolic potential of the feto–placental unit may interfere with levels of exogenous steroid reaching the brain. There are, however, transient changes in the activity of the aromatase system later in pubertal development [19], suggesting that activity of the enzyme can change according to stage of postnatal development. The only study so far to demonstrate a significant inductive effect of androgen on the embryonic brain aromatase system has been carried out in birds in view of the accessibility of the avian embryo for experimental alteration of steroid levels. Work on early embryogenesis of the Japanese quail [20] shows that testosterone influences estrogen formation in the developing brain and the sensitivity of the aromatase system is phasic in that cells containing the enzyme do not respond before a critical period of development. Thus, in quail there is a sudden appearance of sensitivity to androgen around embryonic days 10–12.

One factor that could have a role in determining estrogen production is the action of endogenous aromatase inhibitors. An important difference between birds and mammals is the high level of brain 5β-reductase activity in the former group, especially in early development. The 5β-reduced androstanes appear to be endogenous inhibitors of brain aromatase. In our studies, the effectiveness of 5β-reduction products has been compared to synthetic aromatase inhibitors. The non-steroidal inhibitor, Fadrozole, is effective at concentrations approx. 1000 times lower than 5β-dihydrotestosterone (5β-DHT) and thus more potent. The K_m of the hypothalamic aromatase derived from post-hatching dove chicks is increased by either sample dilution or adding a synthetic aromatase inhibitor such as Fadrozole. Thus, the high K_m value of the developing dove brain (Fig. 1) may be due to the effects of endogenous inhibitors on the enzyme. Also, 5β-DHT increases the K_m of the preoptic area (POA) aromatase with testosterone as substrate *in vitro*. The increase in K_m on adding Fadrozole or 5β-DHT to aromatase with no change in V_{max} indicates competitive inhibition. An important question concerning endogenous inhibitors is whether significant 5β-DHT and/or other inhibitory steroids are present *in vivo*. This has not been tested in the developing brain, but following microinfusion of [^{3}H]testosterone into adult dove POA, ^{3}H-5β-DHT is formed *in vivo*.

The possibility that endogenous inhibitors are involved in regulation of the dove hypothalamic aromatase may explain a further interesting difference between the avian and mammalian brain aromatase systems. In the adult dove, aromatase activity is relatively high, indicating active conversion and the K_m is low implying high substrate and enzyme binding affinity; as compared to early development (embryo and post-hatching chick) where the K_m is higher and the affinity lower. This suggests that the juvenile brain aromatase kinetics are the reciprocal of the adult and the capacity of the enzyme for forming estrogen changes during brain development (Fig. 1). This developmental change may be due to the high level of 5β-DHT or other endogenous inhibitors formed

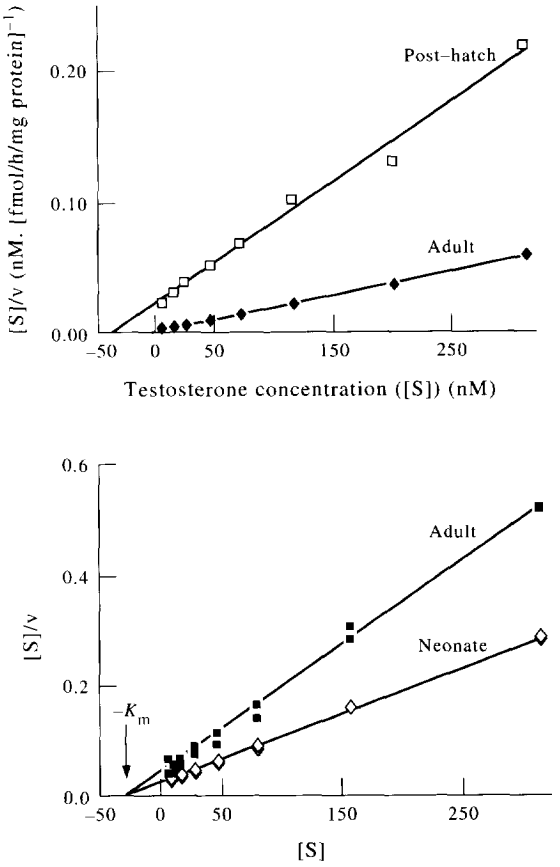

Fig. 1. Species and developmental differences in hypothalamic (preoptic area) aromatase kinetics. Stereospecific formation of ^3H_2O from 1β[^{3}H]testosterone is shown as a function of substrate concentration using Hanes plot derivation. This *in vitro* aromatase microassay minimizes the sample volume required: homogenized aliquots (30 μl) were incubated in a final volume of 50 μl with testosterone (4–320 nM) and NADPH (1.2 mM) in 50 mM KCl, 1 mM EDTA and 100 mM Tris, pH 7.4, for 30 min at 37°C. Reaction was stopped by addition of 160 μl 6% methanol:water, containing 10 μg each of 9 unlabelled androgen standards. Released ^3H_2O was separated from steroids by their extraction onto C 18 (top panel) sorbent. Tissue blanks included 10^{-4} M specific aromatase inhibitor (Fadrozole, Arimidex, etc.) or were boiled. Dove data (upper fig.) represent means of duplicate determinations; all points (lower fig.) shown are for gerbil. Dove kinetic values: post-hatching $K_m = 40$ nM, $V_{max} = 1500$ fmol/h/mg protein; adult $K_m = 4.0$ nM, $V_{max} = 5300$ fmol/h/mg protein. Gerbil kinetic values: neonate $K_m = 30$ nM, $V_{max} = 1250$ fmol/h/mg protein, adult $K_m = 32$ nM, $V_{max} = 670$ fmol/h/mg protein.

in the embryo and post-hatching chick. In mammals (rodents, e.g. mouse, gerbil) the aromatase K_m does not appear to change during development, and the adult enzyme is considerably less active than in adult birds, suggesting a different functional role.

GENETIC EFFECTS ON AROMATASE REGULATION

Is there any evidence that genetic differences between individuals or rodent strains affect formation of estrogen in the brain? Genetic differences in the role of the gonadal steroids have been identified between two selection lines of wild house mice. These strains of wild mice were selected for territorial aggression based on their attack latency [21]. Aggressive short attack latency males (SAL) have higher plasma testosterone levels than less aggressive long attack latency males (LAL). Although testosterone induces aromatase activity via the androgen receptor, aggressive SAL males have lower levels of preoptic aromatase activity despite higher circulating testosterone. Therefore, in adult wild mice, factors other than testosterone determine aromatase activity and regulation of the enzyme.

The general developmental pattern seen in the mouse strains corresponds to that in the rat, where an overall increase in hypothalamic aromatase activity occurs from about 5 days before birth, reaching peak levels 3 days later after which aromatase activity declines sharply. Comparison of aromatase activity in males of the two mouse strains shows that there is an increase in hypothalamic enzyme activity between embryonic days 17 and 18 in LAL males which is correlated with increase in brain weight, but this increase is not seen in SAL males. Although this difference in hypothalamic estrogen formation between SAL and LAL is not absolute in males prenatally, the pattern of development differs within each strain. The effects of selection line in these mice on development of the aromatase system is easier to discern in the amygdala which is known to be involved in aggressive behaviour. Neonatally, SAL males have 50% higher aromatase activity in the amygdala than LAL males [22]. It is likely that genetic effects responsible for strain differences in the brain aromatase system also affect both circulating testosterone level, and maturation of the androgen receptor required for induction of the enzyme.

SEX DIFFERENCES IN DEVELOPING BRAIN AROMATASE ACTIVITY

Gender differences in brain development are likely to be caused in part by formation of estrogen in the brain at specific periods of early ontogeny. To establish this hypothesis, evidence is required which demonstrates that there are cells in the male brain containing the aromatase required to convert androgens to estrogens at specific developmental periods. Apart from answering the question of gender specificity, it is also necessary to know (a) whether the enzyme is localized in developing brain areas which later participate in

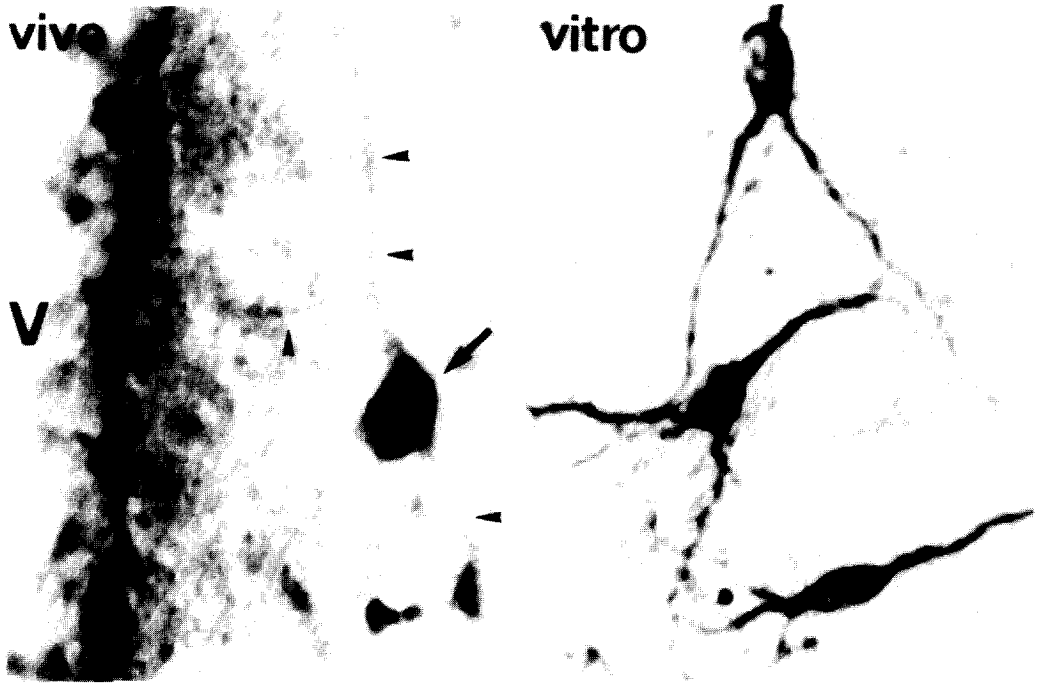

Fig. 2. Photomicrographs illustrate aromatase immunoreactive neurons in male BALB/c hypothalamic cultures (*vitro*) (ED 15, 6 DIV) using a polyclonal, mouse-specific antibody generated against a synthetic 15 amino acid peptide. Both neuronal soma and neurites are stained. Aromatase immunoreactivity was also detected in neuronal processes and soma (*vivo*) in sections of the hypothalamus (V, ventricle).

estrogenic action to regulate the neuroendocrine system and behaviour; (b) which brain cells contain the aromatase and competing enzyme systems; (c) what regulates the aromatase; and (d) whether the activity of the enzyme occurs during specific steroid-sensitive periods of brain development. The mouse is a particularly useful model to study these questions because stages of embryogenesis can be recognized following timed matings and the aromatase gene has been cloned and sequenced [23]. Moreover, the BALB/c mouse strain has an active aromatase system in both the hypothalamus and amygdala. The only practical way to study the developmental origin of cells containing the aromatase system is using tissue culture techniques, so that individual embryonic cells containing the aromatase can be identified.

In our studies, aromatase activity has been compared in tissue homogenates from intact perinatal mouse brains and cells derived from brain tissue culture. Using this design, we have compared hypothalamic and cerebral homogenates of fetuses of known sex (e.g. embryonic day (ED) 13, 15 and 17) and neonates using a sensitive *in vitro* ^3H_2O aromatase microassay. The developmental pattern of aromatase activity can be traced in sex-specific neuronal cell cultures prepared from ED 13 and 15 mouse cerebral hemispheres and hypothalamus. Aromatase activity can also be evaluated in glial-enriched cultures from ED 20 mouse hypothalamus and cortex, as well as ED 15 cultures treated with the neurotoxin kainic acid in order to identify whether aromatase activity is neuronal. In the cortex, aromatase activity is low and sex differences are not apparent irrespective of whether it is measured in ED 15 cultured cells or dissected cortex from fetuses or neonates. However, significant sex differences in aromatase activity are found in hypothalamic cultured cells as early as ED 13. In ED 15 cultured hypothalamic cells from both sexes, aromatase activity is detectable after 3 days *in vitro* (DIV), but there are no sex differences. However, after 6 DIV a sex difference in aromatase activity appears, and activity is significantly increased, indicating that maturation of the hypothalamic aromatase system occurs with time in culture. Male cultured cells always have higher aromatase activity than females. The aromatase is primarily neuronal, since treatment of ED 15 hypothalamic cultures with kainic acid results in a 70–80% decrease in aromatase activity compared to non-treated cultures, and the sex difference seen in hypothalamic cells is no longer present. Astroglial-enriched postnatal hypothalamic cultures exhibit very low aromatase activity after 6 DIV, and no sex differences are present, providing further evidence that aromatase in the developing brain is neuronal. Kinetic studies comparing aromatase activity in male and female hypothalamic cultured cells and homogenates from ED 17 fetuses indicate similar testosterone substrate binding affinities (apparent K_m of ~ 40 nM), suggesting that cultured and intact brain neuronal aromatase share common catalytic properties. Thus, mouse cell culture work demonstrates that aromatase activity in the embryonic brain is neuronal rather than astroglial. There is regional specificity in aromatase activity within the brain, and sex differences are apparent both in the perinatal intact hypothalamus and ED 15 cultured neurones. Since ED 17 hypothalamic neurones show a more marked sex difference in enzyme activity than ED 15 neurones, there is a rapid maturation of the aromatase system at this stage in development which appears to match a peak in circulating testosterone in male fetuses. The developmental profile of aromatase activity in hypothalamic cultures is also of particular interest. Aromatase activity does not differ significantly between the sexes after 3 DIV in ED 15 cells, but exhibits different developmental patterns (male > female) by 6 DIV. At the moment, we are unable to distinguish whether this developmental sex difference reflects a male-specific, cell-intrinsic program, or if it is due to a sex-specific induction of the developing aromatase system before cell culturing on ED 15. The developmental increase in aromatase activity could be due to either more male cells expressing aromatase or increased expression within an existing set of aromatase-containing cells. It is unclear whether the developmental increase in aromatase activity is specifically neuronal or in some other cell type. There may be a dichotomy in source of the enzyme. This possibility depends on whether the aromatase cells seen after 3 DIV are shown to be neuroblasts or developed neuronal soma without processes. The cultures are grown in a medium with undetectable amounts of sex steroids. Therefore, sex differences in aromatase-containing neurons of the early embryonic brain appear to develop in the absence of androgens [24]. However, hypothalamic cells cultured on ED 13 or 15 may be influenced at earlier stages in development by circulating steroids.

IDENTIFICATION OF SEXUALLY DIMORPHIC NEURONS CONTAINING AROMATASE

A polyclonal, mouse-specific aromatase antibody, deduced from the cDNA sequence of mouse ovarian *P*450 aromatase [23] has been developed [24] to identify aromatase-containing cells and their morphological characteristics in embryonic cultures. Aromatase immunoreactivity is localized solely in neurons of the developing mouse hypothalamus and cortex (Fig. 2), but not in astroglial cells or oligodendrocytes. Consequently, estrogen formation in the brain and sex differences in androgen aromatization are restricted to neurons. In contrast, glial cells appear to be involved in other pathways of androgen metabolism, for example 5α-reduction and androstane-3α-diol formation [25]. The neuronal localization of aromatase in mouse cultures contrasts with data on the avian zebra finch

brain in which cultured glial cells express aromatase activity [25].

Hypothalamic cells cultured from ED 13 fetuses contain only a few aromatase-immunoreactive (AR-ir) cells, and there are no immunoreactive processes. Cultures of ED 15 hypothalamic cells form a dense network of microtubuli associated protein (MAP II) positive neurons (60–70% of cells) consisting of neural aggregates of clumped soma and processes [26]. The cultures also contain AR-ir cell aggregates and interconnecting processes which also show immunoreactivity. Double immunofluorescent staining for neurons (MAP II) and aromatase indicates that the AR-ir cells are neurons, probably linked by AR-ir positive processes. These processes do not appear in ED 13 cultured neurons even when the culture period is extended from 6 to 10 days. There is a direct relationship between the number of cells in culture and the enzyme activity measured by radiometric assay. The pronounced increase in aromatase activity between ED 13 and 15 matches the progressive increase in AR-ir cell number. There is also a regional difference between embryonic cortex and hypothalamic aromatase activity, that is matched by the AR-ir neuron number: the number of AR-ir cells are low in the cortex compared to hypothalamus.

HORMONAL REGULATION OF DEVELOPING BRAIN AROMATASE

A key question in understanding the developmental role of the aromatase system is whether estrogen formation in the brain is controlled by the steroid environment of the brain. Since brain aromatase is clearly neuronal, experimental manipulation of steroid level can be studied using the cell culture system. In this way, interference by the feto–placental unit, maternal metabolism, and afferents extrinsic to the fetal hypothalamus can be avoided. Although there are significantly higher numbers of AR-ir neurons which co-stain with MAP II in male cultures compared to female, the aromatase activity expressed per single AR-ir neuron in ED 15 culture does not differ between male and female (capacity for estrogen formation: 0.084 ± 0.01 and 0.080 ± 0.01 fmol/h/mg protein, respectively). Treatment with 10^{-8} M testosterone (for 6 DIV) in cultured male neurons doubles the aromatase activity relative to controls. This increase is specific to hypothalamic cultures and cortical aromatase activity is not increased by testosterone. An interesting result is that female cultured neurons are also hormone sensitive and show increased aromatase activity in response to testosterone. By using the specific aromatase antibody, it is possible to identify hormonal effects on AR-ir cell number. Testosterone increases the number of AR-ir neurons by 70% in cultures of the male hypothalamic cells. Treatment of cultures simultaneously with testosterone and flutamide completely eliminates the testosterone effect. This action of flutamide suggests that the androgen receptor is involved in the induction of neuronal fetal aromatase [26]. There is no evidence so far that testosterone induces proliferation of neuroblasts and neurons expressing aromatase, because MAP II-ir neurons in cultures containing AR-ir neurons do not co-stain for PCNA which is expressed in proliferating cells undergoing cell division [26]. Testosterone is likely to increase aromatase gene expression in hypothalamic cells. However, one possibility that cannot be ruled out is that testosterone may also increase the percentage of cells expressing aromatase by decreasing cell death in the neuronal population of the hypothalamus.

CONCLUSIONS

Estrogens are involved in the development of sex differences in the brain. In the adult, steroid-metabolizing enzymes convert androgens to metabolites effective for brain development, particularly estradiol-17β. Circulating steroid levels and environmental factors influence formation of estrogen by aromatase activity in the brain. A question which still has to be resolved is what determines changes in aromatase activity effective for the differentiation of sexually dimorphic brain development. Aromatization of androgen is important in both fetal and postnatal brain development. In the fetal mouse brain, estrogen formation is neuronal rather than glial. Neurons developing in the embryonic male brain contain higher aromatase activity than the female. This sex difference exists at early stages of embryonic development, whereas the embryonic aromatase system is only regulated later by androgens. Developing aromatase-containing neuroblasts probably form processes which connect to other aromatase cells. The factors that promote the development of neuronal connectivity, and whether there is a steroid-independent period in neuronal ontogeny, remain unknown. Since sex can be identified from an early embryonic age, it is clear that the estrogen-forming capacity of the male hypothalamus has special characteristics and plasticity which develop at certain stages in ontogeny. Future work will identify the aromatase-containing cells in relation to neurotransmitter content as well as other factors involved in the development of sex-specific neuronal connectivity.

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REFERENCES

1. MacLusky N. J. and Naftolin F.: Sexual differentiation of the central nervous system. *Science* **211** (1981) 1294–1303.
2. McEwen B. S., Lieberburg I., Chaptal C. and Krey L.: Aromatization: important for sexual differentiation of the neonatal rat brain. *Hormone Behav.* **9** (1979) 249–263.

3. Garcia-Segura L. M., Baetens D. and Naftolin N.: Synaptic remodelling in arcurate nucleus after injection of estradiol valerate in adult female rats. *Brain Res.* **366** (1986) 131–136.
4. Naftolin F.: Brain aromatization of androgens. *J. Reprod. Med.* **39** (1994) 257–261.
5. Pilgrim C. and Hutchison J. B.: Developmental regulation of sex differences in the brain: can the role of gonadal steroids be redefined? *Neuroscience* **60** (1994) 843–855.
6. Arnold A. P. and Gorski R. A.: Gonadal steroid induction of structural sex differences in the central nervous system. *A. Rev. Neurosci.* 7 (1984) 413–422.
7. Olsen K. L.: Genetic determinants of sexual differentiation. In *Hormones and Behaviour in Higher Vertebrates* (Edited by J. Balthazart, E. Pröve, R. Gilles). Springer-Verlag, Berlin (1983) pp. 138–158.
8. Simpson E. R.: Regulation of expression of the genes encoding steroidogenic enzymes. *J. Steroid Biochem. Molec. Biol.* **40** (1991) 45–52.
9. Hutchison J. B. and Steimer Th.: Androgen metabolism in the brain: behavioural correlates. *Prog. Brain Res.* **61** (1984) 23–51.
10. Balthazart J. and Foidart A.: Brain aromatase and the control of male sexual behaviour. *J. Steroid Biochem. Molec. Biol.* **44** (1993) 521–540.
11. Gahr M. and Hutchison J. B.: Behavioral action of estrogen in the male dove brain: area differences in codistribution of aromatase activity and estrogen receptors are steroid-dependent. *Neuroendocrinology* **56** (1992) 74–84.
12. Hutchison J. B.: Aromatase: neuromodulator in the control of behavior. *J. Steroid Biochem. Molec. Biol.* **44** (1993) 509–520.
13. George F. W. and Ojeda S. R.: Changes in aromatase activity in the rat brain during embryonic, neonatal and infantile development. *Endocrinology* **111** (1982) 522–529.
14. Weisz J., Brown B. L. and Ward I. L.: Maternal stress decreases steroid aromatase activity in brains of male and female rat fetuses. *Neuroendocrinology* **35** (1982) 374–379.
15. Krohmer R. W. and Baum M. J.: Effect of sex, intrauterine position and androgen manipulation on the development of brain aromatase activity in fetal rats. *J. Neuroendocr.* **1** (1989) 265–271.
16. Weaver C. E. and Baum M. J.: Differential regulation of brain aromatase by androgen in adult and fetal ferrets. *Endocrinology* **128** (1991) 1247–1254.
17. Lephart E. D., Simpson E. R. and Ojeda S. R.: Effects of cyclic AMP and androgens on *in vitro* brain aromatase enzyme activity during prenatal development in the rat. *J. Neuroendocr.* **4** (1992) 29–35.
18. Baum M. J., Carroll R. S., Cherry J. A. and Tobet S. A.: Steroid control of behavioural, neuroendocrine and brain sexual differentiation: studies in a carnivore, the ferret. *J. Neuroendocr.* **2** (1990) 401–418.
19. Lephart E. D. and Ojeda S. R.: Hypothalamic aromatase activity in male and female rats during juvenile peripubertal development. *Neuroendocrinology* **51** (1990) 385–393.
20. Schumacher M. and Hutchison J. B.: Testosterone induces hypothalamic aromatase during early development in quail. *Brain Res.* 377 (1986) 63–72.
21. Van Oortmerssen G. A. and Bakker T. C. M.: Artificial selection for short and long attack latencies in wild *Mus musculus domesticus*. *Behav. Genet.* **11** (1981) 115–126.
22. Compaan J. C., Wozniak A., de Ruiter A. J. H., Koolhaas J. M. and Hutchison J. B.: Aromatase activity in the preoptic area differs between aggressive and nonaggressive male house mice. *Brain Res. Bull.* **35** (1994) 1–7.
23. Terashima M., Toda K., Kawamoto T., Kuribayashi I., Ogawa Y., Maeda T. and Shizuta Y.: Isolation of a full-length cDNA encoding mouse aromatase P450. *Arch. Biochem. Biophys.* **285** (1991) 231–237.
24. Beyer C., Wozniak A. and Hutchison J. B.: Sex-specific aromatization of testosterone in mouse hypothalamic neurons. *Neuroendocrinology* **58** (1993) 673–681.
25. Arnold A. P., Amur-Vmarjec S., Campagnoni A. and Schlinger B. A.: Glia express high levels of aromatase in cultures of developing zebra finch telencephalon. *Soc. Neurosci. Abstr.* (1992) 231.
26. Beyer C., Green S. and Hutchison J. B.: Androgens influence sexual differentiation of mouse hypothalamic neurons *in vitro*. *Endocrinology* **135** (1994) 1220–1226.