Pergamon

0960-0760(94)E0065-S

J. Steroid Biochem. Molec. Biol. Vol. 49, No. 4–6, pp. 407–415, 1994

0960-0760/94 $7.00 + 0.00

Brain Formation of Oestrogen in the Mouse: Sex Dimorphism in Aromatase Development

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Steroid sex hormones have an organizational role in gender-specific brain development. Aromatase, converting testosterone (T) to oestradiol-17β (E_2), is a key enzyme in the brain and the regulation of this enzyme is likely to determine availability of E_2 effective for neural differentiation. In rodents, oestrogens are formed very actively during male perinatal brain development. This paper reviews work on the sexual differentiation of the brain aromatase system *in vitro*. Embryonic day 15 mouse hypothalamic culture aromatase activity (AA: mean $V_{max} = 0.9$ pmol/h/mg protein) is several times greater than in the adult, whereas apparent K_m is similar for both (~30–40 nM). Using microdissected brain areas and cultured cells of the mouse, sex differences in hypothalamic AA during both early embryonic and later perinatal development can be demonstrated, with higher E_2 formation in the male than in the female. The sex differences are brain region-specific, since no differences between male and female are detectable in cultured cortical cells. AA quantitation and immunoreactive staining with an aromatase polyclonal antibody both identify neuronal rather than astroglial localizations of the enzyme. Kainic acid eliminates the gender difference in hypothalamic oestrogen formation indicating, furthermore, that this sex dimorphism is neuronal. Gender-specific aromatase regulation is regional in the brain. Oestrogen formation is specifically induced in cultured hypothalamic neurones of either sex by T, since androgen has no effect on cortical cells. Androgen is clearly involved in the growth of hypothalamic neurones containing aromatase. It appears that differentiation of the brain involves maturation of a gender-specific network of oestrogen-forming neurones.

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INTRODUCTION

Early organization of mammalian brain development by steroids, as represented by sexually dimorphic behaviour in rodents, is thought to depend on two separable processes, masculinization and defeminization, which occur at different stages in perinatal development. There is good evidence that oestrogens have an important role in brain sexual differentiation from the use of mutant testicular feminized male (tfm) rats and mice lacking androgen receptors; males are defeminized by postnatal oestrogen despite their insensitivity to androgen [1, 2]. Gonadal steroids, particularly oestrogen [3, 4] regulate the morphology of adult mammalian brain neurones. For example, dendritic spine density of rat hippocampus and ventromedial nucleus is influenced by oestrogen [5]. However, the mechanisms of developmental neurogenesis and cell death linked to steroid effects have not yet been established for the mammalian brain. To link behaviour with structural sex dimorphisms of the brain, rodents have proved to be an interesting model. Preoptic nuclei such as the sexually dimorphic nucleus of the rat develop under the influence of steroids, notably oestrogens [6, 7]. In the gerbil, the volume of the sexually dimorphic area *pars caudalis* (SDApc) is positively correlated with the emission rate of courtship ultrasonic vocalizations [8, 9]. Since both vocal development and SDApc differentiation can be induced in females by treatment of neonates with a synthetic oestrogen, diethylstilboestrol (DES), oestrogen is involved specifi-

Proceedings of the XVI Meeting of the International Study Group for Steroid Hormones, Vienna, Austria, 28 Nov.–1 Dec. 1993.
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cally in brain organization after birth. Oestrogen influences the early development of brain lateralization: thus the left, but not the right, SDApc is correlated with ultrasonic vocal emission [9], providing an indication of an asymmetric link beween oestrogen action, brain structure and vocal mechanisms in the brain. Perception of auditory input in the zebra finch [10] and auditory signals in mice [11] are also lateralized in the brain, suggesting that oestrogen action in early development is related to asymmetry in both motor and sensory brain functions. The avian song control system also provides an example of an early differentiating effect of oestrogen on a neural control system which can be related to behaviour. Gender differences in the size of brain nuclei controlling song are directly related to behaviour in that the male vocal control system is functional in the male, but not in the female [12, 13]. The male zebra finch song control system depends on oestrogens acting on the brain. Oestrogens masculinize the brain in early post-hatching development and result in male-type song in adulthood. Sexual dimorphisms in the brain appear to be due to a reductive cell death process in the genetic female brain which may be due to lack of oestrogen [14]. The oestrogen required for maintenance of the song control nuclei circulating in the male by day 4 post-hatching [15] is probably formed from testosterone (T) in the brain soon after hatching [16]. In mammals, oestrogen also determines the pattern of gonadotrophin release throughout the life of the rat by influencing neurogenesis in the foetus, synaptogenesis in the perinatal and adult rat [17] and synaptic remodelling in the adult [3, 18, 19]. Sex steroids modulate synaptic plasticity: an example is the "phased synaptic remodelling" in the hypothalamic arcuate nucleus of the female rat which occurs in parallel with the oestrus cycle [17].

What is the role of androgen metabolism in the sexual differentiation of the brain? A single 5α-reduced metabolite, 5α-dihydrotestosterone (5α-DHT), is required peripherally for the differentiation of the external genitalia. However, studies of the brain have revealed the complexity of metabolism relating to the physiological action of androgen [20]. An array of metabolites, both biologically active and inactive, are formed in developing brain areas associated later with adult behaviour. Different pathways of androgen metabolism, e.g. 5α-reduction and aromatization, occur in the same hypothalamic areas. In view of the heterogeneity of brain tissue, it is not known yet whether these pathways occur in different cells or co-exist in the same cell type (with possibly different sub-cellular compartmentation); nor if there is gender-specific regulation of the enzymes involved. Brain metabolism of androgen is an important part of the differentiating process and oestrogens formed from gonadal T have a crucial role [21]. Whether there are entirely independent actions of oestrogenic and other steroid metabolites perhaps effective at different stages in early development is still unknown. In rodents (e.g. rat, mouse), oestrogen required for the differentiating processes is formed locally by aromatization of T in the hypothalamus and preoptic area (POA). The conversion is catalysed by an enzymatic complex consisting of cytochrome $P\text{-}450_{arom}$ and the flavoprotein NADPH–cytochrome P-450 reductase.

A major question to be resolved is whether there are gender differences in brain aromatization of androgens, particularly in hormonal regulation of this enzyme pathway during early development. This paper discusses sex differences in the formation of oestrogen in early brain development and how the aromatase is regulated. Experimental work on embryonic oestrogen formation in the mouse will be related to some recent concepts of enzyme regulation in the adult brain derived largely from work on avian and rodent species. Our aim is to understand how early genomic and non-genomic regulation of a key enzyme determines the action of a steroid critical for the sex differentiation of both gonads and brain. At present, there is little information on the early origins of the brain aromatase system due to difficulties in studying metabolism of androgen, particularly in foetal development when differentiation of target nuclei in the hypothalamus and other brain areas is occurring.

REGULATION OF OESTROGEN FORMATION IN THE ADULT BRAIN

T is the major circulating androgen in birds and rodents. Relatively high levels of oestradiol (E_2), measured by radiometric assay in homogenates, are formed in the POA and in the posterior hypothalamus and amygdala (AMYG). In some species, e.g. the ring dove, the preoptic aromatase is active and has a strong substrate-binding affinity (low apparent Michaelis constant, $K_m < 5$ nM). The enzyme activity is regulated by both androgens and oestrogens. Environmental factors such as sexual and photoperiodic stimuli also influence aromatase activity (AA) in POA. The human placental, and chicken and mouse ovarian, aromatase genes have recently been cloned and sequenced [22–24]. The goldfish brain aromatase is the only neural aromatase that has been cloned and sequenced [25]. It is believed that, in each of the species so far studied, oestrogen formed in the brain and ovary is produced by the same translated enzyme. Thus, there appears to be only one aromatase gene per genome, but with tissue-specific gene expression (transcripts) occurring. However, it is not clear what modulates aromatase gene expression in the brain. Kinetic studies [26] show that AA in the dove POA is almost as high as in the ovary, but the substrate-binding affinity constants (K_m) differ between ovarian follicular and brain tissue. Brain and ovarian AA are probably regulated by different mechanisms. Kinetic studies of AA have also revealed differences in binding constants between brain areas [27]. The brain

aromatase in some species such as the male dove appears to have the required characteristics for its suggested role as a regulatory enzyme which confers plasticity in the functional action of T on the adult brain during the reproductive cycle. However, in adult rodents the affinity of the aromatase for substrate appears to be lower and activity less than in the adult avian brain and the role of the enzyme is less clear. The reason for this appears to be that a combination of oestrogen and 5α-reduced metabolites are required for steroid action on target tissues in the mammalian brain.

Evidence for steroid regulation of hypothalamic aromatase came initially from enzyme kinetic studies of the ring dove brain [28, reviewed in 29], where AA was shown to increase markedly in the POA of castrates following T treatment. The maximum AA is increased 5-fold by T, but the K_m does not alter significantly. The increased amount of E_2 formed in preoptic cells of androgen-treated castrates indicates induction of the aromatase, but whether the increase in enzyme activity in brain cells involves activation of existing enzyme, synthesis of more of the same enzyme via gene-related events (induction) or other non-genomic effects involving competing pathways of metabolism [30] has still to be established. These routes of enzyme control exist in the avian brain, but the position is less clear in mammals. It can be assumed that gene expression is involved [31] since changes in mRNA are correlated with circulating steroid level during development. Steroid induction of brain aromatase, demonstrated in a number of avian and mammalian species including rat [32] and Japanese quail [33], has led to general questions about the types of cellular regulatory mechanisms involved, and whether these are specifically a feature of brain neurones. Is the increased activity of the aromatase due to the direct action of T itself, another steroid, or some other factor such as a neurotransmitter in the POA? Is the enzyme neuronal; and does it occur in more than one catalytic form? These questions cannot easily be answered for the developing brain until the cellular localization of aromatase has been established. The evidence available so far suggests that in mammals enhanced aromatization is effected by T or its 5α-reduced metabolite, 5α-DHT. Preoptic AA may also be increased by the product of the reaction (E_2) in the avian brain, suggesting a positive feedback system [29]. Crystalline T implants positioned stereotaxially in the dove brain increase oestrogen formation [34], indicating that there is a direct action of T on cells in the POA probably via genomic effects and not via metabolites formed peripherally.

Non-genomic regulation of the aromatase system by androgenic metabolites is likely to occur in the developing brain. For example, both in the adult and developing dove brain, 5β-reduction of T represents a major steroid inactivation pathway. Theoretically, the action of T on the POA and hypothalamus can be increased by producing more active oestrogen, via increased T supply, or decreased by producing more inactive 5β-DHT. In this case, high 5β-reductase activity may reduce the conversion of T to behaviourally active E_2 by competing with the aromatase for the same substrate (competition hypothesis [29]). However, brain-derived 5α/β-reduced metabolites also inhibit the activity of the aromatase, probably by acting directly on the enzyme (inhibition hypothesis [35]). The latter mechanism potentially represents a rapid non-genomic effect of brain-derived steroid inhibitors. Recent studies have shown that steroids can rapidly modulate the release of neurotransmitters by acting directly on the neuronal membrane [36–38]. Steroids like 5β-DHT in particular, which do not accumulate in the cell nucleus, can rapidly change the sensitivity of brain cells to circulating steroids by directly modulating the activity of intracellular brain enzymes. The possibility of different forms of brain aromatase may complicate interpretation of the inhibitory effects of androgens. One form of aromatase might be sensitive to lower inhibitory steroid concentrations than another. There is evidence supporting distinct regulated forms of the brain aromatase. Two separable brain aromatase systems which differ in their sensitivity to the inductive effects of circulating T have been identified in the ring dove [27]. Study of the androgen receptor deficient tfm rat shows that the activity of the aromatase is modulated by circulating androgens in the POA, but not in the AMYG [39]. These anatomically separable aromatase systems may also show different sensitivities to regulatory steroids. That there are endogenous, brain-derived inhibitors of the aromatase has been indicated from kinetic studies in which the effectiveness of presumed endogenous aromatase inhibitors has been compared to synthetic inhibitors. A new synthetic non-steroidal imidazole inhibitor, Fadrozole, is effective at concentrations approximately 1000 times lower than 5β-DHT and thus more potent. Nevertheless, 5β-DHT and in particular 5α-androstanedione are comparable in potency to the steroidal aromatase inhibitors 4-hydroxyandrostenedione and 1,4,6-androstatrien-3,17-dione [40]. Moreover, in the avian brain the concentration of 5β-DHT is reduced by conversion to inactive metabolites, whereas the synthetic inhibitors are probably not rapidly metabolized. Modulatory effects of 5β-reduced steroids are likely to be equally if not more important in early development, since 5β-reductase activity is higher in embryonic and post-hatching avian brain than in the adult.

In summary, radioisotopic studies, notably of the adult avian and rodent brain, have led to the following views on the role of androgen-metabolizing enzymes. Steroidogenic enzymes activate androgens to effective steroid forms, particularly oestrogens, within the brain. Androgens are also catabolized to biologically inactive forms and the inactivating enzymes influence levels of T available for conversion in target cells during sensitive periods of early development when hormones have

their effects. Brain-derived steroid metabolites which inhibit AA are also formed by competing pathways. Environmental factors such as social stimuli and stress, known to affect brain development, influence metabolic pathways that form both active and inhibitory metabolites from androgen.

AROMATASE IN THE MOUSE BRAIN

AA has been measured in adult mouse brain, but the enzyme has not been localized neuroanatomically [41, 42] until recently. Autoradiographic studies of aromatase distribution using exogenous labelled T and E_2 have been performed on mouse brain [43–45]. However, the inference of localized AA when using labelled substrate requires the assumptions of enzyme and receptor co-localization and no further steroid metabolism. Immunocytochemistry with a polyclonal antibody to human placental aromatase [46] in adult male Jackson and Swiss mice has detected aromatase-containing cells in dorso-lateral parts of the medial and tuberal hypothalamus but not in the usual androgen target areas such as the POA which in other rodent species (e.g. hamster [47]) have AA as detected by radiometric assay. There are species and strain differences in aromatase distribution and the possibility exists that either the mouse has negligible AA in hypothalamic androgen target areas or that mouse strains differ in this respect. However, *in vitro* AA assay has demonstrated significant activity levels in POA and especially AMYG, which is comparable kinetically to ovarian aromatase, in three strains of mice [48]. The POA of sexually active adult male BALB/c, Swiss NIHS and wild mice (*Mus musculus*) contained aromatase of similar activities (mean V_{max} = 172, 251 and 163 fmol/h/mg protein, respectively). BALB/c preoptic AA is significantly higher than in cerebrum (CER). Oestrogen formation in Swiss NIHS mice reveals a similar trend in regional distribution but in AMYG AA is considerably higher (4.6 times) than in POA. It is of note that AA is 4 times higher in the POA-anterior hypothalamus (POA-AH) of the developing (neonatal, day 1) mouse than the adult POA (mean V_{max} = 697 and 163 fmol/h/mg protein, respectively). Both neonate and adult aromatases possess a similar K_m [36 and 31 nM, respectively; Fig. 1(a,b)]. Aromatase in the adult (8-week-old) female BALB/c POA was as active as in the male, but less active than in ovarian follicles (V_{max} = 3 pmol/h/mg protein). Although neonatal POA-AH aromatase is more active than in adult POA, both appear to involve the same enzyme as they have similar K_ms with T as substrate. Since oestrogen is involved in sexual differentiation of the brain and behaviour [34], the neonatal mouse brain may have a greater functional requirement for aromatization than the adult. A similar degree of inhibition found between neonatal and adult POA, POA-AH and AMYG aromatase with Fadrozole (IC_{50} for both BALB/c and *Mus musculus* = 0.5 nM) also suggests a common identity. Thus, the similar effectiveness of Fadrozole as a potent inhibitor of mouse brain aromatase in different strains and stages of development (including adult) suggests a common enzyme. Mouse ovarian AA is higher than in brain, but a similar K_m also implies enzyme similarity in the two tissues.

SEX DIFFERENCES IN DEVELOPING NEURONAL OESTROGEN FORMATION

Despite ample evidence for gender-specific brain AA, the current view from the literature on mammalian brain development suggests that steroids do not directly modify brain AA differentially in the male and female foetuses. In fact, foetal AA appears to be insensitive to experimentally altered levels of maternal steroids. Thus, in the neonatal rat [49] and foetal ferret [50] exogenous steroids do not appear to modify brain AA. These results are difficult to interpret, because the metabolic potential of the foeto–placental unit may interfere with levels of exogenous steroid reaching the brain in mammals. However, there are transient changes in the activity of the mammalian aromatase system later in pubertal development [51]. A regulatory effect of androgen on the developing brain aromatase system has been unambiguously demonstrated in birds (Japanese quail) in view of the accessibility of the avian embryo for experimental alteration of steroid levels. T increases oestrogen formation in the developing quail brain. The sensitivity of the brain aromatase system to T is phasic in that cells containing the enzyme do not respond before a critical period (day 10) of development.

Work on regulation of aromatase in the maturing brain leads to the following questions: (a) Is the developing aromatase system gender-specific in terms of brain cell type expressing the enzyme? (b) Is the enzyme localized regionally in the developing brain and are there sex differences in brain localization of the enzyme? (c) What regulates the enzyme and is the regulation gender-specific? (d) Is aromatase regulation phasic and do sensitive regulatory periods differ between the sexes? The best way to answer these questions as applied to the foetal mammalian brain is by using primary cell culture techniques in which cells containing the enzyme can be identified. Simultaneously, a direct comparison can be made with the intact developing brain using the radiometric assay methods which have previously successfully demonstrated AA sex differences in the perinatal brain [21, 52]. To resolve these questions, the mouse is a particularly useful model because stages of embryogenesis can be recognized following timed matings and the mouse aromatase gene has also recently been sequenced [23].

In order to study the aromatase system in development, it is necessary to compare AA from

microdissected brain areas with identified cells derived from brain tissue culture. Using this procedure, we have compared hypothalamic and cerebral homogenates of both foetuses and neonates of known sex using a sensitive *in vitro* ^3H_2O product formation microassay. The hypothalamic AA measured in dissected brain areas of foetuses (day 19) and neonates (day 1 after birth) shows a highly significant sex difference, with the male having a higher V_{max} than the female (Table 1). The AA detected in cultured cells is primarily neuronal, since treatment of embryonic day (ED 15) hypothalamic cultures with kainic acid results in a 70–80% decrease in AA compared to non-treated cultures. Astroglial enriched cultures from postnatal hypothalamic cells exhibit very low AA. Kinetic studies comparing AA in male and female hypothalamic cultured cells and homogenates from ED 17 foetuses, with T as substrate, indicate similar T-binding affinities (apparent K_m of ~40 nM). Cultured and intact brain neurones evidently contain aromatase with similar catalytic properties [55]. Thus, aromatase from embryonic day 15 mouse hypothalamic primary cultured neuronal cells has an apparent K_m of 30 nM and a V_{max} of approximately 0.9 pmol T converted (^3H_2O formed)/h/mg protein [Fig. 1(c)]. As also shown in this figure, the synthetic non-steroidal inhibitor, Arimidex,

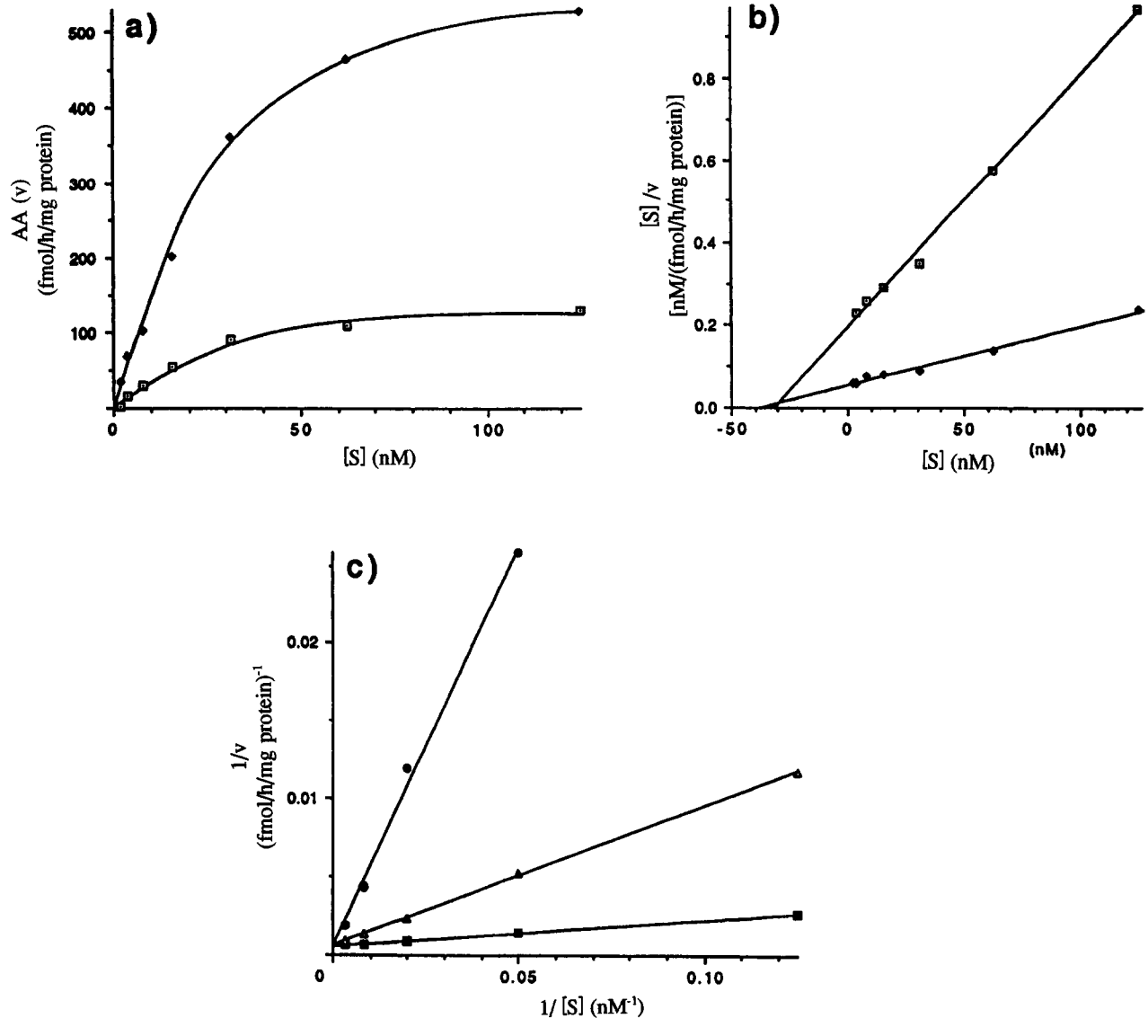

Fig. 1. Aromatase activity (AA) kinetic determinations in mouse brain homogenates of: (a and b) wild mouse (*Mus musculus*) adult male preoptic area (POA) (□) and neonatal preoptic area-anterior hypothalamus (POA-AH) (◆) microdissected tissues; and (c) BALB/c hypothalamic embryonic day 15 primary cell cultures. Pooled tissue ($n = 7$) used. (a) Velocity plots; (b) Hanes plots; (c) Lineweaver–Burk plots with no inhibitor (■); 10^{-9} M (△) and 10^{-8} M (●) Arimidex concentrations. Substrate was 1β-[^{3}H]testosterone at concentrations ([S]) of 3–125 nM. Neonatal and adult aromatase activities (V_{max}) differ significantly, but not substrate affinity (K_m). Mouse aromatase substrate-binding and inhibitory constants from cell cultures and dissected brain samples are similar.

Table 1. Activity and kinetic constants for testosterone (T) aromatization in BALB/c mouse hypothalamic tissue and cell cultures

Brain samples	Sex	K_m (nM)	V_{max} (fmol/h/mg protein)
Neonatal hypothalamic tissue	Male	41 ± 9	1353 ± 190
	Female	43 ± 10	546 ± 95
Embryonic day 17 hypothalamic cultures	Male	36 ± 10	910 ± 187
	Female	40 ± 5	566 ± 80

Pooled cultures (8–10 dishes) and microdissected hypothalami ($n = 6$) were incubated with [1 β-^{3}H]T substrate at different concentrations (10–300 nM).

K_m and V_{max} represent the mean ± SEM of two independent experiments.

increases the aromatase K_m, but V_{max} is unaltered implying that the inhibition is competitive. A plot of the slopes of these lines vs inhibitor concentration (not shown) gives a K_i of 0.2 nM. These kinetic and inhibitory constants, and mode of inhibition, compare favourably with data obtained from direct radiometric assay of preoptic area tissue homogenates microdissected from the embryonic mouse, thus verifying that this model culture system contains a comparably effective aromatase complex. Significant sex differences in AA are found in microdissected hypothalamic area homogenates as early as ED 17, becoming even more distinct in neonates in which AA is always higher in males. There is also an AA sex difference discernible in cultures even earlier in development. In both ED 13 and 15 cultured hypothalami, male cells have the higher AA (Fig. 2). In hypothalamic culture, there appears to be an intrinsic development sequence which does not depend on afferent–efferent neuronal relationships seen in the intact embryonic brain. Thus, in ED 15 cultured hypothalamic cells from both sexes, AA is detectable after 3 days *in vitro* (DIV), but there are no sex differences. However, after 6 DIV AA is significantly increased, indicating that maturation of the cultured hypothalamic aromatase system occurs with time. A significant sex difference is also evident by 6 DIV, with male cultured cells having higher AA. It has not yet been resolved whether this developmental sex difference reflects a male-specific, cell-intrinsic programme, or if it relates to a sex-specific induction of the developing aromatase system before cell culturing on ED 15. The development increase in AA during culture could be due to either more male brain aromatase expressing cells or increased aromatase expression within an existing set of cells. Because cultures are grown in serum with sub-detectable levels of steroids, sex differences in AA of embryonic neurones appear to develop in the absence of androgens. However, hypothalamic cells cultured on ED 13 or 15 could have been influenced earlier by circulating steroids.

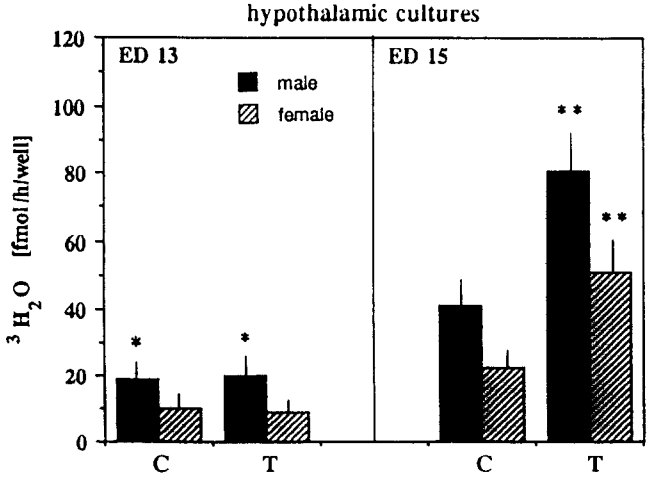

Fig. 2. Effects of testosterone (T) treatment on aromatase activity measured (5 culture wells per gender) in BALB/c mouse embryonic day 13 and 15 hypothalamic cultures grown for 6 days *in vitro*. There is a sex difference (left panel) in AA (*$P < 0.05$). T significantly increases (right panel) AA ($P < 0.01$). Triplicate experiments were carried out (data as mean ± SEM). T has no effect on aromatase activity in ED cultured cells, but has a significant effect in male and female ED 15 hypothalamic cultures. C = control.**

PHASIC DEVELOPMENT OF GENDER-SPECIFIC AROMATASE NEURONES

Given that there is a sex difference in hypothalamic AA which can be demonstrated both in cultured cells and in the intact foetal brain, does steroid environment influence AA specifically during male embryonic development? T treatment (10^{-8} M) during culturing for 6 DIV markedly increases AA (Fig. 2). This effect is specific to hypothalamic cells, since cells derived from cortex do not respond to T treatment. Developmental age is critical in that T is effective specifically in ED 15 cultured cells: there is no measurable effect on ED 13 hypothalamic cells. Although there is an AA sex difference in cultured cells, this apparent induction of the aromatase is not exclusive to the male: hypothalamic cells of both sexes respond to T, although the effect is less pronounced in the female. Further work is required to establish whether there is a threshold difference between the sexes in sensitivity to the inductive effects of T which may occur at different phases in development.

To find out more about how the aromatase system develops in the embryonic brain, cells containing the enzyme need to be identified. For this purpose, we have developed a polyclonal mouse-specific aromatase antibody [53] using the mouse ovarian aromatase cDNA sequence [23]. The antibody binds a single protein with a molecular weight of approximately 55 kDa which corresponds to that previously reported for the aromatase from rat ovary [54], human placenta [55] and avian ovary and hypothalamus [56]; and inhibits AA in male mouse hypothalamic culture homogenates [57].

Aromatase immunoreactivity is localized solely in neurones in the developing mouse hypothalamus and cortex, but not in astroglial cells or oligodendrocytes.

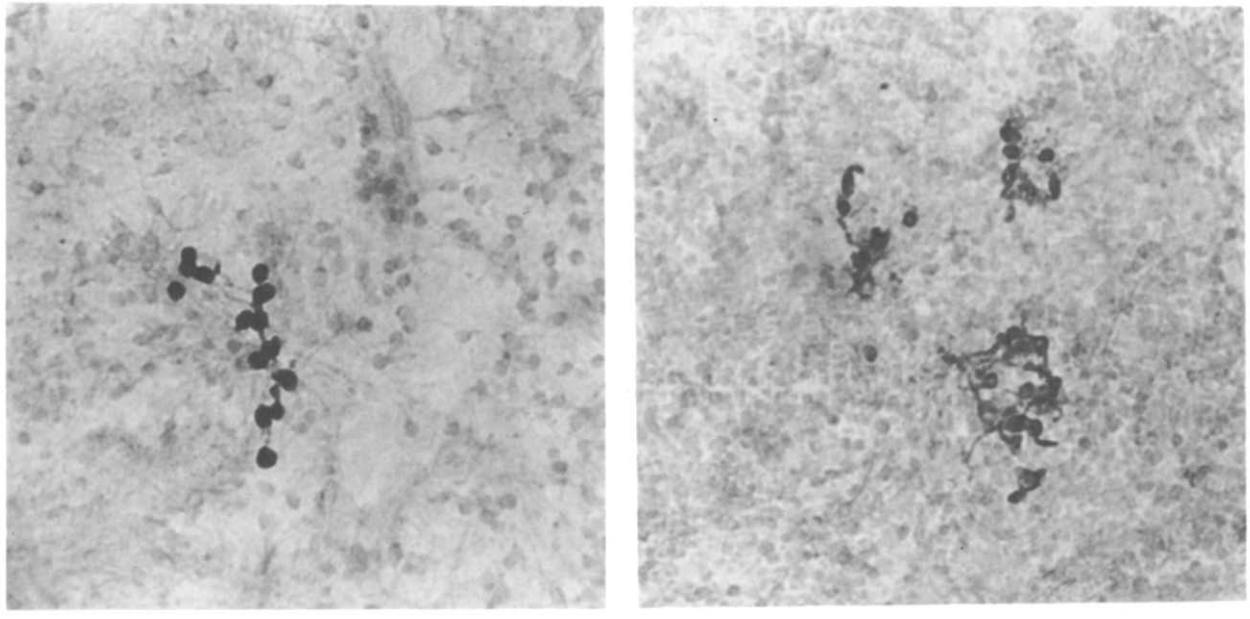

Fig. 3. Photomicrographs illustrating aromatase immunoreactive neurones in male BALB/c mouse hypothalamic cultures (ED 15, 6 days *in vitro*) using a polyclonal, mouse-specific antibody generated against a synthetic 15 amino acid peptide. The peptide corresponds to residues 488–502 of mouse ovarian cytochrome $P\text{-}450_{arom}$ as deduced from the cDNA sequence. Strongly aromatase-immunoreactive clusters of neuronal cell bodies are shown (left plate); both neuronal soma and neurites are stained (right plate).

Figure 3 shows that in aromatase-immunoreactive (AR-IR) neurones both the soma and dendrites contain aromatase. This finding confirms that sex differences in embryonic androgen aromatization is likely to be neuronal. Whether other pathways of embryonic androgen metabolism are also sexually dimorphic, for example 5α-reduction and 3α-diol formation (specific to glial cells [58]), is still unknown. Cultures of ED 15 hypothalamic cells mostly form a dense network of neurones (60–70% of the cells), consisting of clumped soma and processes [53]. AR-IR cells also form aggregates and have interconnecting IR processes (Fig. 3). There is a marked contrast between ED 13 and 15 cultures (corresponding to the developmental differences in AA): thus, hypothalamic neuronal cells cultured from ED 13 foetuses and grown 6 DIV contain only a few AR-IR cells and IR processes are not present even when the culture period is extended to 10 days. The pronounced increase in AA, measured by radiometric assay, between ED 13 and 15 cultured hypothalamic cells, is strikingly matched by a progressive increase in number of AR-IR cells. The clear regional AA difference between the embryonic cortex and hypothalamus is also matched by a difference in number of identified AR-IR neurones: the number of AR-IR cells is low in the cultured cortex compared to the hypothalamus. From these regional and developmental differences it is clear that the oestrogen-forming capacity of aromatase-containing neurones in the male mouse hypothalamus has specific characteristics of sensitivity to steroids which develop at certain stages in ontogeny. Future work will be needed to identify the aromatase-containing cells in relation to regulatory steroids, neurotransmitters and various factors involved in the development of gender-specific neuronal connectivity.

CONCLUSIONS

Oestrogens, formed from androgen, are involved in the sexual differentiation of the male brain in mammals during pre- and postnatal periods of development. Regulation of the brain aromatase is an important step in determining when oestrogen is available to act on the differentiating brain neurones. Foetal formation of oestrogen in the mammalian brain is difficult to study in view of interference by the foeto–placental unit in experimental exposure to factors which directly regulate brain enzyme activity. Direct examination of identified foetal brain cells is required. Using mouse primary cell cultures derived from hypothalamic tissue, AA can be shown to be neuronal rather than glial. There is a sex difference in neuronal oestrogen formation in which the embryonic male hypothalamus has considerably higher AA than the female and a greater number of AR-IR cells. This sex difference is present in the early stages of brain development and is enhanced by androgen during the period immediately before birth; a period which coincides with endogenous testicular T secretion. The androgenic environment of the hypothalamus appears to be instrumental in determining sex differences in a network of aromatase-containing neurones which provide oestrogen required for the differentiation of the male brain.

Acknowledgments—We are grateful to Dr R. E. Hutchison for comments on the manuscript and Ms H. Potter for secretarial assistance. Dr C. Beyer was supported by a DFG–Royal Society Exchange Fellowship. We thank Dr M. Dukes (Zeneca Pharmaceuticals) for providing Arimidex.

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