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BRAIN AROMATASE: ROLES IN REPRODUCTION AND NEUROPROTECTION

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Abstract

It is well established that aromatization constitutes an essential part of testosterone's signaling pathway in brain and that estrogen metabolites, often together with testosterone, organize and activate masculine neural circuits. This paper summarizes the current understanding regarding the distribution, regulation and junction of brain aromatase in mammals. Data from our laboratory are presented that highlight the important function of aromatase in the regulation of androgen feedback sensitivity in non-human primates and the possible role that aromatase plays in determining the brain structure and sexual partner preferences of rams. In addition, new data is presented indicating that the capacity for aromatization in cortical astrocytes is associated with cell survival and may be an important for neuroprotection. It is anticipated that a better appreciation of the physiological and pathophysiological functions of aromatase will lead to important clinical insights.

Keywords

cytochrome P450 aromatase; aromatase; CYP 19; LH; sexual behavior; sexual partner preference; testosterone; estradiol; brain

Introduction

The brain is an important site of estrogen synthesis in vertebrates. This fact was first demonstrated in mammals when Naftolin et al. [1,2] provided direct evidence that central neuroendocrine tissues were capable of converting androgens into estrogens. These results formed the foundation for the classical (original) brain aromatase hypothesis, which has evolved to suggest that many, if not most, of the effects gonadal androgens on neural cell function are mediated by local metabolism to estrogens by cytochrome P450 aromatase (CYP19). More recently, it has been demonstrated that the brain can synthesize estradiol *de novo* from cholesterol and that as such brain-derived estrogen may play a novel role in neuronal and/or glial signaling [3–5]. In other words, aromatase can act to convert circulating androgens to active estrogenic metabolites in specific neural target tissues or may serve as part of endogenous neurosteroid machinery that supplies estrogen directly to specific regions of the brain. The degree of interplay exhibited between these central functions of aromatase is currently not understood.

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Since the first report that the brain can synthesize estrogen, there have been active research efforts by several laboratories to investigate the physiological importance of brain aromatase as a regulator of brain differentiation, neuroendocrine function, and sexual behavior. The present review will discuss research from our laboratory concerning two androgen-signaling functions of aromatase that are important for reproduction: 1) the role of aromatase for the feedback regulation of LH secretion by testosterone in primates, and 2) the role of aromatase in the expression of sexual partner preferences in sheep. Although aromatase is best characterized as an enzyme of the neuroendocrine brain, accumulating data suggest that the induction of aromatase by brain injury or ischemia is part of the program triggered by neural tissue to cope with neurodegenerative insults. Thus, this review also discusses recent data demonstrating that cortical astrocytes derived from female rats are less susceptible than male cells to ischemia, and that this endogenous protection is associated with enhanced aromatase activity in cortical astrocytes derived from females.

Distribution of Brain Aromatase

The distribution of aromatase in the adult and fetal brain has been examined in several species and at multiple levels, including enzyme activity, protein, and mRNA. The predominant sites of aromatase expression at all ages are within hypothalamic and limbic brain regions. Micropunch dissections in adult rats show that aromatase activity is highest within specific subregions of the neuroendocrine brain, including the medial amygdala, cortical amygdala, and bed n. of the stria terminalis [6–10]. Moderate levels are found in the periventricular preoptic n. and medial preoptic n. The ventromedial hypothalamus, anterior hypothalamus, and several other hypothalamic and extrahypothalamic brain regions exhibit low but detectable levels of aromatase.

Neuroanatomical studies using both immunohistochemistry and in situ hybridization have confirmed the general pattern of aromatase distribution in the brain and further refined it within an arc of interconnected nuclei that includes the posteromedial amygdala n., encapsulated region of the bed n. of the stria terminalis, ventrolateral portion of the ventromedial hypothalamic n. and central component of the medial preoptic n. [11–17]. This brain circuit constitutes the medial subdivision of the extended amygdala as described by Heimer and Alheid [18], contains the majority of androgen and estrogen receptor-containing cells [19], and is known to be involved in physiological and behavioral aspects of male reproduction [20]. In addition, various other brain regions, such as the hippocampus, regions of the cerebral cortex, midbrain, sensory afferent neurons, and spinal cord also contain aromatase activity and immunoreactivity suggesting that brain-derived estrogens are involved in other neural functions such as cognition, memory, and sensory perception [3,21–24].

Aromatase is expressed exclusively by neurons under normal conditions in the central nervous system of mammals [25–27]. Neuronal aromatase has been found in cell bodies, processes, and in synaptic terminals of several mammalian and avian species [28]. The recognition that aromatase exists in several cellular compartments, strongly supports the idea that aromatase acts to generate local estrogen that is capable of acting not only through classical receptor-mediated transcriptional mechanisms, but also by direct and rapid effects at neuronal membranes. The best evidence that aromatase supports an estrogen-based signaling pathway at the synapse comes from studies in birds where aromatase can be rapidly regulated by phosphorylation in the brain [29] and is found in neuronal terminals in areas of the brain that are devoid of somal aromatase [22,30].

Aromatase is expressed in astrocytes isolated from the cerebral cortex of neonatal rats and maintained under culture conditions [4,31,32]. Aromatase expression in hippocampal astrocytes is enhanced by different stress conditions such as after serum deprivation or addition

of glutamate to the culture [33]. Brain injury and ischemia in mammals and birds also induces the expression of aromatase in reactive astrocytes [34–36] and these studies together with others suggest that aromatase mediates neuroprotection by promoting local non-gonadal estrogen formation [34,37]. In contrast to mammals, aromatase is highly expressed in radial glial of the brains of teleost fish under normal conditions [38]. It has been speculated that the abundance of aromatase in teleosts may represent an adaptation linked to continual neurogenesis that is known to occur throughout an individual's lifetime among fishes.

Regulation of Brain Aromatase

Aromatase plays an essential role in sexual differentiation of the brain in many mammalian species [39]. Transiently higher levels of aromatase activity and greater circulating levels of testosterone substrate are present in perinatal males than in females suggesting that at critical times males are exposed to higher local levels of estrogen than females [26]. Testosterone and its neural metabolite, estradiol, act in the brain, in part, to restrain cell death during brain development [40,41] and to stimulate the extension of neuronal processes [42] leading to the establishment of sexually dimorphic neural pathways. While it is not understood what factor (s) regulate the expression of aromatase in the fetal brain, it does not appear to be androgen-dependent. In mid-gestation non-human primate fetuses, castration and testosterone treatment did not alter brain aromatase activity [43].

Aromatase expression in the brain declines to low levels after birth and into adulthood. In contrast to the fetus, aromatase activity in the adult brain is regulated primarily by androgens in mammals and by estrogens in birds and fish [44–46]. Our experiments in rats demonstrated that within the preoptic area and hypothalamus, androgens regulate aromatase mRNA via androgen receptor-mediated transcription [6]. In addition, extensive co-localization of androgen receptor and aromatase is observed in the neuroendocrine brain [47] and a potential androgen responsive element and SF-1 site is present at the 5'-end of the brain specific exon 1f of aromatase [48].

In contrast to aromatase expression in the preoptic area and hypothalamus, gonadal steroids do not regulate aromatase in most other brain areas, including the amygdala and hippocampus. This observation suggests that there are at least two populations of aromatase-positive cells in the brain, a steroid-dependent and steroid-independent population. Aromatase exhibits different patterns of promoter usage in different brain areas [23,49,50] supporting evidence that additional factors, including neurotransmitters or neuropeptides [26,27,51], are involved in regulating brain aromatase expression depending on age, sex, specific cell type or population involved. In addition, accumulating evidence suggests that brain aromatase may be rapidly regulated through non-genomic mechanisms involving direct phosphorylation of the aromatase enzyme [45].

Aromatase activity in the preoptic area and hypothalamic nuclei is sexually differentiated in rats and quail [45,52]. These brain areas exhibit a greater capacity for androgen-stimulated aromatase activity in males than in females when both sexes are treated with equivalent concentrations of testosterone [53]. Dose-response studies indicate that these differences are due to a sex difference in the efficacy of testosterone stimulation and are exerted pre-transcriptionally [44]. The mechanism underlying these differences is unclear at present, but could involve sex differences in aromatase-containing cell populations [54], transcriptional control mechanisms [45], or some combination of both. Functionally, the sex difference in the capacity for estrogen synthesis in the brain appears to contribute to the sexually-dimorphic display of T-stimulated male-typical sexual motivation and copulatory behavior [55] and possibly sexual partner preference [56].

Reproductive Functions of Aromatase in the Mammalian Brain

Brain aromatase plays an essential role in both gonadotropin secretion and sexual behavior in adult male mammals. However, the importance of aromatase in these functions differs across species. For instance, a preponderance of evidence in men, non-human primates, sheep, and mice suggests that testosterone must be aromatized in the hypothalamus to completely exert negative feedback control over LH secretion, but no role for aromatase can be demonstrated in rats and guinea pigs [57–60]. The rat and mouse are excellent models to study the essential role of brain aromatase for the activation of male sexual behaviors [61,62]. Sexual behavior in men, non-human primates, sheep, and ferrets is also influenced by estradiol formed from the aromatization of T in the brain [63–65], but aromatase does not appear to play a role in guinea pigs or hamsters [66,67].

a. The role of aromatase for testosterone negative feedback and androgen sensitivity in the non-human primate brain

In non-human male primates, circulating concentrations of LH are stimulated by the pulsatile release of GnRH into the pituitary portal system and inhibited through feedback regulation by testosterone acting directly in the brain [68]. After castration, LH concentrations gradually increase and can be suppressed by either supraphysiological doses of testosterone or a combination of testosterone and estradiol administered at physiological concentrations [69, 70]. These results and others reviewed previously [59] suggest that in non-human primate males, androgens act primarily to restrain pulsatile secretion of GnRH, while estrogens act both to inhibit GnRH secretion and to suppress pituitary responsiveness to GnRH. Evidence that hypothalamic aromatization contributes to LH regulation under physiological condition was obtained by treating adult male rhesus monkeys with the aromatase inhibitor ATD [71]. After 3 weeks of treatment with ATD, mean circulating concentrations of estradiol were suppressed by 30% and hypothalamic aromatase was inhibited by 90%. The concentrations of LH were significantly elevated in ATD-treated monkeys compared to controls in spite of concurrent elevations in circulating concentrations of testosterone indicating that negative feedback was interrupted. Simultaneous treatment of ATD-treated monkeys with small quantities of estradiol restored LH and testosterone levels to normal. Taken together with our subsequent observation that the contents of aromatase activity and GnRH are positively correlated in punch micro dissections from the medial basal hypothalamus of the male rhesus monkey [8], these data strongly suggest that central aromatization of testosterone to estradiol is involved in negative feedback of LH. Clinical studies in men also confirm that hypothalamic aromatase is important for negative feedback [58]. In sheep, LH concentrations were elevated after brain aromatase was inhibited by intracerebral infusion of fadrozole [57]. These results provide direct proof for the concept that central aromatization is required for suppression of LH secretion by testosterone.

In the chronic gonadal state, male rhesus monkeys become unresponsive to the negative feedback actions of testosterone unless estradiol is also administered. This observation led us to ask the question of whether reduced levels of brain aromatase activity could be a reason for why LH suppression becomes refractory to testosterone [72]. To model chronic androgen withdrawal, male rhesus monkeys were castrated and given testosterone replacement immediately or after a delay of 21 days. As expected, immediate replacement suppressed LH levels, whereas delayed replacement did not. The state of androgen resistance was associated with a decreased level of aromatase activity in the preoptic area and medial basal hypothalamus suggesting that deficient capacity for aromatization may lead to androgen insensitivity. Interestingly, the lower aromatase activity observed after delayed testosterone replacement was not associated with reduced aromatase or androgen receptor mRNA concentrations

suggesting that post-transcriptional control of aromatase may be involved in regulating androgen sensitivity.

b. The relationship between the ovine sexual dimorphic nucleus (oSDN) of the preoptic area, aromatase, and sexual partner preferences in sheep

Sexual attraction between opposite sex individuals is essential to successful reproduction for all mammals, yet it is well documented that a minority of individuals in some species of non-human mammals is attracted to same-sex mating partners [73]. Reports indicate that ~8% of domestic rams prefer to approach and mount other rams (i.e. male-oriented rams) as opposed to estrous ewes when given a simultaneous choice between these two alternatives [74]. We are currently studying male-oriented rams in order to understand the degree to which sexual partner preferences in this species are organized by prenatal estrogen exposure through the brain aromatization pathway.

Sexual partner preferences, like other aspects of reproductive behavior are highly sexually dimorphic in mammals. Sexual dimorphisms in reproductive behaviors have classically been ascribed to organizing actions of steroid hormones during a critical period of fetal and/or neonatal development. Animal studies show that exposure to testosterone over an extended, species-specific fetal and/or early neonatal period is able to masculinize the sexual partner preference (and capacity for exhibiting copulatory behavior) of genetic females; presumably because these treatments mimic the action of endogenous testosterone secreted by the developing testes in males [75,76]. Furthermore, studies in rats, mice and pigs suggest that many of the organizational actions of testosterone on the differentiation of a male-typical sexual mate preference actually result from the neural actions of locally synthesized estradiol. Rare syndromes of congenital deficiency of sex steroid synthesis or function in men and women suggest that, although prenatal testosterone and estrogen exposure may play a role, they are not the sole determinants of male-typical psychosexual differentiation in humans and emphasize the complex etiology of human sexual behaviors [76].

Sex differences in reproductive behaviors are thought to derive, in part, from structural and/or chemical differences in the central nervous system. The identification of the sexually dimorphic nucleus (SDN) of the preoptic area of rats was one of the earliest descriptions of a morphological sex difference in the brain [77]. The SDN in rats is 3–4 times larger in males than in females and is, therefore, considered a male-typical feature of the brain. There is also a SDN in male ferrets [78], gerbils [79] and primates including humans [80–83]. The SDN lies at the center of a forebrain circuit that relays sensory information from the periphery and integrates it with information about hormone status to regulate male sexual behaviors [84]. Lesioning of the SDN in male ferrets and rats causes them to prefer to approach and interact with a stud male as opposed to an estrous female [76,85–87]. These results suggest that male-typical sexual partner preferences in ferrets and rats depend on sexually dimorphic processing of sensory cues by the SDN that subsequently elicit a sex-biased behavioral response.

Intriguingly, male- and female-oriented rams appear to either process or respond differently to sexually relevant sensory cues. Perkins and Fitzgerald found that female-oriented rams respond to the presence of an estrous ewe with a characteristic elevation of circulating concentrations of LH and testosterone, but male-oriented rams do not show this neuroendocrine response [88]. Similar results were reported by Alexander et al. when female- and male-oriented rams were given fence line exposure to estrous ewes and stimulus rams 1891. Under these testing conditions, female-oriented rams demonstrated significantly more investigatory and courtship behaviors than male-oriented rams, which may have contributed to the LH response [90]. Despite these behavioral and neuroendocrine differences, Alexander found no differences between male-oriented rams and female-oriented rams in numbers of fos- and fra-

positive immunoreactive neurons in the hypothalamus three hours after fence line exposure [91].

Several approaches have been taken to determine whether there are brain differences between male-oriented rams and female-oriented rams. Perkins et al. found that the estrogen receptor content of the amygdala is sexually dimorphic and that estrogen receptor concentrations in the amygdala are significantly greater in rams that prefer to mount females than in females and rams that prefer to mount other rams [92]. The amygdala, in particular its cortical and medial subdivisions, is part of the sexually dimorphic neuroendocrine brain circuit involved in relaying to the brain hormonal and olfactory information that is important for the control of copulatory behavior [93]. However, it is not yet known whether lesions of the amygdala alter sexual partner preferences in sheep. Resko et al. [94] reported that aromatase activity is significantly higher in the medial preoptic area of female-oriented rams than of male-oriented rams (females were not used in this comparison). Because previous studies in rats demonstrated that the capacity for aromatization in the medial preoptic area is sexually differentiated, Resko speculated that male-oriented rams might have experienced insufficient exposure to testosterone or estradiol during the time of fetal development when sexual partner preference becomes sexually differentiated.

We now have additional evidence demonstrating that morphological features of the medial preoptic area in sheep correlate with sexual partner preferences [56]. Like several other mammals (see above), sheep exhibit a SDN in the medial preoptic - anterior hypothalamus. The ovine SDN (oSDN) is an oblong cell group situated bilaterally adjacent to the 3rd ventricle that can be identified by both its dense Nissl staining and its signature high expression of aromatase mRNA. The oSDN is significantly larger and expresses greater levels aromatase mRNA in female-oriented rams than in male-oriented rams and females. The oSDN also contained more neurons and was longer in female-oriented rams than in male-oriented rams. This was the first demonstration in non-human animals that there is an association between natural variations in sexual partner preferences, hypothalamic structure, and aromatase expression. These results are reminiscent of the original report by LeVay [81] showing that the volume of the third interstitial nucleus of the anterior hypothalamus (INAH3) is significantly greater in presumptive heterosexual men than in homosexual men and women.

Further experiments need to be done that link differences in testosterone/estradiol exposure to differences in sexual partner preference in adults. Previous studies showed that the critical period for sexual differentiation in sheep occurs roughly between gestational day 30 to 90; term ~147 days [95]. Female sheep exposed to testosterone during this time exhibit high levels of mounting behavior, but were never evaluated for sexual partner preferences. The oSDN is present in late gestation fetuses, has roughly the same dimensions as in adulthood, and can be masculinized in genetic females by prenatal testosterone treatments [96]; and our unpublished observations). These results demonstrate that the oSDN is organized prior to birth and structural brain differences exist prior to behavioral expression. Although it is reasonable to propose that aromatization of testosterone to estradiol is necessary for sexual differentiation of the brain in sheep, initial studies do not support this hypothesis [97]. Rams exposed to ATD prenatally from days 50 through 80 of gestation (i.e. the period of maximal sensitivity to the behavioral effects of testosterone) exhibited a modest, but significant, decrease in mounting behavior at 18 mo of age. In contrast, this effect was not associated with significant reductions in the preference of these animals to mount estrous females as opposed to other rams. More research is needed to test whether the timing and dosage of ATD used in this experiment were adequate. Alternatively, prenatal estrogen exposure may not be the principal determinant of male-typical partner preference. Perhaps androgen receptor-mediated mechanisms and/or direct genetic signaling in the developing nervous system contribute to differentiation of male-typical sexual partner preferences. More research is needed to examine these possibilities.

Aromatase and Neuroprotection

Recent evidence demonstrates that the roles that brain aromatase play extend beyond that of mediator of sexual differentiation or modulator of reproductive behaviors and neuroendocrine responses. Aromatase also appears to play a pivotal role in neuroprotection by increasing production of estrogen at the site of injury [34]. Consistent with this hypothesis, aromatase is induced in reactive astrocytes after mechanical and neurotoxic lesions [35,98]. More recently, Carswell et al. showed that aromatase expression is also induced in astrocytes after experimental stroke [36]. Neuronal damage to mechanical and ischemic injury is enhanced in aromatase knockout mice or after pharmacological inhibition of aromatase [34,37]. The capacity of astrocytes to express aromatase was originally demonstrated by cell culture [4, 33]. Recently, Liu reported that astrocytes derived from neonatal female rat brains are less susceptible than male cells to ischemia using oxygen-glucose deprivation (OGD) in vitro, and that the endogenous protection is associated with greater aromatase activity in astrocytes derived from females [32]. To determine whether the difference in aromatase activity mediated the sex-specific response to OGD, cultures were treated with the aromatase inhibitor Arimidex. Inhibitor treatment significantly increased death in female-derived cells and abolished the sex-specific response to OGD. Astrocyte-conditioned media from female cultures protected male cultures exposed to OGD, but transfer from male to female did not alter the female response to OGD. Arimidex treatment abolished the ability of female astrocytes to protect male astrocytes, while pretreatment with estradiol reduced cell death in both sexes. These results strongly suggest that endogenous aromatase and local-estrogen synthesis protect astrocytes from ischemic death. There is increasing recognition that astrocytes are essential for neuronal survival and function, neurogenesis, and neural repair [99]. Thus, these results, showing that female astrocytes are more resistant to ischemic injury and have a higher capacity for estradiol synthesis than male astrocytes, may help explain in part why females are more protected from ischemic brain damage relative to age-matched males [100]. More research is needed to understand the mechanism for the sex difference in aromatase expression by reactive astrocytes and whether reactive astrocytes are capable of producing sufficient testosterone substrate to serve as the source of neuroprotective estradiol.

Concluding comments

Brain aromatase plays an important role to mediate testosterone's effects on the development, neuroendocrine function and sexual behavior. As such, the location, regulation and developmental profile of aromatase expression within the neuroendocrine brain are consistent with these many roles. Aromatase is also localized in neocortical and sensory brain regions and has been recently implicated in cognition, memory, and sensory perception. An exciting new role has been suggested in neuroprotection, since it has been observed that astrocytes respond to several types of brain injury with enhanced aromatase expression. Although not completely understood, the regulation of brain aromatase involves complex transcriptional and post-transcriptional mechanisms suggesting neural and glial cells have the ability, not only to regulate androgen sensitivity, but also to respond to differing demands for local production of estradiol. Accumulating evidence suggests that in addition to metabolizing peripherally derived androgens to estrogens, certain brain areas may be capable of synthesizing estradiol *de novo* from cholesterol suggesting that aromatase may play novel roles in neuronal signaling. Further research is essential for understanding the expanding role of brain aromatase. This information is also crucial to assess the potential impact that the widespread use of aromatase inhibitor therapy for breast cancer could have on brain function.

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