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Brain Aromatization: Classical Roles and New Perspectives

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Abstract

Aromatization of testosterone to estradiol by neural tissue has classically been associated with the regulation of sexual differentiation, gonadotropin secretion, and copulatory behavior. However, new data indicate that the capacity for aromatization is not restricted to the endocrine brain and demonstrate roles for locally-formed estrogens in neurogenesis and in responses of brain tissue to injury. This manuscript summarizes our current understanding of the distribution and regulation of aromatase in the brain and discusses the classical and novel roles it plays. A better understanding of brain aromatization could shed new light on its physiologic and pathologic functions and someday lead to new centrally acting drug therapies.

Keywords

Cytochrome P450 aromatase; CYP 19; LH; sexual behavior; sexual partner preference; brain ischemia

1. Introduction

The idea that estrogens are synthesized locally in the brain and act to amplify and diversify the action of circulating testosterone is considered axiomatic. The evidence that the brain was capable of converting androgen to estrogen, albeit at very low levels, was crucial evidence used in originally formulating the aromatization hypothesis¹. According to the aromatization hypothesis testosterone synthesized by the fetal testis diffuses into the male brain where it is locally aromatized to estradiol and then initiates the process of masculinization. Originally the aromatization hypothesis was advanced to explain the mechanism whereby testosterone acts to organize and sexually differentiate brain structure and chemistry. However, over the intervening 30+ years since it was first proposed, the aromatization hypothesis has been examined from many experimental perspectives in several neural tissues and species. Today, it is well established that specific areas and cell types of the central nervous system synthesize estrogens from precursor androgen. The local synthesis of estrogen in the brain is a dynamic and regulated process that varies with age, sex, and physiologic status. Moreover, it has become increasingly evident that aromatase has more general roles to play in development, protection, and repair of the brain. The current review will present a synthesis of the physiologic importance of aromatase in the central nervous system control of reproduction and neuroprotection.

2. Distribution of brain aromatase

It is generally accepted that under normal conditions the expression of aromatase is restricted to certain neuronal populations in the central nervous system²⁻⁴. The predominant sites of aromatase activity are within brain regions involved in reproductive functions and behaviors such as the hypothalamus and amygdala⁵⁻⁷. Neuroanatomic studies using immunohistochemistry and in situ hybridization have confirmed the general pattern of aromatase distribution and further refined it to encompass an arc of interconnected nuclei that includes the nucleus of the posteromedial amygdala, encapsulated region of the bed nucleus of the stria terminalis, ventrolateral portion of the ventromedial hypothalamic nucleus, and central component of the medial preoptic nucleus. This endocrine brain circuit contains an overlapping distribution of androgen- and estrogen-receptor containing cells⁸. A number of other brain structures contain measurable aromatase activity and/or immunoreactive cells including the excitatory CA1-3 pyramidal cells and dentate gyrus granule cells of the hippocampus, layer II-VI pyramidal cells of the cerebral cortex, midbrain neurons, sensory afferent neurons, as well as cells in the spinal cord and cerebellum⁹⁻¹⁴. Studies on hippocampus demonstrate that locally synthesized estrogen regulates synaptic plasticity to affect the function of excitatory neural circuits¹⁵ and may play a role in neurogenesis within the dentate gyrus of the adult¹⁶.

Aromatase is distributed within several cellular compartments of neurons, including the cell bodies, processes, and pre-synaptic terminals^{17, 18}. This pattern of localization strongly suggests that not only can aromatase generate estrogens to act within target cells, but that the estrogens produced can also act in a paracrine manner on adjacent cells. This latter possibility is supported by reports showing that the incidence of aromatase and estrogen receptor localization is not absolute but ranges from 5% to 80% depending on species and brain area¹⁹⁻²¹. The issue is further complicated by the mounting evidence that, besides their well-known hormonal mode of action at the genetic level, estrogens such as 17 β -estradiol also influence brain function by direct effects on neuronal membranes²². The observation that aromatase is expressed in presynaptic terminals together with the demonstration that aromatase activity can be modulated within minutes by Ca²⁺-dependent phosphorylation eventuating in rapid behavioral responses, has been marshaled to support the hypothesis that brain estrogens display many, if not all, functional characteristics of neuromodulators or even neurotransmitters²³.

For a long time aromatase was only reported in neurons, notably of the diencephalon and limbic system of mammals. However, recent studies have demonstrated that aromatase is expressed in radial glial and intermediate progenitor cells of the developing cortex in the mouse²⁴. Radial glial cells are considered to be a major source of neurons during vertebrate brain development²⁵. It was recently demonstrated that in fish aromatase immunoreactivity is present in actively dividing cells derived from radial glial that differentiate into neurons²⁶.

Although aromatase has been detected within astrocytes of the human temporal cortex²⁷, it appears to be the more general rule that glial cells in the mammalian brain do not express aromatase under normal conditions^{28, 29}. However, cultured astrocytes express aromatase *in vitro* and reactive astrocytes express aromatase following brain injury and ischemia²⁹⁻³². This induction of aromatase decreases areas of gliosis^{33, 34}, and apoptotic degeneration³⁵⁻³⁷, and may mediate neuroprotection and/or enhance neuronal recovery from injury^{29, 30}. Aromatase-expressing reactive astrocytes are observed in all injured brain areas including, cortex, corpus colosum, striatum, hippocampus, thalamus, and hypothalamus indicating astrocytes from most brain areas has the potential for producing estrogen in response to injury²⁹.

3. Regulation of brain aromatase

The conversion of androgens into estrogens in the brain is a key mechanism by which testosterone regulates many physiological and behavioral processes throughout an animal's life. Because of the diverse age- and region-specific actions of testosterone, it is not surprising that the regulation of aromatase in the brain is complex and not completely understood. Recent studies in several species have led to new perspectives on the control of this enzyme by both transcriptional and posttranscriptional mechanisms³⁸.

a. Ontogeny of brain aromatase

Aromatase plays an essential role in the development and sexual differentiation of the brain in many mammalian species³⁹. In short gestation species (i.e. rodents), there is a peak in both activity and mRNA expression of aromatase in the preoptic area/hypothalamus that occurs late in gestation or early neonatal life and corresponds to the critical period for sexual differentiation³. Transiently higher levels of aromatase activity and greater circulating levels of testosterone are present in perinatal males suggesting that males are exposed to higher levels of estrogen than females at these critical times³. More detailed studies in rats show that the temporal pattern of aromatase mRNA expression varies in different subregions of the limbic brain suggesting a more complex system of regulation^{40, 41}.

In long gestation species including non-human primates, sheep, cows, and guinea pigs, the highest levels of aromatase are observed during the first half of gestation, a time when circulating testosterone is also elevated^{42–45}. Although abundant evidence exists for a role of aromatase in sexual differentiation of the brain in short gestation mammals, the evidence in long gestation species is mixed and thought not to be essential in humans and non-human primates⁴⁶. Nonetheless, high levels of aromatase have been measured in the developing brain of all species studied. This finding suggests that local estrogen production could play important developmental roles not limited to sexual differentiation. For instance, estradiol acts in the brain, in part, to restrain cell death during brain development⁴⁷ and to stimulate the extension of neuronal processes⁴⁸ leading to the establishment of important 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 most species³. In mid-gestation non-human primate fetuses, castration and testosterone treatment did not alter brain aromatase activity⁴³.

b. Sex steroid regulation of brain aromatase

Aromatase expression in the preoptic area/hypothalamus 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^{49–51}. Our experiments in rats and nonhuman primates demonstrated that within the preoptic area and hypothalamus, androgens regulate aromatase mRNA via androgen receptor-mediated transcription^{6, 52}. Extensive co-localization of androgen receptor and aromatase is observed in the neuroendocrine brain⁵³ and a potential androgen responsive element and SF-1 site is present at the 5'-end of the human brain specific exon 1f of aromatase⁵⁴. Estrogen has also been found to regulate hypothalamic aromatase in mammals under certain experimental conditions and has been shown to synergize with androgen, but it is not clear whether this effect involves direct transcriptional modulation through the estrogen receptor^{41, 55, 56}. In fish species, estrogen-responsive element (ERE) and androgen-responsive element (ARE) have been identified upstream of the brain AromP450 gene promoter⁵⁷.

c. Region-specific regulation of brain aromatase

The expression of aromatase gene, Cyp19, in various tissues is regulated by the use of tissue-specific promoters, that are, in turn, regulated by different transcription factors and signaling

pathways⁵⁸. Regulation of aromatase expression in the brain is region-specific^{7, 41}. 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 hippocampus⁶. This observation suggests that there are at least two populations of aromatase-positive cells in the adult brain, a steroid-dependent and steroid-independent population. The finding that aromatase exhibits different patterns of promoter usage in different brain areas^{27, 59, 60} supports the idea that region specific regulation could be depend on differential promoter usage, but does not exclude the possibility that other modes of transcriptional and post-transcriptional regulation are involved.

d. Rapid phosphorylation regulation of brain aromatase

Accumulating evidence suggests that brain aromatase may be rapidly regulated through nongenomic mechanisms involving direct phosphorylation of the aromatase enzyme⁵⁰. Aromatase activity in hypothalamic homogenates is rapidly (within minutes) down-regulated by exposure to conditions that enhance protein phosphorylation such as the presence of high concentrations of calcium, magnesium and ATP. Similarly, pharmacological manipulations such as treatment with thapsigargin or stimulation of various neurotransmitter receptors (alpha-amino-3-hydroxy-methyl-4-isoxazole propionic acid (AMPA), kainate, and *N*-methyl-D-aspartate (NMDA)) leading to enhanced intracellular calcium concentrations depress within minutes the aromatase activity measured in quail preoptic explants. The effects of receptor stimulation are presumably direct: electrophysiological data confirm the presence of these receptors in the membrane of aromatase-expressing cells. Inhibitors of protein kinases interfere with these processes and Western blotting experiments on brain aromatase purified by immunoprecipitation confirm that the phosphorylations regulating aromatase activity directly affect the enzyme rather than another regulatory protein. Accordingly, several phosphorylation consensus sites are present on the deduced amino acid sequence of the recently cloned quail aromatase. Fast changes in the local availability of estrogens in the brain can thus be caused by aromatase phosphorylation so that estrogen could rapidly regulate neuronal physiology and behavior⁶¹.

4. Reproductive functions of aromatase in the brain

a. Aromatase and sexual differentiation of the brain

The classic view of sexual differentiation in mammalian species is that sex differences in the brain structure and function are programmed by exposure to testosterone produced by the fetal testis acting during a critical period in perinatal development⁶². This is most profoundly true in brain regions directly involved in reproductive functions. The brain develops as male after exposure to testosterone produced by the developing testis and as female largely in the absence of such exposure⁶³. In most species, masculinization of the brain is evidenced in adults by a capacity to express male-typical sexual behaviors and high levels of aggression. In contrast, the feminized brain is capable of supporting female-typical responses such as ovulation, female-typical receptivity and maternal behavior. Masculinization is usually accompanied by defeminization, and although masculinization can be disrupted by various means it is not a natural part of feminization. There are interesting examples that illustrate the dichotomy between masculinization and defeminization. For instance, adult male non-human primates exhibit tonic gonadotropin secretion but are still capable of responding to estradiol with an LH surge⁶⁴ demonstrating that behavior is masculinized without gonadotropin secretion becoming defeminized.

The paradoxical observation that estradiol was just as effective as testosterone in the masculinization of rat behavior and the reduced nonaromatizable androgen dihydrotestosterone was not, led investigators to ask whether testosterone acts in brain after it is aromatized to

estradiol⁶⁵. We now understand that most sexually dimorphic areas of the rat brain contain substantial levels of both aromatase and high concentrations of estrogen receptors lending strong indirect support to the aromatization hypothesis of sexual differentiation¹. However, the importance of aromatase for brain sexual differentiation differs depending on the specific dimorphic endpoint examined and the species considered. A preponderance of evidence in rats suggests that testosterone must be aromatized to completely masculinize and defeminize male brain structure, function, and behavior^{65, 66}. In ferrets, masculinization is initiated by aromatization prenatally and completed by testosterone itself acting through androgen receptors postnatally⁶⁷. In contrast, androgen receptor mechanisms appear to more exclusively regulate aspects of brain differentiation in non-human primates and guinea pigs, whereas both molecular pathways appear to play roles in mice and sheep^{46, 68}. Much less is known about psychosexual differentiation in humans, but there is little evidence that aromatization is involved⁴⁶.

The concept that *in situ* estrogen synthesis influences neural structures is well illustrated by the development of the sexually dimorphic nucleus (SDN) of the preoptic area of the rat⁶⁹. The SDN is larger in males than in females and forms part of the circuitry that processes sexually relevant sensory cues and formulates appropriate male-typical sexual behavior responses. High levels of aromatase mRNA expression have been observed in the nascent SDN of fetal rats and sheep⁴⁰. In rats, neonatal administration of estrogen or testosterone to genetic females masculinizes the SDN⁷⁰. Direct evidence that neural aromatization is normally essential for the masculinization of SDN was provided by the demonstration that SDN size is significantly reduced compared to controls in males that were treated with an aromatase inhibitor neonatally⁷¹. Sexually dimorphic nuclei exist in other brain regions and analogous structures are found in other species, including humans⁶². In addition to the size of particular brain regions, there are a number of other structural sexual dimorphisms in the brain that are due to the effect of aromatization; such as the extent of dendritic arborization, differences in the density and pattern of synaptic connections, size, number and phenotype of neurons in a particular region and astrocyte morphology^{72, 73}. All of these structural differences are believed to underlie adult sex differences in behavior and neural responsiveness to gonadal hormones in ways yet to be completely understood.

Although the SDN was originally hypothesized to play a role in the expression of copulatory behavior, no strong experimental evidence exists to support this view^{74, 75}. More recent lesion studies suggest that the SDN is critical for male-typical sexual partner preferences in rats and ferrets^{76, 77}. It is well documented that a minority of males in some species are attracted to same-sex mating partner⁷⁸. The domestic ram is one model species currently being studied to understand the relationship between prenatal hormone exposure, brain structure, and sexual attraction because ~8% of domestic rams are sexually attracted to other rams (i.e. male-oriented rams), whereas the majority of rams are attracted to estrous ewes (female-oriented rams)⁷⁹. A SDN (ovine SDN) exists in the sheep preoptic area and is larger in female-oriented rams than in male-oriented rams and ewes⁸⁰. The ovine SDN is organized prenatally by testosterone, but the role of aromatase is not yet known⁸¹.

Structural sexual dimorphisms in the human brain have been described, but many of the reports are controversial and their functional significance is less well established than in animal models. In 1991, LeVay⁸², reported that homosexual men, like heterosexual women, have a smaller interstitial nucleus in the anterior hypothalamus (INAH3) than heterosexual men. Nothing is yet known about the ontogeny of the INAH3 in humans.

Sexual partner preferences, like other aspects of reproductive behavior are highly sexually dimorphic⁸³. Aromatization in perinatal rat brain has been shown to be involved in differentiation of male-typical female-directed sexual partner preferences^{71, 84}. Targeted

disruption of the aromatase gene in mice results in a loss of male sexual behavior, including male-typical sexual partner preferences⁸⁵. Low levels of aromatase activity and mRNA expression in adult rams are correlated with a male-oriented sexual preference^{80, 86}. Taken together these studies indicate that there may be a relationship between aromatase expression in the developing preoptic/anterior hypothalamic and male-typical sexual preferences. However, a recent attempt to prove this causal relationship in sheep was unsuccessful⁶⁸. Moreover, men with congenital aromatase deficiencies are uniformly heterosexual^{87, 88} and gene linkage studies in humans failed to suggest a role for aromatase variation in the development of sexual orientation in men⁸⁹. Thus, the data in humans and sheep appear to conflict with rodent studies emphasizing that the causal factors involved in establishing sexual partner preferences remain to be established.

b. Aromatase and regulation of sexual behaviors

There is clear evidence for the involvement of the aromatization hypothesis in the central activation of sexual behavior in adult male rodents⁹⁰. As noted above, aromatase, androgen receptors, and estrogen receptors are abundant in the brain circuitry that regulates male copulatory behaviors. Estradiol is both necessary and sufficient to maintain or restore most elements of copulatory behavior except for ejaculatory patterns in male rats⁹¹. Estradiol administration reverses the effects of castration on copulatory behavior in male rats, while treatment with aromatase inhibitors and estrogen antagonists inhibits the restoration of copulatory behavior by testosterone administration to castrates^{92–94}. Administration of the aromatase inhibitor fadrozole to testosterone-clamped male rats, decreased anticipatory level changing (i.e. sexual motivation), increased the latencies to mount, intromit, and ejaculate, and decreased the numbers of these behaviors⁹⁵. Replacement with estradiol restored anticipatory behavior, mounts and intromissions, but not ejaculations.

Aromatase knockout (ArKO) mice have been generated by several investigators independently and used to test whether neural aromatization of testosterone contributes to copulatory behavior in male mice^{96–99}. The ArKO mouse model is ideal for studying the contribution of aromatization to both sexual differentiation of the brain and adult behavior because they have functional estrogen receptors. Male copulatory behavior is severely impaired in male ArKO mice consistent with the role for aromatization in both the organizational and activational effects of testosterone. Testosterone administration did not improve behavior in castrated ArKO adults, whereas combined treatment with estradiol and dihydrotestosterone almost completely restored copulatory behavior to levels observed in wildtype males¹⁰⁰. These results suggest that estrogens derived from aromatization of testosterone exert major activational effects on male coital behavior in male C57Bl6 mice.

Species differences exist in the extent to which neural aromatization of testosterone and accompanying activation of neural estrogen receptors are required to maintain male sexual behavior. Nonaromatizable dihydrotestosterone is able to maintain or restore copulation in guinea pigs¹⁰¹ and monkeys¹⁰² suggesting that androgens are sufficient in these species. However, studies in non-human primates demonstrated that sexual motivation and copulatory behaviors also depend in part on aromatized testosterone¹⁰³.

The extent to which testosterone in men acts through aromatization to estradiol is not clear. Gooren¹⁰⁴ found that, in eugonadal men, the estrogen receptor antagonist, tamoxifen, and the aromatase inhibitor, testolactone, had no adverse sexual effects, and that dihydrotestosterone was as effective as testosterone in maintaining sexuality in hypogonadal men arguing that aromatase was not involved. The comparison of two men with congenital aromatase deficiencies, one with accompanying hypogonadism, suggested that testosterone alone allows for a normal sexual activity, but that there is a synergistic effect between testosterone and estradiol derived from aromatization^{87, 88}. These findings suggest that aromatization may be

required in men for completely normal sexual behavior, but androgens are the main steroids involved.

c. Aromatase and regulation of gonadotropin secretion

It is well accepted that the secretion of the gonadotropin, LH, is tonically inhibited by testosterone (i.e. negative feedback) in males of all species, including man. However, the importance of aromatase in this action of testosterone differs across species. A preponderance of evidence in men, non-human primates, sheep, and mice suggests that testosterone must be aromatized in the hypothalamus and periphery to completely exert negative feedback control over LH secretion, but no role for aromatase has been demonstrated for rats and guinea pigs^{52, 105–108}. The most direct evidence for a role of central aromatization in testosterone negative feedback comes from studies in sheep. In sheep, infusion of the aromatase inhibitor, fadrozole, intracerebrally increased LH pulse frequency without effecting plasma estradiol concentrations¹⁰⁵.

Inhibition of aromatase in non-human primates causes a marked increase in LH secretion despite a concomitant increase in endogenous testosterone levels that normally serve to restrain LH secretion¹⁰⁹. Testosterone action in non-human primates is thought to occur in part through central aromatization because it was found that aromatase activity and GnRH concentrations are positively correlated in micropunch dissections of the medial basal hypothalamus¹¹⁰.

A number of studies in men, have demonstrated that aromatization is needed for testosterone negative feedback, whereas other studies provide evidence that testosterone can act independent of aromatization^{111–114}. Discrepancies in this field are due to several issues including, the use of pharmacological sex steroid regimes and aromatase inhibitors with androgenic activity, as well as the difficulty in isolating the hypothalamic and pituitary components that control LH secretion. More recently, a clever human investigative model that employs sex steroid ablation with physiologic sex steroid add back in normal men and GnRH-deficient men treated with fixed GnRH pulses was used to determine the importance of central and peripheral aromatization in the control of gonadotropin secretion^{106, 115}. This model represents a hypothalamic clamp because the doses and frequency of exogenous GnRH are experimentally controlled. Thus, any effect on LH secretion of altering gonadal steroids can reflect only a pituitary site in such men, and can be used to detect a hypothalamic effect when experimental responses differ from normal men. Inhibition of aromatase and comparison of gonadotropin secretion between normal men and hypogonadotropic men revealed that estrogen acts within the hypothalamus to exert negative feedback in men. Moreover, it was shown that testosterone can slow LH pulse frequency in chemically castrated normal men while estradiol levels remain suppressed indicating that testosterone feedback at the hypothalamus can occur through direct androgen action not requiring aromatization. In contrast, testosterone treatment failed to suppress either mean LH levels or LH amplitude in the hypogonadotropic men given pulsatile GnRH indicating that testosterone's negative feedback at the pituitary is mediated by aromatization. These data argue that circulating testosterone together with circulating estradiol generated by peripheral aromatization and estrogen formed locally in the brain all contribute to negative feedback in men.

5. A role for the aromatase in neuroprotection

Emerging data suggest that enhanced aromatase activity near the site of an ischemic or traumatic insult can favorably impact injury outcome, presumably by producing neuroprotective estradiol. The background for this concept arises from two sets of experimental observations. First, it has long been noted that injury outcomes after experimental stroke and trauma are sex-specific. Female rats and mice of various inbred and outbred strains experience smaller tissue damage for an equivalent insult from cerebral ischemia^{116–120} and improved

functional outcome¹²¹. Similarly, male animals sustain greater injury than do age-matched females after traumatic brain injury¹²². Data from these studies suggest that, in part, the female “benefit” is mediated through ovarian steroid availability. Second, exogenous estradiol administration provides robust cerebroprotection in a wide range of neurological disease models, and it is equally effective in both sexes; for reviews, see^{123–126}. These observations set the stage for investigations into how changes in aromatase expression and endogenous estradiol production alter the environment for evolving brain damage.

Early reports confirmed that mechanical trauma or an excitotoxic challenge by intra-cerebral kainic or domoic acid injection leads to increased local aromatase expression and activity^{29, 127, 128}. At the cellular level, aromatase induction after injury occurs in the reactive astrocyte rather than in the neuron as ordinarily occurs under physiological conditions. Treatment with the specific aromatase inhibitor, fadrozole, enhances kainic acid-induced neuronal loss, confirming that aromatase induction has functional consequences¹²⁷. Using a similar paradigm, Veiga et al.¹²⁹ implicated extragonadal estradiol in protecting hippocampal neurons in rodents of both sexes. In addition to aromatase induction in the zone-of-injury, local increases in androgen receptor and estrogen receptor α expression have also been documented¹³⁰. Accordingly, many of the essential mechanisms for neuroprotective signalling by estradiol are engaged after injury in a coordinate manner.

Others have borne out these original observations using more complex, but clinically relevant, models of experimental stroke. Immunohistochemistry in spontaneously hypertensive rats demonstrates that the aromatase protein is elevated at 24 h and at 8 days after focal cerebral ischemia, specifically in peri-infarct zones known as the penumbra³¹. Penumbra regions include tissue which has been injured by ischemia but remains viable. Therefore, the localization pattern for the enzyme likely has significance. Double labeling studies indicated that post-ischemic aromatase expression is associated with astrocytic processes³¹.

We speculated that the female brain is strongly dependent on estradiol production in the ischemic penumbra for favorable outcomes. To provide initial proof of this concept, ischemic injury was evaluated in mice with targeted deletion of the CYP19 gene (ArKOs)³². ArKOs of both sexes have abnormal reproductive phenotypes with plasma estradiol levels below detection by radioimmunoassay and elevated testosterone and gonadotropin levels^{96, 131–133}. ArKOs also demonstrate abnormalities in bone formation, both early and late in life, and heavy accumulation of adipose tissue. In McCullough et al.³², female ArKOs were treated with a standard focal cerebral ischemic insult: reversible middle cerebral artery occlusion followed by 22 hours of reperfusion. ArKOs sustained larger tissue damage than did their wild-type littermates, a result that was replicated by treating wild type female mice with fadrozole, a well-known aromatase inhibitor. Loss of aromatase activity resulted in greater ischemic damage than observed with ovariectomy, suggesting that extragonadal estradiol is vital to female stroke.

In vitro, aromatase is constitutively expressed in astrocytes³⁵, and plays a role in cell viability after oxygen and glucose deprivation (OGD, a commonly used model of “ischemic injury”). In addition, there are significant sex differences in how the aromatase is engaged after OGD in male vs. female astrocytes. For example, we have shown that female astrocytes are more resistant to OGD than male cells. In part, this is due to the higher aromatase mRNA and protein and higher enzyme activity than occurs in female cells under ischemic stress¹³⁴. Furthermore, the aromatase inhibitor, Arimidex, markedly increases cell death in female astrocytes and abolishes the sexual dimorphism in outcome from OGD. Using a bio-assay approach, we observed that astrocyte-conditioned media from female cultures conferred protection against OGD-induced cell death in male cultures. However, when pretreated with Arimidex, conditioned medium from female astrocytes was unable to protect male cells¹³⁴.

To further verify sex-dependent differences in aromatase-mediated mechanisms of protection against cell death, we developed a novel method that employs sex-specific and genotype-specific single pup primary astrocyte cultures from wild-type and ArKO mice. While wild-type female astrocytes are predictably more resistant to OGD than wildtype male cells, this sex difference disappears in ArKO cells. Specifically, post-OGD cell death is increased in ArKO female astrocytes to a level indistinguishable from male ArKO cells¹³⁵. These findings confirm that the aromatase is instrumental in mediating astrocyte survival following OGD and again emphasize that activation of the enzyme leads to cytoprotection. Furthermore, aromatase activity appears to be essential in endogenous neuroprotection in female astrocytes.

The precise mechanisms by which aromatization of steroids protect brain have not been directly studied. However, the prevailing hypothesis is that it is estrogen production is responsible, rather than metabolism and reduction of tissue androgen levels. It has been exhaustively demonstrated that exogenous estradiol reduces ischemic brain injury even in complex animal models with co-morbidities such as hypertension, genetic predisposition to stroke¹¹⁷, diabetes^{136, 137} and in aging¹³⁸. Estradiol's protective mechanisms are thought to be multifactorial and this is the subject of other reviews in this series.

6. Conclusions

Aromatase plays a critical role in brain function under physiological and pathological conditions (Fig. 1). Classically, aromatase has been implicated in the control of reproductive status, sexual development, neuroendocrine function, and sexual behavior. As such, the predominant center of aromatization is within limbic neural circuits that regulate these functions. The regulation of aromatase within these circuits is complex and not uniform in all brain regions. Evidence exists for post-transcriptional control, but sex steroids exert the major transcriptional control. Brain-derived estrogen is necessary for the effects that testosterone exerts on sexual differentiation, gonadotropin secretion and male-typical reproductive behavior, however, its role varies among species playing more essential roles in rodents than in primates. More recently, brain aromatase has been shown to play unanticipated roles in neurogenesis, neural plasticity, neuroprotection, and repair suggesting that local estrogen synthesis has broader roles in brain function than previously appreciated. These exciting new observations hold the promise that someday pharmacologic regulation of brain aromatase could offer therapeutic opportunities for the treatment neurologic disorders and cerebrovascular disease.

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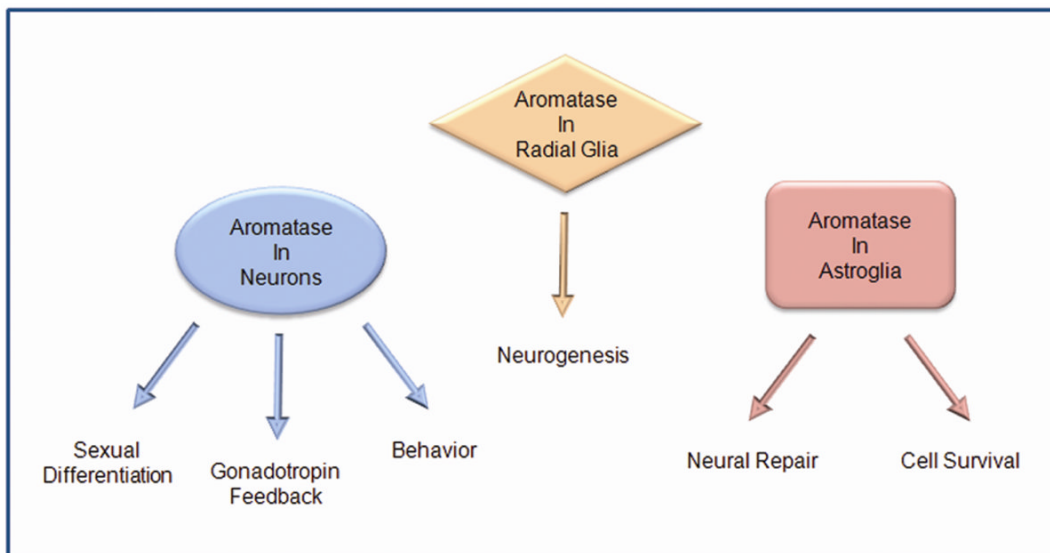


Figure 1.

Summary of classical reproductive and novel nonreproductive actions of brain aromatase. Under physiological conditions aromatase is predominantly expressed in mammalian neurons and radial glia, but under pathological conditions can be expressed in astroglia. Locally formed estrogen acts to alter synaptic activity, stimulate neuronal connectivity, and enhance cell survival. Acting through these mechanisms, brain aromatase affects development, reproduction, behavior, and repair.