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Glial estradiol synthesis after brain injury

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Abstract
Glial cells are important contributors to the hormonal milieu of the brain, particularly following damage. In birds and mammals, neural injury induces the expression of aromatase in astroglia at and around the site of damage. This review describes the progress in our understanding of the incidence, regulation, and function of estrogens synthesized in glia. Following a quick discussion of the landmark studies that first demonstrated steroidogenesis in glia, the author goes on to describe how the inflammatory response following perturbation of the brain results in the transcription of aromatase and the resultant rise in local estradiol. The author ends with several unresolved questions, the answers to which may reveal the precise manner in which neurosteroids protect the brain from injury, both before and immediately after injury.

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Since the discovery of the neurosecretory cell [1], studies in a diverse set of animal models have recognized the brain as a significant source of hormones. In the early 1980s, steroidogenesis in the brain was inferred by a pair of landmark studies on two different steroids by two different laboratories. More specifically, Corpéchot et al. [2] documented the presence of dehydroepiandrosterone sulfate (DHEAS) in the murine brain at levels higher than the periphery, and despite the removal of the adrenals and gonads. The same year, Maclusky and Naftolin [3] described a crucial role for the hypothalamic conversion of androgens to estrogens in the organization and activation of masculine neural circuits of the rodent brain [3]. These studies, taken together, laid the foundation for the discovery of multiple steroids in the CNS of many species across all vertebrate classes [3–12]. These studies, in turn, have fueled the continuing search for the function of neural steroidogenesis under normal and pathological conditions [13–18].

Steroidogenesis in glia

While much research and understanding of neuroendocrine function is steeped in neuronal anatomy and physiology within the central nervous system (CNS), other cell types have more recently been recruited to the set of central neurosecretory cells. Indeed, the secretion of growth factors, cytokines, and other diffusible signals from non-neuronal cells is well established. Among these, astrocytes (types 1 and 2), and oligodendrocytes have emerged as important contributors to the neuroendocrine milieu of discreet brain loci during development, adulthood, and aging. Oligodendrocytes and astrocytes were among the first glial cell types found to concentrate the enzymes 5α-reductase and 3α-hydroxysteroid dehydrogenase [19–21]. These studies were replicated more recently, cementing some glial cells and their precursors as potent metabolizers of pregnenolone, progesterone, and the androgens DHEA and testosterone [22]. Indeed, every enzyme necessary for the synthesis of androgens from cholesterol has been reported in oligodendrocytes or astrocytes [23,24].

The first description of aromatase (*estrogen synthase*) of glial origin came from studies of mixed telencephalic cultures of hatchling zebra finch brain [25]. Notably, aromatase activity seemed to be upregulated in older cultures, a characteristic sustained even after the apparent depletion of neurons. The coincidental demonstration of aromatase transcript in cells of the glial bed *in vitro* strongly supported the neurosteroidogenic capabilities of glial cells [25]. The subsequent documentation of the aromatase transcript, estrone, and 17β-estradiol (E2) by neurons and astrocytes, but not oligodendrocytes in purified, cell-specific cultures of developing rat brain, mirrored the findings in songbirds and suggested that the synthesis of estrogens by astrocytes was generalizable across vertebrates [26].

This conserved property was later contextualized by *in vivo* studies demonstrating the expression of aromatase in astrocytes of murine rodents and songbirds.

Specifically, Garcia-Segura et al. [27], first showed, and Peterson et al. [28,29], later echoed that aromatase expression is dramatically induced in the CNS following excitotoxic or mechanical injury. Second, this induction occurred in astrocytes of the rat, mouse, and zebra finch brain [30,31]. This induction of aromatase following injury in the mammalian and passerine brain is largely but not exclusively restricted to the area immediately around the injury, where estrogenic signals interact with several indices of neural metabolism and cell turnover. Here, the author briefly summarizes the findings that established a neuroprotective role of glial aromatization following brain injury. The author then describes the processes whereby aromatase expression is induced in reactive astrocytes and the crucial role glial estrogens play in the regulation of neuroinflammation. This review will end with unanswered questions and future studies that may further illuminate the role of astrocytic aromatization in the damaged brain.

Brain injury and glial aromatase expression

The expression of the CYP19A1 gene in astroglia appears to be context-dependent in rodents and songbirds. Specifically, while aromatase expression under normal conditions is neuronal, perturbation of the neuropil in rats, mice, and zebra finches results in a dramatic induction of aromatase expression in reactive astrocytes and radial glia around the site of damage [32,33]. As mentioned earlier, induction of glial aromatase is most noticeable immediately around the site of damage but has also been documented far from the primary location of injury [28,34]. This induction is clearly *de novo*, as increases in the aromatase transcript, protein expression, and biochemical activity have all been documented and replicated across numerous experiments on rodents and songbirds by multiple independent reports [35,36] and in brain areas where aromatase is undetectable prior to injury [37,38].

Injury-induced, astrocytic aromatase expression is neuroprotective. In rodents and songbirds, inhibition of aromatase at the site of damage and concomitant replacement with estradiol (E2) exacerbates and mitigates the extent of neural damage, respectively [39–42]. Notably, this effect is discernible following penetrating, excitotoxic, and percussive injury [30,34,43] and appears to result from the inhibition of necrotic and apoptotic degeneration following brain damage [41–44]. In addition to cell death, part if not all the neuroprotective influence of astrocytic E2 provision may occur due to an effect on growth factors [39], gliosis [44], and cytogenesis at and around the injury [45]. Collectively, these processes reflect a dramatic modulation of several indices of cell turnover, perhaps revealing an orchestrated set of events collectively influencing the survival and resilience of neural circuits by glial synthesis of E2. The question remains however, as to what precise aspect or aspects of brain injury regulate the synthesis of E2 in glia. Alternatively, what about brain injury removes a constitutive inhibition of aromatase expression in glia, resulting in the injury-associated synthesis of E2 in astrocytes.

Regulation of glial aromatase expression

As mentioned above, the induction of aromatase in glia occurs in areas devoid of constitutive aromatase expression, suggesting that some consequence of brain injury regulates the transcription of the aromatase gene. Rats, mice, and zebra finches all have a single aromatase gene [46–48]. Thus, the *de novo* expression of aromatase in astrocytes following injury may involve the specific induction of an astrocyte-specific aromatase transcript. Despite the documentation of alternative transcripts from a single aromatase gene in several species [48–51], the data suggest that the injury-induced, astrocyte-specific transcript of aromatase does not differ from that in neurons, at least in zebra finches [52]. While this finding needs to be corroborated in other species, the question of why aromatase is undetectable in astrocytes *in vivo* prior to brain injury remains unanswered. Another question that may provide a similar answer could be what are the factors specific to brain injury are necessary for the expression of aromatase in astrocytes?

Brain injury, inflammatory signaling, and astrocytic aromatase expression

Traumatic brain injury (TBI) of various types consists of primary and secondary stages. The primary stage is the injury itself resulting from concussion, penetration, or twisting. Although there are some differences, a major similarity across all these types of trauma is the activation of the innate immune system, including the infiltration of immune cells into the site of damage and the secretion of cytokines and chemokines [see 43]. Any of all these changes are potential inducers of astrocytic aromatase following brain injury. However, the most revealing regulators of injury-associated aromatization appear to be the inflammatory response initiated by primary damage to the brain.

An interaction between components of the inflammatory cascade and aromatase has long been recognized in peripheral cells and tissues, particularly in disease states. Cytokines and are established as inducers of aromatase expression in malignant breast tissue [53–56]. Additionally, prostaglandin E2 dramatically increases aromatase expression in endometrial uterine cells [50]. Thus, inflammation as a precursor of aromatization may well occur in neural tissue, particularly when injured and disrupted.

Numerous studies have documented increases in multiple components of the inflammatory cascade following brain injury, including but not limited to several cytokines, cyclooxygenase enzymes, and prostanoids. Any of these moieties may serve as signaling molecules in the regulation of aromatase expression in astrocytes. Pedersen et al. [57] directly tested the hypothesis that injury-induced increases in the inflammatory cascade increase the astrocytic aromatase expression. Briefly, using a within-subject experimental design, adult zebra finches of both sexes received identical penetrating injuries in each telencephalic hemisphere. One telencephalic lobe received the COX inhibitor indomethacin, while the contralateral lobe served as a control. Following a 24hr recovery, each telencephalic lobe was homogenized, and the expression of aromatase was measured using qPCR. In both sexes, injury in the presence of COX inhibition resulted in a lower induction of aromatase relative to the control hemisphere, suggesting a potent regulation of astrocytic aromatization by prostaglandin E2 synthesis [36,57]. Further work implicated the receptors EP3 and EP4 in this regulatory effect. More poignantly, a sex difference was found in the mechanism providing a mechanism whereby PGE2 may induce aromatase expression in astrocytes following brain injury. While the induction of aromatase was dependent on EP3 receptors in the male brain, EP4 seemed to be the critical receptor type in females.

Similar mechanisms may operate in the mammalian brain. Saldanha [34] describes one experiment examining the induction of aromatase following penetrating injury in wild-type, interleukin 1 receptor (IL1R) knockouts, and tumor necrosis factor-alpha (TNFα) knockouts. While wild-type and IL1R both showed robust elevations in aromatase transcript one week after the injury, no such induction was evident in TNFα knockouts. The roles of other molecules such as cytokines, chemokines, thromboxanes, and prostacyclins in the induction of glial aromatase, remain to be tested; taken together, the data point to injury-induced elevations of inflammatory markers as one mechanism capable of increasing aromatization.

In finches and mice, increases in glial aromatase expression are well matched by concomitant increases in local E2 levels. Several studies have demonstrated a robust elevation in local E2 following penetrating brain injury in the zebra finch [57–59], and global cerebral ischemia in rats [60–62]. Thus, high levels of local E2 synthesized in glial cells is available to E2-dependent cells and circuits in and around the site of insult.

Functional consequence of glial aromatization following brain injury

As mentioned earlier, estrogen provision by glia has multiple effects on the injured brain, including the inhibition of necrosis, apoptosis, and gliosis [43,44]. It is noteworthy that in the passerine brain, the upregulation of glial aromatase is rapid and dramatic enough to completely obscure the characteristic wave of secondary degeneration [63]. Additionally, estrogens of glial origin increase cytogenesis and neurogenesis following brain injury in birds, effects that suggest a potentially restorative role [45]. Importantly, work in birds and rodents has identified potent anti-inflammatory actions of local aromatization following brain damage. Specifically, increases in neural estrogens after damage lowers the expression of proinflammatory cytokines and PGE2 in finches [59] and decreases microglial activation in rodents [61,62,64]. Thus, the neuroprotective effects of glial estrogens appear multifaceted, involving anti-inflammatory cascades, cell turnover, and possibly the migration of new neurons to the site of damage [29,45]. All these effects in concert may reflect the neuroprotective effects of induced local estrogens following brain injury.

Unanswered questions and future directions

Neuroprotection following brain injury seems to be a conserved role for induced aromatization in glia. However, there are a number of peculiarities that remain to be examined and explained. First, while the effects of glial synthesis of estrogens after brain injury is beyond doubt, there is very little information about the protective role of neural estrogens before the damage. Since peripheral estrogens are known to buffer damage caused by various perturbations, including focal and global ischemic insult [60,65], it is possible that constitutive aromatization may function in a similar manner. One could imagine, for example, that sites of higher constitutive aromatization may be less vulnerable to damage than those devoid of aromatase expression. Alternatively, it is possible that the activation of astrocytes following injury may involve signaling pathways between neurons and glia. Recently, a role for neuronally derived fibroblast growth factor, a known inhibitor of astrocytic activation, has been implicated as a key signaling mechanism in the E2-mediated neuroprotection following ischemia [66]. These data suggest that neuronal signals may regulate not only the activation of astrocytes but also cellular events within activated astrocytes, such as aromatase expression. This hypothesis awaits direct testing. An associated finding is that lipopolysaccharide (LPS) increases neuronal aromatase and biochemical activity in the absence of brain injury, suggesting that constitutive, neuronal aromatase may serve as a mechanism that protects vulnerable targets of infection such as the brain [36]. Finally, what effects, if any, does brain injury have on steroidogenesis in other glial cells. In addition, since females have extremely low levels of circulating androgens, the androgenic substrate for aromatization in glia remains unclear. Finally, the behavioral consequences of glial estrogen provision after brain injury remain to be more fully understood. The basic science of glial aromatization and neural injury may serve as an excellent foundation to inform tractable therapies for various forms of injuries, including concussive, penetrating, ischemic, and anoxic threats to the brain.

Conflict of interest statement

Nothing declared.

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- * of special interest
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