

Role of Astroglia in Estrogen Regulation of Synaptic Plasticity and Brain Repair

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ABSTRACT: Astroglia are targets for estrogen and testosterone and are apparently involved in the action of sex steroids on the brain. Sex hormones induce changes in the expression of glial fibrillary acidic protein, the growth of astrocytic processes, and the degree of apposition of astroglial processes to neuronal membranes in the rat hypothalamus. These changes are linked to modifications in the number of synaptic inputs to hypothalamic neurons. These findings suggest that astrocytes may participate in the genesis of androgen-induced sex differences in synaptic connectivity and in estrogen-induced synaptic plasticity in the adult brain. Astrocytes and tanycytes may also participate in the cellular effects of sex steroids by releasing neuroactive substances and by regulating the local accumulation of specific growth factors, such as insulin-like growth factor-I, that are involved in estrogen-induced synaptic plasticity and estrogen-mediated neuroendocrine con-

trol. Astroglia may also be involved in regenerative and neuroprotective effects of sex steroids, since astroglia formation after brain injury or after peripheral nerve axotomy is regulated by sex hormones. Furthermore, the expression of aromatase, the enzyme that produces estrogen, is induced de novo in astrocytes in lesioned brain areas of adult male and female rodents. Since astroglia do not express aromatase under normal circumstances, the induction of this enzyme may be part of the program of glial activation to cope with the new conditions of the neural tissue after injury. Given the neuroprotective and growth-promoting effects of estrogen after injury, the local production of this steroid may be a relevant component of the reparative process. © 1999 John Wiley & Sons, Inc. *J Neurobiol* 40: 574–584, 1999

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Brain development and activity depend on coordinated functional interactions of glial cells and neurons. Astroglia are a glial cell type characterized by expression of the intermediate filament glial fibrillary

acidic protein (GFAP). Astroglia play a critical role in brain function by providing metabolites, trophic factors, and neuromodulators to neurons, and by the regulation of extracellular ion concentrations and local cerebral blood flow (Kettenman and Ransom, 1995; Castellano et al., 1998). Furthermore, astroglia have a fundamental role in neural signaling. Astro-

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cyte-to-astrocyte signaling is based on intracellular Ca^{2+} variations that can be propagated as intercellular Ca^{2+} waves via gap junctions (Cornell-Bell et al., 1990; Charles et al., 1991; Finkbeiner, 1992; Dani et al., 1992). Astrocytes express receptors for neurotransmitters (Porter and MacCarthy, 1997); therefore, neurons can signal to astrocytes and induce astrocyte Ca^{2+} waves (Dani et al., 1992; Porter and MacCarthy, 1996). In turn, astrocytes can signal to neurons by releasing glutamate which elevates Ca^{2+} levels in neurons (Charles, 1994; Nedergaard, 1994; Parpura et al., 1994; Hassinger et al., 1995), modulating neuronal currents and excitatory and inhibitory synaptic transmission (Araque et al., 1998a,b). Therefore, astrocyte-to-astrocyte communication is regulated by neuronal activity, while neuronal activity is modulated by astrocyte signaling.

Astrocyte–neuron communication also plays an important role in the response of neural tissue to injury. The central nervous system responds to injuries with an increase in the number and size of astrocytes in the proximity of the lesioned area (Eng et al., 1992). These reactive astrocytes, highly immunoreactive for GFAP, express several growth factors and cytokines that may affect neuronal responses to injury (Eddleston and Mucke, 1993; Ridet et al., 1997). This coordinated astroglia–neuron communication during normal and pathological conditions emphasizes the need to consider astroglia as an integral part of the cellular mechanisms involved in hormonal action on brain development and function. Here, we review current evidence that astroglia are affected by gonadal steroids and are actively involved in the organizational and activational effects of these hormones on synapse formation, synapse plasticity, and the response of neural tissue to brain injury.

ASTROGLIA ARE TARGETS FOR SEX HORMONES

Several studies have shown that testosterone and estradiol promote astroglia differentiation and regulate astroglia gene expression *in vitro* and *in vivo*. Most of these studies have focused on the neuroendocrine hypothalamus of rodents. Sex differences in astroglia morphology and in the expression of astroglia cell markers have been described in several hypothalamic regions, including the mediobasal hypothalamus (Tobet and Fox, 1989; Chowen et al., 1995; Mong et al., 1996; Mong and McCarthy, 1999), the supraoptic nucleus (Suárez et al., 1991), and the suprachiasmatic nucleus (Collado et al., 1995). Sex differences in hypothalamic astroglia are likely due to an effect of

sex hormones, since estradiol promotes astroglia differentiation in primary (Garcia-Segura et al., 1989) and explant cultures (Toran-Allerand et al., 1990) from fetal rat hypothalamus. In addition, both estradiol and testosterone regulate astroglia morphology and the expression of the astroglial marker GFAP in the hypothalamus of developing and adult animals (Garcia-Segura et al., 1994, 1995b; Stone et al., 1998; Mong and McCarthy, 1999; Mong et al., 1999).

Effects of gonadal steroids on astroglia are not restricted to the hypothalamus. Sex differences in astrocyte immunoreactivity have been detected in the hippocampus, striatum (Garcia-Segura et al., 1988) and cerebellum (Suárez et al., 1992). Furthermore, GFAP immunoreactivity in the hilus of the dentate gyrus fluctuates during the estrous cycle following the physiological variations in circulating levels of ovarian hormones (Luquin et al., 1993). This change in immunoreactivity may reflect a cytoplasmic redistribution of GFAP, since neither GFAP transcription nor messenger RNA changed in the hilus of the dentate gyrus during the estrous cycle (Stone et al., 1998). GFAP immunoreactivity decreases in the hilus of the dentate gyrus after removal of circulating gonadal steroids by ovariectomy and increases after the pharmacological administration of either 17β -estradiol or progesterone, but not 17α -estradiol, to ovariectomized animals (Luquin et al., 1993). Castration of adult male rats results in elevated levels of GFAP mRNA in the hippocampus (Day et al., 1990). Administration of estradiol to ovariectomized adult rats, castration of newborn males, and testosterone administration to newborn females result in significant changes in the immunohistochemical distribution of GFAP in the striatum as well (Tranque et al., 1987; Garcia-Segura et al., 1998). Finally, testosterone decreases GFAP in the cerebellum of aged male rats (Day et al., 1998).

It is still unclear whether the effects of sex hormones on astroglia are direct or indirect. Among the possibilities, steroid hormones might influence astroglia by rapid nongenomic effects of steroids on glia or neurons (Zhou et al., 1996; Gu and Moss, 1998), by gonadal hormone receptors in glial cells, or by neurons bearing sex hormone receptors. Sex steroids have both genomic and nongenomic actions on neurons that may affect neuronal activity, and this could in turn affect astroglia. In serum-free hypothalamic monolayer cultures, the response of astroglia to estradiol depends on their direct contact with neurons (Torres-Aleman et al., 1992) and on the expression of a specific isoform of neural cell adhesion molecule (NCAM) in the neuronal membranes (Garcia-Segura et al., 1995a). This suggests that estrogen may have

indirect effects on hypothalamic astroglia which are mediated by neurons. However, the possibility of a direct effect of estrogen on astrocytes is suggested by the presence of an estrogen response element in the 5'-upstream region of the GFAP promoter (Stone et al., 1998). Indeed, direct effects of sex steroids on astrocytes have been demonstrated *in vitro* (Melcangi et al., 1996, 1999; Stone et al., 1998). Furthermore, expression of estrogen receptor- α (ER α) has been detected in astroglia in monolayer cultures (Santagati et al., 1994; Ma et al., 1994). However, the expression of estrogen receptors by astroglia *in situ* is still controversial. Most immunohistochemical studies have detected ER α exclusively in neurons. Nevertheless, ER α immunoreactivity has been described with electron microscopy in astrocytes, ependyma, and endothelia of the guinea pig preoptic area and median eminence (Langub and Watson, 1992), and with fluorescence microscopy in rat arcuate nucleus tanycytes (Gudiño Cabrera and Nieto-Sampedro, 1999). The apparent absence of ER α from astroglia in most immunohistochemical studies with conventional light microscopy may reflect a very low expression level of these receptors, only being detectable with electron microscopy or confocal microscopy using highly sensitive fluorescent labels. A similar situation exists for estrogen receptor- β (ER β). ER β immunoreactivity has been detected in a subpopulation of GFAP immunoreactive astrocytes in the rat hippocampal formation using confocal microscopy (Fig. 1), while ER β immunoreactivity is undetectable in rat brain astrocytes by conventional light microscopy (Azcoitia et al., 1999). Further studies with alternative techniques are still needed to clarify this question. Of particular interest is the recent finding of androgen receptor immunoreactivity in a significant fraction of astrocytes and oligodendrocytes, in addition to neurons, in primate prefrontal cortex (Finley and Kritzer, 1999). This observation is in contrast with the exclusive neuronal localization reported by previous studies in rodents.

ASTROGLIA ARE INVOLVED IN THE INTERACTION OF SEX HORMONES AND GROWTH FACTORS

Studies by the group of Ojeda (Ojeda et al., 1992; Ma et al., 1992, 1994; Ojeda and Ma, 1998, 1999) have shown that the release of growth factors by glial cells may be involved in neuroendocrine regulation by sex steroids. Estrogen regulates the production of transforming growth factor- α (TGF- α) by hypothalamic astroglia. This factor regulates luteinizing hormone

releasing hormone (LHRH) release in two steps involving astroglia-to-astroglia and astroglia-to-neuron communication. First, TGF- α activates specific receptors in astrocytes. Then, this activation induces the release of prostaglandin E2. Finally, prostaglandin E2 released by glia acts on prostaglandin E2 receptors in LHRH neurons (Rage et al., 1997; Ojeda and Ma, 1999). Furthermore, astroglia regulate LHRH release in LHRH cell lines (Melcangi et al., 1997) and several growth factors that may be released by astroglia *in vivo*, such as TGF- β or basic fibroblast growth factor (bFGF), regulate the production or release of LHRH in these cells (Melcangi et al., 1995; Wetsel et al., 1996). Another factor involved in the regulation of LHRH neurons is insulin-like growth factor-I (IGF-I) (Hiney et al., 1996; 1998; Zhen et al., 1997; Longo et al., 1998; Wilson et al., 1998). IGF-I immunoreactivity in the hypothalamic arcuate nucleus and median eminence is localized in tanycytes (Dueñas et al., 1994), specialized glial cells that have many ultrastructural and immunological similarities with astrocytes while preserving a radial shape characteristic of the astroglia of submammalian vertebrates. Tanycytes have been grouped together with Schwann cells, olfactory ensheathing cells, pituicytes, pineal glia, retinal Müller cells, and cerebellar Bergmann glia in a new glial cell type, based on the common neuronal growth-promoting properties of these cells and on the expression of several common markers, including estrogen receptors (Gudiño-Cabrera and Nieto-Sampedro, 1999). Sex differences have been observed in IGF-I immunoreactivity in tanycytes of the rat arcuate nucleus, with adult females showing significantly lower IGF-I levels than males of the same age. This sex difference is abolished by early postnatal androgenization of females (Dueñas et al., 1994). Furthermore, IGF-I levels in tanycytes increase in both male and female rats at the time of puberty. Females show an abrupt increase in IGF-I-immunoreactive levels in tanycytes between the morning and afternoon of the first proestrus. Thereafter, IGF-I immunoreactivity fluctuates according to the different stages of the estrus cycle (Fig. 3). IGF-I-immunoreactive levels are high in the afternoon of proestrus after the peak of estrogen in plasma, remain increased in the morning of the following day, and then decrease to basal conditions by the morning of metestrus (Dueñas et al., 1994). In addition, IGF-I levels decrease in tanycytes when gonadal steroid levels are reduced by ovariectomy and increase in a dose-dependent manner when ovariectomized rats are injected with 17 β -estradiol (Dueñas et al., 1994).

The estrogen-induced increase in IGF-I levels in tanycytes is not mediated by a local change in IGF-I

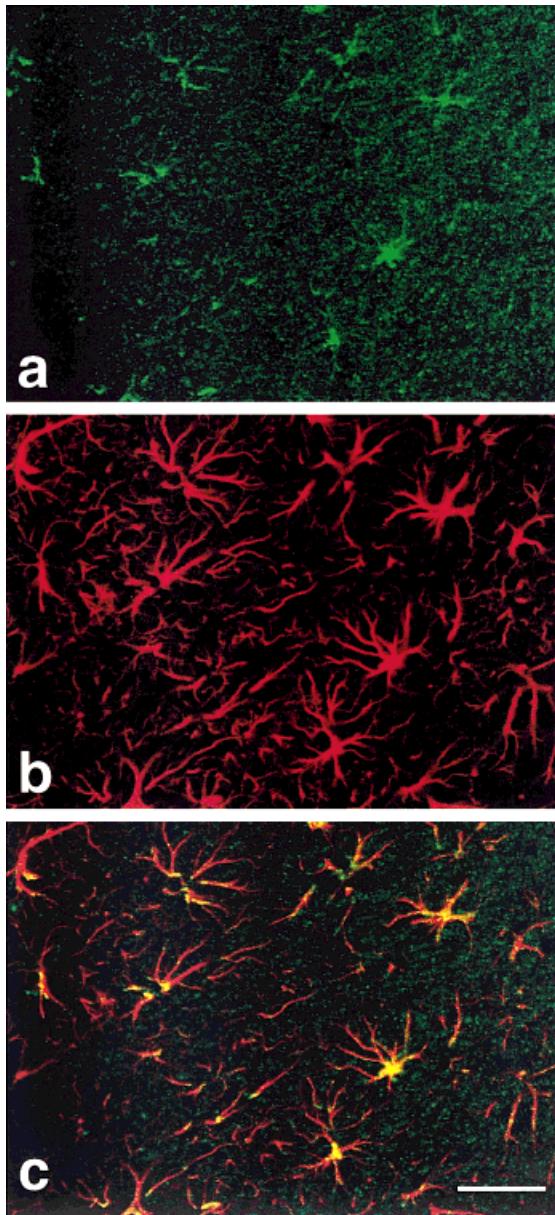


Figure 1 Double localization of estrogen receptor- β -immunoreactivity and glial fibrillary acidic protein (GFAP) by confocal microscopy in the hilus of the dentate gyrus of the hippocampus of an adult male rat. (a) Estrogen receptor- β immunoreactivity recognized using a secondary antibody labeled with Cy2. (b) Localization of GFAP using Cy5 labeling. (c) Double localization of estrogen receptor- β immunoreactivity and GFAP immunoreactivity. Colocalization (yellow) is observed in some cytoplasmic regions. The images are single optical sections. Scale bar = 40 μm .

synthesis, since these glial cells do not express mRNA for IGF-I (Dueñas et al., 1994). However, the modifications in IGF-I levels in tanycytes appear to be the result of hormonal modulation of its accumulation from blood or cerebrospinal fluid. Indeed, tanycytes

do express IGF-I receptors, indicating their ability to interact with this growth factor (Garcia-Segura et al., 1997). When IGF-I labeled with digoxigenin is injected intravenously or in the lateral cerebral ventricle, it is specifically accumulated by various subsets of neurons and glial cells throughout the central nervous system (CNS), including tanycytes (Fernandez-Galaz et al., 1996, 1997, 1998). The accumulation of labeled IGF-I in tanycytes is due to the intracellular internalization of the molecule, as shown by confocal microscopy (Fernandez-Galaz et al., 1998). The internalization of IGF-I is specific and mediated by IGF-I

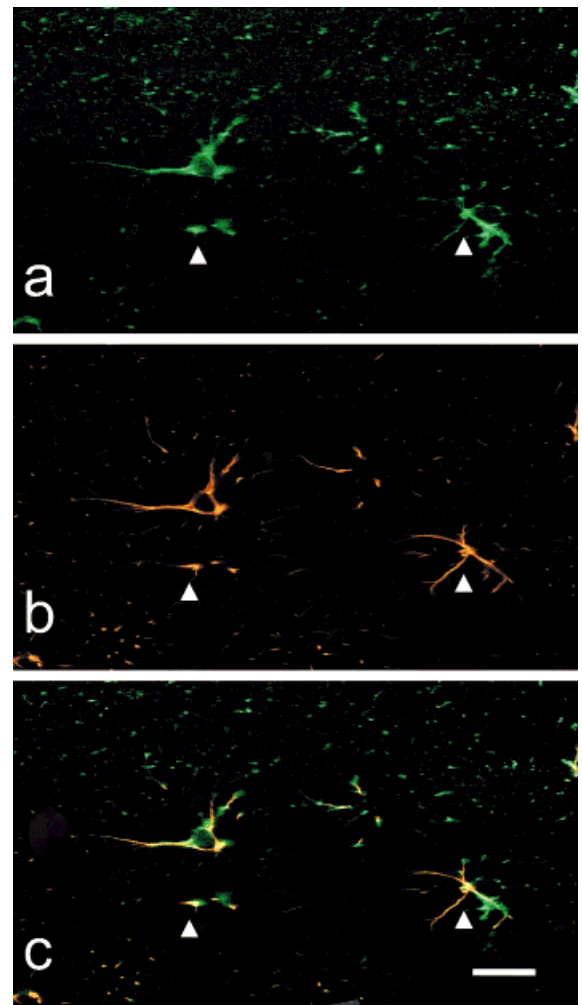


Figure 2 Double localization of aromatase and glial fibrillary acidic protein (GFAP) by confocal microscopy in the stratum radiatum of the CA1 region of the hippocampus of a rat injected intraperitoneally with kainic acid. (a) Aromatase immunoreactivity recognized using a secondary antibody labeled with Cy2. (b) Localization of GFAP using Cy5 labeling. (c) Double localization of aromatase and GFAP immunoreactivity. Colocalization (yellow) is observed in some cytoplasmic regions (arrows). The images are single optical sections. Scale bar = 20 μm .

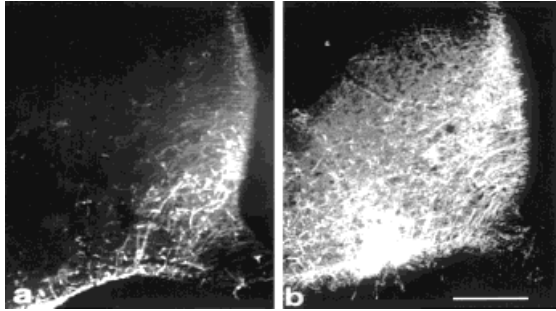


Figure 3 IGF-I immunoreactivity in the arcuate nucleus in (a) the morning of proestrus and (b) afternoon of proestrus. Scale bar = 0.3 mm.

receptors, since it is substantially decreased by the administration of unlabeled IGF-I or unlabeled insulin, which also acts on IGF-I receptors, and is blocked by a specific IGF-I receptor antagonist.

Insulin-like growth factor-I is trafficked by two intracellular routes by astroglia: a degradative endosomal pathway and a nondegradative retroendocytotic pathway that may be involved in transcytosis (Auletta et al., 1992). Therefore, it is possible to postulate that using a nondegradative retroendocytotic pathway tanycytes may translocate IGF-I from extrahypothalamic sources to arcuate neurons and astrocytes. Since the accumulation of IGF-I labeled with digoxigenin in tanycytes after its administration in the lateral cerebral ventricle fluctuates during the different stages of the estrous cycle, tanycytes may mediate sex steroid effects in the mediobasal hypothalamus by regulating local IGF-I levels (Fernandez-Galaz et al., 1996, 1997).

ASTROGLIA MAY BE INVOLVED IN SEXUAL DIFFERENTIATION OF SYNAPTIC CONTACTS

Perinatal androgens affect synaptic formation and promote the appearance of structural sex differences in synaptic connectivity in several brain areas of rodents. Studies conducted in the rat suggest that astroglia may be involved in the genesis of sex differences in synaptic contacts in the hypothalamic arcuate nucleus. The appearance of sex differences in the number of axosomatic synapses is preceded or accompanied by sex differences in the levels of GFAP, the growth of astroglial cell processes and the amount of neuronal membrane surface covered by astroglial cell processes (Chowen et al., 1995; Garcia-Segura et al., 1995b; Mong et al., 1996, 1999). These sex differences in astroglia are dependent on perinatal andro-

gens, with androgen-mediated sexual differentiation of astroglia in the rat arcuate nucleus observed as early as 1 postnatal day after birth (Mong et al., 1996, 1999). Administration of testosterone to newborn females increases GFAP expression, the growth of astroglial cell processes, and the proportion of neuronal membrane covered by glia, while decreasing the final number of axosomatic synapses per neuron to male levels. Castration of newborn males results in the opposite outcome (Chowen et al., 1995; Garcia-Segura et al., 1995b; Mong et al., 1996, 1999). Based on these observations, it has been proposed that testosterone or its metabolite, estradiol, may regulate the generation of the sexually dimorphic pattern of synaptic contacts in the arcuate nucleus by affecting the growth of astroglial processes on neuronal surfaces which affects the amount of neuronal membrane available for the establishment of synaptic contacts (Garcia-Segura et al., 1995b; Mong and McCarthy, 1999; Mong et al., 1999).

ASTROGLIA ARE INVOLVED IN ESTROGEN-INDUCED SYNAPTIC PLASTICITY

In addition to the organizational effects of gonadal steroids, estrogen also promotes synaptic plasticity in the adult brain (Garcia-Segura, 1994a; Naftolin et al., 1996; Woolley, 1998). In adult female rats, there is an estrogen-induced transient disconnection of GABAergic inputs to the somas of arcuate neurons during the preovulatory and ovulatory phases of the estrous cycle. This synaptic remodeling is blocked by progesterone and begins with the onset of puberty (Garcia-Segura et al., 1994a). Astrocytes may play a significant role in these synaptic changes, since modulation of GFAP immunoreactivity and the growth of astrocyte processes occurs during the estrous cycle in this hypothalamic nucleus (Garcia-Segura et al., 1994b). In contrast to what has been observed in the hilus of the dentate gyrus, changes in GFAP immunoreactivity in the arcuate nucleus are accompanied by modifications in the transcription of GFAP mRNA (Kohama et al., 1985; Stone et al., 1998). Synthesis of GFAP is induced in the afternoon of proestrus, and this increase in GFAP is associated with the redistribution of astroglia cytoskeletal components, the growth of astrocyte processes, and the ensheathing of neuronal somas by glial processes. The result of all these changes is the transient disconnection of inhibitory GABAergic synapses from neuronal somas by the interposed glial processes. These changes are also elicited by the

administration of estradiol to adult ovariectomized rats (Garcia-Segura et al., 1994a,b).

The effect of estrogen on synaptic and glial plasticity in the arcuate nucleus may be dependent on growth factor receptor activation. During the estrous cycle, the fluctuations in IGF-I levels in the rat hypothalamic arcuate nucleus occur in parallel with synaptic and astroglia plastic changes. Furthermore, estrogen-induced increases in IGF-I immunoreactivity in the arcuate nucleus are associated with axosomatic synaptic disconnection, the growth of astroglial processes and increased GFAP expression. Since estrogen and IGF-I interact on hypothalamic neuronal differentiation and survival *in vitro* (Dueñas et al., 1996), and since IGF-I is involved in synaptic plasticity in other neuronal systems, we decided to test whether IGF-I is involved in the glial and synaptic changes of the arcuate nucleus. We first tested whether astrocyte activation by estrogen in the arcuate nucleus is dependent on IGF-I receptors (Fernandez-Galaz et al., 1997). Hypothalamic tissue fragments from ovariectomized rats, which contained the arcuate nucleus and the median eminence, were incubated in an artificial cerebrospinal fluid in the presence or absence of estradiol. Surprisingly, the hormone did not induce a significant increase in GFAP-immunoreactive levels, which is in contrast to what is observed *in vivo*. The effect of the hormone was observed, however, in the presence of insulin, which at the concentration used is known to act on IGF-I receptors. Furthermore, the effect of estradiol in the presence of insulin was abolished when the fragments were incubated with a specific IGF-I receptor antagonist. This suggests that the effect of estradiol on arcuate nucleus astrocytes in the presence of insulin depends on the activation of IGF-I receptors. However, insulin alone in absence of estradiol had no effect on GFAP immunoreactivity. This suggests that the activation of IGF-I receptors alone is not enough to stimulate glial cells. This supports the existence of a mechanism involving an interaction between IGF-I receptors and estradiol signaling pathways which activate astroglia in the hypothalamus.

The role of IGF-I receptors in estradiol-induced glial activation and synaptic plasticity *in situ* was assessed by administering into the lateral cerebral ventricle of cycling female rats a specific IGF-I receptor antagonist. In agreement with previous findings, the number of synaptic inputs to arcuate neuronal somas in controls decreased between the morning of proestrus and the morning of estrus. This decline in synaptic inputs, as well as the accompanying increase in glial ensheathing of neuronal somas, was blocked by the IGF-I receptor antagonist (Fernandez-Galaz et

al., 1999). Furthermore, administration of the IGF-I receptor antagonist to ovariectomized females injected with estradiol blocked the effect of estradiol on synapses and astroglia. These findings indicate that IGF-I receptor activation may be involved in the hormone-induced remodeling of arcuate nucleus synapses and astroglia during the estrous cycle.

Further studies are needed to determine the precise mechanism involved in the coordinated actions of IGF-I and estrogen in the regulation of synaptic plasticity. Arcuate neurons express IGF-I receptors (Garcia-Segura et al., 1997) and therefore may be a direct target for IGF-I. IGF-I may affect pre- and/or postsynaptic mechanisms, since ultrastructural studies have shown that IGF-I receptors are present both in a subset of axosomatic presynaptic terminals and a subset of neuronal somas of the rat arcuate nucleus (Garcia-Segura et al., 1997). Arcuate astrocytes are another possible cellular target for IGF-I, since they also express IGF-I receptors (Garcia-Segura et al., 1997). Tanycytes express IGF-I receptors as well (Garcia-Segura et al., 1997) and, as mentioned before, are able to accumulate IGF-I from extrahypothalamic sources and show changes during the estrous cycle in IGF-I immunoreactivity and IGF-I accumulation. Therefore, tanycytes may participate in the regulation of synaptic plasticity by regulating IGF-I levels in the arcuate nucleus. All these possibilities reflect the complex cellular and molecular interactions that should be taken into account to explain the actions of sex hormones in the central nervous system. Figure 4 is a schematic representation of the possible cellular interactions involved in the regulation of synaptic plasticity during the estrous cycle in the arcuate nucleus of rodents.

SEX STEROIDS REGULATE REACTIVE ASTROGLIA

Because of the therapeutical implications of sex steroids as promoters of neural regeneration and prevention of neuronal death, it is important to keep in mind the role that astroglia play in the response to injury. Results from a growing number of experimental studies have shown that estrogen or its precursor, testosterone, promotes axon and synaptic regeneration (Rabbani et al., 1997; Jones et al., 1997b; Tanzer and Jones, 1997), induces the expression of antiapoptotic molecules in neurons (Garcia-Segura et al., 1998), and protects neurons from different stressors *in vitro* (Chowen et al., 1992; Behl et al., 1997; Weaver et al., 1997) and *in vivo* (Sudo et al., 1997; Azcoitia et al., 1998; Chen et al., 1998; Dubal et al., 1998). These

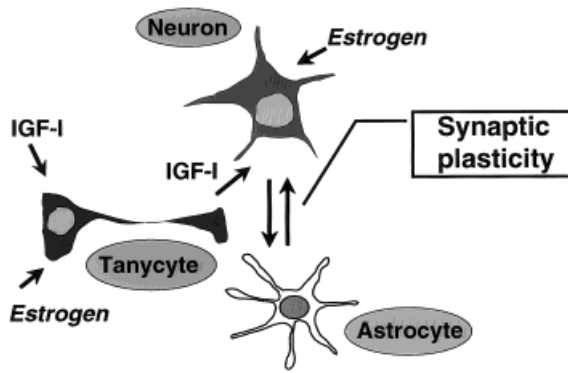


Figure 4 Schematic representation of the possible cellular interactions involved in the regulation of synaptic plasticity during the estrous cycle in the arcuate nucleus of rodents. Estrogen may regulate synaptic plasticity acting on neurons bearing estrogen receptors. In addition, estrogen-induced synaptic plasticity depends on interactions between neurons and astrocytes. Changes in the ensheathing of neuronal somas by astrocytic processes are involved in synaptic changes. Furthermore, estrogen regulates the accumulation of IGF-I by tanycytes. IGF-I from extrahypothalamic sources internalized by tanycytes may be released by transcytosis to arcuate nucleus parenchima, where it may act on neurons and astrocytes expressing IGF-I receptors. The regulation of IGF-I levels by tanycytes may be relevant for synaptic changes, since the activation of IGF-I receptors is necessary for estrogen effects on astrocyte activation and synaptic plasticity.

findings are in good agreement with the reported beneficial effect of estrogen replacement therapy in humans on cognitive functions, regional cerebral blood flow, electroencephalographic (EEG) activity, and severity of dementia in women diagnosed with Alzheimers disease (Paganini-Hill and Henderson, 1994; Tang et al., 1996).

Astrocyte activation is a general response observed after a brain lesion. Reactive astrocytes are characterized by an increase in cell size and GFAP expression. Furthermore, many different molecules, including growth factors and their receptors, cytokines, eicois-anoids, antigen presentation molecules, adhesion molecules, and a variety of enzymes involved in different functions are up-regulated in reactive astrocytes (Edleston and Mucke, 1993; Ridet et al., 1997). It is assumed that these molecules play an important role in the adaptation of neural tissue to injury and in the processes of brain repair. On the other hand, reactive astroglia form a physical barrier that impedes axon growth and are a source of molecules, such as proteoglycans, that inhibit central neurite growth (Bovolenta et al., 1992; Le Roux and Reh, 1996). The recovery of neural tissue after an injury is probably in part the result of an equilibrium between the negative

effects of reactive astroglia on axon growth and the beneficial properties of the substances released by these reactive astroglia that may promote neuronal survival.

Sex steroids may affect the brain's responses to pathological conditions by regulating reactive gliosis (Garcia-Estrada et al., 1993). Sex differences in the formation of reactive astroglia may be related to the sex differences in CNS response to injury and immune responses (Barna et al., 1996). Sex steroids may regulate the expression of molecules in reactive astroglia that are involved in their response to injury. For instance, estrogen increases the expression of apolipoprotein E, a molecule involved in neural regeneration after injury, in astrocytes and microglia (Stone et al., 1997). In addition, sex steroids attenuate the injury-induced increase in GFAP that is one of the most characteristic responses of reactive astrocytes (Day et al., 1990a,b). Finally, sex steroids may regulate the proliferation of astrocytes after injury and the accumulation of reactive astrocytes in the lesioned brain area (Garcia-Estrada et al., 1993). These effects of sex steroids on gliosis may be a contributing factor for neural regeneration. This is also suggested by studies of the central astrocytic response to peripheral nerve injury in the presence of testosterone. In adult male hamsters, testosterone propionate administration reduces the increase in GFAP mRNA in the facial nucleus after facial nerve axotomy (Jones et al., 1997b), attenuates glial-mediated synaptic stripping of axotomized motoneurons (Jones et al., 1997a), and increases facial nerve regeneration (Kujawa et al., 1991). The relationship between reduced astroglia in the facial motor nucleus and the increased axon regeneration is still unknown. However, the results strongly suggest that hormonal regulation of astroglia may be part of the regenerative mechanisms mediating effects of testosterone on facial nerve regeneration (Jones et al., 1999).

IS ESTROGEN PRODUCED BY REACTIVE ASTROGLIA INVOLVED IN BRAIN REPAIR?

Another mechanism by which reactive astroglia may participate in the regenerative actions of sex steroids is by producing the enzymes for their synthesis. We have recently shown that brain lesions induced by the systemic administration of kainic acid or by introducing a cannula in the brain parenchima of rats and mice induces aromatase expression in glial cells (Garcia-Segura et al., 1999). In agreement with previous findings (Lephart, 1996), we observed that aromatase

expression in intact rodent brain is restricted to neurons. No aromatase-immunoreactive glial cells were observed in any brain area of control animals. In contrast, aromatase-immunoreactive astrocytes (Fig. 2) were observed in all injured brain areas, including the cortex, corpus callosum, striatum, hippocampus, thalamus, and hypothalamus. In general, the distribution of aromatase-immunoreactive astrocytes was reminiscent of the distribution of reactive astrocytes after a penetrating brain injury. Indeed, many aromatase-immunoreactive astrocytes had the morphological appearance of reactive astrocytes, including a hypertrophic cytoplasm. The injury-induced expression of aromatase in astrocytes was accompanied by a significant increase in aromatase enzymatic activity in lesioned brain areas.

Since aromatase expression is induced in astrocytes after brain insults that involve neuronal degeneration, it is tempting to speculate on the possible role of local glial estrogen formation on brain repair. Estrogen formed by astrocytes from precursor androgens may be released as a trophic factor for damaged neurons and could be involved in the compensatory restructuring of the injured brain tissue. Since aromatase-immunoreactive glial processes are observed in the neuropil and in contact with the basal lamina of capillaries (Garcia-Segura et al., 1999), it is possible that estrogen released by astroglia may locally affect synaptic function, neural regeneration, and cerebral blood flow, all of which could facilitate neuronal recovery and reduce neuronal death.

CONCLUDING REMARKS

Our knowledge of the important and diverse functions that glial cells perform in the developing and adult brain has expanded dramatically in recent years. These cells, once thought to be only "neuroglue," are active participants in the development, survival, maintenance, and normal functioning of neurons and their synaptic connections. One mechanism by which they accomplish this is through mediating the actions of specific substances, including growth factors and sex steroids. Exposure to sex steroids during either the neonatal or postnatal periods can modulate astroglial morphology in specific brain regions, and this is coincident with axosomatic synaptic changes. Some of these sex steroid-induced changes are dependent on the presence of IGF-I, since an antagonist to the IGF-I receptor blocks the effects of estrogen. Furthermore, sex steroids modulate the accumulation of IGF-I in specific brain regions, and this accumulation is at least in part a function of glial cells. Glial cells are also

known to be involved in the response to brain lesions. Estrogen, which is a neuroprotector and promotes axon and synaptic regeneration, may modulate this glial response. Furthermore, reactive astroglia may participate in the production of sex steroids in the area of the brain lesion, affecting recovery. Hence, it is apparent that glial cells are intimately involved in the mechanisms of action of sex steroids on brain physiology and pathology.

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