MINIREVIEW

Astrocytes and Brain Function: Implications for Reproduction

KRISHNAN M. DHANDAPANI, VIRENDRA B. MAHESH, AND DARRELL W. BRANN[1]

Institute of Molecular Medicine and Genetics, Program in Neurobiology, and Department of Neurology, Medical College of Georgia, Augusta, Georgia 30912

Recent evidence suggests that astrocytes have important neuroregulatory functions in addition to their classic functions of support and segregation of neurons. These newly revealed functions include regulation of neuron communication, neurosecretion, and synaptic plasticity. Although these actions occur throughout the brain, this review will focus on astrocyte–neuron interactions in the hypothalamus, particularly with respect to their potential contribution to the regulation of gonadotropin-releasing hormone (GnRH) secretion and reproduction. Hypothalamic astrocytes have been documented to release a variety of neuroactive factors, including transforming growth factors-α and -β, insulin-like growth factor-1, prostaglandin E2, and the neurosteroid, 3α-hydroxy-5α-pregnane-20-one. Each of these factors has been shown to stimulate GnRH release, and receptors for each factor have been documented on GnRH neurons. Astrocytes have also been implicated in the regulation of synaptic plasticity in key areas of the hypothalamus that control GnRH release, an effect achieved by extension and retraction of glial processes (i.e., glial ensheathment). Through this mechanism, the number of synapses on GnRH neurons and GnRH regulatory neurons can potentially be modulated, thereby influencing the activation state of GnRH neurons. The steroid hormone 17β-estradiol, which triggers the GnRH and luteinizing hormone surge, has been shown to induce the astrocyte-regulated changes in hypothalamic synaptic plasticity, as well as enhance formation and release of the astrocyte neuroactive factors, thereby providing another potential mechanistic layer for astrocyte regulation of GnRH release. As a whole, these studies provide new insights into the diversity of astrocytes and their potential role in reproductive neuroendocrine function. Exp Biol Med 228:253–260, 2003

Key words: astrocyte; reproduction; GnRH; transforming growth factor; estrogen; hypothalamus; LH; neurosteroid; synaptic plasticity; neuron

The preovulatory surge of the gonadotropin hormones, luteinizing hormone (LH) and follicle-stimulating hormone, is a tightly controlled process responsible for the regulation of reproduction in mammals. This process is principally controlled by the ovarian steroid hormone, 17β-estradiol (17β-E_2), which modulates the release of the hypothalamic decapeptide, gonadotropin-releasing hormone (GnRH). GnRH in turn stimulates the release of the gonadotropin hormones, follicle-stimulating hormone, and LH from the anterior pituitary gland to achieve ovulation. Most studies to date have failed to demonstrate estrogen receptors in GnRH neurons, although several recent studies have suggested some GnRH neuronal subpopulations may be estrogen receptor positive (1–4). Nevertheless, it is generally believed that estrogenic control of GnRH release occurs in an indirect manner. This control likely involves the actions of excitatory and inhibitory neurotransmitters that impinge upon and regulate the activity of GnRH neurons (for review, see Refs. 5–7).

In addition to a role for inhibitory and excitatory transmitter-containing neurons, recent studies suggest that glia, a class of non-neuronal cells in the brain, may participate in the control of GnRH secretion. As the most abundant cell type in the brain, glial cells outnumber neurons by a 9:1 ratio. The majority of the work in this area has focused on a particular subset of glial cells, called astrocytes, which are so named because of their star-like shape. Traditionally, astrocytes have been relegated primarily to a supportive or structural role in the brain. However, there is a growing literature that suggests astrocytes are also an important source for neuroactive substances, such as growth factors,

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[1] To whom requests for reprints should be addressed at Institute of Molecular Medicine and Genetics, Program in Neurobiology, Medical College of Georgia, 1120 15th Street, Augusta, GA 30912. E-mail: dbrann@mail.mcg.edu

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eicosanoids, and neurosteroids, which may subsequently influence neuronal development, survival, and neurosecretion (8–11). Although astrocyte–neuron interactions have important implications for all areas of the brain, this review will focus on the hypothalamus and the potential importance of such interactions in the control of GnRH secretion and reproduction.

Astrocyte-Derived Neuroactive Factors and GnRH Release

Work from a number of laboratories has shown that hypothalamic astrocytes release factors that can stimulate GnRH release (9, 12–15). This GnRH releasing ability is illustrated in Figure 1, in which work from our laboratory showed that conditioned media collected from purified hypothalamic astrocytes markedly stimulated GnRH release from GT1-7 neurons, an immortalized GnRH neuronal cell line (14). A similar GnRH stimulatory effect has been reported with conditioned media from cerebral cortical astrocytes (12). In further confirmation of the GnRH regulatory ability of astrocytes, Marchetti and co-workers (13, 15) have demonstrated that co-culture of hypothalamic astrocytes and GT1-7 neurons leads to both enhanced GnRH release and increased neurite outgrowth.

Although many groups have shown that astrocytes can regulate GnRH release, the identity of the astrocyte-active factor(s) mediating this effect is still controversial. It is known that astrocytes can secrete a variety of neuroactive factors, many of which can stimulate GnRH secretion. Along these lines, work by several laboratories has identified transforming growth factor-β1 (TGF-β1) as a neuroregulatory factor secreted by astrocytes that can modulate GnRH secretion (12, 14, 16, 17). Additionally, elegant work by Ojeda and co-workers (9, 18–20) has provided evidence in support of a neuroregulatory role for astrocyte-derived transforming growth factor-α (TGF-α). Furthermore, neuroactive steroid metabolites have recently been shown to be produced and released by astrocytes, and like the growth factors, the steroid metabolites are capable of stimulating the release of GnRH both *in vitro* and *in vivo* (11, 21–23). The following sections of the review will thus focus on:

1. The specific neuroactive factors released by astrocytes.
2. The evidence supporting their potential role in the regulation of GnRH release.
3. The role of estrogen in the regulation of astrocytes and astrocyte-derived neuroactive factors.
4. The potential importance of glial ensheathment in hypothalamic synaptic plasticity and GnRH release.

TGF-β

Several laboratories, including our own, have provided evidence that one of the astrocyte-active factors responsible for stimulating GnRH release is a soluble, heat-labile factor that is greater than 10 kDa in size (12, 14, 17). Astrocyte-conditioned media was determined to possess GnRH-releasing activity only after freezing or heating, suggesting that the active factor may be synthesized as a larger, biologically inactive precursor, which is subsequently activated for biological activity. As shown in Figure 2, the filtration of hypothalamic astrocyte conditioned media with a 10-kDa cutoff filter essentially eliminated the GnRH-releasing activity of the conditioned media, further suggesting the active factor is a protein greater than 10 kDa. These unique properties raised the possibility that the active factor could be TGF-β1, which is known to be released in a latent form that is greater than 10 kDa (25–30 kDa) and which can be activated by processes such as freezing and heating. To assess whether TGF-β1 is released by astrocytes and whether its levels display a correlative relationship to the GnRH-releasing activity of astrocyte-conditioned media, Buchanan

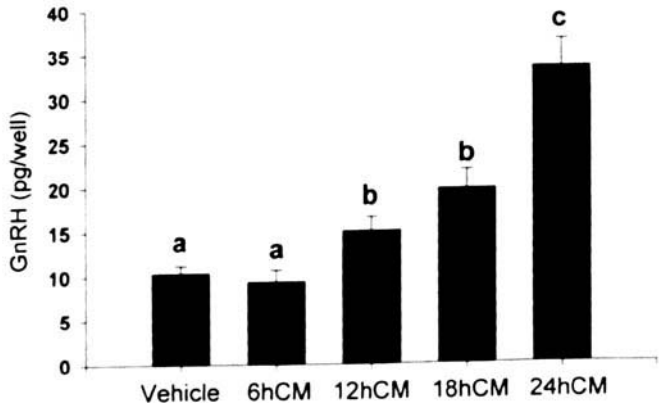

Figure 1. Effect of hypothalamic astrocyte 6-, 12-, 18-, and 24-hr conditioned media on GnRH release from GT1-7 neurons. Media was exposed to hypothalamic astrocytes for the times indicated, then collected, and applied to GT1-7 neurons for a 1h incubation period, after which the media was collected and assayed for GnRH. The control group was GT1-7 neurons exposed to media straight out of the bottle (i.e., media not exposed to astrocytes). Groups with different subscripts are significantly different at $P < 0.05$. From Reference 14 with permission.

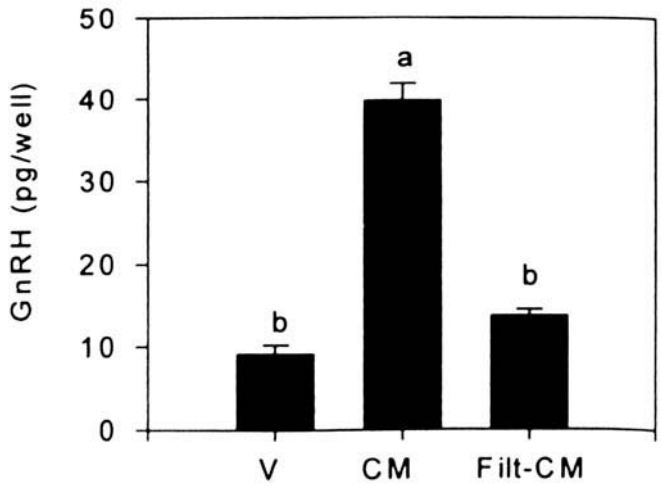

Figure 2. Effect of ultrafiltration on the stimulatory effect of hypothalamic astrocyte 24-hr conditioned media on GnRH release from GT1-7 neurons. Ultrafiltration was performed with 10-kDa cutoff filters. Groups with different subscripts are significantly different at $P < 0.05$. From Reference 14 with permission.

et al. (14) measured TGF-β1 levels in hypothalamic astrocyte-conditioned media and compared these levels with the GnRH-releasing activity of the astrocyte-conditioned media. As shown in Figure 3, TGF-β1 levels increased in a time-dependent manner in astrocyte-conditioned media, and there was a strong correlative relationship between the TGF-β1 levels and the GnRH-releasing activity of the conditioned media (compare with Fig. 1). In contrast with the correlative changes observed for TGF-β1, another growth factor, TGF-α was not detectable in the astrocyte-conditioned media. However, this does not rule out a regulatory role for TGF-α, as subsequent work discussed in the next section has shown that estrogen treatment is required to induce TGF-α gene expression in hypothalamic astrocytes.

To confirm that the correlative relationship for TGF-β1 and GnRH release was causative, studies were performed by two different laboratories using a pan-specific TGF-β antibody for immunoneutralization of the astrocyte-conditioned media and the effect on the ability of the astrocyte-conditioned media to stimulate GnRH release was examined (12, 14). As shown in Figure 4, immunoneutralization with different concentrations of the pan-specific TGF-β antibody resulted in a concentration-dependent attenuation of the ability of the astrocyte-conditioned media to stimulate GnRH release from GT1-7 neurons, with the highest concentration essentially eliminating the GnRH-releasing effect (14).

To be a target of TGF-β1, the GnRH neurons would need to express TGF-β receptors. This was confirmed using a variety of approaches both *in vitro* and *in vivo*. For instance, Messi *et al.* (24) and Buchanan *et al.* (14) using both reverse-transcription polymerase chain reaction (RT-PCR) and Western blot analysis demonstrated that GT1-7 neurons express both the mRNA transcript and protein for the type I and type II TGF-β receptor, which allow signal tranduction following activation by TGF-β isoforms. *In vivo* studies using rodent animal models has also confirmed that the TGF-β type 1 receptor is colocalized in native GnRH neurons (25). The colocalization of the TGF-β type II receptor in GnRH neurons *in vivo* has not been examined as yet. Nevertheless, these studies support the concept that GnRH neurons are targets for TGF-β1 regulation. In further support of this suggestion, exogenous TGF-β1 application has been shown to stimulate GnRH release from GT1-7 neurons *in vitro* at a dose range of 5–25 ng/ml (12, 14). This range is slightly higher than the TGF-β1 levels measured in the astrocyte conditioned media (14), which may suggest the presence of co-factors or additive factors that enhance or facilitate TGF-β1 actions on GT1-7 neurons. Indeed, in several neural systems, TGF-β1 has been shown to require the presence of cofactors/additional factors to achieve a full physiological response (26, 27).

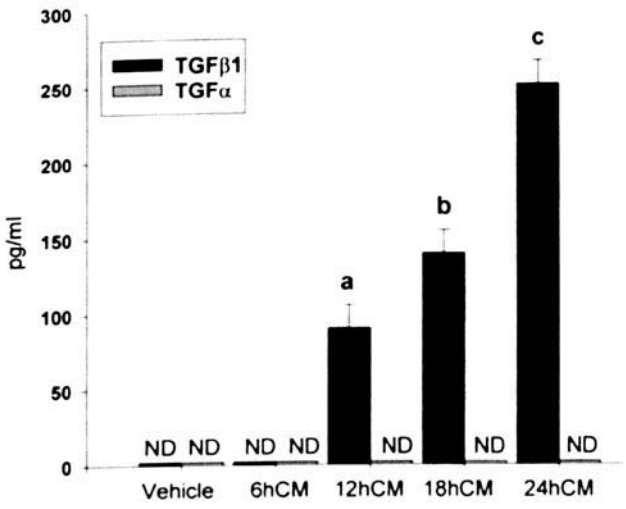

Figure 3. TGF-α and TGF-β1 content in hypothalamic astrocyte 6-, 12-, 18-, and 24-hr conditioned media. Control is media straight from the bottle (i.e. never exposed to hypothalamic astrocytes). ND = nondetectable. Groups with different subscripts are significantly different at $P < 0.05$. From Reference 14 with permission.

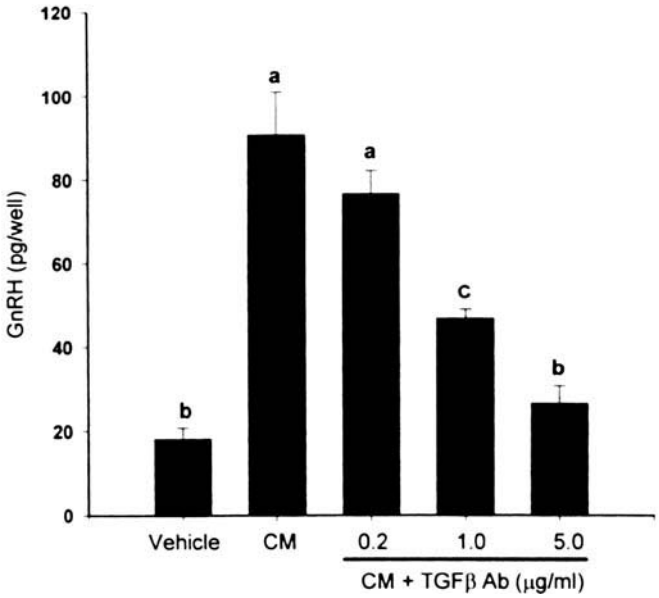

Figure 4. Effect of increasing doses of a panspecific TGF-β-neutralizing antibody (Ab) on the stimulatory effect of hypothalamic astrocyte 24-hr conditioned media on GnRH release from GT1-7 neurons. Groups with different subscripts are significantly different at $P < 0.05$. From Reference 14 with permission.

In addition to influencing GnRH release, astrocyte-conditioned media also influence GnRH gene expression. For instance, recent work has shown that astrocyte-conditioned media increase GnRH gene expression after 1 hr of treatment in GT1-7 neurons, with a subsequent suppression at 6 and 24 hr (16). Further work suggests that this ability of astrocytes to regulate GnRH gene expression may be caused, at least in part, by TGF-β1 (16). In support of this possibility, TGF-β1 has been shown to exert an almost identical regulation over GnRH gene expression as astrocyte-conditioned media; with the only exception being it has a longer stimulatory phase lasting from 1–6 hr followed by suppression at 24 hr. This regulatory ability may extend to other TGF-β isoforms as well, as TGF-β2 also induced a similar up-regulation of GnRH gene expression *in vitro* (16).

Although compelling *in vivo* data for the role of astrocyte-derived TGF-β1 in the physiological regulation of ovulation has been difficult to obtain because of the technical limitations of studying neuron-astrocyte interactions in the *in vivo* situation, TGF-β1 mRNA has been shown to fluctuate in the adult rat hypothalamus during the estrous cycle (28). Of significant interest, TGF-β1 gene expression peaked on the morning of proestrous, before the increased release of GnRH and subsequent ovulatory LH surge. These changes in TGF-β1 gene expression may be caused by steroid regulation, as administration of 17β-E_2 to ovariectomized animals was found to significantly increase TGF-β1 mRNA levels in the hypothalamus (28, 29). The increased TGF-β1 gene expression on the day of the GnRH and LH surge suggests a possible contributory role for TGF-β1 in the generation of the proestrous GnRH and LH surge. However, further studies using either antisense technology or transgenic knockout mice are needed to confirm the putative role of astrocyte-derived TGF-β in the GnRH and LH surge.

Regarding steroid feedback control, it is interesting to note that hypothalamic astrocytes have been reported to express estrogen receptors both *in vitro* and *in vivo* (14, 30–33). Along these lines, Buchanan *et al.* found that estrogen receptor-α and -β mRNA and protein are expressed in hypothalamic astrocytes (Fig. 5; Ref. 14). The estrogen receptor appears functional, as estrogen treatment increased TGF-β1 release directly from purified hypothalamic astrocytes *in vitro* (14). Important work by Zwain and coworkers further demonstrated that estrogen treatment of astrocytes *enhanced* their ability to stimulate GnRH release from GnRH neurons (17). This effect appears to be caused by estrogen-induced TGF-β1 release from the astrocytes, as immunoneutralization with a TGF-β antibody was found to abolish the stimulatory effect of estrogen. These *in vitro* studies on estrogen regulation of TGF-β1 have recently been extended to the *in vivo* situation by Melcangi and co-workers, who demonstrated that estrogen treatment *in vivo* induces the TGF-β1 gene in the hypothalamus of ovariectomized female rats (28, 29).

Taken as a whole, TGF-β1 appears to be a key mediator of astrocyte ability to modulate GnRH release, although it most likely is not the sole mediator. Interestingly, TGF-β1 has also been implicated in regulating neurite outgrowth and neuronal survival in regions such as the cortex and hippocampus (34–37). These neurite-extending and prosurvival effects of TGF-β1 have recently been extended to GnRH neurons using the GT1-7 neuronal model (38). Of significant note, astrocyte-conditioned media also caused a similar induction of neurite outgrowth and reduced serum deprivation-induced cell death of GT1-7 neurons, an effect that was blocked by immunoneutralization with a TGF-β antibody (38). Thus, in addition to regulating neurosecretion by GnRH neurons, astrocytes may also regulate other critical GnRH neuronal functions, such as neural connectivity and survival via the release of the neuroactive growth factor, TGF-β1. These additional potential regulatory actions of astrocytes upon GnRH neurons will certainly be an area for future focus, along with additional *in vivo* confirmations of the role of TGF-β1 and astrocytes in GnRH secretion.

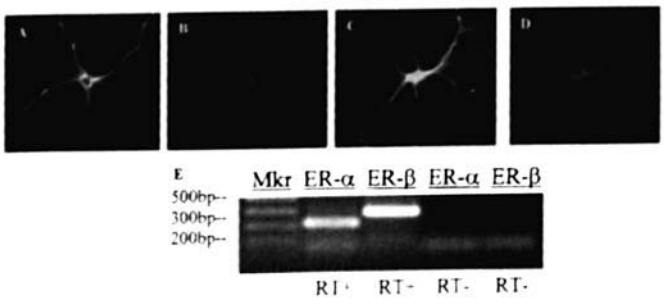

Figure 5. Double immunostaining for GFAP (A,C), ER-α (B), and ER-β (D) in hypothalamic astrocytes *in vitro*. (E) RT-PCR analysis for the expression of ER-α and ER-β in hypothalamic astrocytes in the presence (+) or absence (–) of reverse transcriptase. The RT-PCR products of 220 bp and 292 bp representing ER-α and ER-β, respectively, were detected in hypothalamic astrocytes. From Reference 14 with permission.

TGF-α

In addition to a role for TGF-β1 in regulation of GnRH neuronal activity, work by Ojeda and co-workers has shown that astrocyte-derived TGF-α, a member of the epidermal growth factor family, may have a significant role in the regulation of GnRH secretion and puberty (9, 18, 19, 39, 40). In very elegant studies, Ojeda and co-workers have demonstrated that exogenous TGF-α stimulates GnRH release, an effect that may be indirect as GnRH neurons lack EGF receptors (EGFR), which elicit the biological effects of TGF-α *in vivo* (18, 41–43). Although EGFR appears not to be expressed in GnRH neurons, it is expressed in astrocytes (42, 44); this observation has led to the suggestion that astrocytes may mediate the stimulatory actions of TGF-α on GnRH release (9, 39, 42). In addition to expressing the EGF receptor, hypothalamic astrocytes have also been shown to express the gene for TGF-α (18). Further work showed that TGF-α stimulates TGF-α gene expression in astrocytes, which suggests that TGF-α may act in a paracrine/autocrine manner to stimulate astrocyte expression of factors that may in turn enhance GnRH secretion.

In support of this possibility, Ma *et al.* (19) demonstrated that TGF-α increases the release from hypothalamic fragments of a well-known GnRH secretogogue, prostaglandin E_2 (PGE_2). Furthermore, TGF-α and EGF both were capable of inducing PGE_2 from rat hypothalamic astrocytes *in vitro*. Inhibition of EGFR signaling by administering an EGF receptor antagonist (RG-50864), blocked the stimulatory effect of TGF-α on PGE_2 release, suggesting TGF-α stimulates PGE_2 release by activation of the EGFR and subsequent tyrosine kinase-signaling pathway. Additionally, the EGF receptor antagonist inhibited the ability of TGF-α-treated astrocyte-conditioned media to stimulate GnRH release. The effect of the EGF receptor antagonist appeared to be at the level of the astrocyte, as it had no effect on basal GnRH release (19). Immunoneutralization of

PGE_2 also blocked the stimulatory effect of TGF-α-treated astrocyte-conditioned media on GnRH release from GT1-1 cells. Further work showed that PGE_2 was unable to stimulate GnRH secretion when placed into astrocyte defined medium, but was able to restore the GnRH secretory ability of the immunoneutralized astrocyte-conditioned medium. Because the astrocyte-conditioned medium allowed a normally ineffective dose of PGE_2 to stimulate GnRH secretion, these findings have been interpreted to mean that astrocytes release a co-factor that enhances the stimulatory action of PGE_2, although the identity of this co-factor remains to be identified. Finally, additional studies revealed that estrogen and progesterone treatment enhances TGF-α and EGFR (erbB) gene expression in the hypothalamus of the rat, thus providing critical linkage of the astrocyte-TGF-α-erbB pathway to the steroid positive feedback trigger of the GnRH and LH surge (9).

Although the EGF family member, TGF-α has clearly been implicated in the control of GnRH release, it may not be the only EGF family member so involved. Very recent work by Ojeda and co-workers has identified another member of the EGF family that can enhance GnRH release from the hypothalamus, the neuregulins (45). Neuregulins are a family of proteins that have EGF-like motifs and activate EGF receptors. At least four genes have been identified that code for neuregulins (neuregulin I–IV). In keeping with being an EGF family member, neuregulins appear to have a mode of action similar to TGF-α. Along these lines, neuregulins appear to stimulate GnRH secretion via the erbB-2 receptor on astrocytes, which leads to enhanced PGE_2 release from astrocytes and subsequent stimulation of GnRH neurosecretion. A physiological role for neuregulins in the achievement of puberty has also been recently suggested based on the finding that intracebroventricular administration of erbB-2 antisense oligonucleotides significantly delays the initiation of puberty in female rats (45). Thus, paracrine/autocrine signaling by astrocytes involving EGF family members and subsequent astrocyte release of the neuroactive eicosanoid, PGE_2, provides an additional mechanistic layer whereby astrocytes can influence GnRH neuronal function and reproduction.

Neurosteroids

Within the past several years there has been a growing interest in the ability of the brain to generate "neurosteroids." Particular attention has been devoted to the progesterone metabolite, 3α-hydroxy-5α-pregnane-20-one (3α,5α-THP), which can be produced by astrocytes and has been shown by several laboratories to possess GnRH stimulating activity (21–23). Along these lines, Brann *et al.* (21) demonstrated that *in vivo* administration of 3α,5α-THP stimulated LH release and this effect appeared to be mediated by the $GABA_A$ receptor, as it was blocked by a $GABA_A$ receptor antagonist but not by a progesterone receptor antagonist. Sim *et al.* (23) recently confirmed this observation and extended it by using patch clamp electrophysiological studies to demonstrate 3α,5α-THP acts directly upon GnRH neurons to activate the GnRH neuron. Further studies have also shown that 3α,5α-THP stimulates GnRH release from immortalized GnRH (GT1-7) neurons *in vitro*, and that these neurons express $GABA_A$ receptor subunits (14, 22). These observations are of interest because 3α,5α-THP has been shown to be produced primarily by astrocytes in the brain from the substrate pregnenolone (11). Thus, astrocyte-derived 3α,5α-THP could be an important regulator of GnRH neuronal activity. Whether astrocyte-derived 3α,5α-THP exerts a physiologically relevant regulatory role over GnRH neuronal secretion remains unclear. Interestingly, studies by Buchanan *et al.* (14) have shown that 3α,5α-THP release by hypothalamic astrocytes is correlated with the astrocyte-conditioned media's ability to stimulate GnRH release from GT1-7 neurons. However, because ultrafiltration of astrocyte-conditioned media with a 10-kDa filter (which removes proteins ≥10 kDa, but would not be expected to remove 3α,5α-THP) essentially eliminated the GnRH releasing ability of the conditioned media (see Fig. 2), it is doubtful that 3α,5α-THP plays a major role in the astrocyte-induced GnRH release *in vitro*. Nevertheless, an alternative explanation is that filtration removed cofactors necessary for the 3α,5α-THP stimulatory effect. It is also possible that the astrocyte-derived 3α,5α-THP acts in a permissive fashion to enhance the effect of a neuronal-derived messenger, which would not be present in the highly purified astrocyte system. This is especially true when one considers that 3α,5α-THP has nanomolar affinity for the $GABA_A$ receptor and is a potent allosteric enhancer of $GABA_A$ receptors (22). Thus, although astrocytes can produce and secrete neurosteroids, definitive conclusions on the role and contribution of such astrocyte-derived hormones in GnRH secretion are difficult to reach with the limited evidence available. Clearly, further studies in this interesting and potentially important area are needed.

Astrocytes and Hypothalamic Neuronal Plasticity

In addition to releasing neuroactive factors, astrocytes may also be capable of controlling GnRH neuronal function via regulation of hypothalamic synaptic plasticity. Although this effect also can be caused by the release of neuroactive factors, it can also occur through a process called glial ensheathment, in which the extension and withdrawal of astrocytic processes serve to modulate synaptic contacts of both GnRH neurons and regulatory interneurons. Along these lines, work by Witkin *et al.* (46) revealed that GnRH neurons are intimately associated with glia in the medial basal hypothalamus (MBH) and preoptic area (POA) of the female Rhesus monkey. Further work showed that ovariectomy results in an increased apposition of glial processes to the perikarya membranes of the GnRH-producing neurons in both hypothalamic regions and a corresponding decreased innervation of GnRH neurons. This effect was partially reversed by ovarian steroid replacement, suggesting alterations in circulating gonadal steroid levels influence the

morphology and function of glial cells in the regions surrounding GnRH neurons. By decreasing the glial ensheathment of GnRH neurons, estrogen and/or progesterone may facilitate activation of the GnRH neuron and subsequent GnRH release. Additional work revealed that glial ensheathment of GnRH neurons in the POA and MBH was high in early- to mid-pubertal female rhesus monkeys, although the functional importance of this action has not been resolved (47, 48).

It is not clear whether the glial ensheathment observed in primates extends to the rodent model, which is extensively used in reproductive studies. Prevot *et al.* (49) have reported that there is increased contact of GnRH nerve terminals with the perivascular space in the median eminence on proestrus, following peak estrogen levels. However, it was not clarified whether this increased contact was due to GnRH neurite extension, endothelium outgrowth and/or withdrawal of glial ensheathment. Nevertheless, in the neighboring arcuate nucleus of the rat, Garcia-Segura and co-workers (50–54) have shown that estrogen induces a transient disconnection of axo-somatic *inhibitory* synapses during the preovulatory and ovulatory stages of the estrous cycle. A similar regulation of synaptic plasticity by estrogen has been observed in the arcuate nucleus of the monkey (55). Astrocytes appear to play a role in these synaptic changes, as estrogen increases glial fibrillary acidic protein (GFAP), an important component of the astrocyte cytoskeleton, on proestrus (56–60). The synthesis of GFAP is accompanied by the growth of glial processes that ensheath the neuronal membrane and displace the synaptic terminals. Glia-derived insulin-like growth factor-1 (IGF-1) may play a role in the estrogen-induced synaptic plasticity changes in the arcuate nucleus, as the surface density of IGF-1-like immunoreactivity increases on proestrus and administration of an IGF-1 receptor antagonist blocks the estrogen-induced synaptic decrease in the arcuate nucleus (60–62). Thus, in the arcuate nucleus of the rat, there appears to be an estrogen-dependent extension of glial processes, which decreases inhibitory axo-somatic synaptic contacts. Because the arcuate nucleus is known to be important in the control of GnRH secretion and the LH surge, the resultant reduction of inhibitory synaptic contacts in this nucleus has been suggested to aid in the activation of GnRH neurons by relieving inhibition of excitatory GnRH-regulatory neurons in the MBH. Thus, in addition to the release of GnRH-stimulating neuroactive factors, astrocytes have, as an additional potential regulatory mechanism, the ability to modulate the number of synapses on both GnRH neurons and GnRH regulatory neurons in the hypothalamus.

Summary

The previous prevailing viewpoint that glia serve only structural and support functions in the brain needs revision. Emerging evidence from both *in vitro* and *in vivo* studies demonstrate that glia, particularly astrocytes, can communicate and regulate the activity of neurons. As shown in the summary diagram (Fig. 6), the regulatory ability of glia is caused in part by the release of neuroactive substances, such as the growth factors discussed herein, but also to morphological properties in which extension and withdrawal of glial processes modulate synaptic plasticity. Of the neuroactive substances released by astrocytes, TGF-β1 and TGF-α have moved to the forefront and have been implicated by a number of groups to be involved in the control of GnRH secretion. Thus, three parallel avenues exist for potential modulation of GnRH secretion by glia:

- neuroactive factor-induced GnRH neurosecretion;
- modulation of inhibitory/excitatory synapses on GnRH or GnRH-regulatory neurons; and
- modulation of access of GnRH terminals to the perivascular space in the median eminence.

These glial regulatory actions, while parallel, have one thing in common—they are regulated by estrogen. This estrogen regulation may be direct on the astrocyte, as estrogen receptors have been demonstrated on hypothalamic astrocytes *in vitro* and *in vivo*. Alternatively, the effect of estro-

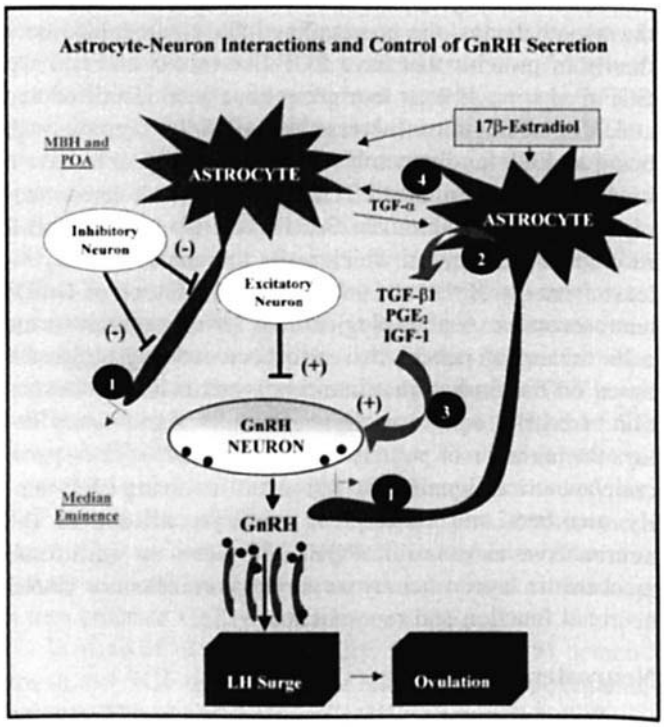

Figure 6. Simplified diagram illustrating postulated astrocyte regulation of synaptic density and GnRH release in the hypothalamus. (1) Estrogen-induced glial ensheathment in the MBH/POA disconnects inhibitory synapses to GnRH neurons and GnRH regulatory neurons, thereby facilitating activation of GnRH neurons. Estrogen-induced withdrawal of glial ensheathment in the median eminence may facilitate GnRH terminal contact with the perivascular space, thereby facilitating GnRH release into portal vessels. (2, 3) Estrogen stimulates release of astrocyte-derived factors such as TGF-β1, PGE_2 and IGF-1, which enhances GnRH release. (4) Estrogen enhances release of TGF-α from astrocytes, which in a paracrine/autocrine fashion enhances release of the GnRH-stimulatory factor, PGE_2, from astrocytes. Through these postulated steps, astrocytes are envisioned to participate with the classical transsynaptic inhibitory and excitatory inputs to facilitate GnRH release, and the subsequent LH surge and ovulation. See text for further description.

gen could also be indirect through active factors released from neurons. Further work is needed to resolve this question. It is envisioned that antisense approaches and cell-type specific knockout mouse technology will be particularly useful in further confirming the role of astrocytes in GnRH neuronal function and in elucidating the mechanisms whereby estrogen modulate astrocyte activity. In this vein, astrocyte specific ablation mouse models may prove quite useful, although care will be needed in interpretation from such models. In closing, although much work is still needed to fully understand the role of astrocytes in reproductive biology, astrocytes have at least moved center-stage in the research arena. It is hoped that the current spotlight on astrocytes will serve as a vital springboard for future efforts in this important research area and that our understanding of the diversity, scope and importance of astrocyte functions in the hypothalamus and the brain will continue to be advanced.

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