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Role of Glia in the Regulation of Gonadotropin-Releasing Hormone Neuronal Activity and Secretion

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Key Words

Gonadotropin-releasing hormone neuron · Astrocyte · Tanycyte · Preoptic area · Median eminence · Puberty · Estrous cycle

Abstract

Gonadotropin-releasing hormone (GnRH) neurons are the final common pathway for the central control of reproduction. The coordinated and timely activation of these hypothalamic neurons, which determines sexual development and adult reproductive function, lies under the tight control of a complex array of excitatory and inhibitory transsynaptic inputs. In addition, research conducted over the past 20 years has unveiled the major contribution of glial cells to the control of GnRH neurons. Glia use a variety of molecular and cellular strategies to modulate GnRH neuronal function both at the level of their cell bodies and at their nerve terminals. These mechanisms include the secretion of bioactive molecules that exert paracrine effects on GnRH neurons, juxtacrine interactions between glial cells and GnRH neurons via adhesive molecules and the morphological plasticity of the glial coverage of GnRH neurons. It now appears that glial cells are integral components, along with upstream neuronal networks, of the central control of GnRH neuronal function. This review attempts to summarize our current knowledge of the mechanisms used by glial cells to control GnRH neuronal activity and secretion.

Introduction

Reproductive function is centrally controlled by a group of specialized neurosecretory neurons that produce the neuropeptide gonadotropin-releasing hormone (GnRH). These neurons, which in rodents are located in the preoptic region of the hypothalamus, send their neurosecretory axons to the median eminence of the hypothalamus, where GnRH is released into pituitary portal blood vessels for delivery to the anterior pituitary gland. Within the adenohypophysis, GnRH elicits the secretion of luteinizing hormone (LH) and follicle-stimulating hormone, which in turn promote gonadal development and support reproductive physiology. While GnRH neurons are in place within the hypothalamus at birth, they are not mature until puberty. From this point on, in females, the coordinated and timely activation of GnRH neurons on the day of proestrus triggers a peak in the release of GnRH into the portal blood, which in turn induces a surge in LH and, subsequently, ovulation [1].

The activity of GnRH neurons is finely regulated by a complex network of excitatory and inhibitory transsynaptic inputs [2–4]. In addition to their neuronal afferents, GnRH neurons are in close contact with glial cells (fig. 1). Astrocytes enwrap GnRH perikarya, while tanycytes, which are specialized ependymoglial cells, interact selectively with GnRH axons and terminals at the level of the median eminence [5–13]. A growing body of evidence

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now suggests that glial cells are far from being passive structural elements, and actually play a critical role in the regulation of GnRH neuronal activity and secretion.

This review will summarize our current knowledge regarding the mechanisms used by glial cells to regulate GnRH function. While astrocytes may also be implicated in the early development of the GnRH system [14, 15] and in the control of neuronal circuits regulating the activity of GnRH neurons, notably in the arcuate nucleus (see [16] for review), here we will focus only on the direct molecular and cellular interactions that occur between glial cells and GnRH neurons during postnatal development.

Cellular and Molecular Mechanisms Involved in the Glial Control of GnRH Neuronal Activity and Secretion

Glial cells use a variety of molecules and strategies to modulate distinct aspects of GnRH neuronal physiology. These mechanisms include the secretion of paracrine factors, juxtacrine interactions between glial cells and GnRH neurons via adhesive molecules, and the structural remodeling of the glial coverage of GnRH neurons.

Glial Cell-Derived Paracrine Factors Involved in Communication between Glia and GnRH Neurons

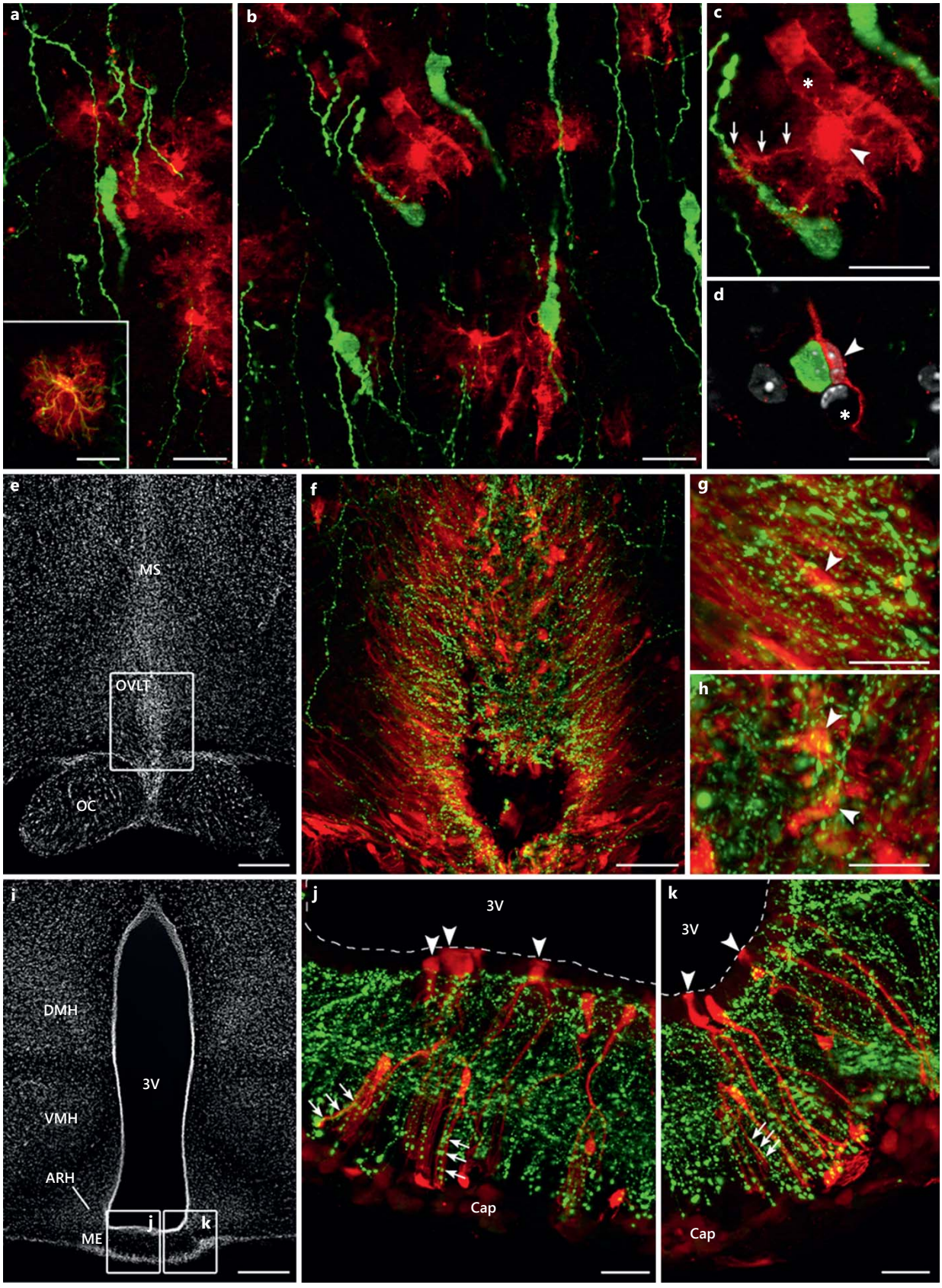
The best-characterized glial cell-derived factors that act on GnRH neurons in a paracrine manner to modulate their function are prostaglandin E_2 (PGE2) and peptide growth factors of the transforming growth factor (TGF)-β family (fig. 2, 3).

Glia-to-GnRH-Neuron Communication via PGE2

PGE2 is a phospholipid-derived molecule that has long been known to be a potent stimulator of the GnRH system (reviewed in [17]). In the hypothalamus, glial cells, both astrocytes and tanycytes, have been identified as primary sources of PGE2.

A highly potent signaling system capable of triggering PGE2 release from hypothalamic astrocytes involves the epidermal growth factor (EGF)-related family of peptides and their receptors, the erbB tyrosine kinases [18]. In rodent hypothalamic astrocytes, which express 3 of the 4 erbB receptors, namely erbB1 (or EGF receptor; EGFR), erbB2 and erbB4 [19–21], exposure to transforming growth factor-alpha (TGFα) and the neuregulins (NRGs) activates erbB1/erbB2 and erbB4/erbB2 heterodimers, respectively, leading to the release of PGE2, which in turn stimulates the release of GnRH from GnRH-producing cells and median eminence explants [20–23]. Importantly, as GnRH neurons have been shown to lack erbB receptors [21, 24, 25], the stimulatory effect of EGF family peptides on GnRH release necessarily involves a glial intermediary. Moreover, this astrocyte-to-neuron signaling pathway has been shown to be set in motion by glutamate. The concomitant activation of ionotropic and metabotropic glutamate receptors located on astroglial cells stimulates erbB

Fig. 1. Glial cells interact closely with both GnRH neuronal cell bodies and processes. Fluorescent photomicrographs of the adult mouse hypothalamus showing GnRH (**a–k**, green) and GFAP (**inset** in **a**, green) immunoreactivity, and expression of the glia-specific protein GLAST (red). To visualize glial cells, transgenic mice expressing the tamoxifen-inducible CreERT2/loxP system under the control of the sodium-dependent glutamate/aspartate transporter (Glast) promoter (Tg(Glast-CreERT2)45–72 line [108]) were crossed with mice expressing the ACTB-tdTomato reporter (http://jaxmice.jax.org/strain/007676.html). Adult bigenic mice were subcutaneously injected with tamoxifen (4 mg from a stock of 20 mg/ml in 1:9 ethanol/sunflower oil), sacrificed two days later and subjected to immunofluorescent detection of GnRH or GFAP following previously described procedures [109]. **a–d** Within the preoptic area, GnRH neuronal cell bodies morphologically interact with astrocytes. **Inset** in **a** While the cell body and the highly branched, 'bushy', structure of astrocytes can be visualized using GLAST-Tomato mice (red), GFAP immunoreactivity (green) only reveals the cell body and the major cell processes. **c** (high-magnification view from **b**), **d** GnRH neurons, astrocytes (arrowheads) and blood-brain barrier capillaries (asterisks) make intimate contacts. Little arrows in **c** point to an astrocytic process that contacts a GnRH neuron. Hoechst-stained nuclei are shown in **d** (white). **e** Low-magnification view of the organum vasculosum of the lamina terminalis (OVLT) after counterstaining of the nuclei with Hoechst (white). The boxed area indicates the site of the image shown in **f**. **f–h** In the OVLT, a circumventricular organ where GnRH neurons were recently shown to extend highly branched dendritic trees [110], GnRH neurites exhibit a strong association with tanycyte-like elements [111]. **g**, **h** High magnification views from **f**. Arrowheads point to tanycyte-like cell bodies abundantly apposed by GnRH dendritic elements. **i** Low-magnification view of the median eminence (ME) after counterstaining of nuclei with Hoechst (white). Boxed areas indicate the site of the images shown in **j** and **k**. **j**, **k** Tanycytes, whose cell bodies line the ventricular wall (arrowheads), send processes toward the capillaries (Cap) of the pituitary portal blood system. GnRH axonal fibers are closely apposed to tanycytic processes in the external zone of the ME (arrows). 3V = Third ventricle; ARH = arcuate nucleus of the hypothalamus; DMH = dorsomedial hypothalamus; MS = medial septum; oc = optic chiasm; VMH = ventromedial hypothalamus. Scale bars: 200 μm (**e**, **i**), 50 μm (**f**), 25 μm (main panel in **a**, **b**, **j**, **k**) and 20 μm (**inset** in **a**, **c**, **d**, **g**, **h**).



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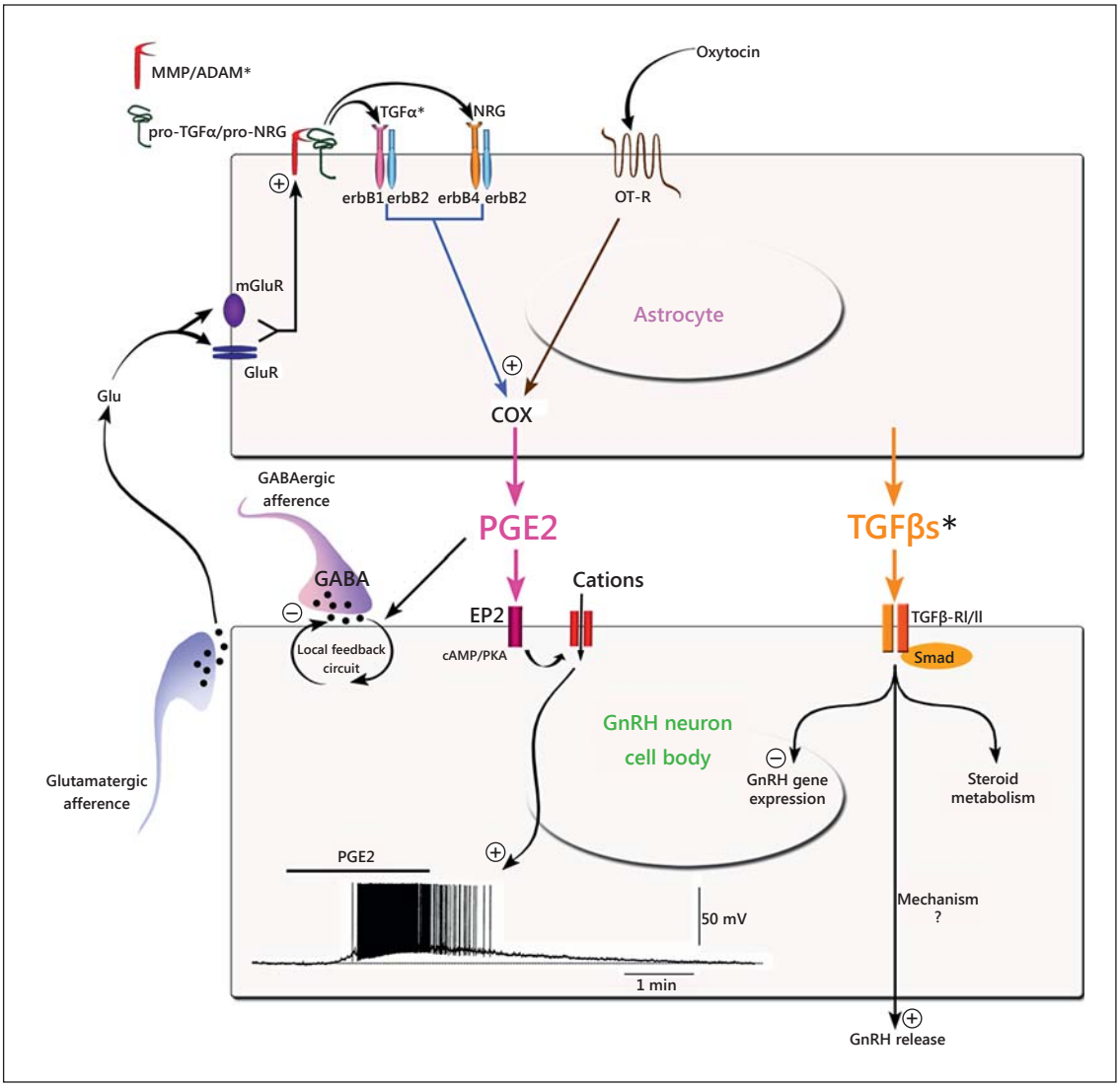

Fig. 2. Glia-to-GnRH-neuron paracrine communication at the level of GnRH cell bodies. Astrocytes modulate GnRH function via the release of PGE2 (left) and growth factors of the TGFβ family (right). In hypothalamic astrocytes, two signaling systems have been shown to trigger PGE2 release, the glutamate/EGF peptides/erbB signaling system and the oxytocinergic signaling system. The concomitant activation of metabotropic (mGluR) and AMPA-type glutamatergic receptors (GluR) in astrocytes by neuronally released glutamate (Glu) stimulates the activity of zinc-dependent MMP of the ADAM (a disintegrin and metalloproteinase) family and promotes the recruitment of erbB1, erbB4, and their pro-ligands to the cell membrane. MMPs catalyze the release of mature TGFα and NRG peptides from their respective membrane-anchored precursors, pro-TGFα and pro-NRG. In particular, the metalloproteinase ADAM17, also known as TACE is involved in the processing of pro-TGFα. TGFα and NRG then activate erbB1/erbB2 and erbB4/erbB2 heterodimers, respectively, leading to the production of PGE2 by COX and its subsequent release from astrocytes [19, 20, 22, 26]. PGE2 is also released upon the activation of the G protein-coupled oxytocin receptors (OT-R), which are located on hypothalamic astrocytes. Notably, oxytocin is less potent than TGFα in eliciting PGE2 release [33]. Astrocyte-derived PGE2 activates EP2 receptors (EP2) and the subsequent mobilization of a cAMP/PKA pathway in GnRH neurons, leading to the activation of a nonselective cation current that promotes membrane depolarization and initiates spike firing [44]. Moreover, the depolarization of GnRH neurons induces the short-term inhibition of their GABAergic afferents via a local feedback circuit that requires the presence of astrocyte-derived PGE2 [47]. Astrocytes also release TGFβs [39, 48–51], which activate type I (TGFβ-RI) and type II (TGFβ-RII) serine/threonine kinase TGFβ receptors located on GnRH neuronal cell bodies [58, 59]. These receptors use Smad proteins as intracellular transducers to regulate transcriptional events (for review see [112]). In GnRH neurons, TGFβs affect steroid metabolism, downregulate GnRH mRNA expression, and stimulate GnRH release [50–55, 58] via an as yet unidentified mechanism thought to occur at the level of GnRH perikarya. Asterisks indicate the molecular components shown to be positively regulated by gonadal steroids in primary cultures of hypothalamic astrocytes (see main text for details).

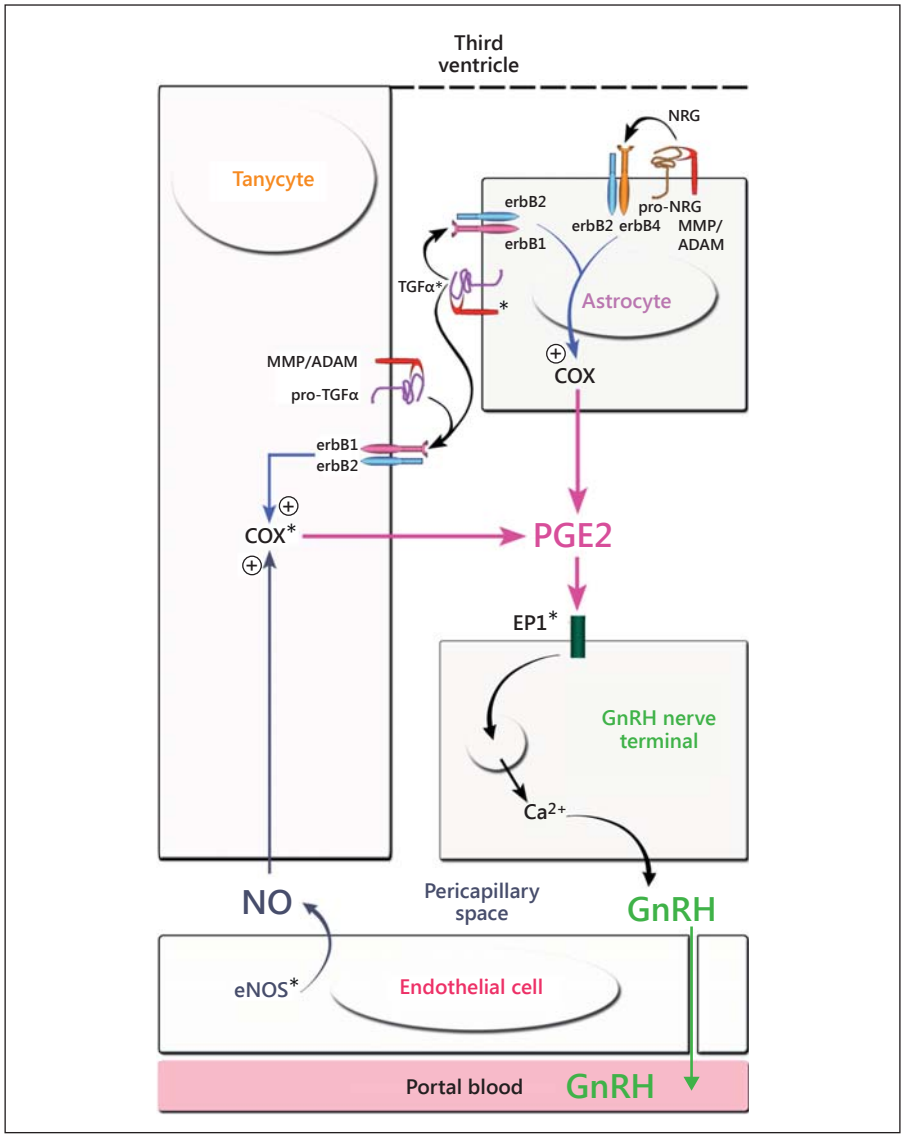

Fig. 3. Glia-to-GnRH-neuron paracrine communication at the level of the GnRH nerve terminals. Within the median eminence, TGFα, which is produced by both tanycytes and astrocytes [27, 39, 79], stimulates erbB1/erbB2 heterodimers in tanycytes, leading to the production of PGE2 [39]. The release of PGE2 by tanycytes is also promoted by a cell-cell mechanism involving the endothelial cells of the fenestrated capillaries of portal blood vessels. Upon activation of eNOS, endothelial cells release the gaseous messenger nitric oxide (NO), which rapidly diffuses to neighboring tanycytes to stimulate the enzymatic activity of COX and the subsequent release of PGE2 [40, 41]. Astrocytes are also abundant within the median eminence and provide another source of glial paracrine factors (see legend of figure 2 for details). The importance of median eminence astrocytes in eliciting GnRH release via the production of PGE2 is demonstrated by experiments performed on median eminence explants from transgenic mice with deficient erbB4 signaling in astrocytes. Indeed, at the level of the median eminence, erbB4 is only present in astrocytes [20] and its disruption abrogates the NRG-induced release of GnRH from median eminence explants, an effect that is reversed by exogenous PGE2 [21]. Notably, since mechanistic insights into the release of paracrine factors from hypothalamic astrocytes were obtained using in vitro preparations of isolated cells from whole hypothalami, it is not presently known whether astrocytes of the preoptic area differ from those of the median eminence. Moreover, while TGFβs act as glia-derived paracrine factors in the preoptic area due to the presence of their receptors on GnRH cell bodies (fig. 2), they are unable to do so in the median eminence since GnRH nerve terminals do not express TGFβ receptors [58]. Upon its release, glia-derived PGE2 is thought to stimulate EP1 receptors (EP1) on GnRH nerve endings, leading to the mobilization of intracellular calcium stores and the subsequent release of GnRH from neurosecretory terminals [42, 45, 46]. Asterisks indicate the molecular components shown to be positively regulated by gonadal steroids in primary cultures of astrocytes, tanycytes, endothelial cells and in the GnRH-producing cell line GT1–1. Note that the stimulatory effect of estradiol on EP1 expression in GT1–1 cells is not direct but requires astrocytic mediation (see main text for details).

signaling by favoring the redistribution of TGFα-erbB1 and NRG-erbB4 complexes to the cell membrane and the subsequent metalloproteinase (MMP)-dependent transactivation of receptors [19, 26]. This link between glutamate receptors and erbB signaling in astrocytes could represent a mechanism used by the neuroendocrine brain to coordinate the enhanced glutamatergic neurotransmission and glial back-signaling to GnRH neurons required for the timely onset of mammalian puberty [2]. The in vivo significance of the EGF signaling pathway in controlling GnRH function is suggested by experiments showing that the expression of TGFα, erbB1, erbB2, erbB4 and the enzymatic activity of tumor necrosis factor-α-converting enzyme (TACE), a metalloproteinase involved in the ectodomain shedding of TGFα and the subsequent activation of erbB1 in response to glutamatergic inputs [26], increase in the hypothalamus at the time of puberty [20, 24, 26–28]. Moreover, the in vivo deregulation of TGFα/erbB1 [23, 27, 29–32] or erbB2 signaling [20], or the inhibition of TACE activity [26], perturbs the onset of female puberty. The crucial role of astrocytic erbB signaling in the control of reproductive function has been demonstrated in transgenic mice expressing a dominant-negative erbB4 receptor in astrocytes, which selectively blocks the ligand-dependent activation of erbB4 and erbB2 receptors in these cells [21]. Mice carrying the transgene exhibit delayed sexual maturation and diminished reproductive capacity in early adulthood. Moreover, erbB1 and erbB4 work in a coordinated fashion to control reproductive function, since mutant mice in which both erbB1 and erbB4 signaling are disrupted exhibit further delays in the onset of puberty and a striking decrease in reproductive capacity in adulthood in comparison to mice deficient in either erbB1 or erbB4 alone [23].

Another environmental cue capable of triggering the release of PGE2 from hypothalamic astrocytes is oxytocin [33]. Oxytocin is a potent stimulator of GnRH secretion from hypothalamic explants of sexually mature animals [34, 35], and accelerates the pulsatile GnRH secretion required for sexual maturation in both males and females [33, 36]. In line with these data, the expression of oxytocin [37] and its receptor [38] increases in the hypothalamus at the time of puberty. Blocking PGE2 synthesis inhibits the increase in the GnRH pulse frequency elicited by oxytocin in hypothalamic explants from prepubertal female rats. The observation that oxytocin receptors are not detected in GnRH neurons but in adjacent astrocytes [33] suggests that, as for EGF-related peptides, the stimulatory effect of oxytocin on GnRH neurons requires glial mediation through the release of PGE2.

Tanycytes are another source of PGE2 at the level of the median eminence. As in hypothalamic astrocytes, erbB1 ligands activate erbB1/erbB2 heterodimers in tanycytes, resulting in the production of PGE2 [39]. Another mechanism of PGE2 release involves the endothelial cells of the fenestrated capillaries of the portal blood vessels. The activation of endothelial nitric oxide synthase (eNOS) in these cells leads to the production of the gaseous messenger nitric oxide (NO). NO then rapidly diffuses to tanycytes to directly stimulate the enzymatic activity of the cyclooxygenases (COX), which catalyze the synthesis of PGE2 [40, 41].

Once synthesized by glial cells, PGE2 immediately diffuses away to activate specific E-prostanoid receptors (EP) located on GnRH neurons [42–44]. Experiments conducted on median eminence explants and GnRH-producing cells indicate that PGE2 induces the release of GnRH from nerve endings, probably through the activation of EP1 receptors and the downstream mobilization of intracellular calcium stores [42, 45, 46]. PGE2 also acts onto GnRH neurons at the level of the cell body, and appears to be an important regulator of the electrical activity of these neurons. Indeed, PGE2 has recently been shown to exert a powerful excitatory effect on GnRH neurons, involving an EP2-protein kinase A (PKA) signaling pathway that leads to the activation of a nonselective cation conductance. Importantly, the selective inhibition of astrocyte metabolism by fluoroacetate or the impairment of astrocytic PGE2 production due to defective erbB signaling in astrocytes suppresses the spontaneous firing activity of GnRH neurons in brain slices [44]. These results thus identify PGE2 as a novel gliotransmitter, and point to astrocytes as important regulators of GnRH neuronal excitability. Moreover, astrocytic-derived PGE2 has recently been implicated in the regulation of a local feedback circuit involving GnRH neurons and their GABAergic afferents [47]. However, how the glial modulation of GnRH neuronal electrical activity affects neuropeptide secretion at their nerve endings is still unknown.

Glia-to-GnRH-Neuron Communication via TGFβs

A series of in vitro studies have shown that growth factors of the TGFβ family, which are produced and released from astrocytes [39, 48–51], modulate GnRH expression, stimulate its release [50–54] and affect steroid metabolism [55] in GnRH-producing cells, which express TGFβ receptors and their downstream effectors – the Smad proteins [50, 53, 56]. In support of these in vitro studies, $TGF\beta_1$, whose levels fluctuate in the hypothalamus across the estrous cycle [57], is selectively detected in astrocytes sur-

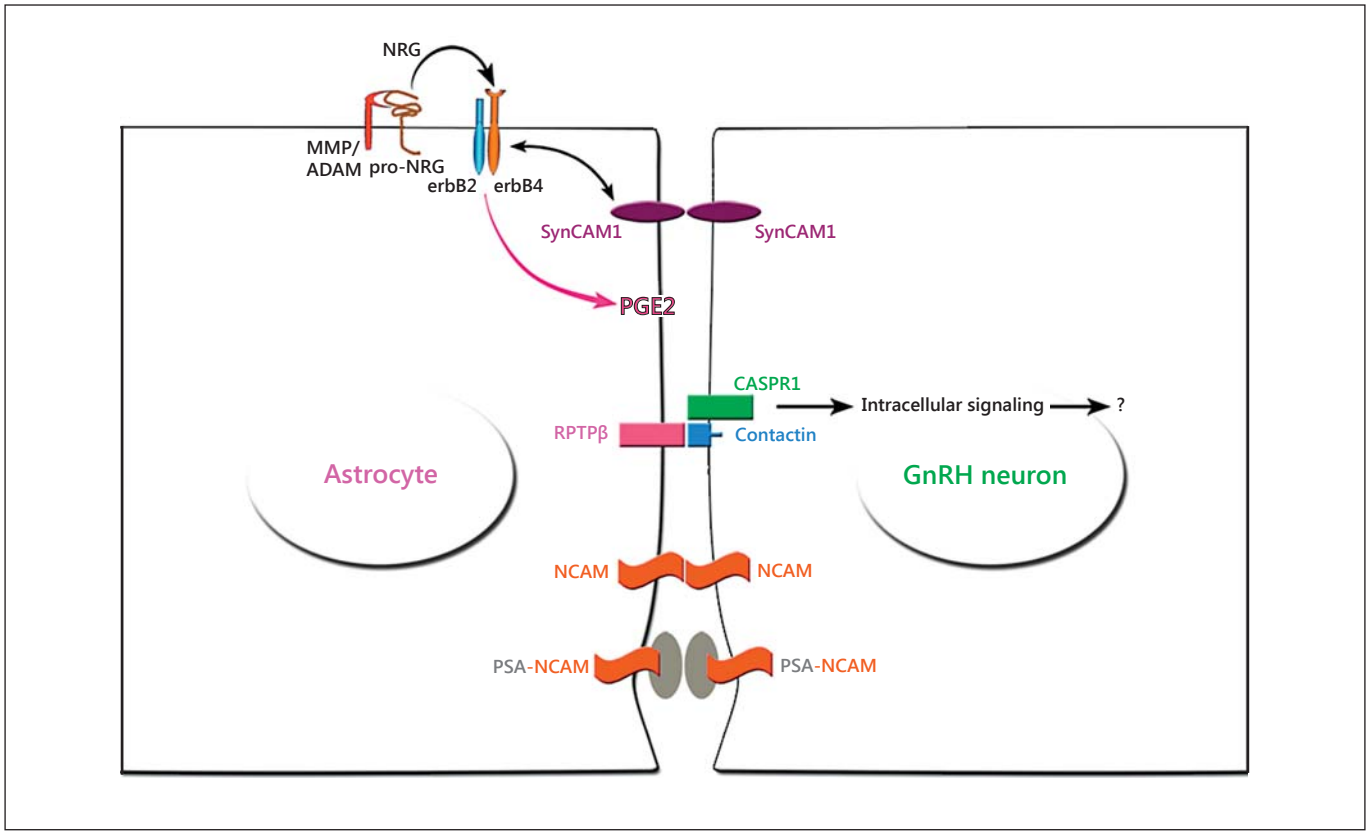

Fig. 4. Juxtacrine interactions between GnRH neurons and glial cells. Synaptic cell adhesion molecule 1 (SynCAM1), which is expressed by astrocytes and GnRH neurons, mediates cell-cell adhesion via homophilic extracellular domain-mediated interactions. The in situ detection of SynCAM protein expression suggests that these interactions occur both at the level of GnRH neuronal perikarya and nerve endings. The SynCAM1 and erbB4 signaling pathways are functionally coupled in hypothalamic astrocytes. The ligand-induced activation of erbB4 receptors promotes the physical interaction of erbB4-SynCAM1 through their intracellular domains and stimulates the adhesive behavior of SynCAM1. SynCAM1 is in turn necessary for neuregulin-dependent erbB4 activation to elicit PGE2 release from astrocytes and GnRH release from nerve terminals [66, 68]. Hypothalamic astrocytes and GnRH neurons also use the RPTPβ/contactin/CASPR1 complex for mutual interactions. This ternary complex exhibits both adhesive and signaling properties. The glial transmembrane protein RPTPβ, which possesses intracellular tyrosine phosphatase domains, binds *in trans* the glycosylphophatidylinositol-anchored neuronal protein contactin. Contactin, which lacks a cytoplasmic domain, interacts *in cis* with the transmembrane protein CASPR1 that recruits and activates intracellular signaling pathways thanks to its cytoplasmic proline residues, which serve as binding sites for the SH3 domains of signaling proteins [113]. The in situ detection of contactin suggests that this ternary complex promotes astrocyte-GnRH-neuron interactions at the level of their nerve terminals (both those reaching the organum vasculosum of the lamina terminalis in the preoptic region and those in the median eminence) but not their perikarya, which seem to lack contactin expression [65]. Both astrocytes and GnRH neurons express PSA-NCAM [62], a plasma membrane-associated cell adhesion glycoprotein. It is worth noting that while RPTPβ/contactin and SynCAM1 complexes promote cell-cell adhesiveness, the sialylation of NCAM attenuates its adhesive properties, allowing structural remodeling and the movement of cellular elements [61]. The functional consequences of RPTPβ/contactin and PSA-NCAM interactions between hypothalamic astrocytes and GnRH neurons, in terms of morphological remodeling and/or intracellular signaling, are still to be uncovered. In addition, it is not known at present whether PSA-NCAM, RPTPβ and SynCAM1 are also expressed at the surface of tanycytes.

rounding GnRH neurons, while TGFβ receptors and Smad proteins are expressed in GnRH perikarya within the rat preoptic region [58, 59]. Moreover, the incubation of preoptic area explants with $TGF\beta_1$ induces the downregulation of GnRH expression in individual neurons [58]. In apparent contrast with in vitro studies, however, $TGF\beta_1$ does not directly trigger GnRH release from GnRH nerve terminals [60], which are devoid of TGFβ receptors [58], suggesting that any in vivo stimulatory effect of $TGF\beta_1$ on GnRH release occurs at the level of GnRH neuronal cell bodies.

Juxtacrine Interactions between GnRH Neurons and Glial Cells: Role of Adhesion Molecules

PSA-NCAM

The polysialylated form of the neural cell adhesion molecule (PSA-NCAM), a plasma membrane-associated cell adhesion glycoprotein primarily expressed in regions capable of morphological or physiological plasticity [61], is abundantly expressed in GnRH neurons and astrocytes in adult female rodents (fig. 4), with higher levels in proestrous, when estrogen levels peak, than in diestrous, when estrogen levels are low [62, 63]. While these observations suggest the possible involvement of PSA-NCAM in the structural neuroglial plasticity modulating GnRH secretion, as elaborated below, the role of this molecule in the regulation of GnRH neuronal activity and/or secretion and its expression by glial cells remain unclear.

In the search for novel communication molecules involved in female sexual maturation, the group of Ojeda has recently identified cell-cell communication complexes consisting of adhesion/signaling proteins that mediate glial cell adhesiveness to GnRH neurons (fig. 4).

RPTPβ/Contactin/CASPR1 Complex

In vitro and in vivo studies performed in nonhuman primates and mice have shown that hypothalamic astrocytes and GnRH neurons use the receptor protein tyrosine phosphatase-β (RPTPβ)/contactin/contactin-associated protein-1 (CASPR1) complex for mutual adhesion. Hypothalamic astrocytes express the transmembrane protein RPTPβ, which interacts with the contactin/CASPR1 dimer present at the surface of GnRH neurons [64, 65]. Notably, contactin is expressed in GnRH nerve terminals but is absent from GnRH perikarya. While the functional consequences of these adhesive interactions on GnRH secretion remain unknown, the observation that RPTPβ mRNA levels increase selectively in the female mouse hypothalamus during the period preceding the onset of puberty, while remaining unchanged in the cerebral cortex [65], raise the possibility that increased interaction between astrocytes and GnRH axons via the RPTPβ/contactin/CASPR1 complex may be part of the neuronal-glial communication mechanisms involved in sexual maturation.

SynCAM1

Astrocytes and GnRH neurons both express synaptic cell adhesion molecule 1 (SynCAM1), which mediates cell-cell adhesion via homophilic extracellular-domain-mediated interactions [66]. In nonhuman primates, SynCAM1 expression increases in the hypothalamus at the time of female puberty [67], and the selective disruption of SynCAM1-dependent intracellular signaling in astrocytes delays puberty, disrupts estrous cyclicity and reduces fecundity in mice [68]. Importantly, the SynCAM1 and erbB4 signaling pathways are functionally coupled in hypothalamic astrocytes. The ligand-induced activation of erbB4 receptors promotes the physical interaction of erbB4 and SynCAM1 through their intracellular domains, activates SynCAM1 gene transcription and stimulates its adhesive behavior [66, 68]. SynCAM1 is in turn necessary for neuregulin-dependent erbB4 activation to elicit PGE2 release from astrocytes and GnRH release from nerve terminals [68]. It appears, therefore, that erbB4 and SynCAM1 form a signaling complex in hypothalamic astrocytes that is activated at puberty and promotes female reproductive capacity by jointly regulating adhesive and paracrine communication between astrocytes and GnRH neurons.

Plasticity of the Glial Coverage of GnRH Neurons

Glial cells are known to undergo highly dynamic morphological changes in response to a wide range of stimuli, which ultimately result in the modification of neuronal function [69]. A spectacular example of such morphological plasticity has been described at the level of the median eminence during the ovarian cycle (fig. 5). Ultrastructural studies have revealed that under conditions of low gonadotropin output, such as in diestrus, tanycytes envelop neurosecretory GnRH nerve terminals and prevent them from directly contacting the perivascular space via their end-feet. Intriguingly, this arrangement appears to be specific to GnRH neurons, and is not as pronounced for the other neuroendocrine systems terminating in the median eminence [70–75]. During the preovulatory surge on the day of proestrus, tanycytes undergo structural remodeling that removes this physical barrier and permits the establishment of direct contact between GnRH nerve endings and basal lamina of the pericapillary space [11, 76], thus favoring the release of GnRH into the portal blood. Notably, a decrease in membrane apposition between GnRH terminals and glial cells has been noted in older female rats [13, 77], raising the possibility that altered structural neuroglial plasticity within the median eminence participates in the mechanisms underlying reduced GnRH output during reproductive aging.

Interestingly, the molecular components involved in the paracrine communication between astroglia and GnRH neurons are also involved in regulating this morphological plasticity, but through distinct mechanisms.

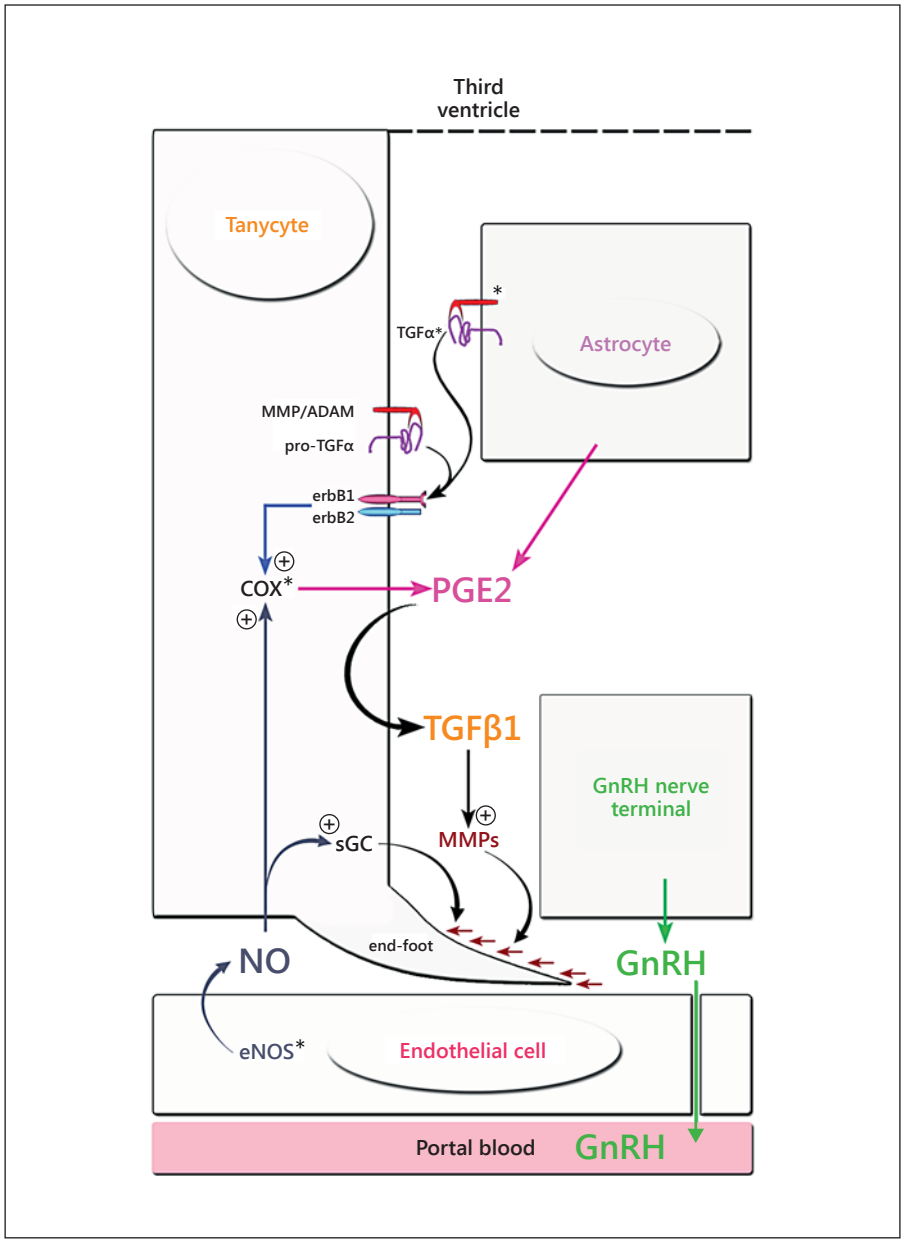

Fig. 5. Morphological plasticity of the glial coverage of GnRH neurons in the median eminence. Under conditions of low gonadotropin output such as in diestrus, tanycytes envelop neurosecretory GnRH nerve terminals and, through their end-feet, prevent them from directly contacting the perivascular space and releasing GnRH into the vascular compartment. TGFα, which is produced by tanycytes and astrocytes [27, 39, 79], activates erbB1/erbB2 heterodimers to stimulate the production of PGE2 and the subsequent PGE2-dependent release of TGFβ$_1$ from tanycytes. Astrocytes also provide a source of PGE2 in response to EGF peptides (fig. 3). Tanycyte-derived TGFβ$_1$ acts in an autocrine manner to induce the retraction of tanycytic processes through the MMP-mediated degradation of extracellular matrix [39]. Endothelial cells also regulate the morphological plasticity of tanycytes via the release of NO, which diffuses to tanycytes to stimulate the enzymatic activity of COX and of the NO-sensitive soluble guanylyl cyclase (sGC) [40]. Notably, the mechanism by which the activation of sGC promotes actin cytoskeleton remodeling in tanycytes remains unknown. The retraction of tanycytic end-feet enables GnRH nerve endings to make direct contact with the pericapillary space (i.e., the space delineated by the parenchymatous basal lamina on one side and by the endothelial basal lamina on the other side; not shown). The released GnRH then enters the portal blood via the fenestrations of endothelial cells. Asterisks indicate the molecular components shown to be positively regulated by gonadal steroids in primary cultures of astrocytes, tanycytes and endothelial cells (see main text for details).

Indeed, the EGF and TGFβ signaling systems converge in tanycytes to regulate their structural remodeling. Tanycytes express both erbB and TGFβ receptors, as well as their respective ligands [20, 24, 27, 39, 58, 59, 78]. TGFα, which is produced by tanycytes and astrocytes [27, 39, 79], activates erbB1/erbB2 heterodimers to stimulate the production of PGE2 and the subsequent release of $TGF\beta_1$ from primary tanycyte cultures. Notably, this stimulatory mechanism is specific to tanycytes because, while they produce both PGE2 and $TGF\beta_1$, hypothalamic astrocytes do not respond to TGFα by further increasing the release of $TGF\beta_1$. Tanycyte-derived $TGF\beta_1$ acts in an autocrine manner to induce the retraction of tanycytic processes through the metalloproteinase-mediated degradation of the extracellular matrix [39]. The observation that hypothalamic TGFα expression increases before that of $TGF\beta_1$ during the period encompassing the preovulatory surge of gonadotropins [27] suggests that the sequence of events identified in vitro [80, 39] may also exist in vivo.

The morphological plasticity of tanycytes is also regulated by endothelial signals. In vitro studies using purified cultures of tanycytes and endothelial cells of the median eminence show that endothelial cells promote rapid actin cytoskeleton remodeling in tanycytes through the release of NO [40, 41]. NO exerts its effects on tanycytes by stimulating the enzymatic activity of both the NO-sensitive guanylyl cyclase and the COX enzymes [40], which produce cGMP [81, 82] and prostaglandin [83], respectively. In agreement with a physiological role for endothelial NO in regulating GnRH secretion via the modulation of tanycytic plasticity, the activation of endogenous NO secretion in median eminence explants promotes the establishment of direct junctions between GnRH nerve terminals and the vascular compartment, with very few tanycytic processes remaining around those GnRH nerve endings that have direct access to the pericapillary space [40]. Moreover, the targeted inhibition of NO production in the median eminence of adult female rats disrupts estrous cyclicity, with a reduction in the percentage of days in proestrus and estrus, when GnRH nerve endings form direct neurovascular junctions with the pituitary portal blood system [40].

A few studies suggest that the remodeling of the glial coverage of GnRH neurons may also occur at the level of their cell bodies, in response to varying levels of gonadal steroids. Morphometric changes in the surface area of astrocytes apposed to GnRH cell bodies have been described across natural [84] and artificial [85] estrous cycles in female rats. Notably, these studies rely on immunolabeling for the cytoskeletal glial fibrillary acidic protein (GFAP), which does not give a true representation of the highly complex, 'bushy' structure of astrocytes (fig. 1a, inset). An ultrastructural study performed in female rhesus monkeys has shown that ovariectomy induces an increase in the apposition of glial processes to GnRH perikarya, associated with a decrease in their synaptic inputs, while steroid replacement reduces the glial ensheathment of GnRH neurons and increases their innervation [6]. Further studies are needed to determine whether the remodeling of the astrocytic coverage of GnRH neuronal cell bodies affects their electrical activity and, ultimately, their neurosecretory output.

Glial Cells: Sensors and Effectors of Sex Steroid Actions in the Neuroendocrine Brain

Most of the above-described mechanisms for the glial control of GnRH neurons have been shown to be under the regulatory influence of gonadal steroids, whose circulating levels rise dramatically at the onset of the preovulatory GnRH/LH surge [86]. The in vivo manipulation of sex steroid levels demonstrates their stimulatory effect on the hypothalamic expression of TGFα [27], erbB2 and erbB4 [20], oxytocin [37, 87, 88], the oxytocin receptor [89, 90], $TGF\beta_1$ [57] and PSA-NCAM [62]. At least some of these effects may be mediated by a direct action of gonadal steroids on astrocytes, which express steroid receptors [91], since the in vitro treatment of hypothalamic astrocytes with estradiol increases the expression of TGFα [92] and the enzymatic activity of TACE [26]. Estradiol further enhances the efficacy of the TGFα/erbB1/PGE2 signaling system by stimulating the expression of prostaglandin receptors in GnRH-producing cells through the secretion of glial signals [42]. In addition, hypothalamic astrocytes respond to progestogens and estradiol by increased $TGF\beta_1$ gene expression [93] and release [50, 54], respectively. Tanycytes also express estrogen receptors in situ and in vitro [41, 94]. Estradiol may regulate tanycytic morphological plasticity by controlling eNOS and COX protein expression in median eminence endothelial cells and tanycytes, respectively, and thus the dialogue set in motion between these non-neuronal cell types [41]. However, astroglia may not just be passive sensors of peripheral steroid hormones. Work from the group of Micevych (reviewed in [95]) suggests that hypothalamic astrocytes play a critical role in regulating positive feedback by estrogen. Indeed, they have shown that rising estradiol levels initiate a membrane-associated estradiol signaling cascade involving the transactivation of metabotropic

glutamate receptor type 1a (mGluR1a) in hypothalamic astrocytes, which ultimately leads to the synthesis of progesterone, critical for initiating the LH surge. Moreover, primate studies have identified rapid excitatory effects of estradiol on GnRH neuronal activity and suggest that estradiol locally synthesized in the hypothalamus may play a significant role in the control of the preovulatory surge and/or pulsatile release of GnRH [96]. Since data from rodents and humans suggest that astrocytes are a source of estrogens in the brain [97, 98], these observations raise the possibility that astrocytes could contribute to the regulation of GnRH neuronal activity and secretion via the local synthesis of neuroestrogens.

Do Glial Cells Regulate the GnRH System in the Human Brain?

While most of the data concerning the glial regulation of GnRH neurons have been obtained from rodents and nonhuman primates, studies conducted in humans suggest that at least some of these mechanisms are also at work in our species. A neuroanatomical study has shown that GnRH neurons in the adult human hypothalamus intimately interact with glial cells both at the level of their cell bodies and their nerve terminals, as they do in rodents [12]. Interestingly, the observation that some hypothalamic hamartomas, non-neoplastic hypothalamic tumors associated with precocious puberty, are rich in astrocytes expressing TGFα and its receptor erbB1 [99], associated with the demonstration that human hypothalamic astrocytes express a functional erbB signaling system [79], raise the possibility that the activation of erbB receptors in human astrocytes may set in motion glia-to-neuron signaling capable of stimulating GnRH neurons.

Metabolic magnetic resonance imaging studies performed on young women have revealed sex-steroid-driven plasticity in the hypothalamus in vivo [100]. During the pill-free period, when the hypothalamus is active and normal early-follicular-phase pulsatile LH release occurs, there is higher water molecule diffusion and lower levels of choline [100], a metabolite enriched in astrocytes [102, 103], than in the pill-supplemented period, when the hypothalamic-pituitary-gonadal axis is fully inhibited [101]. Although the underlying mechanisms remain to be determined, the increase in water diffusion and reduction in choline levels may reflect a decrease in the tortuosity of the extracellular space due to diminished astroglial cell size, as shown for other neuroendocrine systems [104]. Importantly, such modifications are not observed in the thalamus, a brain structure that is unrelated to reproductive control. These data raise the possibility that transient changes in glial microstructure participate in the control of GnRH secretion in humans.

Notably, even though some of the mechanisms used by rodent astrocytes to control GnRH secretion appear to be conserved in humans, divergences certainly exist. Importantly, clear differences have been described in the neural and hormonal control of the preovulatory GnRH/LH surge between rodents and higher primates [96, 105]. In addition, astrocytes in the human cerebral cortex exhibit increased size, structural complexity, diversity and speed of Ca^{2+} wave propagation than their rodent counterparts [106]. At the molecular level, even though they express the same set of erbB receptors (i.e. erbB1, erbB2 and erbB4), human hypothalamic astrocytes appear to respond to the neuregulins through the activation of erbB4/erbB4 homodimers [79], while rodent astrocytes recruit erbB4/erbB2 heterodimers [20]. Although the functional consequences of these signaling differences remain to be determined, these observations suggest that human hypothalamic astrocytes exhibit unique features that need to be explored in future studies.

Concluding Remarks

From the body of data reviewed here, it now appears that glial cells, in addition to transsynaptic neuronal control, are critical regulators of GnRH neuronal activity and secretion. Glial cells are an abundant source of cellular and molecular strategies to control the electrical and neurosecretory activity of GnRH neurons, both at the level of their cell bodies and at the level of their nerve endings. In vitro studies have provided significant insights into some of the molecular determinants of glia-to-GnRH-neuron communication systems, and the in vivo deregulation of these signals, some of them targeting astrocytes, have demonstrated their relevance to the central control of reproduction. Nevertheless, the development of additional transgenic animals in which the molecules involved in glia-to-GnRH-neuron communication can be specifically targeted both in space (i.e. in astrocytes and/or tanycytes) and time (at the onset of puberty or at critical periods of the estrous cycle), will enable us to refine our understanding of the role of glia in the control of GnRH function. In addition, a still-mysterious aspect of GnRH system physiology is the mechanism by which these neurons, which are scattered within

a vast hypothalamic area, synchronize to generate the pulsatile release of neuropeptide. Given the network organization of astrocytes, which provides them with the potential ability to coordinate distant neuronal units via the multidirectional spread of signaling molecules [107], glial cells may also contribute to the mechanisms underlying the control of the GnRH system at a network level. Future studies addressing these issues promise to generate exciting new data.

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