Prostaglandin E_2, Gliotransmission and the Onset of Puberty

Vincent Prevot and Jerome Clasadonte

Abstract Over the past four decades it has become clear that prostaglandin E_2 (PGE_2), a phospholipid-derived signaling molecule, plays a fundamental role in modulating the gonadotropin-releasing hormone (GnRH) neuroendocrine system and in shaping the hypothalamus. In this chapter, after a brief historical overview, we highlight studies revealing that PGE_2 released by astrocytes is intimately involved in the active control of GnRH neuronal activity and the acquisition of reproductive competence.

Introduction

Sexual development, puberty and adult fertility are achieved by events that are initiated within the central nervous system and require the maturation and function of a neural network that transmits both homeostatic and external cues to the discrete hypothalamic neuronal population that releases gonadotropin-releasing hormone (GnRH) from neuroendocrine terminals within the median eminence into the pituitary portal vessels to control gonadotropin [luteinizing hormone (LH) and follicle stimulating hormone (FSH)] secretion (Donato et al. 2011; Herbison and Neill 2006; Malpaux 2006; Ojeda and Skinner 2006; Plant 2006; Terasawa and Fernandez 2001). In turn, these gonadotropins act on the ovaries and testis to regulate the secretion of sex steroids and the production of eggs and sperm.

Accumulating evidence over the past two decades has indicated that, in addition to neurons, glial cells, and in particular astrocytes, contribute to the neural network that converges onto GnRH neurons to control reproduction. Both the neuronal and glial elements of this GnRH neural network are subject to the direct modulatory influence of gonadal steroids (Bellefontaine et al. 2011; Christian and Moenter

V. Prevot (✉) • J. Clasadonte
Jean-Pierre Aubert Research Center, U837, Development and Plasticity of the Postnatal Brain, Inserm, Lille, France

School of Medicine, University of Lille, F-59000 Lille, France
e-mail: vincent.prevot@inserm.fr

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2010; Garcia-Segura and McCarthy 2004; Mong and Blutstein 2006; Prevot et al. 2010a; Ronnekleiv and Kelly 2005; Wintermantel et al. 2006). Although neuronal elements regulate the activity of GnRH neurons through a complex array of excitatory and inhibitory synaptic inputs, glial cells communicate with GnRH neurons via the activation of specific growth factor-dependent signaling pathways (reviewed in Sharif et al. 2013).

The main glial population in the brain consists of astrocytes that ensheathe synapses and are in contact with blood vessels. They regulate blood flow, provide much-needed energy to neurons, and supply the building blocks for neurotransmitters at the synapses, in addition to dynamically contributing to information processing within the central nervous system (Di Castro et al. 2011; Eroglu and Barres 2010; Halassa and Haydon 2010; Haydon and Carmignoto 2006; Iadecola and Nedergaard 2007; Martineau et al. 2006; Panatier et al. 2011; Pfrieger 2010), including the hypothalamus (Gordon et al. 2009; Hatton and Wang 2008; Oliet and Bonfardin 2010; Panatier 2009; Theodosis et al. 2008). As integrative hubs, astrocytes likely play a fundamental role in shaping and regulating the GnRH system.

Here, we will review recent findings that illustrate the remarkable interplay between glia and neurons within the hypothalamo-hypophyseal-gonadal axis. We will mainly restrict our focus to the roles of hypothalamic astrocytes subserved by the release of prostaglandin E_2 (PGE_2), a molecule that has long been known to regulate GnRH neuronal function and has recently been identified as a gliotransmitter.

PGE_2 Is Involved in the Hypothalamic Control of Reproduction

PGE_2 is one of a number of prostanoids synthesized from arachidonic acid, which is produced from membrane phospholipids by a phospholipase A_2. Arachidonic acid is converted to bioactive prostanoids by the cyclooxygenases (COX-1 and COX-2) and a class of terminal synthases (see for review Bosetti 2007). Several studies suggest that PGE_2 is mainly derived from the COX-2 pathway (Brock et al. 1999; Sang et al. 2005; Vidensky et al. 2003). PGE_2 signaling is propagated by four G-protein-coupled receptors, EP1-EP4 (see for review Coleman et al. 1994; Fig. 1).

For more than 35 years, PGE_2 has been known to play a role in the central control of reproduction. The first indication that PGE_2 was involved in the process of GnRH secretion was provided by experiments showing that, when PGE_2 was injected into the third ventricle of the rat brain, it induced the release of LH into the general circulation (Harms et al. 1973) and the release of GnRH into the pituitary portal blood vessels (Eskay et al. 1975; Ojeda et al. 1975b). A similar stimulatory effect of PGE2 on GnRH release has been documented using push-pull perfusion in conscious monkeys (Gearing and Terasawa 1991). To bring about the activation of the GnRH axis, PGE_2 acts at two main hypothalamic sites: the preoptic-anterior

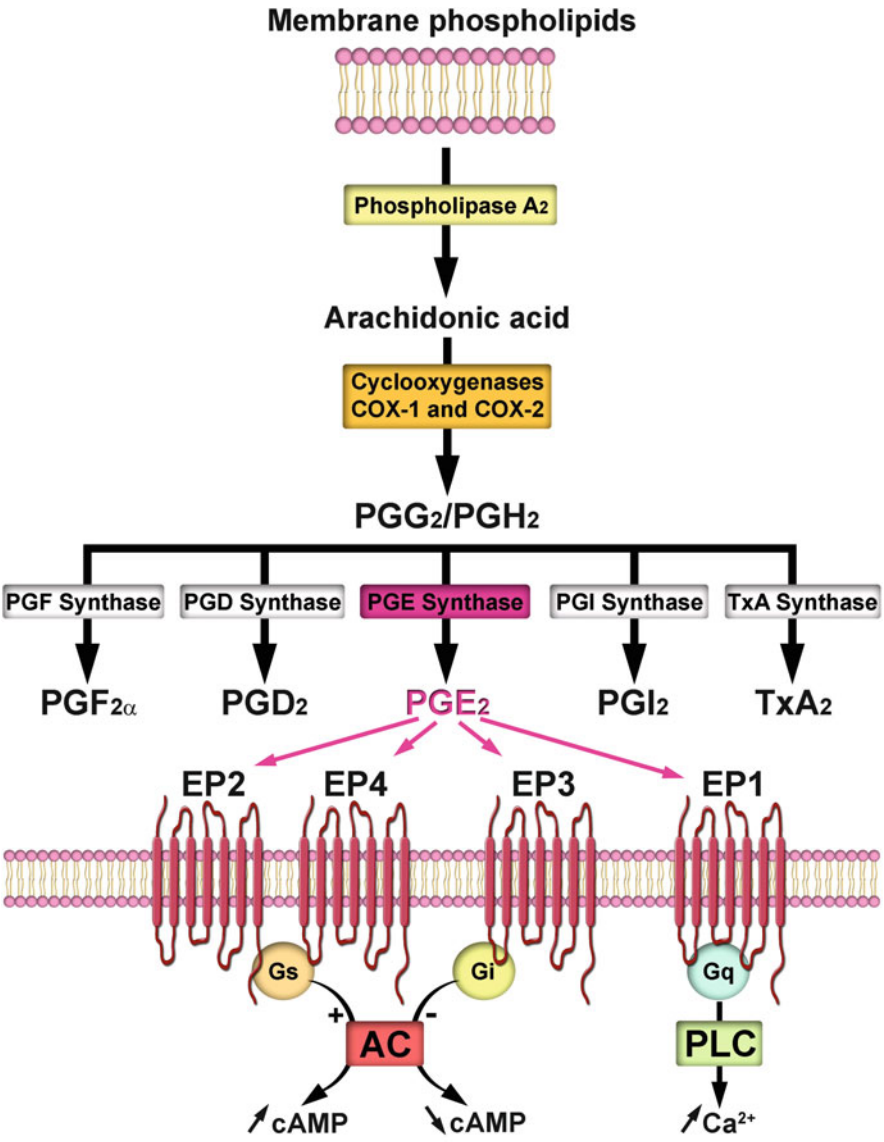

Fig. 1 Prostaglandin E2 (*PGE2*) biosynthesis and signaling. Upon its release from plasma membrane phospholipids by phospholipase A2, arachidonic acid is converted to the unstable endoperoxide intermediates, prostaglandin G2 (*PGG2*) and prostaglandin H2 (*PGH2*), by the cyclooxygenases (COX-1 and COX-2, encoded by separate genes). Both COX isoforms catalyze the same reactions, but while COX-1 is constitutively expressed, COX-2 is rapidly and transiently upregulated by cytokines and growth factors. Terminal synthases convert both PGG2 and PGH2 into prostaglandins [PGE2, PGD2, PGF2α, prostacyclin (PGI2)], and thromboxane (TxA2). Once synthesized, PGE2 immediately diffuses away and activates its specific E-prostanoid receptors (EP1-4), which belong to the family of 7-transmembrane G-protein-coupled receptors. EP2 and EP4 are coupled to Gs and stimulate the adenylyl cyclase (*AC*)-cyclic adenosine monophosphate (*cAMP*)-protein kinase A (*PKA*) pathway. In contrast, EP3 is coupled to Gi and inhibits AC activation, resulting in decreased cAMP concentrations. EP1 is thought to be coupled to the

hypothalamic region in which GnRH cell bodies reside and the tuberal region of the hypothalamus, which contains the median eminence and GnRH-releasing neuroendocrine terminals (Ojeda et al. 1977). The use of COX inhibitors such as indomethacin has provided further support for a physiological role of the prostaglandins in the control of GnRH release. Indomethacin administration suppresses the LH surge induced by estradiol during anestrus in ewes (Carlson et al. 1974) and during the early follicular phase in rhesus monkeys (Carlson et al. 1977). In rats, the intraventricular or intrahypothalamic administration of indomethacin inhibits both pulsatile LH release and the LH discharge induced by ovarian steroids (Ojeda et al. 1975a). Other studies have demonstrated that the microinjection of either aspirin, a non-steroidal COX inhibitor, or N-0164, a prostaglandin and thromboxane antagonist, into the tuberal region of the rat hypothalamus results in the suppression of ovulation (Botting et al. 1977; Labhsetwar and Zolovick 1973). Finally, experiments conducted using hypothalamic explants in vitro have revealed that PGE_2 is an effective stimulator of GnRH release from median eminence nerve terminals (Gallardo and Ramirez 1977; Ojeda et al. 1979, 1986b).

Evidence implicating PGE_2 as a physiological component of the GnRH system during postnatal development arises from findings showing that PGE_2 can induce the release of GnRH long before puberty in both mice and rats (Ojeda et al. 1986a; Prevot et al. 2003). As puberty approaches, the increasing output of estradiol from the developing ovaries induces a preovulatory surge of GnRH/LH. Biochemical analyses at this last phase of sexual maturation have demonstrated that the capacity of the reproductive hypothalamus to metabolize arachidonic acid through the COX pathway leads to a specific increase in PGE_2 synthesis (Fig. 1), particularly during the first proestrus (Ojeda and Campbell 1982). This effect appears to be estrogen-dependent since it is mimicked by the treatment of juvenile animals (early post-weaning period) with estradiol at doses capable of inducing a preovulatory surge of LH (Ojeda and Campbell 1982). More recent studies have shown that an estradiol-induced increase in hypothalamic PGE_2 levels can be seen even in newborn rats (Amateau and McCarthy 2002). Intriguingly, experiments showing that estradiol treatment upregulates both COX-2 mRNA and protein synthesis in the hypothalamus of female rats during postnatal development (Amateau and McCarthy 2004) raise the possibility that estrogens may act on COX-2 expression to promote PGE_2 synthesis at puberty.

Fig. 1 (continued) Gq-phospholipase C (*PLC*) pathway, leading to an elevation of free cytosolic calcium concentrations (Milatovic et al. 2011). Notably, an examination of the capacity of the hypothalamus to metabolize arachidonic acid through the COX pathway has revealed a pubertal increase in the formation of PGE2, particularly during the first proestrus (Ojeda and Campbell 1982). Intriguingly, the increase in PGE2 synthesis is not associated with changes in the formation of PGF2α, PGI2, PGD2 or thromboxane A2 from exogenous arachidonic acid, suggesting that it is a specific event directly associated with the peripubertal activation of the reproductive hypothalamus (Ojeda and Campbell 1982). Such a selective synthesis of PGE2 has also been shown to be triggered by estrogens during early postnatal development (Amateau and McCarthy 2002)

Astrocytes Appear to Be the Main Source of PGE_2 in the GnRH Neuroendocrine System

Although PGE_2 was initially postulated to be an intracellular messenger produced by the binding of neurotransmitters to receptors located on GnRH neurons and acting within these neurons (Gearing and Terasawa 1991; Ojeda et al. 1982; Rettori et al. 1992), this concept has been revisited following studies showing that the actions of PGE_2 on GnRH release are initiated by its binding to specific membrane receptors (Coleman et al. 1994) expressed by GnRH neurons (Rage et al. 1997) and the recognition that astrocytes represent a major source of PGE_2 in the brain (Bezzi et al. 1998; Hirst et al. 1999; Ma et al. 1997). Two decades ago, seminal studies by Ojeda and colleagues revealed that the PGE_2-mediated activation of GnRH neuronal secretory activity triggered by estrogen at the time of puberty required the activation of growth factor-dependent glial signaling pathways involving receptor tyrosine kinases of the erbB family (Junier et al. 1991; Ma et al. 1992; Ojeda et al. 1990).

Of the four known members of the erbB family (Fig. 2), three – erbB1, erbB3 and erbB4 – bind and are activated by cognate ligands. In contrast, erbB2 has no known ligand, and functions primarily as a modulator of the other members of the family (Hynes and Lane 2005). While erbB receptors do not appear to be expressed

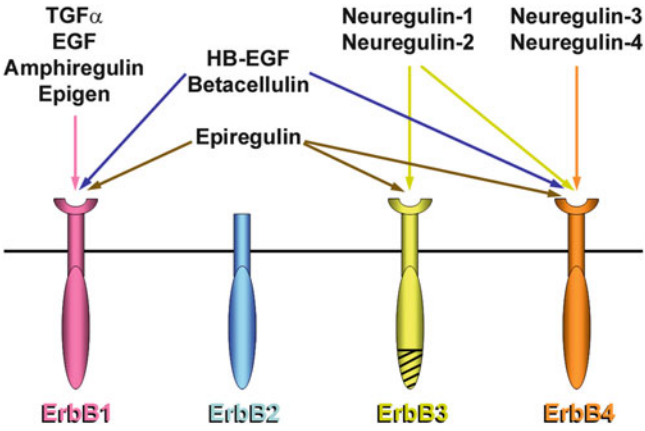

Fig. 2 The erbB family of receptors and their ligands. ErbB1 (or EGFR, epidermal growth factor receptor) and erbB4 are fully functional receptors that possess an extracellular ligand-binding domain and a cytoplasmic protein tyrosine kinase domain and can function as homo- or heterodimers. In contrast, erbB2 (or neu), which lacks a ligand-binding domain, and erbB3, which is defective in its intrinsic tyrosine kinase activity (*dashed lines*), must heterodimerize with another member of the erbB family for signal transduction. The different EGF-like growth factors exhibit different binding specificities for the erbB receptors. While TGFα, EGF, amphiregulin, epigen, neuregulin-3 and neuregulin-4 are specific for a single member of the receptor family, the five other EGF-like ligands can bind two or three receptors each. *EGF* epidermal growth factor, *HB-EGF* heparin binding-EGF, *TGFα* transforming growth factor α

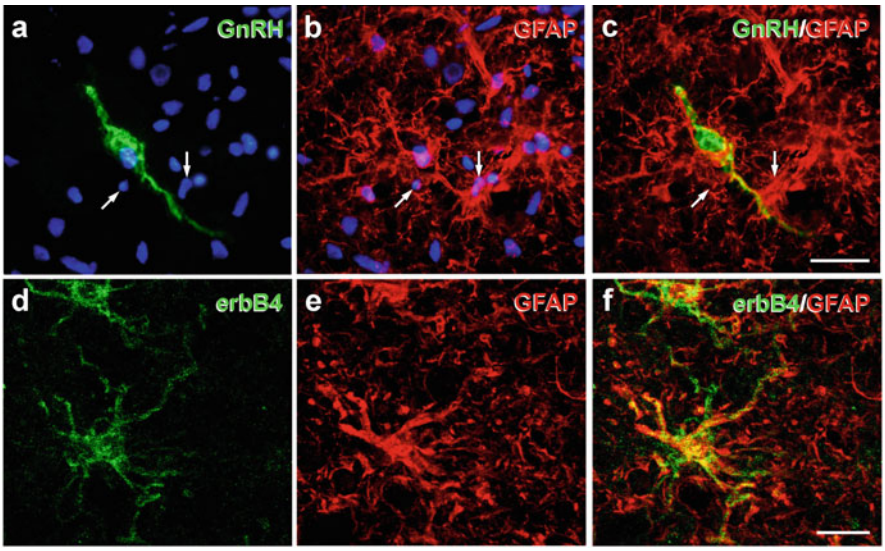

Fig. 3 Astrocytes morphologically interact with GnRH neurons and express erbB4 receptors in the tuberal region of the human hypothalamus. (**a**–**c**) Photomicrographs showing a GnRH neuronal cell body (*green*) to which the processes of glial fibrillary acidic protein (*GFAP*)-immunoreactive astrocytes (*red*, *arrows*) are abundantly apposed. Cell nuclei are stained with Hoechst (*blue*) (Adapted from Baroncini et al. 2007 with permission). (**d**–**f**) GFAP-immunoreactive astrocytes (*red*) of the tuberal region of the human hypothalamus express erbB4 receptors (*green*) (M. Baroncini, V. Prevot, unpublished data). Scale bars = 20 μm (**c**), 10 μm (**f**)

in GnRH neurons Ma et al. 1994b, 1999; Prevot et al. 2003; Voigt et al. 1996), erbB1, erbB2 and erbB4, but not erbB3, are expressed in hypothalamic astrocytes, known to morphologically and physically interact with GnRH cell bodies (Baroncini et al. 2007; Cashion et al. 2003; Sandau et al. 2011b; Witkin et al. 1995) both in rodents and humans (Figs 3 and 4; Ma et al. 1999; Prevot et al. 2003; Sharif et al. 2009). In addition, hypothalamic astrocytes express the erbB1 ligand, transforming growth factor alpha (TGFα; Fig. 4) and several forms of the erbB4 ligand, neuregulin (Ma et al. 1992, 1994a, 1999; Sharif et al. 2009). Importantly, gonadal steroids have been found to induce dramatic increases in the expression levels of the erbB receptors and their ligands within the hypothalamus at puberty; no such changes are seen in the cortex or other brain regions unrelated to reproductive control (Ma et al. 1992, 1994b, 1999).

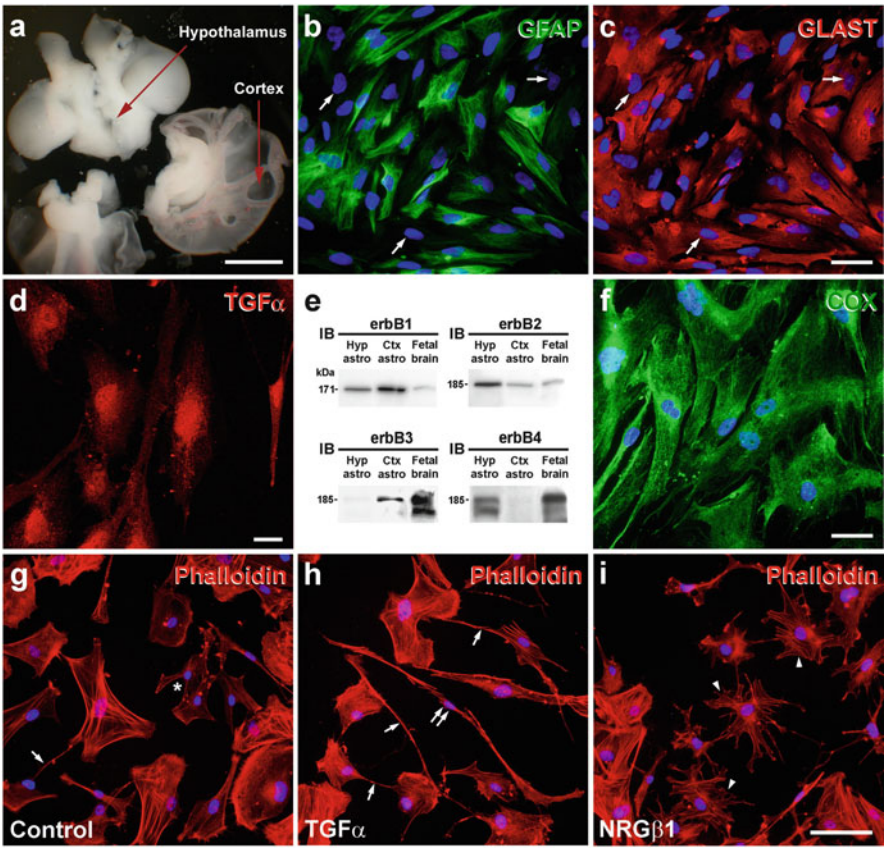

Fig. 4 Human hypothalamic astrocytes express the molecular components required for a glia-to-neuron communication through the erbB-prostaglandin signaling system. Primary cultures of human hypothalamic astrocytes prepared from 9- to 12-week-old human fetuses (**a**). The cultures are composed 98 % of cells immunopositive for the astrocytic markers GFAP (**b**, *green*) and the glutamate-aspartate transporter GLAST (**c**, *red*). Note that cells that express GFAP at low to undetectable levels are nevertheless strongly immunopositive for GLAST (*arrows*). (**d**) Human astrocytes in culture express TGFα protein (*red*). (**e**) Western blot analysis of erbB receptor expression in primary cultures of human cortical and hypothalamic astrocytes. While all four erbB receptors are expressed in the fetal brain, hypothalamic astrocytes (Hyp astro) express erbB1, erbB2, and erbB4, but not erbB3, and cortical astrocytes (Ctx astro) express erbB1, erbB2, and erbB3 but not erbB4 receptors. IB, immunoblot. (**f**) Human hypothalamic astrocytes in culture are immunopositive for COX (*green*). (**g–i**) EGF ligands induce profound morphological rearrangements of human hypothalamic astrocytes in vitro. Cell morphology was examined by visualization of the actin cytoskeleton using Alexafluor 568-conjugated phalloidin (*red*). Hypothalamic astrocytes exhibit heterogeneous shapes under control conditions, i.e., polygonal cells, cells with short and thick extensions (*asterisk*) or long and thin processes (*arrow*) (**g**). TGFα (50 ng/mL for 3 days) stimulates the extension of long and thin processes (*arrows*) and the apparition of bipolar cells (*double arrows*) (**h**) whereas treatment with the neuregulin-1 HRGβ1 (50 ng/mL for 3 days) increases the number of multipolar cells with thick processes (*arrowheads*). (**i**) Nuclei are counter-stained with Hoechst (**b**, **c**, **f**, **g**, **h**, **i**, *blue*). Scale bars = 3 mm (**a**), 50 μm (**b**, **c**, **f**), 20 μm (**d**), 100 μm (**g–i**) (Adapted from Sharif et al. 2009 with permission)

The pharmacological or genetic inhibition of erbB1, ebB2 and/or erbB4 receptors delays the onset of puberty (Apostolakis et al. 2000; Ma et al. 1992; Prevot et al. 2003, 2005) and alters adult reproductive function in rodents (Prevot et al. 2005). In vitro studies using either hypothalamic explants or primary cultures of hypothalamic astrocytes with a GnRH-producing neuronal cell line have shown that erbB receptor ligands can stimulate GnRH release from the explants or neuronal cells, but do so indirectly by inducing astrocytes to secrete PGE_2 (Ma et al. 1997, 1999; Ojeda et al. 1990; Prevot et al. 2003, 2005). In addition, ligand activation of erbB receptors has been shown to promote morphological rearrangements in hypothalamic astrocytes (Fig. 4g–i; Sharif et al. 2009) thus raising the possibility that erbB signaling may also influence the astrocytic coverage of GnRH neurons in vivo (see for review Prevot et al. 2010b).

In vitro experiments suggest that erbB signalling in hypothalamic astrocytes is functionally connected to the neuronal glutamatergic system, the primary mode of excitatory transsynaptic communication used by hypothalamic neurons (van den Pol and Trombley 1993), and one that is known to increase GnRH secretion (Claypool et al. 2000; Donoso et al. 1990) and accelerate the initiation of puberty in both rodents and primates (Plant et al. 1989; Urbanski and Ojeda 1987, 1990). In hypothalamic and non-hypothalamic astrocytes alike (Bezzi et al. 1998; Zonta et al. 2003a, b), transmitter spillover from nearby synaptic activity results in an elevation of PGE_2 release (Glanowska and Moenter 2011; McCarthy et al. 2008). For example, neuronally released glutamate can engage biochemical signaling in astrocytes through the co-activation of AMPA and metabotropic glutamate receptors to cause a ligand-dependent increase in astrocytic erbB signaling and PGE_2 release (Dziedzic et al. 2003), which, in turn, signals back to GnRH neurons (Fig. 5), facilitating neuroendocrine development and adult reproductive function (Prevot et al. 2003, 2005).

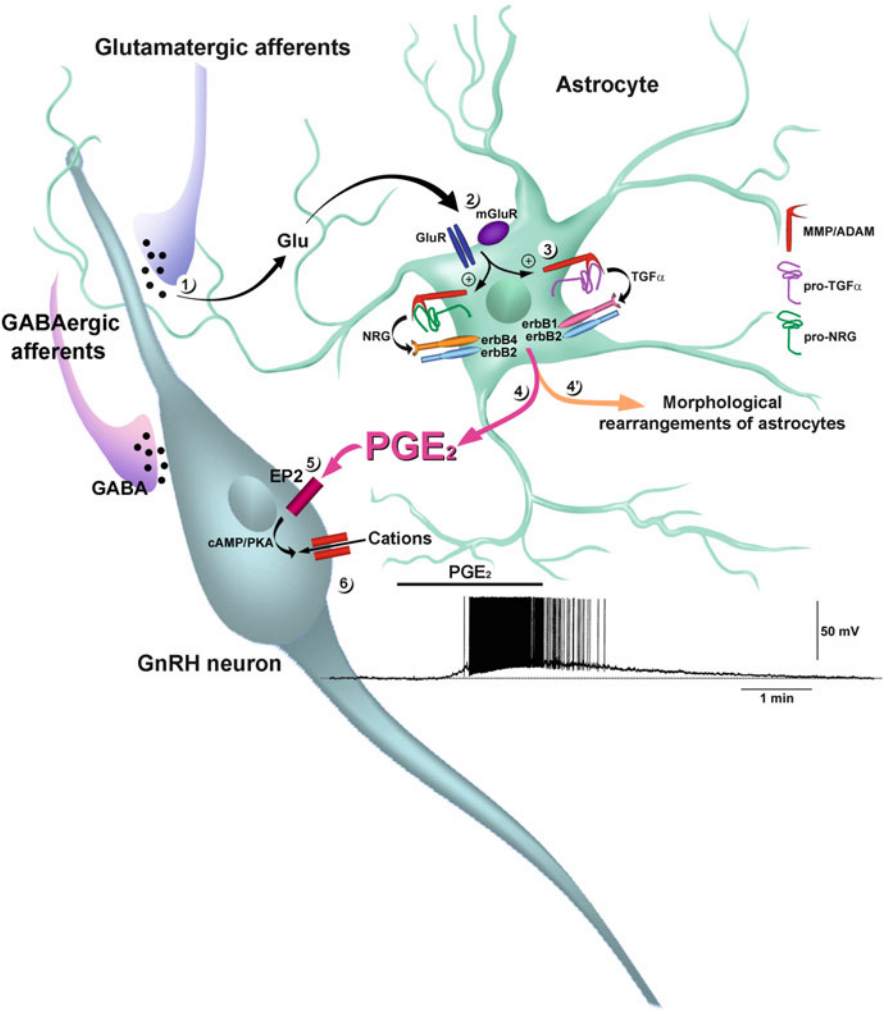

Fig. 5 PGE2 acts as a gliotransmitter to stimulate GnRH neuron electrical activity. Neuronally released glutamate (Glu) (*1*) co-activates metabotropic glutamatergic (*mGluR*) and AMPA glutamatergic receptors (*GluR*) in astrocytes (*2*), stimulating the activity of zinc-dependent matrix metalloproteinases (*MMPs*) of the ADAM (a disintegrin and metalloproteinase) family (*3*). The MMPs catalyze ectodomain shedding of the pro-EGF ligands pro-TGFα and pro-NRG (pro-neuregulin). In particular, the processing of pro-TGFα has been shown to involve the metalloproteinase ADAM17, also known as tumor necrosis factor α converting enzyme (TACE). The subsequently released mature TGFα and NRG activate erbB1/erbB2 and erbB4/erbB2 heterodimers, respectively (Dziedzic et al. 2003). The co-activation of glutamatergic receptors induces the recruitment of erbB1, erbB4 and their pro-ligands to the cell membrane, where multiprotein complexes form, as demonstrated by the direct physical association of glutamatergic and erbB receptors (not shown). The activation of erbB receptors in hypothalamic astrocytes promotes profound morphological changes, including the retraction of cytoplasm and the elongation and stellation of processes (see Fig. 4g–i) (*4'*). The activation of erbB receptors also promotes the release of PGE2 (Dziedzic et al. 2003; Ma et al. 1997, 1999) (*4*), which stimulates a cAMP/protein kinase A (PKA) pathway in GnRH neurons through the mobilization of EP2

Does Glial PGE_2 Control Dendritic Spine Plasticity in the GnRH Neural Network?

GnRH neurons exhibit a simple bipolar morphology with one or two very long dendritic processes that can extend up to 1 mm (Campbell et al. 2005, 2009). Intriguingly, recent studies have demonstrated that the density of spines along these dendrites is subject to robust increases during sexual development in immature animals (Cottrell et al. 2006). Although sexual maturation have been shown to require the neuronal expression of sex-steroid receptors (Mayer et al. 2010; Raskin et al. 2009; Wintermantel et al. 2006), studies suggesting that astrocytic mechanisms might control the stabilization of individual dendritic processes and their subsequent maturation into spines (Nishida and Okabe 2007), together with the demonstration that specific juxtacrine signaling pathways are involved in sculpting astrocyte-dendritic spine interactions (Murai et al. 2003), raise the possibility that astrocytes play a role in the physiological changes of synaptic structure underlying GnRH neuronal maturation and function. PGE_2 release by astrocytes could be central to this process, and PGE_2 has in fact been shown to mediate the dramatic neuronal spine plasticity induced by estrogens in the developing preoptic region (Amateau and McCarthy 2002, 2004; Wright and McCarthy 2009). This effect involves the activation of AMPA and metabotropic glutamate receptors (Amateau and McCarthy 2002; Wright and McCarthy 2009), which are known to promote erbB-dependent PGE_2 release in hypothalamic astrocytes (Dziedzic et al. 2003), as well as the EP2/PKA signaling pathway (Amateau and McCarthy 2002) recently found to be functional in native GnRH neurons (Clasadonte et al. 2011). Importantly, estrogens, which have long been known to regulate neuronal spine plasticity in the adult hippocampus (Woolley and McEwen 1992, 1994), have also been shown to promote comparable changes in the immature hippocampus (Amateau and McCarthy 2002). However, in the hippocampus, the underlying mechanisms do not appear to require PGE_2 synthesis (Amateau and McCarthy 2002), suggesting that increases in PGE_2 synthesis are selectively used by estrogens to promote dendritic spine plasticity in the developing preoptic region.

Fig. 5 (continued) receptors (EP2-R; Clasadonte et al. 2011) (*5*). Activation of this signaling pathway induces a reversible membrane depolarization of GnRH neurons, leading to the initiation of spike firing via a postsynaptic effect involving the activation of a nonselective cation current (Clasadonte et al. 2011) (*6*)

PGE_2 Acts as a Gliotransmitter in the GnRH Neuroendocrine System

Even though PGE_2 has been known to trigger GnRH release from the hypothalamic neurons controlling reproduction for almost 40 years, it is only very recently that it has been identified as a potent excitatory regulator of GnRH neuronal activity in both male and female mice (Clasadonte et al. 2011). Using patch-clamp recordings in brain slices from transgenic mice expressing green fluorescent protein (GFP) under the control of the GnRH promoter, we showed that PGE_2 induced a reversible membrane depolarization of GnRH neurons leading to the initiation of spike firing via the postsynaptic effect involving activation of a nonselective cation current (Fig. 5; Clasadonte et al. 2011) reminiscent of the ones recently described in GnRH neurons by other groups (Roland and Moenter 2011; Zhang et al. 2008). Although GnRH neurons are known to express both the EP1 and EP2 subtypes of prostaglandin receptors in vivo (Jasoni et al. 2005; Rage et al. 1997), the excitatory effect of PGE_2 on GnRH neuronal activity was selectively mimicked by the EP2 receptor agonist butaprost (Clasadonte et al. 2011), previously shown to promote GnRH release in the GnRH-producing neuronal cell line, GT1-7 (Rage et al. 1997). The PGE_2-mediated membrane depolarization of GnRH neurons was also shown to require the cAMP/protein kinase A (PKA) pathway (Clasadonte et al. 2011), which is known to be coupled to the EP2 receptor (Fig. 1; Coleman et al. 1994; Sang et al. 2005) and to underlie the stimulatory effect of PGE_2 on GnRH secretion (Fig. 5; Ojeda et al. 1985).

As alluded to above, the selective disruption of erbB4 signaling in astrocytes by the overexpression of a dominant-negative erbB4 receptor under the control of the human GFAP promoter leads to diminished PGE_2 release in response to ligand-dependent erbB4 activation; this in turn leads to reduced GnRH release, delayed puberty, and disrupted adult reproductive function (Prevot et al. 2003, 2005). Intriguingly, electrophysiological analyses have shown that the spontaneous activity of GnRH neurons in these animals is decreased and that this deficiency is mimicked by the bath application of either fluoroacetate, an inhibitor of astrocyte metabolism (Fonnum et al. 1997; Henneberger et al. 2010), or the COX blocker indomethacin, to slices of the preoptic region from wild-type animals (Clasadonte et al. 2011). The fact that GnRH neuronal activity in all these conditions can be rescued by exogenous PGE_2 (Clasadonte et al. 2011) strongly suggests that glial PGE_2 is an important component of the homeostatic mechanism controlling GnRH neuronal excitability. The role of glia in the control of GnRH neuronal activity is further supported by a recent study demonstrating that glial prostaglandins may regulate the efficacy of GABAergic inputs to GnRH neurons in ovariectomized mice (Glanowska and Moenter 2011). Using GnRH-GFP transgenic mice and patch-clamp recordings in brain slices, the authors demonstrated that the repeated action potential-like depolarization of a GnRH neuron caused a short-term reduction in the frequency of spontaneous GABAergic postsynaptic currents in the same neuron, suggesting the presence of local circuit interactions between GnRH neurons

and their GABAergic afferents (Chu and Moenter 2005; Glanowska and Moenter 2011). It is important to note that, in this local circuit, the activation of $GABA_A$ receptors exerts a depolarizing action that can trigger action potential firing due to the elevated chloride levels maintained in adult GnRH neurons (DeFazio et al. 2002; Han et al. 2002; Herbison and Moenter 2011). Consequently, this represents a negative feedback loop in which depolarized GnRH neurons reduce the activity of their own excitatory GABAergic afferents. In addition to being steroid-dependent and under the influence of both glutamatergic and endocannabinoid signaling mechanisms via the activation of presynaptic metabotropic glutamate receptors and cannabinoid CB1 receptors, respectively (Chu and Moenter 2005; Glanowska and Moenter 2011), this local negative feedback loop also requires the action of glia-derived prostanglandins (Glanowska and Moenter 2011). Indeed, the incubation of brain slices with indomethacin, the broad-spectrum prostaglandin receptor antagonist AH 6809, or fluorocitrate, which like fluoroacetate, is a specific blocker of astrocyte metabolism, prevents the depolarization-induced suppression of GABAergic transmission in GnRH neurons (Glanowska and Moenter 2011). Since GABA exerts a depolarizing action in this local circuit, we could envisage that glial prostaglandins, by suppressing excitatory drive, would reduce GnRH neuronal activity. Estradiol could also differentially influence this local inhibitory feedback to exert its positive or negative feedback effects (Glanowska and Moenter 2011). Thus, in addition to exerting a direct postsynaptic excitatory action on the cell body of GnRH neurons, prostaglandins released from astrocytes can participate in mechanisms that regulate the activity of their GABAergic presynaptic inputs (Fig. 5). Thus in the GnRH system, PGE_2 fulfils all the criteria that qualify a compound as a "gliotransmitter" (Parpura and Zorec 2010): (1) it is synthesized by astrocytes, (2) its regulated release is triggered by physiological stimuli, (3) it acutely activates the firing of GnRH neurons and modulates the activity of their GABAergic afferents, and (4) it plays a role in an important physiological function, i.e., the neuroendocrine control of reproduction, which is vital to species' survival.

Conclusions

Several observations made over the last two decades have demonstrated that PGE_2, which has been known for almost 40 years to play an important role in the regulation of the hypothalamic-pituitary-gonadal axis, is a transmitter released by astrocytes and intimately linked with GnRH neuronal function in the preoptic region, where the cell bodies of GnRH neurons in rodents are located. However, many mysteries regarding the underlying mechanisms remain unsolved. For example, even though recent studies suggest that GnRH neurons can directly communicate with neighboring astrocytes via juxtacrine signaling pathways (Sandau et al. 2011a, b), a true understanding of how these GnRH neurons interact with hypothalamic astrocytes to modulate PGE_2 gliotransmission is missing. Are these

communication processes involved in sculpting astrocyte-dendritic spine interactions and in promoting the physiological changes in synaptic structure that underlie GnRH neuronal maturation? How is PGE_2 released from hypothalamic astrocytes?

Now that a general strategy for the application of molecular genetics to the study of neuron-glia interactions and gliotransmission has been elucidated, the next several years should provide an opportunity to begin to address these questions.

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