Neuroendocrine Control of Female Puberty: Glial and Neuronal Interactions

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Emerging evidence suggests that, in addition to neuronal inputs, growth factors of glial origin are also important in the control of mammalian puberty via a cell-cell interaction that ultimately affects the neurons that release gonadotropin-releasing hormone (GnRH), a neurohormone controlling sexual development. Among these growth factors, transforming growth factor-α (TGFα) appears to be one of the physiologic components that controls the onset of female puberty by affecting GnRH neuronal activity in a glia-mediated autocrine/paracrine manner. Specifically, TGFα induces glia to produce bioactive substances, such as prostaglandin E_2 (PGE_2). In turn, PGE_2 directly acts on GnRH neurons to stimulate the release of GnRH. Furthermore, the neuregulin of glial origin neu differentiation factor (NDF) was found to facilitate the action of TGFα, suggesting that other growth factors may exert their biologic effects on GnRH neuronal function via a glia/neuron interaction. Another indication that glial cells may be involved in the regulation of neuroendocrine function is the presence of estrogen receptors on hypothalamic astrocytes. Thus, region-specific glial cells appear to play an integral role in the regulation of neuroendocrine function. ***Key words: astrocytes/growth factors/receptors/GnRH. Journal of Investigative Dermatology Symposium Proceedings 2:19–22, 1997***

The onset of mammalian female puberty depends on the enhanced secretory activity of a specialized group of neurons that produce gonadotropin-releasing hormone (GnRH), a key neurohormone for sexual maturation and reproductive function. The regulation of GnRH neuronal function has been a focal issue for understanding the cellular and molecular mechanisms controlling sexual development and reproductive biology. It is believed that neuron to neuron transsynaptic inputs, both excitatory (Brann and Mahesh, 1994; Crowley *et al* 1995) and inhibitory (Terasawa, 1995), contribute to the regulation of GnRH secretory activity. A combination of a decrease in inhibitory tone and an increase in excitatory input has been generally considered to bring about the initiation of puberty. The main excitatory inputs include neuronal systems that utilize norepinephrine (Ramirez *et al* 1984), neuropeptide Y (Crowley *et al* 1995), and excitatory amino acids (Ojeda, 1991; Brann and Mahesh, 1994; Plant, 1994). The inhibitory components, on the other hand, are γ-aminobutyric acid (Masotto and Negro-Vilar, 1987; Terasawa, 1995) and opioid peptides (Bhanot and Wilkinson, 1983).

Although this neuronal regulation of the GnRH neuronal network is well-documented, recent studies have raised the intriguing possibility that glial cells may also play a role in the control of GnRH neuronal activity (Ojeda and Ma, 1995). Anatomical studies revealed that the glial ensheathment of GnRH cell bodies in primates is prominent, as glial processes surround the GnRH cells (Witkin *et al* 1991). Such a structural relationship suggests a direct glial influence on GnRH neuronal activity. This hypothesis is further supported by the evidence that glial cells produce a variety of bioactive molecules and growth factors able to affect the activity of GnRH neurons. Furthermore, glial apposition to GnRH nerve terminals in the median eminence of the hypothalamus is yet another specialized structural feature in both rats and primates. The predominant cells in the median eminence are astrocytes and modified ependymal glia known as tanycytes (Kobayashi and Matsui, 1969). Interestingly, these specialized glial cells that line the wall of the third ventricle send their processes toward the endothelial wall of the portal blood vasculature, neighboring the GnRH nerve terminals (Kobayashi and Matsui, 1969; Kozlowski and Coates, 1985). More intriguingly, sex steroids appear to modulate this structural arrangement as demonstrated by the finding that the space between the GnRH-nerve terminals and the basal lamina of the portal vasculature decreases after ovariectomy, presumably due to retraction of the glial cells. These structural changes may lead to an increase in the access of GnRH terminals to the portal vessels. The evidence that about 50% of glial cells in the median eminence have estrogen receptors (Langub and Watson, 1992) further supports the notion that a direct action of estrogen on glial cells is region-specific (Ma *et al*, 1994a). Because glial-neuronal synaptoid specializations are common structural features in the median eminence (Kobayashi and Matsui, 1969), the concept of a direct glial involvement in the control of GnRH release via cell-cell interactions with GnRH nerve terminals was proposed. In this article, we will discuss some recent results obtained by our laboratory that underscore the emerging role of glial cells in the regulation of GnRH neuronal function.

We demonstrated that glia-derived transforming growth factor alpha (TGFα), a mitogenic polypeptide member of the epidermal growth factor (EGF) family (Derynck, 1988; Carpenter and Cohen, 1990; Massague, 1990), plays an important role in the glia-neuron–mediated control of GnRH secretory activity (Ma *et al* 1992, 1994a,

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Abbreviations: EGFR, epidermal growth factor receptor.

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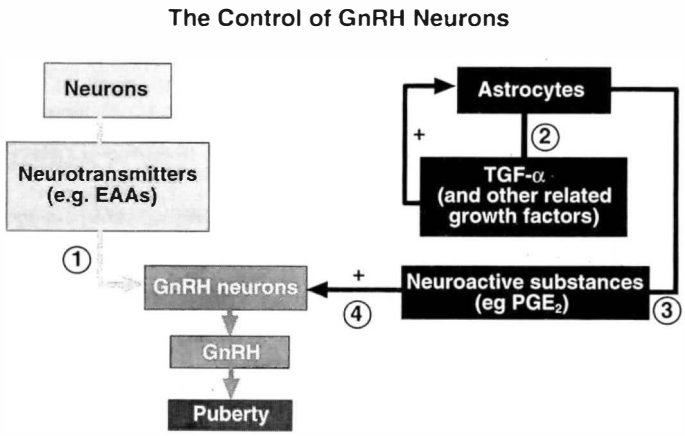

Figure 1. A simplified schematic illustration of the postulated control of GnRH neuronal function. *(1)* Transsynaptic inputs regulate GnRH release. For detailed description, see text. *(2)–(3)* Glial-derived TGFα and other related growth factors stimulate the secretion of neuroactive substances (i.e.. PGE_2) in a paracrine/autocrine fashion. *(4)* Neuroactive substances act on GnRH neurons to enhance secretion. See text for further description.

1994b, 1994c, 1994d; Ojeda, 1994) (see **Fig 1**). Other distant members of the EGF family, neuregulins of glial origin—including heregulin (Holmes *et al*, 1992) and neu differentiation factor (Wen *et al*, 1992), the alternatively spliced products from a single gene (Wen *et al*, 1992; Marchionni *et al*, 1993;)—appear to be also involved in the regulation of GnRH release via a mechanism by which neu differentiation factor facilitates the action of TGFα (YJ Ma, DF Hill, M Costa, SR Ojeda, unpublished observation). Like other members of the EGF family, TGFα is synthesized as a transmembrane precursor. It is important to note that this precursor is able to interact with neighboring cells endowed with its receptors, without the need of releasing the mature peptide. This type of cellular action is designated "juxtacrine" (Massague, 1990) and represents an additional cellular signaling mechanism of membrane-anchored growth factors in the central nervous system.

The biologic actions of TGFα, like other members of the EGF family, are initiated by binding to a family of membrane-spanning receptors with protein kinase activity for cellular signaling (Schechter *et al*, 1985; Carpenter, 1987; Kraus *et al*, 1989; Plowman *et al*, 1990, 1993). The receptor for TGFα (as well as EGF, amphiregulins, and others) is a 170-kDa glycosylated protein known as the EGF receptor (EGFR) that has an extracellular domain containing the amino acid motif essential for specific ligand recognition, a single transmembrane domain, and an intracellular enzyme catalytic element containing the tyrosine kinase activity. Other EGFR-related members (encoded by different proto-oncogenes) including HER-2 (neu) receptor (Karunagaran *et al*, 1996), HER-3 (named ERBB-3) (Kraus *et al*, 1989; Plowman *et al*, 1990), and HER-4 (Plowman *et al*, 1993) also have similar structural features. These EGFR congeners, however, do not directly bind to TGFα, EGF, and amphiregulin, but instead recognize other members of the EGF family. Recent studies have suggested that the heterodimers (HER-2/HER-3 or HER-2/HER-4) and homodimer (HER-4/HER-4) are the functional receptor units required for the signal transduction of neuregulins (for review see Lemke, 1996). Furthermore, HER-2 can be transmodulated by the activation of EGFR (Stern and Kamps, 1988), suggesting interactions between EGFR and HER-2. This view is supported by the observation that HER-2 is able to function as an auxiliary component that enhances the affinity of EGFR by stabilizing dimerization of the receptor (Karunagaran *et al*, 1996).

In our studies of both rats (Ma *et al*, 1992) and nonhuman primates (Ma *et al*, 1994b), we found that the TGFα gene and its protein product are predominantly expressed in astroglial cells of certain hypothalamic nuclei involved in the control of GnRH neuronal function (Ma *et al*, 1992), such as the suprachiasmatic and arcuate nucleus. Both TGFα mRNA and protein levels are also abundant in tanycytes and glial cells of the median eminence, and the levels are significantly elevated around the time of puberty (Ma *et al*, 1992, 1994b). Furthermore, EGFR in the median eminence is colocalized with TGFα (Ma *et al*, 1994d), and—at least in monkeys (Ma *et al*, 1994b)—its developmental expression profile parallels that of TGFα, indicating the involvement of the glia-derived growth factor and its receptor in neuroendocrine regulation. That TGFα may be involved in the control of GnRH neuronal activity was demonstrated by the finding that TGFα is able to stimulate the release of GnRH from median eminence fragments in a dose-response-related manner (Ojeda *et al*, 1990). Subsequent studies further supported the notion that a TGFα/EGFR system contributes, at least in part, to the normal initiation of mammalian female puberty because overexpression of TGFα in transgenic mice induced precocious puberty (Ma *et al*, 1994c). In contrast, blockade of EGFR tyrosine kinase activity by RG-50864 (Yaish *et al*, 1988) targeted to the median eminence resulted in a delayed onset of puberty (Ma *et al*, 1992), suggesting that activation of the EGFR-mediated signaling pathway is critical for the initiation of reproductive competence. Our most recent evidence (YJ Ma, DF Hill, M Costa, SR Ojeda, unpublished data) show that glia-derived neu differentiation factor is able to enhance the stimulatory action of TGFα on GnRH release via activation of an HER-2/HER-4 receptor complex, indicating that other members of the EGF family may also be involved in the process of sexual development. This view is further supported by the evidence (YJ Ma, DF Hill, M Costa, SR Ojeda, unpublished observation) that disruption of HER-2 synthesis via an anti-sense oligonucleotide attenuated the stimulatory effect of TGFα on GnRH release.

The effect of TGFα on GnRH secretion does not appear to be direct, as GnRH neurons do not express EGFR. The GnRH terminals in the median eminence, however, are in close association with glial cells that express TGFα and EGFR (Ma *et al*, 1992, 1994d), suggesting that these glial cells mediate the effect of TGFα. This view is supported by the recent findings (Ma *et al*, 1997) that isolated hypothalamic astrocytes respond to TGFα with the release of prostaglandin E_2 (PGE_2), a bioactive substance that stimulates GnRH release. That PGE_2 (although one cannot rule out other bioactive substances), in turn, acts on GnRH neurons to stimulate the release of GnRH is indicated by the ability of conditioned medium from hypothalamic astrocytes treated with TGFα to enhance GnRH release from the GnRH-producing cell line GT1–1. These cells express no EGFR and are unresponsive to TGFα itself (Voigt *et al*, 1996). Moreover, blockade of PGE_2 action by immunoneutralization in the conditioned medium abolished the effect of the conditioned medium on GnRH release (Ma *et al*, 1997).

The biologic actions of PGE_2 are initiated via the activation of its receptors, including at least four subtypes known as EP1, EP2, EP3, and EP4 (Sugimoto *et al*, 1992; Honda *et al*, 1993; Watabe *et al*, 1993). Activation of EP1 leads to increased calcium flux, whereas EP2 enhances and EP3 attenuates adenylate cyclase activity, suggesting that the diversity of actions of PGE_2 in different circumstances may depend on the type of PGE_2 receptors expressed. By ribonuclease protection assay, we observed the presence of mRNAs encoding EP1 and EP3 in the immortalized GnRH neuronal GT1–1 cell line. Utilizing *in situ* hybridization combined with immunocytochemistry, we further observed that EP1 mRNA is expressed by GnRH neurons in mouse hypothalamus (F Rage, BJ Lee, YJ Ma, SR Ojeda, unpublished data). These studies provided cellular and molecular evidence supporting the view that GnRH secretion is regulated by biomolecules produced by glial cells in response to the action of TGFα.

The importance of glial-neuronal interactions in the regulation of GnRH neuronal activity is further indicated by the finding that glutamic acid, a major excitatory amino acid neurotransmitter in the neuroendocrine brain (van den Pol *et al*, 1990), is released from astrocytes and affects neuronal activity (Parpura *et al*, 1994). We

found that glutamate increases TGFα mRNA expression in hypothalamic astroglia via activation of metabotropic glutamate receptors located on the astrocytes.[1] Thus, specific metabotropic glutamate receptor subtypes may be involved in the process by which excitatory amino acids control glial TGFα production and, hence, facilitate GnRH release. Other excitatory neurotransmitters were also shown to influence glial function (Chiu and Kriegler, 1994; Glowinski *et al*, 1994).

It is well-documented that estradiol is important in regulating GnRH neuronal function (for review, see Ojeda and Urbanski, 1994; Kalra, 1986). The action of estradiol on GnRH release, however, is indirect, as GnRH neurons themselves do not contain detectable estrogen receptors (Shivers *et al*, 1983), indicating that other cell types expressing the receptors mediate this effect. That hypothalamic astrocytes, in addition to other neurons, may contribute to mediating the action of estradiol is indicated by the finding that estrogen receptor mRNA was detected in hypothalamic astrocytes (Ma *et al*, 1994a). Estradiol is able to enhance TGFα gene expression in hypothalamic, but not cerebellar (i.e., non-neuroendocrine), astrocytes (Ma *et al*, 1994a). Moreover, the culture medium of astrocytes exposed to estradiol increases PGE_2 receptor EP1 gene expression in GT1–1 cells. No change in EP1 mRNA levels was observed, however, in GT1–1 cells directly treated with estradiol (F Rage, BJ Lee, YJ Ma, SR Ojeda, unpublished data). These findings suggest that astrocytes of the neuroendocrine brain are indeed able to mediate the effect of gonadal steriods on LnRH release.

In summary, mammalian puberty is initiated within the brain by multiple but well-coordinated events that ultimately affect the activity of the GnRH neuronal network. These events include: (i) an increase in transsynaptic excitatory inputs such as excitatory amino acids, norepinephrine, and neuropeptide Y; (ii) a decrease in the transsynaptic γ-aminobutyric acid-inhibitory regulation, and (iii) increased glial production of growth factors able to stimulate the production of neuroactive substances, such as prostaglandins. It appears that members of the EGF family, such as TGFα and its distant relatives neuregulins, are factors contributing to this glial involvement. Finally, it is of great interest to determine whether these glial influences are also essential for the regulation and maintenance of normal reproductive function during adulthood. Future experiments are needed to address this important issue.

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