REVIEW ARTICLE
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GnRH nerve terminals, tanycytes and neurohaemal junction remodeling in the adult median eminence: functional consequences for reproduction and dynamic role of vascular endothelial cells

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Running head: Neuronal-glial-endothelial interactions and GnRH release

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Abstract

Although coordinated actions of several areas within the hypothalamus are involved in the secretion of gonadotropin releasing hormone (GnRH), the median eminence of the hypothalamus, where the nerve terminals are located, plays a particularly critical role in the release of GnRH. In adult females, prior to the preovulatory surge of GnRH, the retraction of specialized ependymoglial cells lining the floor of the third ventricle named tanycytes allows for the juxtaposition of GnRH nerve terminals with the adjacent pericapillary space of the pituitary portal vasculature, thus forming direct neurohaemal junctions. These morphological changes occur within a few hours and are reversible. Such remodeling may promote physiological conditions to enhance central release of GnRH and potentiate estrogen-activated GnRH release. This plasticity involves dynamic cell interactions that bring into play tanycytes, astrocytes, vascular endothelial cells and GnRH neurons themselves. Underlying signaling pathways responsible for these structural changes are comprised of highly

diffusible gaseous molecules such as endothelial nitric oxide (NO) and paracrine communication processes involving receptors of the erbB tyrosine kinase family, transforming growth factor beta 1 (TGFβ1) and eicosanoids such as prostagladin-E_2 (PGE_2). Some of these molecules, because of their ability to diffuse within the median eminence, may also serve as synchronizing cues allowing for the occurrence of functionally meaningful episodes of GnRH secretion by coordinating GnRH release from the GnRH neuroendocrine terminals.

Introduction

As the projection field of neuroendocrine gonadotropin releasing hormone (GnRH) neurons, the median eminence of the hypothalamus is poised to play a crucial role in the precise regulation of GnRH release, and is therefore central to the control of the reproductive axis. The median eminence, which is located ventral to the third ventricle in the tuberal region of the hypothalamus, is one of the seven so-called circumventricular organs and primarily contains neurosecretory axon terminals [1]. It constitutes a window of exchanges between the hypothalamus and the periphery that is facilitated by the presence of permeable brain capillaries featuring fenestrated endothelium [2,3]. Thus it appears that the most important function associated with the lack of blood-brain barrier in this region is that it permits the release of neurohormones produced by neuroendocrine cells from terminals into the pituitary portal circulation. It is also important to acknowledge that the cellular processes through which neuroendocrine terminals release their neuropeptides into the circulation could be subjected to the direct modulatory influence of blood-born factors acting on this region. The peculiar cytoarchitecture of the median eminence is mainly conferred by tanycytes, which are specialized unciliated ependymoglial cells that form a belt lining the floor of the third ventricle [1]. One dominant feature of tanycytes is their marked polarization; although tanycyte cell bodies line the border of the third ventricle, they also send processes to the vascular walls, where they make contact through “end-feet” specializations. In addition, tanycytes were

recently shown to express efficient tight junction complexes at their apex that bestow them with properties of the blood-brain barrier [3]. Although tanycytes are the dominant cell type, astrocytes also reside within the internal zone of the median eminence (Figure 1).

Intriguingly, early *in vivo* experiments showed that deafferentation of the tuberal region of the hypothalamus does not inhibit the pulsatile release of luteinizing hormone (LH) in primates [4] or in rats [5]. While in primates, including humans, GnRH neuron cell bodies are diffusely distributed in the forebrain and are particularly abundant in the preoptic region and in the tuberal region of the hypothalamus; in rats they are not present in the latter region. Deafferentation studies in rodents, together with work showing that release of GnRH from hypothalamic explants is pulsatile [6,7], thus led to the concept that at least part of the mechanisms synchronizing GnRH secretion may reside within the tuberal region of the hypothalamus. These synchronizing events could even occur directly within the median eminence as median eminence explants were also shown to release GnRH in a pulsatile mode *in vitro* [8,9]. Equally intriguing are the findings showing that plastic events taking place within the median eminence modulate the direct access of GnRH neurons to the pituitary portal blood vessels and that these structural changes are directly correlated to the endocrine status of the individual, e.g. in rats, direct neurohaemal junctions are visualized at the onset of the preovulatory surge of GnRH when estrogens levels are highest [10-12].

The present article will review recent findings that have unveiled some of the cell-cell communication processes involving non-neuronal cells such as tanycytes, astrocytes and vascular endothelial cells, that locally regulate both GnRH neurohaemal junction formation and GnRH release within the median eminence. These modulatory mechanisms use signaling molecules that may represent some of the synchronizing cues to coordinate GnRH release from the scattered GnRH neuroendocrine terminals that may allow for the occurrence of functionally meaningful episodes of GnRH secretion.

Endocrine status-promoted morphological plasticity at the neurohaemal interface for GnRH neurons

Over the past decade, it has been established that fluctuating physiological conditions during the ovarian cycle have the power to reversibly alter structural relationships among the various cell types of the median eminence that specifically interact with nerve terminals containing GnRH [13-16]. Median eminence dynamics involve coordination of neuroendocrine axons, tanycytes and the parenchymatous basal lamina, the last structure secreted neurohormones must cross in order to enter the blood [17-19]. During the ovarian cycle, under conditions of low gonadotropin output, GnRH neuroendocrine terminals are completely enwrapped by tanycyte end-feet, which prevent direct access to the pericapillary space and thus create a diffusion barrier hampering GnRH entry into the pituitary portal circulation [10,11]. As predicted by Koslowski and Coates more than 20 years ago [17] by analogy to the neuro-glial remodeling that occurs in the neurohypophysis after parturition [20-22], a structural rearrangement of tanycytes occurs during the preovulatory surge resulting in the release of the engulfed neuroendocrine terminals and the establishment of direct neurohaemal contacts between GnRH neurons and the pituitary portal blood (Figure 2) [10]. In parallel to tanycytic endfeet retraction, GnRH axon terminals are frequently seen to sprout new terminals towards the pericapillary space and thus appear to be attracted by the endothelial wall which they eventually contact (Figure 2) [10]. Similarly, electron microscopic studies performed in gonadectomized rats, an experimental condition that results in increased GnRH release, showed that the distance of GnRH axon terminal from the pericapillary space was positively correlated to the plasma levels of LH [23]. Interestingly, microstructural changes in the median eminence have also been seen in the Japanese quail, a seasonal breeder, in response to the changing environmental context, e.g. changes in photoperiod. Indication that glial cells of the median eminence are involved in the photoperiodic control of GnRH release in birds was first suggested by c-Fos expression studies [24]; Yamamura and collaborators demonstrated that during long-day conditions (when GnRH secretion is induced) GnRH axon terminals were seen in close contact to the pericapillary space, whereas during short-day conditions (when GnRH secretion is inhibited),

GnRH nerve terminals were enclosed in tanycytic processes and located far from the basal lamina delineating the vascular wall [12].

Whether similar structural changes occur within the human brain during the menstrual cycle remains unknown. Recent studies showed that GnRH axon fibers were abundantly apposed to tanycytic processes in the human median eminence, raising the possibility that, as in rodents and seasonal breeders, putative physiological condition-induced plastic changes involving morphological interaction could play a role in the neuroendocrine control of GnRH secretion in humans [25]. As in the female rodent, the hypothalamic-pituitary responses to gonadal steroid feedback during the ovarian cycle in women are both dose and time dependent [26]. Acute or chronic exposure to low concentrations of gonadal steroids inhibits gonadotropin secretion [27], whereas a progressive increase in estrogen over a period of several days stimulates LH secretion [27,28]. With the advancement of magnetic resonance imaging (MRI) techniques such as diffusion MRI (measurement of water diffusion coefficient that provides information about the cellular structure of tissue) and proton MR spectroscopy (measurement of a range of cerebral metabolites including N-acetyl-aspartate, choline and creatine that provides information about tissue metabolism) tissue structure can now be probed and imaged on a microscopic scale *in vivo* [29,30]. A noninvasive longitudinal study monitoring sex steroid hormone-controlled plasticity in women recently evidenced that structural changes actually occur within the hypothalamus during an artificial menstrual cycle [31]. In this study, female volunteers were subjected to diffusion and spectroscopy MRI at two stages of their artificial menstrual cycle: 13 days after initiating oral contraception, i.e., when the hypothalamic-pituitary-gonadal axis is fully inhibited [32] and at the end of the pill-free interval, i.e., when most of the steroidogenic negative feedback effects wear off and normal early follicular phase LH pulse pattern is found [32]. Results showed that removal of the oral contraceptive-mediated gonadal steroid negative feedback on the reproductive axis dramatically and selectively favors diffusion in the hypothalamus and is associated with variations in the release of choline (the precursor of phosphatidyl choline, the core phospholipid in the cell membrane), which is a metabolite mainly released by glial cells

[33,34] when changes in cell-membrane turnover occur [35]. Similar to studies conducted in brain slices showing that changes in the astrocytic coverage of neurons modify extracellular space geometry and diffusion parameters [36], these human data raise the possibility that the microstructural changes monitored during the pill free period (increased diffusivity of water molecules) in the female hypothalamus could be due to the retraction of glial cell processes [31].

Functional significance of structural remodeling in the median eminence

Even though definite evidence for the involvement of morphological plasticity in the control of GnRH release has not yet been provided, several arguments strongly suggest that it plays a key role in the control of reproduction. Tanycyte engulfment of GnRH axons and terminals, associated with basal levels of peptide release, suggests an inhibitory role for tanycytes under these conditions. As mentioned in the previous paragraph, predominant occupation of the basal lamina by the tanycyte endfeet may serve in part as a diffusion barrier to peptides entering the pericapillary spaces. This barrier is removed when activation of the system causes these glial endfeet to retract from the basal lamina and enable a subpopulation of GnRH neurons to directly contact the pericapillary space. In support to this interpretation, preincubation of median eminence for 30 min with the precursor of nitric oxide (NO), L-arginine, or prostaglandin E_2 (PGE_2), which induce tanycyte movement and reconfiguration (see bellow), has been shown to enhance GnRH release [37-42]. Conversely, local infusion of nitric oxide synthase (NOS) or cyclooxygenase (COX, enzyme involved in prostaglandin synthesis) inhibitors into the median eminence arrests the ovarian cycle in either the diestrus or the estrus phase [43,44] when GnRH release is low and GnRH neuroendocrine terminals are enclosed by tanycyte endfeet [10,11]. Furthermore, recent studies aiming at studying the effect of aging, a physiological condition where both GnRH release and the responsiveness of the GnRH neural network to estrogens are diminished [45,46], on the GnRH nerve terminal microenvironment suggested that alterations of the relationship between neuroendocrine terminals and tanycyte processes may contribute to the senescence of the hypothalamic-pituitary-gonadal axis [47,48].

Glial cells, vascular endothelial cells and GnRH neurons: a ménage à trois in the control of GnRH neurohaemal junction formation

A role for Glia in the release of GnRH and median eminence functional plasticity.

Insights into the mechanisms by which the glia contribute to morphological plasticity in the median eminence came initially from studies showing that transforming growth factor alpha (TGFα), an epidermal growth factor (EGF)-related peptide expressed by tanycytes and astrocytes of the median eminence [49] was able to stimulate GnRH release from median eminence explants [41]. Interestingly, TGFα does not stimulate GnRH release directly; instead it does so via a paracrine mechanism that involves PGE_2 release, which subsequently acts on GnRH neurons to induce GnRH secretion [50,51], but also triggers acute tanycytes retraction both in cultured tanycytes (Figure 3A) and in hypothalamic explants (Figure 3B) [43]. *In situ* and *in vitro* studies both showed that the TGFα receptor, erbB1, was expressed in tanycytes [52-54]. Importantly, injection of estrogen and progesterone was shown to increase TGFα mRNA expression in premature rats and blockade of TGFα action in the median eminence with tyrphostins, erbB-1 inhibitors, delayed the occurrence of the first GnRH/LH preovulatory surge at puberty [49]. Because tanycytes of the median eminence express estrogen receptors [43,55], estrogen may act directly on these cells to promote both TGFα expression and release on the day of proestrus. *In vitro* studies conducted in primary cultures of tanycytes showed that 12-h TGFα treatment promotes the release of PGE_2, and a PGE_2-dependent release of TGFβ1 [54], a growth factor also known to be involved in the glial control of GnRH secretion [56-58]. Morphometric studies *in vitro* showed that both TGFα and TGFβ1 had dramatic but opposite effects on tanycyte morphology [54]. When tanycytes monolayers are treated with TGFα, during the first 16-h of treatment, TGFα-erbB1 signaling acts on tanycytes to first promote outgrowth of their processes and then to elicit a PGE_2-dependent production of TGFβ1 [54]. Subsequently, TGFα-induced TGFβ1 release induces retraction of the tanycyte processes during the

following 6-8 h [54]. This sequence of events appears to recapitulate the estrogen-dependent changes in growth factor expression and morphology displayed by tanycytes during the preovulatory surge of GnRH. TGFβ1-mediated cell retraction in tanycytes, which were shown to express TGFβ receptors *in vivo* [58,59] requires the activity of matrix metalloproteinases [54] that were also shown to be expressed in the median eminence [60]. In contrast to the aforementioned effect of PGE_2 that promotes tanycyte end-feet retraction by promoting actin cytoskeleton remodeling (within 30 min) [43], TGFβ1-mediated tanycytes retraction involves digestion of the extracellular matrix that causes substrate adhesion loss for tanycytes as shown by time-laps experiments [54]. These two mechanisms mediating tanycyte retraction thus appear highly complementary.

Intriguingly, recent data suggest that TGFα signaling may also be a key component of the cell-cell communication pathways used by the neuroendocrine brain to regulate structural changes between tanycytes and GnRH nerve terminals during the photoperiodic control of reproduction in the Japanese quail [61].

Do median eminence astrocytes also play a role in modulating GnRH release and/or tanycyte plasticity?

Hypothalamic astrocytes, in addition to expressing TGFα and its receptor, also express neuregulins, other peptides members of the EGF family, and their erbB4 receptor [52,62], an erbB receptor that is not expressed in tanycytes [54]. Like TGFα, neuregulins were shown to stimulate GnRH release [42,52] and the neuregulin-stimulated release of GnRH requires astrocyte intermediacy and PGE_2 release [52]. The key involvement of median eminence astrocytes in the control of GnRH release was demonstrated using transgenic mice in which a dominant negative form of the erbB4 receptor, lacking the intracellular domain, was specifically targeted to astrocytes [42,63]. The mutant astrocytes exhibited a blunted PGE_2 response to neuregulin stimulation, a reduced GnRH response to neuregulin treatment in median eminence explants, diminished plasma gonadotrophin levels and delayed onset of

the first preovulatory surge at puberty, all of these in the face of normal erbB1-dependent function [42]. PGE_2 originating from median eminence astrocytes following erbB receptor activation could, in addition to stimulating GnRH neurons themselves, also modulate median eminence plasticity either by promoting actin cytoskeleton remodeling [43] and/or TGFβ1 expression [54] in tanycytes. Importantly, erbB4 expression within the hypothalamus is regulated by estrogens and its expression levels are maximal at the time of proestrus [52].

Glia-to-glia and Neuron-to-glia interactions in the control of PGE_2 *release*.

Paracrine communication between astrocytes, tanycytes and neurons in the median eminence may play a major role in the integration of neuronal and nonneuronal stimuli that these cells receive under a series of varying physiological situations across the estrous cycle. Neuron-to glia signaling and cross-communication between glial cells in the median eminence may be modulated, at least in part, through the control of erbB signaling. In agreement with this hypothesis are the data showing that concomitant activation of metabotropic and AMPA glutamate receptors on hypothalamic astrocytes results in activation of erbB receptors, recruitment of their ligands to the glial cell membrane and release of PGE_2 [64]. It was further demonstrated that metabotropic and AMPA glutamate receptor agonists together induce the phosphorylation of both erbB1 and erbB4 via a transactivation mechanism, requiring proteolytic activity [64] and presumably leading to the release of both erbB1 receptor ligands and erbB4 ligands from their membrane-bound precursors. These results suggest that the availability of EGF-like peptides in the extracellular matrix may be a key regulatory point for neuron-to-glia and glia-to-glia interactions. EGF-like peptides are membrane-anchored and are released upon cleavage of the ectodomain [65-67]. The shedding of the ectodomain of these factors is controlled by a class of cell surface proteolytic enzymes, termed metalloproteinases [68,69]. Matrix metalloproteinases (MMPs) and ADAMs (a disintegrin and metalloproteinase) are two subfamilies of zinc-dependent-metalloproteinases involved in extracellular proteolysis (see for review [70]). While MMPs, by their capacity to degrade the components of the extracellular matrix, play a pivotal role in

modulating interactions between cells and their microenvironment [71], ADAM proteins, which mediate both adhesive interactions and proteolysis [72], have been shown to participate in the cleavage of transmembrane proteins. Interestingly, ADAM17, also known as TACE (tumor-necrosis-factor-alpha-converting enzyme) is one of the molecules involved in the shedding of both TGFα [73] and neuregulins [65] the main ligands of erbB receptors in the brain and has recently been shown to be expressed in astrocytes in the median eminence [74]. TACE activity increases selectively in this region at the time of the first preovulatory surge of GnRH/LH. Importantly, inhibition of TACE activity targeted to the median eminence decreases GnRH secretion and delays the occurrence of the first preovulatory surge of gonadotropins [74].

A dynamic role for vascular endothelial cells in the release of GnRH and median eminence morphological plasticity.

The recognition that vascular endothelial cells of the median eminence play a key role in modulating neuronal-glial remodeling and GnRH release emanated from recent studies using immunopanning methods to purify endothelial cells of the median eminence and co-culture experiments with isolated tanycytes [43,44]. These studies showed that median eminence endothelial cells promote acute actin cytoskeleton reorganization in tanycytes (within 30-min coculture) via the release of the highly diffusible and labile mediator nitric oxide (NO). NO is a gaseous transmitter that travels readily across biological membranes and that is formed by oxidation of L-arginine to L-citrulline by NOS [75]. Inhibition of endothelial NO production by pre-incubating vascular endothelial cells with NOS inhibitors or infecting them with an adenoviral vector expressing a dominant negative form of endothelial NOS (eNOS) abrogates endothelial cell-promoted morphological changes in tanycytes [43,44]. In contrast, tanycyte treatment with physiological doses of NO, using NO donors, mimics the coculture effects [43,44]. Downstream effectors of endothelial NO-meditated plasticity in tanycytes were shown to be both soluble guanylyl cyclase and cycloxygenases (COX) [43,44]. Interestingly, when high non-toxic doses of NO donors are applied, or, increased NO

production by endothelial cells is elicited by L-arginine, the precursor of NO, it triggers acute tanycyte retraction *in vitro* [43]. Strikingly, treatment of median eminence explants with L-arginine also causes tanycytic processes surrounding GnRH nerve terminals to undergo acute retraction, enabling GnRH neuroendocrine terminals to establish direct neurovascular junctions, as shown by electron microscopy [44]. The physiological significance of these NO-mediated cell-cell communication processes for reproduction is highlighted by studies showing that the blockade of NO release from the median eminence both inhibits GnRH secretion on the afternoon of proestrus [76] and results in disruption of estrous cyclicity [44]. Within the median eminence NO can be produced by two different constitutive enzymes that have different spatial distributions: neuronal NOS (nNOS), which is confined to neuronal fibers projecting to the neural lobe of the pituitary that are segregated from GnRH axonal processes [77] and eNOS, which is expressed in endothelial cells of the portal blood vessels lying only a few micrometers away from tanycytic endfeet and GnRH nerve terminals (Figure 4C) [78]. Development of amperometric methods to selectively measure NO release in real time demonstrated that NO is spontaneously produced within the median eminence [76]. In female rats, NO secretory pattern appears to be both pulsatile and cyclic in nature (Figure 4A). The pulse frequency of spontaneous NO efflux (one pulse every 32 ± 1 min) is strikingly similar to that of pulsatile GnRH release from median-eminence explants (one pulse every 33 ± 8 min) [7]. The amplitude of NO pulses varies across the estrous cycle reaching peak values on proestrus [76], concomitantly with the increase in GnRH pulse amplitude observed in vivo [79]. These observations together with the finding that GnRH release at the time of the onset of the preovulatory GnRH surge on the afternoon of proestrus can be blocked with L-NIO (Figure 4B), a selective endothelial NOS inhibitor, demonstrate that NO secretion and GnRH release are causally related in the median eminence during the estrous cycle [76]. Although controversial, estrogens may be capable of targeting both tanycytes and endothelial cells within the median eminence [43,55,80-82] and may play an important role in regulating endothelial-glial-neuronal interactions during the estrous cycle. Amperometric experiments coupled to radioimmunoassays first demonstrated that estrogens promote both

acute and long-term endothelial NO-stimulated GnRH release [76,83]. Recent work from our laboratory suggested that estrogens also regulate the dynamic control of tanycyte plasticity by vascular endothelial cells in the median eminence [43]. Estradiol was indeed shown to enhance endothelial-to-glial communication by causing endothelial cell-promoted retraction of tanycytic processes, involving endothelial NO [43]. Estrogens stimulate eNOS expression in endothelial cells [43,84] and up regulate both COX 1 and COX 2 expression in tanycytes while leaving unchanged the expression of soluble guanylyl cyclase [43], the other target of NO in this cell type [44]. Because PGE_2 mimicked, in simple tanycyte cultures, the estrogen-induced acute cellular retraction of tanycytes seen in cocultures with endothelial cells [43], altogether these results provide evidence for a major role for a COX product in the estradiol-induced tanycyte retraction mediated by endothelial NO. Noticeably, treatment of median eminence explants with PGE_2, at concentration known to stimulate GnRH release [40,42], caused the advancement of GnRH neuroendocrine terminals towards the pericapillary space, a phenomenon that probably results from the retraction of tanycyte endfeet [43]. Local infusion of COX inhibitors into the median eminence *in vivo* markedly impairs the ovarian cycle [43] thus highlighting the physiological importance of eicosanoids in the cell-cell communication processes regulating GnRH release.

How does GnRH neuron enter into the play?

Electron microscopic data showing that, on the day of proestrus GnRH axons appear to sprout new terminals and/or phyllopodia towards the pericapillary space with which they eventually contact [10], suggest an active participation of GnRH neurons in the plastic remodeling of the external zone of the median eminence that takes place during the ovarian cycle. Corroborating this hypothesis are the data showing that GnRH neurons actually express intrinsic markers of axon plasticity such as the growth-associated protein 43 (GAP-43) [85] and growth factor receptors known to participate in GnRH axon elongation such as bFGF receptors [86,87]. Interestingly, the GAP-43 mRNA content of individual GnRH neurons varies during the estrous cycle and is maximal on the day of proestrus [85].

Conceivably, an accumulation of GAP-43 in GnRH nerve terminals on proestrus could enhance the responsiveness of individual GnRH nerve endings to growth promoting factors [88,89] produced locally in the median eminence, and promote their sprouting towards the pericapillary space. Molecular determinants involved in such communication processes have yet to be identified. Screening for molecular cues known to play a role in the control of axon guidance indicated semaphorin (SEMA) 3A as a candidate [90]. Robust SEMA 3A mRNA expression was detected in the capillary zone of the median eminence, while only scant signal was found in the nervous tissue using *in situ* hybridization [91]. Consistent with these data, SEMA 3A immunoreactivity appears to be restricted to portal blood capillaries of the median eminence. GnRH nerve terminals may indeed be able to sense SEMA 3A as neuropilin-1, its receptor, is expressed in GnRH neurons. The proportion of GnRH perikarya expressing neuropilin-1 mRNA significantly varied during the rat estrous cycle and was maximal on proestrus, a phase of the estrous cycle when GnRH nerve terminals have direct access to the portal vasculature. Accordingly, strong neuropilin-1 immunoreactivity was detected in the external zone of the median eminence and was found to colocalize with GnRH-immunoreactive fibers [91]. Using electron microscopy, further experiments demonstrated that activation of Sema3A/Neuropilin-1 signalling in the median eminence promoted rapid GnRH axon sprouting towards the pericapillary space of the pituitary portal blood vessels [91]. Taken together these results suggest that SEMA 3A may be a chemotropic factor secreted by endothelial cells to induce GnRH axon plasticity within the median eminence at key stages of the ovarian cycle.

Conclusion

That tanycytes, astrocytes and vascular endothelial cells contribute actively in the regulation of GnRH neuronal function locally within the median eminence of the hypothalamus during the reproductive cycle has become increasingly clear. Tanycytes physically interact with GnRH neuroendocrine terminals by apposing processes to GnRH axonal membrane in a highly dynamic fashion, subjected to short and/or long-range paracrine regulation by

endothelial and glial signaling molecules, of which are under the control of gonadal steroids (Figure 5). This is notwithstanding the importance of transsynaptic influences in the control of GnRH neuron activity [92-94] and the influence that gonadal steroids exert on the GnRH neuronal network [95-98]. As also illustrated in this review, the median eminence of the hypothalamus, by its physiological capacity to undergo dynamic transformations that affect morphology of the neuroendocrine terminals it contains, tanycytes and specialized neuronal junctions, can serve as a useful model to dissect out the fine modalities of these phenomena and their functional consequences.

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Figure 1. Schematic representation of the cell types (tanycytes, astrocytes and endothelial cells) and neuronal elements (neuroendocrine terminals) that reside within the median eminence of the hypothalamus. The median eminence of the hypothalamus is the brain structure forming the floor of the third ventricle (3V). The median eminence, which is one of the circumventricular organs of the brain is capable of conveying information from the brain to the periphery via the release of neurohormones into the circulation and, conversely sensing information reaching the brain via the bloodstream.

Figure 2. Electron micrographs illustrating the dynamic changes occurring in the external zone of the median eminence that control the direct access of GnRH nerve terminals to the pericapillary space during the reproductive cycle in the rat. **Left panel,** Electron micrograph of GnRH-immunoreactive terminals (large arrowhead) in the external zone of the median eminence in close proximity of the fenestrated capillaries (Cap) of the portal vasculature. At most stages of the reproductive cycle, GnRH nerve terminals (labeled with 15-nm gold particles) are entirely embedded in tanycytic endfeets (Tan), which prevent them from contacting the pericapillary space (p.s.) delineated by the parenchymatous basal lamina (arrow). Arrowhead, endothelial basal lamina; short arrows, fenestration of the endothelium. Scale bar: 0.5 mm. **Right panels,** On proestrus, the time of the occurrence of the preovulatory GnRH/LH surge, a significant fraction of GnRH nerve endings (large arrowhead) directly contact the pericapillary space (p.s.) either through filopodial extension of the nerve terminal (arrows) (Bottom right panel) or (Top right panel) by evaginations of the parenchymatous basal lamina (small black arrowheads) that allows the pericapillary space (p.s., asterisk) to penetrate into the nerve parenchyma. In the top right panel note the presence of numerous small clear synaptic vesicles (white vesicles of small size, white

arrowhead) and the fusion of secretory granules (large-sized black vesicles) with the axoplasmic membrane of the GnRH nerve terminal in direct apposition with the parenchymatous basal lamina (small arrows). The penetration of the pericapillary space into the nerve parenchyma on the day of proestrus may result from the morphological remodeling of tanycytic end-feets (tan) anchored to the parenchymatous basal lamina through hemidesmosomes seen as dark thickenings within the tanycytic processes in apposition with the basal lamina, small white arrowhead. Scale bar: 0.5 μm. From [10,11] with permission.

Figure 3. The COX product PGE_2 causes cell retraction in tanycytes *in vitro* and promotes neuronal-glial plasticity in hypothalamic explants containing the median eminence, causing the advancement of GnRH neurosecretory terminals towards the pericapillary space. **A,** The addition of PGE_2 (280 nM, 30 min), one of the most biologically active of COX products, caused acute tanycyte retraction. Tanycytes were stained with Alexa-conjugated phalloidin to visualize filamentous actin (red) and with Hoechst to stain nuclei (blue). Scale bar: 10 μm. **B,** Representative electron micrographs of GnRH immunoreactive axon terminals (15 nm gold particles; long black arrowhead) from female rat median eminence explants incubated for 30 min in the presence (PGE_2) or in the absence (Control) of PGE_2 (1 μM). Under basal unstimulated conditions, GnRH nerve endings (long black arrowhead) were maintained at a distance from the brain basal lamina (white arrow) delineating the pericapillary space (p.s.), by thick enclosing tanycyte end-feet (Tan.). PGE_2 treatment caused the advancement of GnRH axon terminals (long arrowhead) toward the brain basal lamina (white arrow) and the apparent retraction of most of the astroglial sheath (black arrows) from those neurosecretory terminals that were separated from the fenestrated (small arrowhead) portal capillaries (cap.) by only a few nanometers. end., endothelium. Scale bar, 1 μm. From [43] with permission.

Figure 4. In the median eminence of the hypothalamus, endothelial nitric oxide (NO) secretion may represent one of the synchronizing cues that by coordinating GnRH release

from GnRH neuroendocrine terminals that are distributed over 2 mm within the median eminence allows the occurrence of functionally meaningful episodes of GnRH secretion. (A) Real time amperometric measurement of spontaneous NO release from median eminence explants at different stages of the rat estrous cycle. DiII, diestrus II; PRO, proestrus; E, estrus. (B) On the afternoon of proestrus, the preovulatory GnRH/NO release is blocked with L-NIO, an NOS inhibitor selective for eNOS at 0.5 μM. * and a, significantly different from treated samples, p , 0:05: AUC: area under the curve during a 30 min period. (C) Photomicrograph showing GnRH axonal fibers in the external zone of the median eminence (green fluorescence, arrows) in close apposition to eNOS-immunoreactive portal vasculature (red fluorescence, arrowheads). 3V, third ventricle. The dotted lines outline the third ventricle. Scale bar: 75 μm. (A, B) Reproduced with permission from [76]; (C) reproduced with permission from [78].

Figure 5. Schematic representation of neural-glial-endothelial interactions involved in the control of GnRH neurosecretion in the median eminence. Glial-neuronal interactions in the median eminence involve the production of epidermal growth factor (EGF)-related peptides, TGFα and neuregulins (NRG), by tanycytes and astrocytes. The binding of TGFα to tanycytic and/or astrocytic erbB-1 receptors, as well as the binding of NRGs to astrocytic erbB-4 receptors results in the recruitment of erbB-2 co-receptors and signal transduction. The downstream signaling of erbB receptors leads to the secretion of bioactive molecules, such as prostaglandin E2 (PGE2), which are in turn able to directly stimulate GnRH release at the nerve endings. In addition, ligand-dependent activation of erbB-1 receptors in tanycytes results in biphasic plastic changes characterized by an initial phase of tanycytic outgrowth and a secondary phase of retraction. Although the initial outgrowth is independent of the TGFβ1 system, the subsequent retraction requires PGE2 synthesis, a PGE2-dependent increase in the production of TGFβ1 and matrix metalloproteinase activity (MMP). Endothelial-neuronal interactions at the level of the median eminence involves the production

of nitric oxide (NO) by endothelial cells of fenestrated capillaries of the portal blood vessels. Upon its secretion, NO diffuses from its source, where it not only stimulates the release of GnRH from the neighboring GnRH neuroendocrine terminals but also promotes their access to the blood stream by inducing cytoarchitectural changes in tanycytic end-feet. In addition, because GnRH neurons express intrinsic markers of axon plasticity such as the growth-associated protein 43 (GAP-43) and are known to sprout new terminals towards the pericapillary space in proestrus, it is conceivable that individual GnRH nerve endings may be responsive to growth promoting factors produced locally within the median eminence. Estrogens are likely to be the key humoral factors involved in the orchestration of the glia-to-neuron communication that allows GnRH neurons to directly contact the pituitary portal blood vessels on the day of proestrus. eNOS, endothelial nitric oxide synthase. Adapted from [15] with permission.

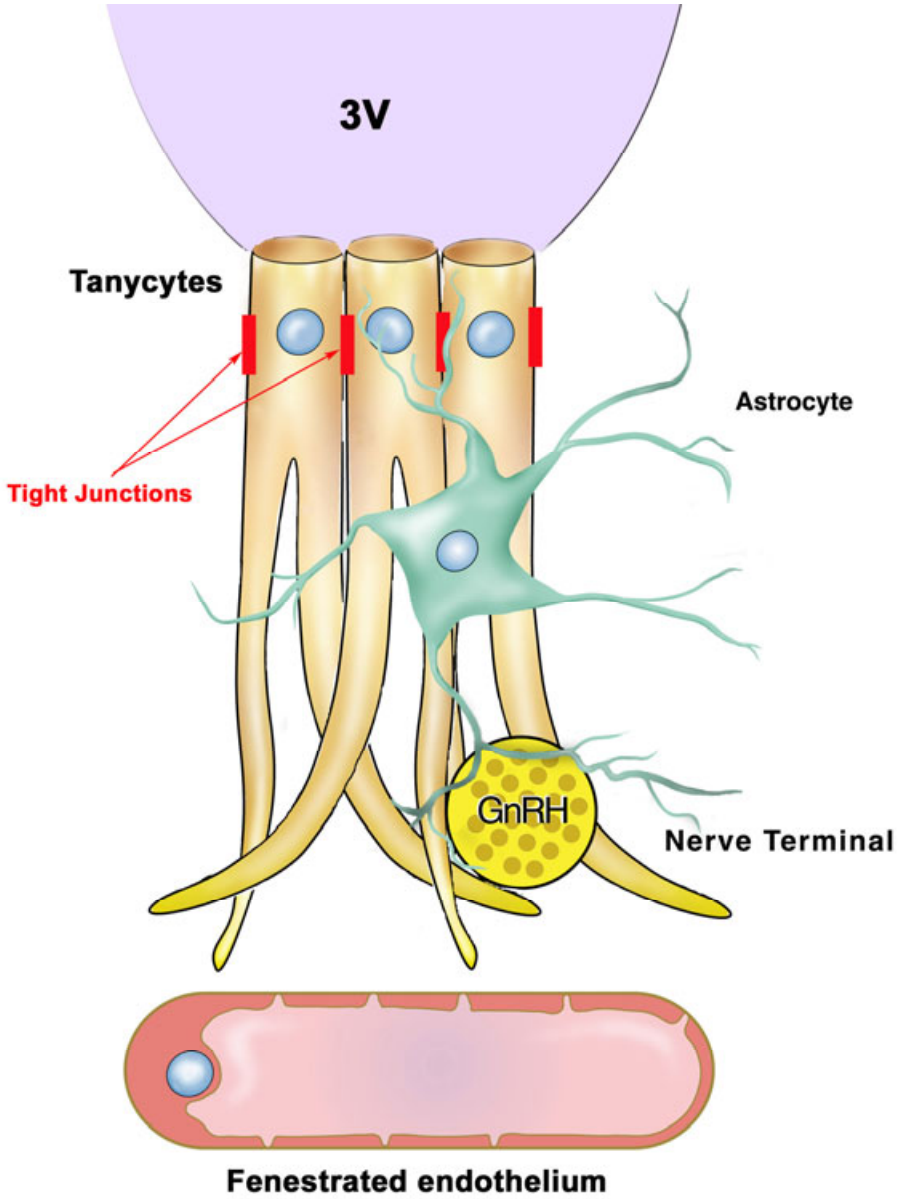
3V
Tanycytes
Tight Junctions
Astrocyte
GnRH
Nerve Terminal
Fenestrated endothelium

Figure 1

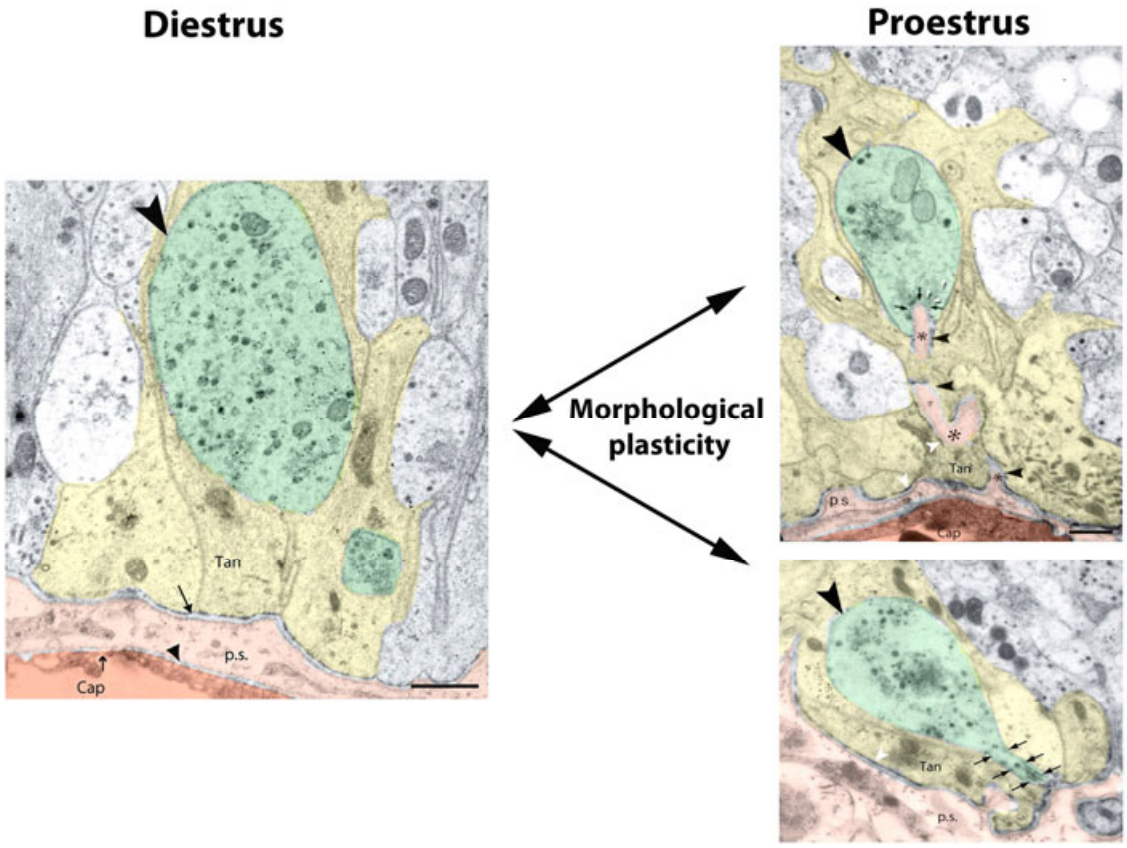
Diestrus
Proestrus
Morphological plasticity
Tan
p.s.
Cap
Tan
p.s.
Cap
Tan
p.s.

Figure 2

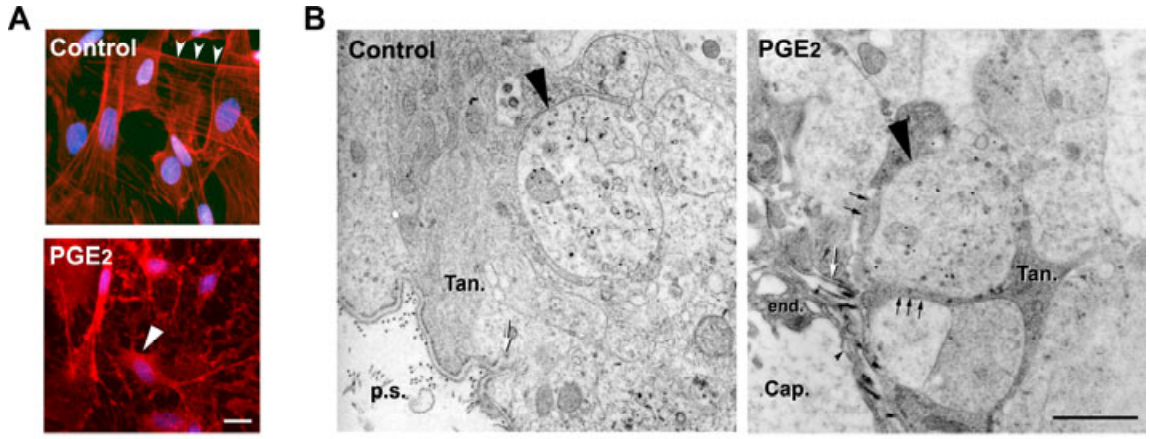

Figure 3

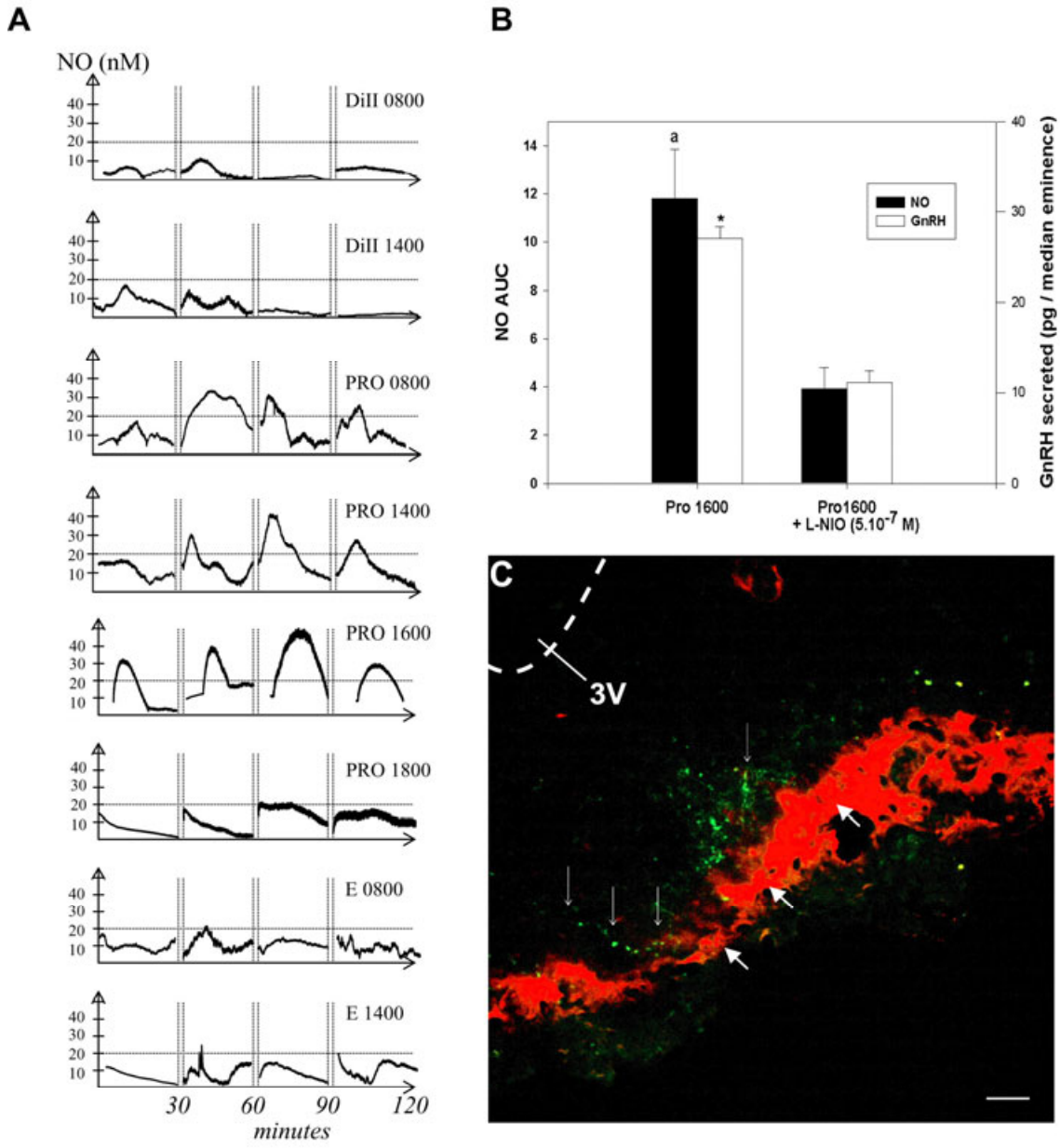
A
NO (nM)
DiI 0800
DiI 1400
PRO 0800
PRO 1400
PRO 1600
PRO 1800
E 0800
E 1400
40
30
20
10
30
60
90
120
minutes
B
NO AUC
GnRH secreted (pg / median eminence)
NO
GnRH
a
*
Pro 1600
Pro1600
+ L-NIO (5.10-7 M)
C
3V

Figure 4

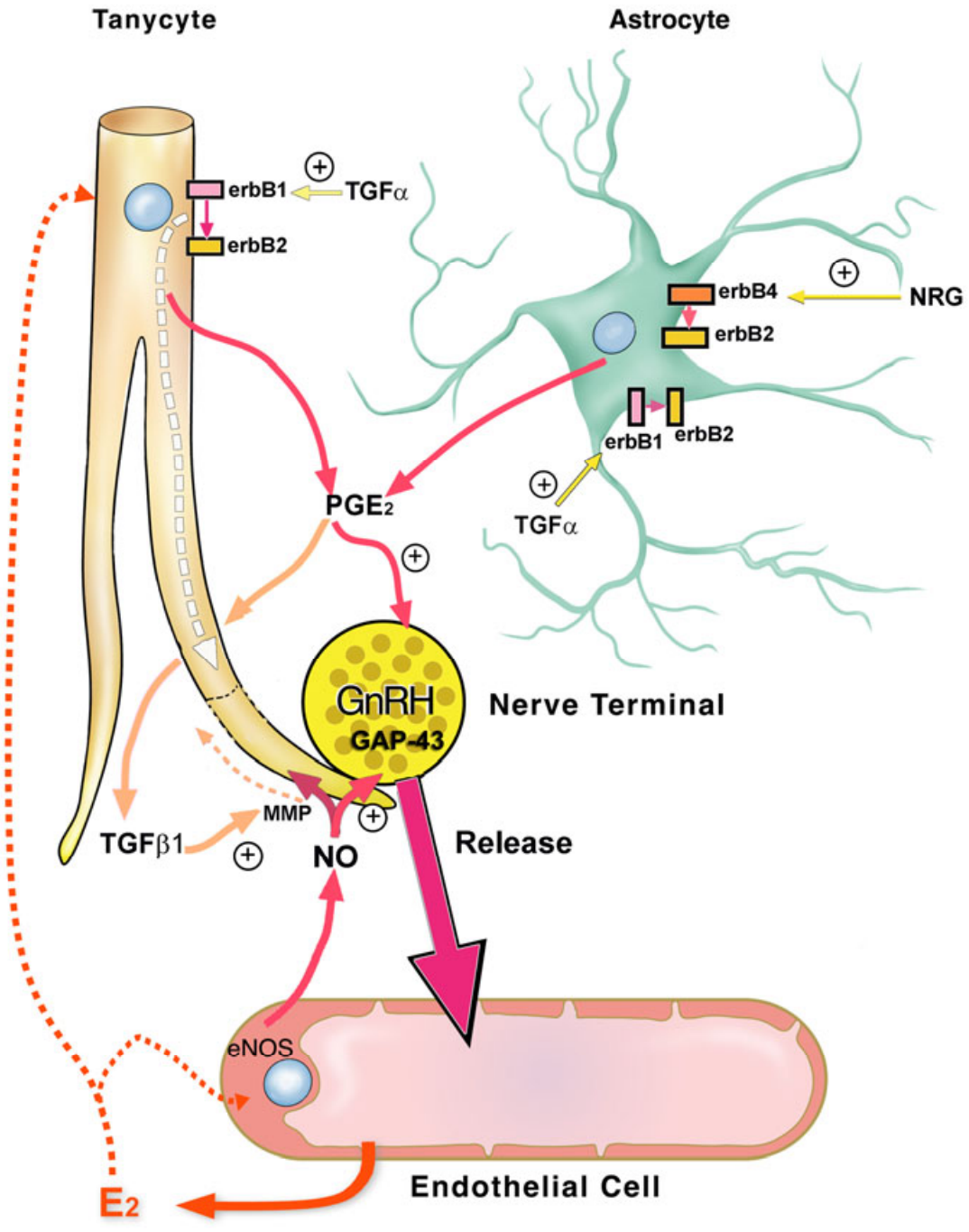
Tanycyte
Astrocyte
erbB1
TGFα
erbB2
erbB4
NRG
erbB2
erbB1
erbB2
TGFα
PGE2
GnRH
GAP-43
Nerve Terminal
TGFβ1
MMP
NO
Release
eNOS
Endothelial Cell
E2

Figure 5