Published in final edited form as:

Steroids. 2019 February ; 142: 77–83. doi:10.1016/j.steroids.2018.01.010.

Membrane-Initiated Estrogen Signaling via Gq-coupled GPCR in the Central Nervous System

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Abstract

The last few decades have revealed increasing complexity and depth to our knowledge of receptor-mediated estrogen signaling. Nuclear estrogen receptors (ERs) ER α and ER β remain the fundamental dogma, but developing research targeting membrane-bound ERs urges for a more expanded view on ER signaling. ER α and ER β are also involved in membrane-delineated signaling alongside membrane-specific G protein-coupled estrogen receptor 1 (GPER1), ER-X, and the Gq-coupled membrane ER (Gq-mER). Membrane ERs are responsible for eliciting rapid responses to estrogen signaling, and their importance has been increasingly indicated in central nervous system (CNS) regulation of such functions as reproduction, energy homeostasis, and stress. While the Gq-mER signaling pathway is well characterized, the receptor structure and gene remains uncharacterized, although it is not similar to the nuclear ER α / β . This review will describe the current knowledge of this putative membrane ER and its selective ligand, STX, from its initial characterization in hypothalamic melanocortin circuitry to recent research exploring its role in the CNS outside of the hypothalamus.

Keywords

17 β -estradiol; Gq-mER; hypothalamus; hippocampus; reproduction; energy homeostasis

Introduction

Estrogen receptors (ER) ER α and ER β were initially discovered through their regulation of gene expression via action of their main endogenous estrogen, 17 β -estradiol (E2) [1, 2]. Over the past 30 years, ER have been thought to signal primarily through long-term transcriptional regulation of the brain, mediating neuronal circuitry formation during developmental stages, or through targeted activation of gene expression in the adult brain

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later in life [3]. These transcriptional events, often called the “classical” ER signaling pathway, are initiated by the formation of ER α and ER β steroid binding–dependent hetero- or homodimers. These dimers translocate to the nucleus to bring about transcriptional activation through their interaction with the DNA binding site known as the estrogen response element (ERE) or with other DNA-bound transcription factors such as activator protein 1 (AP-1) and specificity protein-1 (SP-1) [4–8].

However, it has become clear that there also exists a rapid, membrane-initiated, E2-mediated actions independent of the classic nuclear signaling pathway. Even before the discovery of ER α and ER β , these rapid actions of E2 were observed in the uterus and the hypothalamus, both sites in which it is important for there to be rapid responses to hormonal signaling [9–11]. Only within the last 15 years have these fast-acting E2 actions been mechanistically investigated and shown to have physiological significance [12–17]. Membrane-mediated effects of E2 signaling triggers various intracellular cascade pathways, including protein kinase C (PKC), protein kinase A (PKA), phosphatidylinositol-3 kinase (PI3K), and mitogen-activated protein kinase (MAPK) leading to protein phosphorylation, gene transcription, and regulation of ion channel and neuronal excitability [18–22].

The focus of this review will be on a specific, putative membrane ER that is a Gq protein-coupled receptor called the Gq-mER, which has been extensively characterized in hypothalamic proopiomelanocortin (POMC) neurons [14, 23–25]. In guinea pig and mouse POMC neurons, E2 attenuates the baclofen response (GABA-B receptor activation) within minutes. This rapid action of E2 is mimicked by E2 conjugated to bovine serum albumin (E2-BSA), indicating a membrane-initiated mechanism of disinhibition, and is blocked by the ER antagonist ICI 182,780, indicating that the signaling is mediated by an ER. Baclofen, as a GABA-B receptor agonist, has an inhibitory effect on neuronal excitability by activating a G-protein inward-rectifying potassium (GIRK) channel; thus, E2’s inhibition of the baclofen response reduces the inhibitory GABAergic tone. Furthermore, STX, an agonist for the Gq-mER, can activate the same Gq protein-phospholipase C (PLC)-PKC-PKA pathway activated by E2 (See Figure 1). Together, these results demonstrate that STX as a specific agonist of the Gq-mER capable of eliciting electrophysiological changes in POMC excitability that mimic E2.

Since the characterization of STX in hypothalamic POMC neurons, focus has shifted to other hypothalamic and extrahypothalamic neurons to determine if the E2- and STX-sensitive Gq-mER controls neurological functions elsewhere in the brain and to identify the physiological consequences of the Gq-mER activation. Over the past decade, research has shown direct effects of Gq-mER activation on energy homeostasis [14, 21, 25–27], thermoregulation [26], reproductive behavior [28, 29], regulation of gonadotropin releasing hormone (GnRH) neurons [30–32], and corticotropin releasing hormone (CRH) neurons [33] as well as other neurological functions in the central nervous system (CNS).

Gq-mER signaling in the hypothalamic melanocortin circuitry

E2 is generated by the ovaries and other peripheral tissues [20] and travels to the hypothalamus to interact with neuronal populations to control homeostatic functions. The

primary role of E2 is to control reproduction by controlling output of the gonadotropin releasing hormone (GnRH) neurons in the preoptic area (POA) of the hypothalamus. However, E2 also has a number of secondary functions, most notably in energy homeostasis and temperature regulation (previously reviewed in [34, 35]). E2 is anorectic, and estrogen deficiency is strongly correlated with decreased energy expenditure and increased weight gain [36–38]. In fact, decreased fertility and altered menstrual cycle patterns are linked with obesity [39, 40], indicating that sex hormones, specifically E2, play a role in energy homeostasis. Decreased levels of E2 are implicated in causing the accumulation of fat in postmenopausal women [41]. Furthermore, mutations resulting in dysfunctional ERs show distinct patterns of obesity, hyperinsulinemia, and type 2 diabetes in humans [42]. Increased feeding behaviors and weight gain are also observed in ovariectomized rodents, which are reversible with supplementation of E2 to pre-ovariectomy levels [36, 43]. Likewise in male rodents, a decrease in E2 signaling observed in ER α knockout (KO) rodents results in similar patterns of fat accumulation [44, 45]. However, conditional ER α KO mice that express an ER α lacking a functional DNA-binding (ERE targeting) domain exhibit a phenotype similar to wild-type females [46]. This suggests that substantial nongenomic E2 signaling is important in energy homeostasis.

POMC and neuropeptide Y (NPY) neurons are necessary in mediating the balance between anorectic and orexigenic signaling in energy homeostasis, respectively [47, 48]. E2 control of energy homeostasis is, in part, a CNS-mediated process [38, 49]. Specifically, E2 has important interactions with arcuate POMC and NPY neurons [50, 51]. POMC mRNA decreases in postmenopausal women [52], and in studies with ovariectomized rodents, *Pomc* mRNA is increased following E2 treatment, leading to an increase in the expression of the POMC-derived peptide, β -endorphin [27, 53]. Furthermore, the increase in NPY mRNA and peptide expression in rodents post-ovariectomy is reversed after E2 dosing [54]. These findings indicate that the arcuate POMC and NPY neurons are important targets in the anorectic signaling pathways of E2.

While genomic E2 pathways certainly play a significant part in mediating its anorectic effect, there is mounting evidence supporting the existence and role of the Gq-mER in mediating the response to E2. Genomic pathways typically require a time frame of hours to days for effects to be observed in mammals; however, E2 dosing directly into the third ventricle results in an attenuation of food intake in just 4–6 h in fasted, ovariectomized rodents [55, 56]. This suggests the presence of a fast-acting ER in E2 signaling. The Gq-mER was characterized using both guinea pig and mouse models using whole-cell patch-clamp techniques to record hypothalamic neuronal activity in brain slices [14, 23]. These studies found a significant attenuation of the activity of a GABA-B receptor agonist, baclofen, within minutes after E2 perfusion. When administered alone, baclofen-induced activation of the GABA-B receptor initiates a K⁺ efflux through the GIRK channel in hypothalamic neurons. In POMC neurons, GABA-B signaling acts to inhibit neuronal excitability, hence the attenuation of the baclofen response following E2 perfusion acts to disinhibit GABA-B tone and increase the excitability of POMC neurons, potentially augmenting in the anorectic effects of E2. E2-BSA perfusion recapitulates E2's attenuation of the baclofen response [23]. In addition, an ER-specific antagonist ICI 162,780 blocks the effects of E2 at subnanomolar affinity [23], suggesting that the receptor is not ER α , which

has been shown to activate ER α 36 in some breast cancer cells [57]. Furthermore, the estrogenic attenuation of the baclofen response is mimicked by stimulating G protein-coupled downstream signaling (activation of adenylyl cyclase) and by direct activation of PKA. Inhibition of PKC and PKA blocks the Gq-mER pathway [19, 23]. Lastly, PI3K has also been indicated in this pathway as inhibitors wortmannin and LY294002 significantly reduced the baclofen response in POMC neurons (see Figure 1) [58].

To selectively target this pathway and its role in neurophysiology, Kelly and colleagues [23] developed a selective Gq-mER agonist called STX. STX is a diphenylacrylamide that is structurally similar to 4-hydroxytamoxifen and does not bind to either ER α or ER β . STX mimics E2 attenuation of the baclofen response (at ~20x greater potency; STX: EC₅₀ = 2.6nM, E2: EC₅₀ = 46nM) in wild-type (WT) and ER α or ER β KO mice and was blocked by ICI 164,384, indicating that STX acts through an ER that is not ER α or ER β . Lastly, PKA and PKC inhibitors all blocked the attenuation of the baclofen response by STX [14]. Together, these results characterize STX as a specific agonist of a novel Gq-mER capable of mimicking E2 in eliciting electrophysiological changes in POMC excitability.

Orexigenic NPY neurons also demonstrate a rapid response to E2, in part through the actions of a complementary Gq-mER pathway [59]. NPY neurons will respond to baclofen in the same way that POMC neurons do; however, the effect of E2 on the baclofen response in NPY neurons is variable. Interestingly, some NPY neurons show an attenuated baclofen response similar to POMC neurons, while other NPY neurons exhibit an opposing, hyperpolarizing effect. When explored further, it was revealed that NPY neurons express a rapid ER α -mediated signaling pathway in addition to the Gq-mER signaling pathway that impinges on GIRK-GABA-B interactions [59]. Perfusing NPY neurons with propyl pyrazole triol (PPT), a potent ER α agonist, resulted in an attenuation of the baclofen response in ovariectomized mouse brain slices. In contrast to PPT, STX elicited an augmentation of the baclofen response in NPY neurons that is eliminated with co-administration of ICI 164,384 (see Figure 1) [59]. These results suggest that rapid E2 signaling in NPY neurons has two modes of action through either the membrane-bound ER α or Gq-mER and that the individual NPY neuronal response to E2 signaling, either a suppression or augmentation of GABA-B tone, may be dependent on the relative expression of ER α or Gq-mER, respectively. One possible explanation for this differential response is that Gq-mER is responsible for the control of energy homeostasis, while ER α is involved in a different E2-mediated process such as reproduction or thermoregulation [59].

Gq-mER is involved in the hypothalamic control of reproduction

Arcuate POMC neurons are well known for regulating energy homeostasis, but a subpopulation of the cells is also involved in mediating aspects of female sexual behavior. These POMC neurons project to the median preoptic nucleus (MPN) and release the opioid peptide β -endorphin. β -endorphin activates μ -opioid receptors expressed in MPN neurons signaling for inhibition of sexual receptivity (lordosis) [60, 61]. E2 is known to regulate β -endorphin expression and release [53, 61–63], and recent research proposes that this effect is also mediated by membrane-initiated estrogen signaling.

The opioid receptor-like (ORL) 1 receptor is a Gi-coupled receptor that is highly expressed in the hypothalamus and in arcuate POMC neurons. Orphanin FQ, also known as nociceptin (OFQ/N), the endogenous ligand for the ORL1 receptor, regulates cell excitability by increasing postsynaptic potassium currents, inhibiting postsynaptic calcium currents, and by diminishing presynaptic neurotransmitter release [64–68]. OFQ/N signaling via ORL-1 receptors inhibits POMC neurons, and Conde *et al.* (2016) hypothesized that E2 regulates POMC excitability by attenuating inhibitory ORL-1 signaling that induces a large outward potassium currents to hyperpolarize and reduce neuronal excitability in POMC neurons. This inhibitory effect is attenuated with pre-treatment with E2, as well as E2-BSA, demonstrating E2's inhibitory effect on ORL-1 signaling. This effect was blocked by ICI 182,780 and mimicked by both PPT and STX. This indicates that the signaling is ER-mediated, initiated at the membrane, and involves ER α and Gq-mER, alone or simultaneously. Furthermore, inhibiting PI3K eliminated the attenuation of ORL-1 induced currents, and inhibitors of PLC, PKC, and PKA blocked the estrogenic effect. In contrast, activation of PLC, PKC, or PKA all recapitulated E2's attenuation of ORL-1 signaling [25]. These data indicate the role of membrane-bound ER α as well as Gq-mER in attenuating the inhibitory effects of OFQ/N signaling in POMC neurons, which project to the MPN and release β -endorphin to inhibit lordosis.

Gonadotropin-releasing hormone (GnRH) neurons, localized to the preoptic area in the rodent, coordinates and integrates hypothalamic signals to directly regulate reproduction. GnRH neurons secrete GnRH into the capillaries of the hypophyseal portal system, which transport the neurohormone to the anterior pituitary to stimulate release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the gonadotropic cells. LH and FSH travel to the gonads to control gametogenesis and steroid production [69]. E2 and progesterone signal back to the hypothalamus as negative feedback throughout the estrous cycle, thus compromising the hypothalamic-pituitary-gonadal (HPG) axis. During the proestrous stage, elevated E2 produced by the dominant follicles stimulates a positive feedback loop to induce the GnRH and LH surges and initiate ovulation. Steroidal regulation of the GnRH pulse generator is essential for fine-tuning reproductive cyclicity and function [69, 70].

The pulsatile action of GnRH neurons is an innate characteristic, as immortalized GnRH (GT1-7 cells) in culture also show timed bursts of secretion and activity [71]. This is further emphasized in studies wherein ovariectomized females, released from steroidal feedback, exhibit a consistent, basal pulsatile GnRH release approximately every 30–40 min in rodents and up to 1 hour in sheep and monkey models [72–76]. The exact mechanism that controls this innate pulsatility is not yet known, but calcium signaling seems to be key. Oscillations of intracellular calcium concentrations ($[Ca^{2+}]_i$) correlate with GnRH secretion and other neuronal activity; however, differences are reported depending on the model [77]. Overall, the idea is that depolarization of GnRH neurons activates voltage-sensitive, T-type Ca^{2+} channel influx, and the rise in $[Ca^{2+}]_i$ stimulates bursts of GnRH release [69, 70, 77–79]. This current is produced by 3 channel subunits encoded by the *Cacna1g* (Cav3.1), *Cacna1h* (Cav3.2), and *Cacna1i* (Cav3.3) genes. All three genes are expressed in GnRH neurons with an expression profile of Cav3.3 > Cav3.2 > Cav3.1 based on single-cell RT-PCR in ovariectomized female mice [80].

Reproduction is often considered a long process, not in need of the fast-acting responses to hormonal control of such behaviors like feeding. However, recent research indicates that despite this nature, the rapid signaling of Gq-mER plays a role. Early studies showed that GnRH neurons do not contain nuclear ER α [81], leading to a hypothesis that estrogenic control over GnRH activity is mediated through presynaptic neurons that do express nuclear ER α and produce kisspeptin and other regulators of GnRH neurons [31]. However, the recent discovery that ER β is expressed in GnRH neurons [82] and the exploration of membrane-initiated E2 signaling are overturning the old dogma that GnRH neurons do not directly sense circulating E2.

Over the past decade, GnRH neuronal excitability has been demonstrated using calcium imaging and other techniques to show that E2 has both excitatory and inhibitory effects via either direct or indirect (presynaptic) mechanisms [83–88]. Some of these effects are rapid, membrane-initiated E2 responses potentially through the Gq-mER. E2-BSA was able to mimic an increase in GnRH neuronal activity and synchronization and was reversed by co-perfusion with ICI 182,780 or the pertussis toxin, which blocks G-protein coupled receptors (GPCR), indicating that this effect was produced by a membrane-bound estrogenic GPCR [84]. Using similar approaches, other studies were able to show varying responses to E2, and the variability in effect indicates that GnRH neurons may have specific subpopulations, depending on their differential expression of ER types [83, 84, 87, 88]. Expression profiles within the neurons may determine through which receptor signaling pathway (ER β , GPER, Gq-mER, etc.) E2 regulates GnRH neuronal excitability and synchronization.

The mechanism by which the excitability and synchronization of GnRH neurons is regulated is believed to be through calcium signaling. Influx of Ca²⁺ into the cell via T-type Ca²⁺ ion channels is mediated by E2. E2 regulates expression of T-type channel subtypes (Cav3.1, Cav3.2, and Cav3.3) within the arcuate nucleus and increases peak T-type Ca²⁺ current density in arcuate neurons [89, 90]. Regulation of Cav3.1 expression is dependent on ER α , and regulation of Cav3.2 is dependent on ER α and ER β [90]. Furthermore, E2 regulates channel activity and subunit expression in GnRH neurons using patch clamp electrophysiology coupled with single-cell type quantitative real-time PCR from EGFP-tagged GnRH transgenic mice. Interestingly, STX mimicked E2's regulation of subunit expression (an increase in Cav3.3), indicating that the Gq-mER also plays a role in the activity of T-type channels in the hypothalamus in addition to ER α and ER β (see Figure 1) [80]. STX also modulates GnRH neurons from primate models. Treatment with 10 nM STX increased [Ca²⁺]_i oscillation frequency and synchronized the frequency of these oscillations. STX also increased the percentage of stimulated cells and augmented GnRH release, although at lower magnitude than E2. Additionally, ICI 182,780 and the PLC inhibitor U73122 blocked the STX-induced [Ca²⁺]_i oscillation, further indicating the role of Gq-mER [91].

GnRH is a vital hormone in regulating the reproduction cycle and sexual receptivity. Because STX and the Gq-mER pathway were shown to be involved in regulating GnRH neurons, the next step is to determine whether the receptor has a physiological effect on reproduction. E2 is an essential hormone in regulating reproduction, most often acting as negative feedback in the HPG axis but also having rapid, local effects on sexual behaviors.

In males, sexual motivation has been shown to be rapidly regulated by E2, in a time period less than most protein production [92, 93]. This indirectly suggests that E2 is acting via a nongenomic pathway to regulate male sexual motivation. However, STX was shown to have no effect in both rats and quails [28, 94]. GPER and ER α are also not involved. This effect on male sexual motivation appears to be mediated primarily through ER β and its interaction with the metabotropic glutamate receptor 1a (mGluR1a) [94]. In female rats, however, STX stimulated sexual receptivity and induced activation and internalization of μ -opioid receptors in the medial preoptic nucleus, an action necessary for producing lordosis. If an antagonist to mGluR1a was pre-administered, internalization of μ -opioid receptors were not seen [28, 29]. This suggests that the putative Gq-mER interacts with mGluR1a to rapidly activate cellular signaling to augment lordosis behavior.

Gq-mER activity in corticotropin-releasing hormone and extrahypothalamic neurons

In a recent study from our laboratory [33], activation of the Gq-mER in paraventricular corticotropin-releasing hormone (CRH) neurons increased neuronal excitability in female mice. CRH neurons play a key role in the hypothalamus-pituitary-adrenal (HPA) axis, which mediates hormonal adrenal action, and E2 directly modulate these neurons [95, 96]. Through use of whole-cell electrophysiology, Hu *et al.* (2016) found a rapid attenuation of the M-current (a voltage-dependent, inwardly rectifying K⁺ current) in CRH neurons after perfusion of exogenous E2. The M-current is a constitutively active hyperpolarizing current that suppresses neuronal excitability, so inhibition of the M-current by E2 leads to an excitation of CRH neurons. Co-administration of E2 with inhibitors of the Gq-PLC-PKC-PKA signaling pathway blocked E2's attenuation of the M-current. Furthermore, a selective ER inhibitor also blocked inhibition, and STX mimicked the actions of E2 (see Figure 1). This study additionally examined the *in vivo* effects of Gq-mER signaling by injecting ovariectomized mice with E2 or STX, which elicited an increase in c-fos mRNA expression in CRH neurons and a corresponding rise in plasma corticosterone. This evidence indicates that Gq-mER signaling is involved in the regulation of CRH neurons and the HPA axis and may participate in E2's observed effect on HPA-associated mood disorders [97, 98].

Sex differences in pain perception and analgesic drug efficacy are present in both animal models and humans and in the prevalence of pain-related diseases such as fibromyalgia, migraines, and arthritis [99–104]. The mechanistic nature of these differences is not well known, but estrogens may be an important factor. E2 attenuate GPCR-mediated antinociception [105, 106]. Recent evidence indicates that fast-acting, membrane-bound receptors such as the Gq-mER may contribute to this effect [107]. Nag *et al.* (2014) found that STX rapidly attenuated antinociception induced by the α 2-adrenoceptor agonist, clonidine, as measured by the tail flick test in ovariectomized female rats in a dose-dependent manner. STX and other drugs were intrathecally administered 5 min prior to clonidine, and the resulting decrease in latency observed with STX treatment indicates an inhibition of antinociception or an increase in pain perception. The ER antagonist ICI 182,780 blocked this effect and E2-BSA mimicked the effects of STX. Furthermore, STX increased spinal cord levels of phosphorylated extracellular signal regulated kinase (ERK),

and *in vivo* inhibition of ERK phosphorylation with U0126 blocked the attenuation effect of STX on antinociception. PKA and PKC phosphorylation were altered by STX, indicating that ERK is the primary mediator of the STX-initiated, Gq-mER signaling cascade that inhibits the antinociceptive actions of clonidine.

Accumulating evidence has shown that estrogens have important beneficial effects in the aging body that protect against cardiac incidents, ischemic injury recovery, and neurodegeneration [108–110]. Consequently, this led to the idea of using synthetic estrogens as a hormone replacement therapy for postmenopausal women, who experience a decline in estrogen production. However, there is considerable controversy about the use of hormone replacement therapy as prolonged exposure to estrogens increases risk of breast cancer and thrombosis [111, 112]. Lebesgue *et al.* (2010) hypothesized that non-classical estrogenic ligands, such as STX and the GPER selective agonist G1, might exhibit these neuroprotective effects of E2, while reducing or eliminating the deleterious side-effects of synthetic estrogens that target nuclear ER. In that study, middle-aged rats were subjected to 10 min of global ischemia followed immediately by reperfusion eight weeks after ovariectomy. Rats were immediately injected with either E2, G1, or STX directly into the lateral ventricle. Hippocampal neuronal survivability was assessed one week later. All estrogenic ligands were neuroprotective (55–60% survivability vs. 15% in the controls). A single systemic injection of E2 was also shown to be protective (~50%). This work suggests that activation of estrogen-responsive GPCR (using STX or G1) might be a useful replacement for standard hormone therapy and reduce the susceptibility to stroke in postmenopausal women.

In a recent paper by Gray *et al.* (2016), the neuroprotective effects of STX were examined in the context of amyloid β ($A\beta$) toxicity in neuroblastoma cell lines as well as primary hippocampal neurons from wild-type and Alzheimer model Tg2576 mice [113]. STX reduced cell death, mitochondrial dysfunction, dendritic simplification, and synaptic loss from levels seen in control $A\beta$ -exposed cells. In primary neurons, STX also increased ATP as well as mitochondrial gene expression in both genotypes. This paper indicated that STX can be neuroprotective against $A\beta$ toxicity and may also prove to be useful outside this Alzheimer model, as protective effects were reported in the absence of $A\beta$. Supporting these data are reports of Gq-mER-initiated PKA, ERK, and PI3K signal manipulations increasing mitochondrial activity and protecting against the impaired bioenergetic states caused by $A\beta$ toxicity (see Figure 1) [114, 115]

Lastly, cortical neurons in primary culture from rat pups sorted by sex, 5-min E2 pretreatment protected against glutamate toxicity 24 h later in neurons from females, but from males [116]. ER α and ER β were expressed in these cultures and the ER α -selective agonist PPT replicated these effects while ER α antagonist methyl piperidino pyrazole (MPP) blocked them. The ER β selective agonist diarylpropionitrile (DPN) exhibited a small protective effect in both female- and male-derived neurons. Membrane-delineated receptor mechanisms were also tested via STX and G1 administration. STX was neuroprotective against glutamate toxicity in both female- and male-derived cortical neurons, while G1 had no significant effect. Interestingly, E2-BSA was also shown to not have an effect. These results indicate that the sexually dimorphic neuroprotective effect by estrogenic compounds

is primarily mediated by ER α , and not by other ER receptors (ER β or GPER), while the Gq-mER is neuroprotective against glutamate toxicity in both female- and male-derived cortical neurons.

Conclusions

Research continues to accumulate supporting the significance of membrane-initiated estrogen signaling, and it is becoming increasingly evident that these receptors play more of a role than previously assumed. Also, their involvement in neurophysiology is complex and interlaced with “classical” ER α/β nuclear-initiated and membrane-initiated signaling. As the compound becomes more readily available, STX is proving to be an essential tool for analyzing how the Gq-mER modulates the well-characterized melanocortin pathway, as well as extra-hypothalamic neurons. While the effects of STX and Gq-mER activation are easily examined, the structure and gene sequence of the putative Gq-mER are still unknown. Therefore, receptor identification is of paramount importance as it will provide another tool with which to explore the expanding topic of rapid estrogen signaling.

Acknowledgments

This research was supported by funds from USDA-NIFA (NJ06107) and from the National Institutes of Health (R00DK083457, R00DK083457-S1, and P30ES005022). G.V. was supported by the National Institute of Environmental Health Sciences (T32ES007148).

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Figure 1.

A model cell illustrating the Gq-mER signaling pathway in hypothalamic neurons. (1) E2 (or STX) activates a membrane-associated ER (mER) that is Gq-coupled. The Gαq protein activates PLC to catalyze the hydrolysis of membrane-bound PIP₂ into IP₃ and DAG. (2) DAG activates PKCδ to augment adenylyl cyclase (AC_{VII}) activity and generate cAMP, which in turn activates PKA. (3) In POMC neurons, activation of PKA attenuates the GABA-B receptor-mediated activation of GIRK channels, while in NPY neurons, PKA activation potentiates GIRK channel activity. (4) In CRH neurons, PKA phosphorylates KCNQ subunits to inhibit the M-current, which may also be inhibited by the hydrolysis of PIP₂ in the membrane into free IP₃ and DAG. (5) PKA activation may also lead to the phosphorylation of AMPAR subunits augmenting membrane trafficking and recruitment to rapidly increase the amplitude of AMPAR currents in CRH neurons. (6) STX also increases the expression of Cav3 (T-type Ca²⁺) channels in GnRH neurons, presumably through the PKA-mediated phosphorylation of pCREB, leading to gene regulation through the cAMP response element (CRE). (7) Activation of the Gq-mER has also been associated with PI3K and ERK signaling pathways in hypothalamic and hippocampal neurons.