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Physiological consequences of membrane-initiated estrogen signaling in the brain

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1. ABSTRACT

Many of the actions of 17beta-estradiol (E2) in the central nervous system (CNS) are mediated via the classical nuclear steroid receptors, ERalpha and ERbeta, which interact with the estrogen response element to modulate gene expression. In addition to the nuclear-initiated estrogen signaling, E2 signaling in the brain can occur rapidly within minutes prior to any sufficient effects on transcription of relevant genes. These rapid, membrane-initiated E2 signaling mechanisms have now been characterized in many brain regions, most importantly in neurons of the hypothalamus and hippocampus. Furthermore, our understanding of the physiological effects of membrane-initiated pathways is now a major field of interest in the hypothalamic control of reproduction, energy balance, thermoregulation and other homeostatic functions as well as the effects of E2 on physiological and pathophysiological functions of the hippocampus. Membrane signaling pathways impact neuronal excitability, signal transduction, cell death, neurotransmitter release and gene expression. This review will summarize recent findings on membrane-initiated E2 signaling in the hypothalamus and hippocampus and its contribution to the control of physiological and behavioral functions.

2. INTRODUCTION

Estrogen receptors (ERs), ERalpha and ERbeta, were initially identified as estrogen-responsive nuclear transcriptions factors to modulate gene expression (1,2). For most of the past 30 years, the actions of estrogens (17beta-estradiol, E2) were thought to mediate only long-term transcriptional effects in the brain by either organization of neurogenesis and neural circuitry during embryonic and neonatal development or activational control of gene expression during the later stages of the life cycle (3). However, it has become quite clear that E2 has rapid, membrane-delimited effects that are independent of ER transcriptional control. These rapid effects were first identified in the uterus and hypothalamus decades ago (4,5,6), but only in the past 10-15 years has the physiological significance of these acute E2 effects been investigated (7,8,9,10,11,12).

Rapid membrane-mediated effects of E2 have been identified in the hippocampus and in various hypothalamic nuclei (7,13,14) but may occur throughout the central nervous system. Membrane-initiated estrogen signaling involves the rapid activation of various protein kinase pathways including protein kinase C (PKC), protein kinase A (PKA), phosphatidylinositol-3 kinase (PI3K), and

mitogen-activated protein kinase (MAPK), to modulate signal transduction, protein phosphorylation and cation channel activity (15,16,17,18). In the hypothalamus, membrane signaling has been implicated in gonadotropin secretion and sexual behavior (19,10,20). While many aspects of reproduction are controlled by nuclear-initiated estrogen signaling (21,22), it appears that membrane E2 signaling plays a vital, modulatory role in these reproductive functions. Other hypothalamic homeostatic functions modulated by membrane signaling include energy homeostasis and thermoregulation, which appear to be modulated by a Gq-coupled membrane ER functionally characterized in hypothalamic neurons (arcuate, preoptic area). In the hippocampus, membrane E2 signaling has been implicated in the neuroprotective effects of E2 in rodent ischemia models and may have a role in memory and cognition (23,24).

3. MEMBRANE E2 SIGNALING

Nuclear-initiated estrogen signaling controls cellular functions and gene expression via the classical ERs binding to the estrogen response element (ERE) or to other promoter sites through protein-protein interactions such as Sp-1 and Fos-Jun (AP-1) and activating transcription of important estrogen responsive genes (25). Briefly, E2 can activate transcription via complexes with other transcription factors through protein-protein interactions including pCREB, STATs, Elk-1-SRF, ATF-2-Jun and NFkappaB inducing transcription via their respective promoter sites. In membrane-initiated estrogen signaling, E2 can activate a host of rapid signaling cascades that affect cell function and modulate gene expression through other transcription factor promoter sites using membrane-associated ERs or novel G-protein coupled E2 receptors (26,27,28,29). E2 through these receptors activates multiple signaling pathways including PI3K, phospholipase C (PLC), MAPK, extracellular signal-regulated protein kinase (ERK) and protein kinase pathways (PKA, PKC, etc.) (26,27,28,29,30).

While ERalpha and ERbeta can be associated with the membrane (31), the steroid nuclear receptors do not have extracellular domains that are common for growth factor, cytokine and G protein-coupled receptors (GPCRs). In heterologous cell systems, the classical ERs are associated with the membrane via lipid anchors that attach them to membrane components or via associations with GPCR. ERs can be modified with lipids (palmitoylated) to attach to the plasma membrane and thereby interact with the membrane proteins such caveolin-1 to initiate signal transduction pathways (32,33). Another mechanism characterized in the hypothalamus is the association of ERs to other GPCRs or tyrosine kinase receptors to initiate cell signaling. Primary examples of this novel type of cell signaling are the ligand-initiated association of classical ERs with metabotropic glutamate receptors (mGluRs) (34) or interaction with the IGF-1 receptor (35) to facilitate female reproductive behavior. These associations, between ERs and other receptors, have implications for sexual behavior and positive feedback effects of E2 on GnRH release.

There are several potential candidates for novel membrane ERs including ER-X and two G-protein-coupled receptors, GPR30 and Gq-mER (36,37,38,11,30,39), which offer several targets for E2 to rapidly alter cell functions in the brain. ER-X was identified as a high-affinity, saturable estrogen receptor with sequence homology to the classical ERs that is associated with caveolar-like microdomains in developing neocortical neurones (40). E2 induces tyrosine phosphorylation of both ERK1 and ERK2 in organotypic explants of the developing cerebral cortex (36). Interestingly, the steroid pharmacology of ER-X is distinct in that 17alpha-estradiol is equipotent as E2 in activating the MAPK/ERK pathway and the receptor is insensitive to the anti-estrogen ICI 182,780 (15,40). GPR30 (GPER1), a GPCR, which binds E2 with nanomolar affinity, is involved in the rapid actions elicited by E2 in peripheral reproductive tissue (41,38) and activates the ERK pathway independently of ERalpha or ERbeta (42,30). GPR30 is expressed in the hypothalamus especially in the paraventricular nucleus, supraoptic nucleus and the preoptic area (43,44). However, the pharmacology of GPR30 in these neurones has not been characterized.

We have generated compelling electrophysiological and physiological evidence supporting the hypothesis of a hypothalamic, Gq-coupled membrane ER (Gq-mER). Using whole cell recordings from guinea pig and mouse hypothalamic slices, we have characterized a Gq-mER that activates a PLC-PKC-PKA pathway in response to E2 to significantly attenuate the potency of mu-opioid and $GABA_B$ agonists in activating an inwardly rectifying K^+ (GIRK) conductance at low nanomolar concentrations (EC_{50} = 8 nM) in arcuate POMC neurons (See Figure 1A) (11,37,45). In GnRH neurons, E2 (EC_{50} = 0.6 nM) activates K_{ATP} channels via the same signaling pathway (46). Therefore, the putative Gq-mER is a novel E2-activated membrane receptor in hypothalamic (POMC, GnRH) neurons that awaits definitive characterization by the cloning of the gene.

4. MEMBRANE E2 SIGNALING AND REPRODUCTION

E2 is a key player in the positive and negative feedback loops on GnRH and LH secretion during the ovulatory cycle. In rodents, during negative feedback, low levels of E2 inhibit GnRH neuronal output during most of the estrous cycle. However, on the afternoon of proestrus, E2 production from maturing ovarian follicles is elevated and these peaking levels of E2 in combination with a circadian signal stimulates GnRH neurons to produce the surge of LH from pituitary gonadotropes (47). Therefore, E2 positive feedback is crucial for the LH surge to initiate ovulation. The development of an ERE-independent ERalpha signaling mouse model is a technological advance in understanding the role of nonclassical (non-ERE) signaling in E2-mediated negative and positive feedback (21). This mouse model has lost the capacity for ERalpha to bind to the ERE due to mutations in the DNA binding domain of ERalpha. Using this mouse model, the effects of E2 on positive feedback were determined to depend upon ERE-dependent signaling of ERalpha in neurons (21)

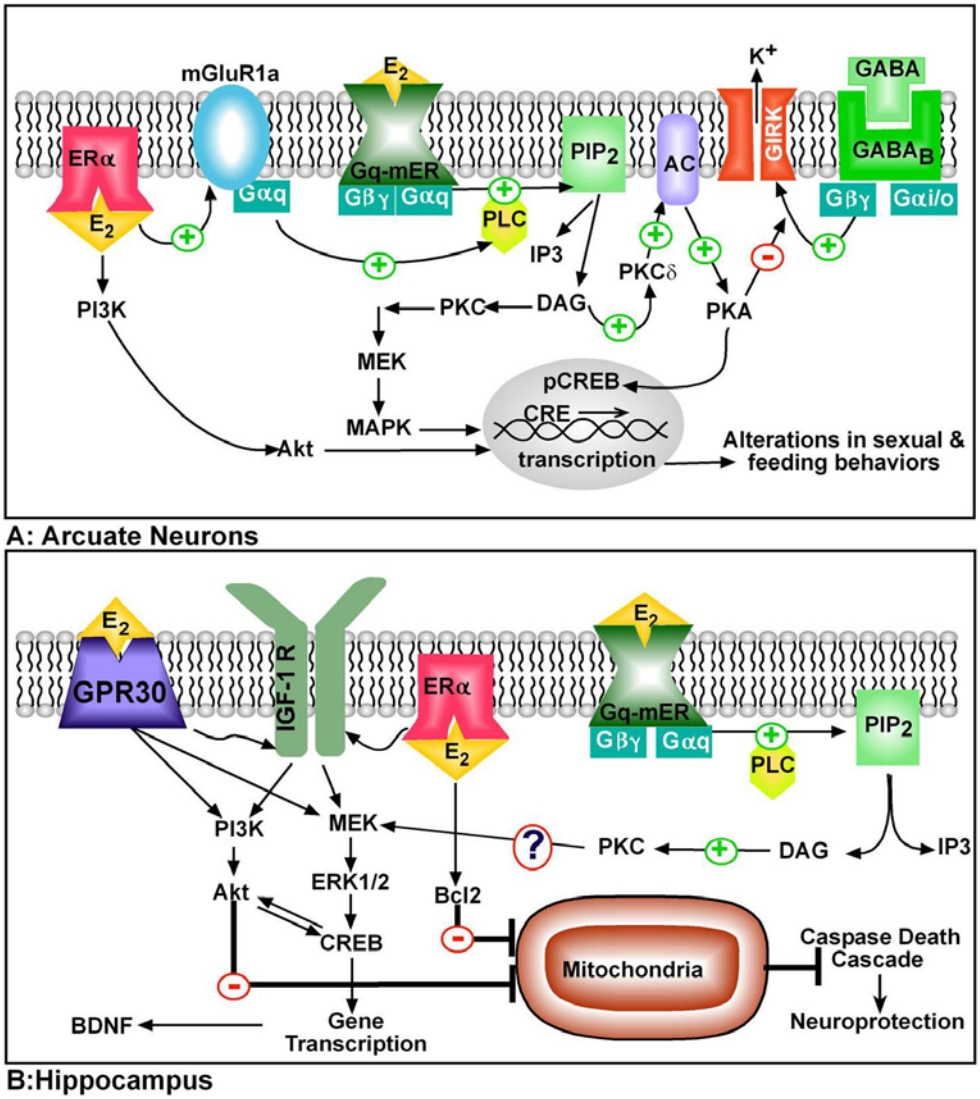

Figure 1. Cellular models of membrane-initiated E2 signaling pathways in the brain. A: In arcuate neurons including those that express POMC, dopamine, and GABA, E2 activates the Gq-mER leading to activation of PLC-PKC-PKA pathway. Activation of the receptor initiates the hydrolysis of membrane-bound phosphatidylinositol 4,5-biphosphate (PIP2) to IP3 and diacylglycerol (DAG) via PLC. DAG activates protein kinase Cdelta (PKCdelta), which through phosphorylation, upregulates adenylyl cyclase VII (AC) activity. The generation of cAMP activates PKA, which can rapidly uncouple $GABA_B$ receptors from their effector system through phosphorylation of a downstream effector molecule (e.g., G protein-coupled, inwardly rectifying K^+ (GIRK) channel). The mER-mediated modulation of kinase pathways reduces the capacity of inhibitory neuromodulators such as GABA via $GABA_B$ receptor (and opioid peptides via mu-opioid receptor, not shown) to reduce neuronal excitability. The Gq-mER-mediated activation of PKA can lead to phosphorylation of cAMP-responsive element binding protein (pCREB), which can then alter gene transcription through its interaction with CREs on genes. Also, in arcuate (NPY?) neurons, ERalpha interacts with metabotropic glutamate receptor 1a (mGluR1a) to initiate Gq stimulation of PLC, which leads to MAPK-induced CREB phosphorylation. Membrane-associated ERalpha can activate PI3K/Akt signaling. B: Membrane-initiated E2 signaling treatment may act through multiple cellular pathways for its neuroprotective actions. Acute E2 administration may bind to either classical membrane ERs (ERalpha is shown) and/or GPR30. Through the membrane-associated ERs, E2 can either directly control Bcl-2 (anti-apoptotic protein) gene expression or by transactivation of IGF-1 receptors for a sustained activation of the ERK/MAPK pathway. GPR30 may block apoptotic cascades through the direct activation of either ERK/MAPK or PI3K/Akt pathways via the transactivation of receptor tyrosine kinases (IGF-1 and/or Trk-B). BDNF, a target of activated CREB, is thought to bind the receptor tyrosine kinase Trk-B to activate both the MAPK and PI3K pathway to promote neuroprotection. Stimulation of the PI3K/Akt pathway by BDNF would inactivate the pro-apoptotic proteins to halt the caspase death cascade. STX treatment also offers neuroprotection from ischemia via an unknown mechanism, but a possible pathway is the PKC-activation of MAPK signaling.

presumably presynaptic to GnRH neurons since ERalpha is not expressed in GnRH neurons. However, during negative feedback, E2, in part, utilizes ERE-independent signaling to inhibit GnRH and/or LH secretion, but not synthesis (gene expression), through a rapid, p21-activated kinase (PAK1)-mediated pathway (21,48,49). Interestingly, the expression of arcuate kisspeptin, a major excitatory drive for GnRH neurons, is inhibited by E2 via an ERE-independent signaling mechanism during the negative feedback period (50).

4.1. Membrane E2 signaling and GnRH secretion

An acute direct effect of E2 on GnRH neuronal activity was first described over twenty-five years ago (5). Although the effects of membrane E2 signaling on GnRH neurons in the hypothalamus have been extensively reviewed (14,7,51,52), new insights into the multiple signaling events involved in GnRH neuronal activity and release suggest that E2 has multiple pathways to rapidly modulate these vital reproductive neurosecretory cells (53,46,54).

Studies from our lab have previously shown that in guinea pigs E2 will rapidly hyperpolarize GnRH neurons (5,55,46). Recently, in mouse GnRH neurons, high physiological doses of E2 enhanced action potential firing by modulating the intrinsic afterhyperpolarizing and afterdepolarizing potentials in PKA-dependent mechanisms involving ERbeta. Conversely, in these neurons, low physiological doses of E2 had an effect on neuronal activity via GABAergic and glutaminergic inputs suggesting that some of the inhibitory inputs of E2 occur via presynaptic mechanisms (54). In addition, an acute effect of sub-nanomolar E2 potentiates K_{ATP} channel activity via PKC and PKA pathways to maintain hyperpolarization. The K_{ATP}-controlled hyperpolarization is potentially involved in recruitment of excitatory channels that are critical for burst firing of GnRH neurons including the T-type calcium channel (53,46). Furthermore, E2 via activating ERbeta and/or GRP30 receptors rapidly potentiates high-voltage-activated Ca^{2+} currents (L- and R-type Ca^{2+} channels) suggesting that Ca^{2+} homeostasis is a target for membrane signaling in the GnRH neurons (56). In the primate, E2 rapidly increases firing frequency, spike density and burst duration through a membrane-delimited pathway in monkey placode GnRH neurons; however, since this study did not block presynaptic inputs with TTX, the effects of E2 may not be directly on GnRH neurons (57). These studies support the hypothesis that one of the important signaling effects of E2 on GnRH function is to modulate neuronal excitability by modulating intrinsic cation channel activity.

Another membrane-initiated effect of E2 is the modulation of Ca^{2+} oscillations in primate and mouse GnRH neurons. Ca^{2+} oscillations in GnRH neurons synchronize with a periodicity of approximately 60 minutes (58,59,60), which is similar to the pulsaltile rhythm of GnRH peptide release (61,58,62). Furthermore, perfusion of primate GnRH neurons with nanomolar concentrations of E2 alters the patterns of Ca^{2+} oscillations via a membrane-delimited (E2-dendrimer) mechanism (63). The E2 signaling mechanism modulating the Ca^{2+} oscillations in primate GnRH neurons is suppressed by pertussis toxin treatment and by knockdown of GPR30 mRNA, and mimicked by the GPR30 agonist, G1 (44). In the mouse, Ca^{2+} oscillations are blocked by ICI 182,780 and mimicked by E2-BSA (59,60). The actions of E2 are also abrogated by PTX treatment. Wray and colleagues hypothesize that the effects of E2 are ERbeta-mediated, whereas Terasawa and colleagues hypothesize that it is GPR30-mediated since ICI 182,780 does not block the actions of E2.

Membrane-initiated E2 signaling may be involved at multiple levels of GnRH release into the median eminence and LH secretion from the pituitary during the ovulatory cycle. In the primate GnRH study, E2 stimulates the release of GnRH from cultured primate GnRH neurons via a membrane-initiated mechanism within 20 min of administration (44). At the level of the median eminence, membrane signaling events (by using E2-BSA) are implicated in the E2-induced release of GnRH from nerve terminals via activation of nitric oxide from the median eminence vascular endothelial cells (64). Conversely, membrane-initiated signaling mechanisms, potentially via ERalpha, are required for the acute suppression of LH *in vitro* from ovine pituitary cultures after GnRH stimulation (19,65). In an immortalized GnRH cell line, E2 via a Gi-coupled, membrane receptor-mediated mechanism inhibits cAMP production and GnRH release at picomolar concentrations (20). Collectively, these data suggest that membrane signaling is necessary for many of the pre- and postsynaptic mechanisms regulating GnRH neurons to ultimately control reproduction.

4.2. Membrane E2 signaling and sexual behavior

Both nuclear-initiated and membrane-initiated E2 signaling are involved in the regulation of sexual behavior, and specifically, lordosis, the female response to intromission by the male characterized by the arching of the spine. Lordosis behavior is heightened during estrus and is controlled by E2 through a complex hypothalamic circuitry involving the arcuate, the VMH and medial preoptic area (mPOA) (66,34). Systemic administration of E2 in ovariectomized rats activates IGF-1 receptors and induces the association between Insulin-like Growth Factor-1 (IGF-1) receptors and ERalpha in the mPOA (67,68,69). There is an interaction between the p85 subunit of phosphatidylinositol 3-kinase (PI3K) and ERalpha within 1-3 h, which leads to activation of protein kinase B/Akt, a serine/threonine kinase that has multiple downstream targets (68,69). The E2-induced activation of IGF-1 receptors augments alpha-1-adrenergic receptor signaling, and blockade of IGF-1 receptors prevents E2-induced increases in alpha-1-adrenergic receptor binding density as well as IGF-1 enhancement of noradrenergic receptor signaling, which is critical for expression of reproductive functions (67,70). The cross-talk between the E2 and IGF-1 receptor signaling pathways appears to contribute to synaptic remodeling and neuronal plasticity during the estrous cycle. Moreover, intracerebroventricular (i.c.v.) infusion of a selective competitive blocker of IGF-1 autophosphorylation (JB-1) inhibits the E2-induced LH surge and sexual behavior in ovariectomized, E2-treated

rats (70). In addition, co-administration (i.c.v.) of inhibitors of PI3K (wortmannin) and MAPK (PD98059) inhibit the long-term (48 h) effects of E2 to induce the LH surge and facilitate lordosis behavior (71). Therefore, facilitation of female sexual behavior by E2 appears to involve activation of both PI3K and MAPK signal transduction pathways. The importance of growth factors for female sexual behavior is further highlighted by observations that epidermal growth factor (EGF) and also IGF-1 can, in the absence of E2 and progesterone (within 1-4 h of i.c.v. administration) induce mating behavior in rats and mice, in part, through an ERalpha-dependent mechanism (72). This relatively rapid, ligand-independent ER action is in striking contrast to the well established finding that E2 priming over a period of at least 24 h is needed for progesterone induction of female reproductive behavior (73). Therefore, the ability of both IGF-1 and E2 to induce female sexual behavior may involve complex interactions between ERalpha, the IGF-1 receptor and the PI3K p85 subunit.

Furthermore, the lordosis neural circuitry involves the actions of membrane E2 signaling via the initiation of an ERalpha-mediated pathway in the arcuate nucleus leading to the release of beta-endorphin (beta-END) in the mPOA and an internalization of the mu-opioid receptor (MOR). The internalization of MOR occurs rapidly (< 30 min of E2 treatment) and is correlated with an initial inhibition of the lordosis behavior in females (74,75). Membrane-impermeant E2 administered into the arcuate nucleus also initiates the MOR internalization (76). The short latency for the E2-induced release of beta-END and subsequent inhibition of lordosis indicates that these actions are initiated at the membrane and/or occur in the cytoplasm and not by gene expression. The transient inhibition of lordosis by E2 appears to be necessary for full sexual receptivity measured 30 h after treatment (76). Interestingly, in MOR knockout mice, females exhibit a diminished lordosis response even after E2 treatment (74,77). One question that remains is how does the transient, E2-induced activation and internalization of MOR in the mPOA facilitate full sexual receptivity 30 h later? One possibility is that activation of MOR produces a transient inhibition of mPOA neurons that rebound from a hyperpolarized state with a facilitated firing and drive to the neurons regulating sexual behavior.

Furthermore, the internalization of MOR and the inhibition of lordosis behavior by membrane-associated ERalpha-mediated signaling depend on mGluR1a signaling. ERalpha and mGluR1a are co-expressed and co-immunoprecipitated using membrane fractions from primary, hypothalamic astrocyte cultures or in HEK cells transfected with both ERalpha(-EGFP) and mGluR1a (76,78). In cultured hypothalamic astrocytes, the membrane-initiated actions of E2, at picomolar concentrations, increases free cytoplasmic Ca^{2+} levels from intracellular calcium stores within seconds by a membrane-associated, ERalpha-mediated mechanism interacting with mGluR1 receptors (79,80,78). The mGluR1a antagonist, LY367385 ((S)-(+)-a-Amino-4-carboxy-2-methylbenzeneacetic acid) blocks the actions of E2 in astrocytes, indicating that the interactions between ERalpha and mGluR1 are necessary for the E2-induced Ca^{2+} flux (76,81).

As in the blockade of E2's actions in astrocytes, LY367385 prevents the E2-induced MOR internalization and the inhibition of lordosis behavior and an mGluR1a agonist, DHPG ((S)-3,5-dihydroxyphenylglycine), mimics the E2-induced activation/internalization of MOR in the mPOA (76). The activation of an ERalpha-mGluR1a complex initiates multiple signaling pathways including PLC-PKC and PKA, which was shown previously to stimulate lordosis behavior (82,12,83). This is similar to STX-activated Gq-mER pathway in arcuate neurons (37,11). These data collectively suggest that the membrane-initiated interactions of the ERalpha-mGluR1a complex via a PKC-mediated pathway are a component of the E2 control of MOR internalization in the mPOA and the regulation of lordosis behavior.

Finally, the membrane-mediated effects of E2 potentially modulate male sexual behavior (84). In rodents, rapid or membrane-mediated effects of E2 on male sexual behavior were initially identified in castrated rats. Peripheral injection of E2 at high doses increased genital sniffs and total mounts while decreasing the latency to mounting within 35 min of administration (85). Membrane-delimited E2-BSA, when chronically administered via cannulation directly into the medial preoptic area, significantly increased mounting behavior, intromissions and ejaculations and decreased the latency to mounting and ejaculation in castrated, dihydrotestosterone-treated rats (86) but not when chronically administered into the medial amygdale (87). In wild-type male mice, acute peripheral injections of E2 attenuate the inhibition of mounting frequency and intromission frequency by aromatase inhibitors within 10 minutes of administration. Furthermore, in aromatase knockout male mice, E2 can increase intromission frequency within 10 minutes of administration and increase mounting frequency within 30 minutes (88). Therefore, E2, within 10-30 minutes, can control male sexual behaviors potentially using similar membrane-mediated cellular mechanisms as in females.

5. MEMBRANE E2 SIGNALING AND HOMEOSTASIS

5.1. Membrane E2 signaling and energy balance

E2 controls multiple hypothalamic homeostatic functions including the regulation of energy homeostasis and core body temperature. The nuclear-initiated signaling of ERalpha is a necessary component of the regulation of energy homeostasis by E2 (defined as the balance between energy intake and energy expenditure) (89). However, there is little or no direct evidence suggesting a role for the activation of membrane-associated ERalpha signaling pathways in the control of energy homeostasis. E2 attenuates food intake within 6 h of administration into the third ventricle via cannula after an overnight fast compared to saline in mice (90). In fed rats, E2 reduces food intake in the period between 4 and 14 h after administration (91). The short-term E2-induced effects on food intake do

suggest that membrane-mediated signaling events may be involved in the estrogenic regulation of energy homeostasis. Furthermore, there is compelling evidence for a novel mechanism that modulates different aspects of energy homeostasis.

A Gq-mER signaling pathway has been elucidated in hypothalamic arcuate neurons (POMC, GABA, dopamine) and was initially characterized in female guinea pigs (37,11), but has also been functionally examined in ERalpha and ERbeta knockout both male and female and in male ERalpha-beta double-knockout mice (11) as well as GPR30 knockouts (92). E2 stereospecifically activates a Gq-mER pathway and is blocked by the anti-estrogen ICI 182,780 (93,45). The steroid stereospecificity of the Gq-mER suggests a pharmacologically distinct receptor compared to ER-X. As described above, the Gq-mER activates a PLC-PKC-PKA pathway that attenuates the activation of GIRK channels by both $GABA_B$ receptors and MOR receptors. The attenuation of the inhibitory presynaptic inputs increases the excitability of anorectic POMC neurons. To target this E2 pathway, a selective agonist (STX), which is structurally similar to 4-OH tamoxifen, was developed to have no measureable binding affinity for the classical ERs (94). In fact, STX has a greater affinity (~20-fold) for the Gq-mER than E2 and has no affinity for ERalpha/beta (11).

Using a series of pharmacological agents, the signaling pathway that is activated by E2 and STX in hypothalamic neurons has been identified (See Figure 1A). The signaling mechanism begins with the binding of the ligand to a Gq-mER and the activation of G-alpha-q. Activated G-alpha-q initiates the hydrolysis of PIP_2 by PLC to liberate DAG. Free DAG stimulates PKCdelta, and PKCdelta activates adenylyl cyclase (VII) to elevate cAMP levels and stimulate PKA. PKA, through phosphorylation, uncouples the inhibitory $GABA_B$ and MOR receptors from activation of GIRK channels (Figure 1A) (37). Furthermore, PKA will phosphorylate CREB (cAMP response element-binding protein) to initiate gene expression via cAMP response element (CRE) and IP_3 may release calcium through the IP_3 receptor on the endoplasmic reticulum. Other transcriptional pathways that the putative Gq-mER may activate include the MAPK, calcium-activated and PI3K pathways (95).

The ability of STX to mimic E2's modulation of POMC activity led to the hypothesis that the putative Gq-mER has a role in energy homeostasis. Compelling, reproducible evidence demonstrates that STX peripheral administration mimics the effects of E2 on energy homeostasis using whole animal physiological studies (11,96,97). The E2 (STX)-induced increase in POMC neuronal activity is predicted to reduce food intake and, subsequently, the post-ovariectomy body weight gain. Indeed, STX inhibits food intake in ovariectomized guinea pigs by reducing meal frequency similarly to E2 treatment and a subsequent reduction in abdominal fat accumulation (97). Furthermore, STX administration generates new transcription in the arcuate nucleus of the STX-treated female guinea pigs (96). Several of the regulated arcuate genes are involved in the control of energy homeostasis (e.g., neuropeptide Y) and neuronal activity (e.g., Cav3.1). Therefore, Gq-mER may function in the estrogenic control of energy homeostasis, presumably through activation of POMC neurons in the arcuate nucleus, although other hypothalamic neurons may be involved.

5.2. Membrane E2 signaling, thermoregulation and bone remodeling

Another homeostatic function that E2 has a role in is the maintenance of core body temperature (Tc). In a recent publication, peripheral administration of STX reduced Tc significantly compared to the vehicle control similarly to E2 (97). The exact cellular mechanism for E2's control of Tc is not known. A potential mechanism is the direct action of E2 on thermosensitive (GABAergic) neurons in the preoptic area of the hypothalamus (98,99,100). Previous studies in female guinea pigs have shown that mPOA GABAergic neurons respond to acute E2 treatment via a membrane-initiated pathway to attenuate $GABA_B$ autoinhibition leading to increased neuronal activity (101). It is not known if these E2-sensitive neurons are also the same warm-sensitive neurons characterized by Boulant and colleagues (102).

The Gq-mER is similar to other Gq-coupled GPCRs involved in thermoregulation such as the 5HT receptors (5HT2A/2C), which activate signaling pathways to lower Tc and are implicated in thermoregulation dysfunction caused by ovariectomy (103,104). Both the Gq-mER and 5HT2C receptors attenuate inhibitory GABAergic signals in POMC neurons (90). Selective serotonin reuptake inhibitors elevate endogenous serotonin levels and are efficacious for treating hot flushes (105) and can significantly decrease the effects of post-ovariectomy thermoregulatory dysfunction in rodents (106). These similarities imply that serotonin and E2 via a Gq-mER have similar targets sites in the hypothalamus to control Tc and other neuroendocrine and autonomic functions.

An exciting recent discovery is the efficacy of STX to mimic the effects of E2 on tibial bone density in the ovariectomized guinea pigs (97). E2 has direct effects on the osteoclast/osteoblast cells involved in bone remodeling (107,108). However, E2 via a Gq-mER may reduce bone loss, a hallmark of hypo-estrogenic states, in part, by controlling the central mechanism of bone remodeling in the hypothalamus. It is known that the preautonomic paraventricular (PVN) neurons that drive sympathetic activity are involved in bone remodeling of other central and peripheral signals (109,110). E2, potentially via the putative Gq-mER, can either directly act on these neurons or indirectly on the neurons via pathways involving arcuate POMC neurons or VMH neurons both of which are known to synapse on the preautonomic paraventricular (PVN) neurons. The sympathetic nervous system in turn controls bone remodeling via the beta 2 adrenergic receptor (Adr-beta 2) activity (110). In fact, there is an increase in bone formation and a decrease in bone reabsorption in Adr-beta $2^{-/-}$ mice (111). Unlike in wild-type mice, gonadectomy of Adr-beta $2^{-/-}$ mice does not alter bone mass or bone resorption parameters, indicating that increased

sympathetic activity may be responsible for the bone loss in hypo-estrogenic states.

6. MEMBRANE E2 SIGNALING IN THE HIPPOCAMPUS

6.1. Memory & learning

The effects of membrane E2 signaling on hippocampal function and memory have been previously reviewed (14,24,112). Long-term potentiation (LTP) is the activity-dependent enhancement of synaptic activity (glutamate transmission) in the hippocampus (113) that can last for hours and is considered a cellular model of memory storage (112). The induction of LTP is by brief, high frequency trains of stimuli to afferents (Schaffer collaterals) of hippocampal CA1 pyramidal neurons and requires the activation of NMDA receptors. The subsequent potentiation of the strength of the synaptic signal is via non-NMDA (AMPA) receptors augmentation. E2 augments LTP rapidly (< 30 min) suggesting a membrane E2 signaling mechanism (18,114). The E2 enhancement of LTP is blocked by a tyrosine kinase inhibitor PP2 ((4-amino-5-(4-chlorophenyl)-7-(dimethylethyl)pyrazolo[3,4-*d*]pyrimidine), which also blocks the E2-dependent phosphorylation of NMDA receptors (115). E2, via activation of a cAMP/PKA pathway, also potentiates the non-NMDA (kainate)-mediated excitation of hippocampal CA1 pyramidal neurons (116,117) in wild-type and in ERalpha KO mice suggesting that either ERbeta or another membrane-associated E2 receptor is involved in the augmentation of LTP (118).

Furthermore, E2 exerts a positive influence on memory and higher cognitive functions both chronically (119,120,121) and acutely (122,123,124,125). Long-term hormone replacement therapy in human females either experiencing surgical or natural menopause protects against memory loss in both verbal and non-verbal memory tests and attention (126,121). In nonhuman primates, cyclical E2 replacement reversed the age-related impairment of spatial working memory and recognition memory observed in ovariectomized females (120). In acute models, E2 injection, either peripherally or centrally, in ovariectomized females will enhance various measurements of memory consolidation. Injections of E2 (5.0 micrograms/0.5 microliters) immediately after training, but not 2 h after training, significantly potentiated memory retention after a 24 h intertrial delay potentially via interactions with cholinergic circuitry (124).

Furthermore, after a shorter intertrial delay (4 hr), E2 treatment administered immediately after training enhanced visual (object), placement memory (122,123) and inhibitory avoidance task which mimics the effects of LTP (125). Luine and colleagues (122,123) suggest that these short-term effects are the result of an "extranuclear" receptor-mediated mechanism because of the short time course (<4 hr) and the rapid effects of E2 (<30 min) on monoamine neurotransmitter levels and metabolism. However, these data are not definitive evidence for membrane-initiated estrogen signaling. The measurements of memory occur 4 hr after injection of E2, which is long enough for nuclear-initiated signaling to occur. Therefore, further experiments using membrane-delimited E2 (E2-BSA, EDC) and/or novel ligands for E2-responsive GPCR (G1, STX) are needed.

Regardless, some of the effects of E2 on these hippocampal functions are, in part, via either direct enhancement of glutamate inputs on the CA1 pyramidal cells (as described above) or indirect attenuation of GABAergic inputs (116,117,127). Although the nuclear-initiated E2 signaling events are necessary for many of these effects in the hippocampus (112), membrane E2 signaling can acutely mimic these actions on hippocampal neurons (128,129,130,117,18,114,112). In addition, E2 may modulate a host of signal transduction pathways including mobilization of calcium and increased phospholipase and protein kinase activities via G-protein coupled mechanisms (24). Membrane E2 signaling has also recently been identified as involved in the E2-induced enhancement of memory consolidation in the dorsal hippocampus via activation of ERK signaling pathway (131,132). Therefore, there are multiple mER-mediated mechanisms that contribute to learning and memory.

6.2. Neuroprotection

Due to the reduction in serum E2 after menopause, there is a significant gender difference in the incidence of stroke with the incidence of stroke higher in women as compared to men each year while premenopausal women are less likely to suffer a stroke compared to men (133,134,135). Hormone replacement, depending upon the length of time a woman spends in a hypo-estrogenic state, may reduce the risk of stroke in post-menopausal women (134,135). Thus, E2 may have neuroprotective effects against ischemic injury and disease in the aging brain. There are multiple pathways involved in E2-mediated neuroprotection (136,137,138). Briefly, in the hippocampus, E2 employs both nuclear- and membrane-mediated mechanisms to augment signal transduction pathways such as PKC, PI3K/Akt and MAPK/ERK and gene expression relevant for cell survival (139,140,141,142,143). The genomic mechanisms involve both ERE-dependent and -independent transcriptional modulation that converge with the membrane-initiated effects on signaling proteins to modulate factors involved in cell death and survival such as the caspase death cascade (141,138).

While both ERalpha and ERbeta have been implicated in the neuroprotective effects of E2 (144,145) via both nuclear- and membrane-initiated mechanisms, the role of novel E2-responsive GPCRs in the neuroprotective effects of E2 has been recently examined. Using selective ligands for GPR30 (G1) and the Gq-mER (STX), Lebesgue et al. (2010) reported neuroprotective effects of both ligands in hippocampal CA1 neurons administered after a global ischemia insult during reperfusion in middle-aged rats eight weeks after ovariectomy (23). E2, G1 and STX were neuroprotective after immediate infusion via a lateral ventricle cannula with over 50% of CA1 neurons surviving compared to only 15% in the vehicle controls. G1 also

mimicked potentiation of field excitatory postsynaptic potentials (LTP) by E2 in hippocampal slices from ovariectomized females. The role of GPR30 in E2's neuroprotective effects in the hippocampus is supported by the recent findings demonstrating that robust neuroprotection is exerted by membrane-delimited E2 (E2-BSA and EDC) via the activation of an ERK-Akt-CREB-BDNF signaling mechanism (146). The ERK signaling pathway is a known effector of GPR30 actions in the hippocampus (42,30). The membrane-mediated neuroprotective effects from global ischemia are correlated with a preservation of cognitive functions (146). These data suggest that the neuroprotective effects of E2 via E2-responsive GPCRs promote cell survival after injury (See Figure 1B).

7. SUMMARY

In addition to the nuclear-initiated E2 signaling mechanisms that are often the primary driver of E2's effects, it is now clear that membrane E2 signaling plays a modulatory role in the CNS control of physiology and behavior including reproduction, feeding, thermoregulation and learning and memory. The membrane signaling mechanisms include modulating cation channel functions and thereby neuronal excitability through multiple signaling pathways, as well as inducing changes in calcium mobilization and other signaling pathways that impinge on gene expression. There are many examples in the hypothalamus, hippocampus and other brain regions for an E2-induced up-regulation of PKC, PKA, PI3K and MAP kinase activity leading to altered neuronal functions. One of the major obstacles to our full understanding of these events is the identity and characterization of the multiple receptor types (membrane-associated ERalpha and ERbeta vs. novel GPCR) that mediate the pleiotropic effects of E2. Once all of the membrane receptors are characterized, there will be even greater progress towards understanding all of the effects of E2 on neuronal function, physiology and behavior.

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Abbreviations: Abbreviations: AC, Adenylate cyclase; ATF-2-Jun, activating transcription factor-2-Jun; Bcl-2, B-cell lymphoma 2; BDNF, Brain-derived neurotrophic factor; CaM, calmodulin; cAMP; cyclic adenosine monophosphate; CRE, cAMP response element; CREB, cAMP response element binding protein; DAG, diacylglycerol; E2, estradiol; E2-BSA, estradiol-bovine serum albumin; EDC, estradiol-dendrimer; ELK-1-SRF, ETS domain-containing protein-serum response factor; EGF, epidermal growth factor; ER, estrogen receptor; ERE, estrogen response element; ERK, extracellular-signal regulated kinase; GABA, gamma-aminobutyric acid; GPCR, G protein-coupled receptor; GIRK, G-protein-coupled inwardly rectifying K^+ channel; GnRH, gonadotropin releasing hormone; Gq-mER, STX-activated, membrane estrogen receptor; GPR30/GPER-1, G-protein-coupled estrogen receptor 1 (IUPHAR designation); IGF-1, insulin growth factor 1; IP_3, inositol 1,4,5-triphosphate; K_{ATP}, ATP-sensitive K^+ channel; LTP, long term potentiation; LH, luteinizing hormone; mPOA, medial preoptic area; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; mGluR1a, metabotropic glutamate receptor 1a; MOR, mu-opioid receptor; NFkappaB, nuclear factor kappa-light-chain-enhancer of activated B cells; NPY, neuropeptide Y; PI3K, phosphatidylinositol 3-kinase; PIP_2, phosphatidylinositol 4,5-bisphosphate; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PLC, phospholipase C; POMC, proopiomelanocortin; STAT, signal transducers and activator of transcription; VMH, ventromedial hypothalamus.

Key Words: Estrogen, Membrane Receptor, Reproduction, Energy Homeostasis, Neuroprotection, Hypothalamus, Hippocampus, Review

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