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Research Report

A selective membrane estrogen receptor agonist maintains autonomic functions in hypoestrogenic states

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ABSTRACT

It is well known that many of the actions of estrogens in the central nervous system are mediated via intracellular receptor/transcription factors that interact with steroid response elements on target genes. But there is also a compelling evidence for the involvement of membrane estrogen receptors in hypothalamic and other CNS functions. However, it is not well understood how estrogens signal via membrane receptors, and how these signals impact not only membrane excitability but also gene transcription in neurons. Indeed, it has been known for sometime that estrogens can rapidly alter neuronal activity within seconds, indicating that some cellular effects can occur via membrane delimited events. In addition, estrogens can affect second messenger systems including calcium mobilization and a plethora of kinases within neurons to alter cellular functions. Therefore, this brief review will summarize our current understanding of rapid membrane-initiated and intracellular signaling by estrogens in the hypothalamus, the nature of receptors involved and how these receptors contribute to maintenance of homeostatic functions, many of which go awry in menopausal states.

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1. Membrane-initiated signaling of estrogens

It has been known for a number of years that 17β-estradiol (E2) has acute, membrane-initiated signaling actions in the brain (Kelly and Rønnekleiv, 2002; Rønnekleiv and Kelly, 2005; Micevych and Dominguez, 2009). A decade ago the nature and physiological significance of these actions were a matter of debate, but it is now widely accepted that some of the actions

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of E2 are quite rapid and cannot be attributed to the classical nuclear-initiated steroid signaling of ERα or ERβ. Importantly, ERα and ERβ can associate with signaling complexes in the plasma membrane (Razandi et al., 1999; Boulware et al., 2005; Pedram et al., 2006; Szegõ et al., 2006; Dewing et al., 2007; Bondar et al., 2009). In addition, many of the rapid effects of E2 can be induced by selective ERα or ERβ ligands, antagonized by the ER antagonist ICI 182,780 or are absent in animals bearing mutations in ERα and/or ERβ genes (Couse and Korach, 1999; Singer et al., 1999; Dubal et al., 2001; Wade et al., 2001; Abraham et al., 2003; Boulware et al., 2005, 2007). However, it is also evident that E2 can activate *bona fide* G-protein coupled receptors (GPCRs), the most notable GPR30 and a Gαq-coupled membrane ER (Gu et al., 1999; Toran-Allerand, 2004, 2005; Qiu et al., 2003, 2006; Noel et al., 2009; Zhang et al., 2010; Kenealy et al., 2011).

Substantial evidence has been generated in the support of a novel Gαq-coupled membrane ER (Gαq-mER). Intracellular sharp electrode and whole cell patch recording from guinea pig and mouse hypothalamic slices have been used to characterize this Gαq-mER (Lagrange et al., 1997; Qiu et al., 2003, 2006). These hypothalamic slice studies established that E2 acts rapidly and stereospecifically within physiologically-relevant concentrations (EC_{50}=7.5 nM) to significantly reduce the potency of μ-opioid and $GABA_B$ agonists (i.e., desensitize) to activate an inwardly rectifying K^+ conductance (Lagrange et al., 1997; Qiu et al., 2003). Estrogenic desensitization of μ-opioid and $GABA_B$ receptors is mimicked either by stimulation of adenylyl cyclase with forskolin or by direct protein kinase A (PKA) activation with the non-hydrolyzable cAMP analog Sp-cAMP, in a concentration-dependent manner (Lagrange et al., 1997; Qiu et al., 2003). Furthermore, the selective PKA antagonists KT5720 and Rp-cAMP block the effects of E2. As one would predict from the extensive literature on desensitization of GPCRs (Gainetdinov et al., 2004), PKA is downstream in a signaling cascade that is initiated by a Gαq-coupled membrane ER that is linked to activation of phospholipase C (PLC)–protein kinase C (PKC)–PKA (Qiu et al., 2003, 2006). Importantly, E2 does not alter the affinity of the μ-opioid and $GABA_B$ receptors for their respective receptors (Cunningham et al., 1998). Furthermore, the ER antagonists ICI 164,384 and ICI 182,780 block the actions of E2 with subnanomolar affinity (K_i=0.5 nM) that is similar to K_i for antagonism of ERα (Weatherill et al., 1988; Lagrange et al., 1997). These pharmacological findings clearly argue for a novel G-protein-coupled membrane receptor with high selectivity for E2.

In view of the differences between circulating levels of E2 and working concentrations of E2 used for *in vitro* analysis, it is important to clarify the pharmacological analysis of *in vitro* E2 physiological responses: When applying E2 in a bath superfusing hypothalamic slices, the physiological actions depend on the pharmacokinetics of E2 in the slice as it does for all other tissues. Therefore, it is important to do a dose–response to establish the potency and efficacy of E2. The potency (EC_{50}) of an agonist is the concentration required to produce 50% of the maximum effect in an experimental preparation. The value is obtained from a mathematical curve fitted to experimental data points. The potency (EC_{50}) is dependent on the binding affinity, the efficacy of agonist, the receptor reserve in the tissue or cell, and the ability of the agonist (i.e., E2) to penetrate to the site of action (Furchgott, 1978). As discussed above, the EC_{50} value for E2 to rapidly attenuate (desensitize) the μ-opioid response is 7.5 nM (Lagrange et al., 1997), whereas the EC_{50} for E2 to augment the K_{ATP} channel activity in GnRH neurons is an order of magnitude lower (0.6 nM), which is probably reflective of the receptor reserve in GnRH neurons versus POMC neurons (Zhang et al., 2010). Most importantly, critical requirement for establishing a specific receptor-mediated response is the blockade by a selective antagonist. Indeed, selective ER antagonists block the actions of E2 in POMC neurons and GnRH neurons with subnanomolar affinity (K_i=0.5 nM) similar to K_i for inhibition of E2 binding to ERα (Weatherill et al., 1988; Lagrange et al., 1997). Therefore, based on all of the criteria for establishing a physiological response, these rapid actions of E2 are physiological. The importance of this rapid response in physiological systems is discussed below.

About a decade ago a diphenylacrylamide compound, STX, that does not bind ERα or ERβ (Qiu et al., 2003, 2006) was developed to selectively target the Gαq-mER and its downstream signaling cascade – phospholipase Cβ-protein kinase Cδ-protein kinase A pathway – that mediates μ-opioid and $GABA_B$ desensitization in hypothalamic neurons. The design arose out of studies in which we found that E2 stereospecifically (17α-estradiol is not active) activates the Gαq-mER signaling pathway (see above), and these actions were blocked by the ER antagonist ICI 182,780 (Lagrange et al., 1997; Qiu et al., 2003, 2006). The results from these physiological and pharmacological experiments led to the design of STX, which is structurally similar to 4-OH tamoxifen, to target the Gαq-mER signaling pathway (Qiu et al., 2003). As predicted, STX had greater affinity (~20-fold) for the Gαq-mER than E2 and does not bind to ERα or ERβ (Qiu et al., 2006; Tobias et al., 2006). Most importantly, both STX and E2 activated this Gαq signaling pathway in mice lacking both ERα and ERβ and in GPR30-knockout mice (Qiu et al., 2006, 2008). Definitive characterization (i.e., cloning) of this novel Gαq-mER is currently a work in progress.

Parallel studies initiated in hippocampal slices some 20 years ago showed that E2 enhanced N-methyl-D-aspartate (NMDA)-mediated excitatory postsynaptic potentials (EPSPs) and long-term potentiation (LTP) following Schaffer (collateral) fiber stimulation (Wong and Moss, 1992; Foy et al., 1999; Rudick and Woolley, 2003). Also, E2 potentiated non-NMDA (kainate)-mediated excitation of hippocampal CA1 pyramidal neurons via activation of a cAMP/PKA pathway (Gu and Moss, 1996, 1998). Importantly, these rapid actions of E2 on glutamate excitation of hippocampal neurons were still present in animals deficient in ERα (from Dr. Ken Korach, NIH), suggesting a novel mechanism (receptor) for the rapid actions of E2 in the hippocampus (Gu et al., 1999). In addition E2 and E2-BSA (E2 conjugated to bovine serum albumin to limit membrane penetration) applied acutely to the hippocampus in ovariectomized animals produced a sustained reduction of the slow after hyperpolarization current (I_{AHP}) in CA1 pyramidal neurons (Carrer et al., 2003). This provided further evidence for the involvement of a membrane ER, although, the mechanism by which E2 regulates Ca^{2+} influx into CA1