HHS Public Access

Author manuscript

*Brain Res*. Author manuscript; available in PMC 2017 May 15.

Published in final edited form as:

*Brain Res*. 2013 June 13; 1514: 75–82. doi:10.1016/j.brainres.2013.03.020.

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

**Martin J. Kelly**[a,b,*] and **Oline K. Rønnekleiv**[a,b,c,1]

[a]Department of Physiology and Pharmacology, L334, Oregon Health & Science University, 3181 S.W. Sam Jackson Park Road, Portland, OR 97239-3098, USA

[b]Division of Neuroscience, Oregon Regional Primate Research Center, Oregon Health & Science University, Beaverton, OR 97006, USA

[c]Department of Anesthesiology and Perioperative Medicine, Oregon Health & Science University, Portland, OR 97239, USA

## 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.

### Keywords

Proopiomelanocortin (POMC); Gαq-mER; $GABA_B$ and μ-opioid receptors; G protein-coupled; Inwardly rectifying $K^+$ (GIRK); channel; Desensitization; Obesity; Hot flash; Osteoporosis

## 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

*Corresponding author at: Department of Physiology and Pharmacology, L334, Oregon Health & Science University, 3181 S.W. Sam Jackson Park Road, Portland, OR 97239-3098, USA. Fax: +1 503 494 4352. kellym@ohsu.edu (M.J. Kelly), ronnekle@ohsu.edu (O.K. Rønnekleiv).

[1]Fax: +1 503 494 4352.

significance of these actions were a matter of debate, but it is now widely accepted that some of the actions 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 down-stream 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 neurons is currently unknown. More recent studies from Catherine Woolley's laboratory have shown that E2 via ERβ also has presynaptic effects to enhance glutamate release (increased vesicle release probability) and hence, excitation of CA1 neurons (Smejkalova and Woolley, 2010). In addition, E2 through the association of ERα with the metabotropic glutamate receptor 1 (mGluR1) attenuates GABA-A mediated inhibitory postsynaptic currents (IPSCs) (Huang and Woolley, 2012). The ERα-mediated effects are via mGluR1-dependent mobilization of the endocannabinoid anandamide that

retrogradely suppresses GABA from CB-1 receptor-containing presynaptic GABAergic boutons. Interestingly, this signaling pathway exists in females but not males, which is congruent with the findings from Boulware et al. (2005, 2007)) who showed in female and not male hippocampal CA3–CA1 neuronal cultures that E2 rapidly stimulates MAPK-dependent cAMP-responsive element binding protein (CREB) phosphorylation. This modulation of CREB activity also occurs via ERα interactions with mGluR 1. The protein–protein interaction between the "classical" ERs and mGluRs has not been fully elucidated, but ERα and GluR1a have been co-immunoprecipitated in membrane fractions from both astrocytes and neurons (Dewing et al., 2007; Kuo et al., 2009; Dominguez and Micevych, 2010).

Recently, the orphan GPCR GPR 30 has received notoriety because of its role in mediating E2's effects in the CNS (Noel et al., 2009; Lebesgue et al., 2010; Kenealy et al., 2011). GPR30 exhibits the signaling characteristics of a bonafide ER (Thomas et al., 2005; Prossnitz et al., 2008). In breast cancer cells that are transfected with GPR30, E2 activates the mitogen-activated protein kinases (MAPK), ERK1 and ERK2, and these actions are independent of ERα or ERβ (Filardo, 2002; Filardo and Thomas, 2005). Accordingly, E2 activates Gβγ-subunits that promote the release and activation of an epidermal growth factor precursor (proHB-EGF). The active HB-EGF binds to the EGF receptor (ErbB) to facilitate receptor dimerization and downstream activation of ERK (Filardo et al., 2000, 2002; Filardo, 2002). Interestingly, the selective estrogen receptor modulator (SERM) tamoxifen and ER antagonist ICI 182,780 both promote GPR30-dependent transactivation of the EGF receptor and subsequent MAPK activation. It is important to note that the Gαq-mER-mediated response to E2 in arcuate neurons is still present in GPR30 KO mice (Qiu et al., 2008). GPR30 has been localized in the brain and E2 binds to this receptor (Funakoshi et al., 2006; Bologa et al., 2006; Brailoiu et al., 2007; Prossnitz et al., 2007), and recent studies from the Terasawa lab have shown that GPR30 is involved in mediating the rapid actions of E2 in monkey olfactory placode GnRH neurons (see below). Therefore, it is evident that estrogen can rapidly alter cell function through ERα, ERβ and/or novel GPCRs that bind E2.

## 2. Membrane-initiated signaling of E2 and hypothalamic control of autonomic functions

Besides its quintessential role in the feedback control of the reproductive axis, E2 modulates a number of hypothalamic-regulated autonomic functions, most notably energy homeostasis and temperature. E2 signaling via ERα is a critical component in the hypothalamic regulation of energy balance (Geary et al., 2001). In rodents, hypo-estrogenic states are clearly associated with decreased activity and an increase in body weight (Czaja and Goy, 1975; Butera and Czaja, 1984; Czaja, 1984; McCaffrey and Czaja, 1989; Jones et al., 2000; Asarian and Geary, 2002; Qiu et al., 2006; Clegg et al., 2006, 2007). In humans, a loss-of-function mutation in ERα has a clear metabolic phenotype in man with expression of type 2 diabetes, hyperinsulinemia and obesity (Smith et al., 1994). However, global reinstatement of an ERα that is lacking the ERE targeting domain is sufficient for "rescuing" the metabolic deficits in mice (Park et al., 2011). These findings suggest an important role for non-ERE mediated E2 signaling. Moreover, brain-specific knockout of ERα causes

hyperphagia and hypometabolism (Musatov et al., 2007; Xu et al., 2011), and selective knockdown of ERα in POMC neurons appears to recapitulate the hyperphagic phenotype in female mice (Xu et al., 2011). However, there are at least two caveats that impact the interpretation of gene deletion experiments. Firstly, ERα is a transcription factor affecting the expression of hundreds of genes important for cell signaling, and many of these genes are essential for mER initiated responses that contribute to POMC excitability and hence control of energy homeostasis (Hewitt et al., 2003; Malyala et al., 2004). Secondly, POMC-Cre (mice utilized in Xu et al. (2011)) is also expressed in progenitor neurons that are destined to become NPY neurons and perhaps other hypothalamic and extrahypothalamic neurons (Padilla et al., 2010, 2012). Therefore, ERα knockout spreads well beyond the single neuron phenotype as originally proposed. Hence, one must be cautious in interpreting ERα knockout (global or targeted) experiments.

Experiments dating back three decades determined that the anorectic effects of E2 in rodents are mediated through CNS sites of action since direct injections of E2 into the paraventricular nucleus of the hypothalamus (PVH) or arcuate/ventromedial nucleus are effective to reduce food intake, body weight and increase wheel running activity in females (Colvin and Sawyer, 1969; Ahdieh and Wade, 1982; Butera and Czaja, 1984). This is due, in part, to the actions of E2 on arcuate POMC and neuropeptide Y (NPY) neurons, the former being anorexic and the latter being orexigenic (Roepke, 2009). Thus, a number of experiments have shown that E2 upregulates the expression of the peptide β-endorphin in POMC neurons in ovariectomized female guinea pigs and increases the mRNA expression of POMC in both mice and guinea pigs (Thornton et al., 1994; Bethea et al., 1995; Pelletier et al., 2007; Roepke et al., 2008). Furthermore, there is a decrease in hypothalamic POMC mRNA levels in postmenopausal women (Abel and Rance, 1999). In addition, E2 reverses the ovariectomy-induced increase in arcuate NPY protein and mRNA expression in rodents (Crowley et al., 1985; Shimizu et al., 1996; Pelletier et al., 2007). Therefore, it appears that the arcuate nucleus and particularly POMC and NPY neurons are major targets for the anorectic actions of estrogen, which underscores their importance in the control of energy homeostasis. In addition, POMC neurons are critical for the regulation of feeding behavior and are also involved in the rewarding aspects of food intake (Hayward et al., 2002; Appleyard et al., 2003).

### 2.1. Energy homeostasis and obesity

The ability of STX to robustly mimic the effects of E2 on POMC neuronal activity led to the hypothesis that the Gαq-mER may have a role in the control of energy homeostasis. Indeed, in translational animal experiments we have demonstrated that peripheral administration of STX mimics the effects of E2 in controlling energy homeostasis (Qiu et al., 2006; Roepke et al., 2008, 2010). As predicted from the E2- or STX-induced increase in POMC neuronal activity, both E2 and STX reduce food intake and, subsequently, the post-ovariectomy body weight gain. STX and E2 inhibit food intake in ovariectomized guinea pigs by reducing meal frequency, and there is a subsequent reduction in abdominal fat accumulation (Roepke et al., 2010). In support of a hypothalamic site of action, treatment with STX, similar to E2, induces new gene transcription in the arcuate nucleus (Roepke et al., 2008). Many of the regulated genes in the arcuate nucleus are involved in the control of neuronal excitability

(e.g., Cav3.1) and intracellular signaling in POMC neurons (Roepke et al., 2008). For example, the PI3 kinase regulatory subunits are regulated by E2 and STX: PI3 kinase p55γ mRNA is increased by E2 treatment (Malyala et al., 2008) and PI3 kinase p85α mRNA is upregulated by STX (Roepke et al., 2008). Therefore, Gαq-mER appears to function in the estrogenic control of energy homeostasis through activation of POMC neurons in the arcuate nucleus, although other hypothalamic neurons may be involved via synaptic input from POMC neurons and/or via direct actions of E2.

### 2.2. Core body temperature and hot flashes

Another critical homeostatic function modulated by circulating estrogens is the maintenance of core body temperature (Tc). In fact, hot flashes affect 75–85% of perimenopausal and postmenopausal women (Moline et al., 2003). Hot flashes are characterized by periods of sweating and peripheral vasodilation and are often associated with increased environmental temperature (Rapkin, 2007). Therefore, a hot flash can be defined as an exaggerated heat dissipation response initiated by the preoptic temperature sensitive neurons. Although the mechanism behind this response is not known, repeated observations have found that the majority of hot flashes are preceded by elevation in Tc independent of peripheral vasoconstriction or elevated metabolic rate (Freedman et al., 1995; Freedman, 2005). Therefore, it has been postulated that elevated Tc may serve as one trigger of menopausal hot flashes (Freedman and Blacker, 2002; Rapkin, 2007). In general, there is compelling experimental evidence that the incidence of hot flashes in hypo-estrogenic females is decreased by E2 treatment (Tankersley et al., 1992; Brooks et al., 1997; Freedman and Blacker, 2002). The estrogenic reduction in the naloxone-induced rise in tail skin temperature of morphine-dependent ovariectomized rats has been the industry standard for a hot flash model (Simpkins et al., 1983; Merchenthaler et al., 1998; Komm et al., 2005). The morphine-dependence, naloxone-precipitated withdrawal rat model has a number of drawbacks because of the adverse autonomic reactions independent of an elevation in core and skin temperature. But the model does highlight the involvement of CNS opioid effects (e.g., from POMC neurons) directly or indirectly on preoptic temperature sensitive neurons (Yoshida et al., 2009).

We have established a guinea pig “hot flash” model based on the hypothesis that the expression of vasomotor symptoms in menopausal women is due to the reduced thermoneutral zone in core body temperature (Freedman, 2005). In the ovariectomized female guinea pig, both E2 and STX significantly reduce Tc compared to animals receiving vehicle injections (Roepke et al., 2010). The exact cellular target for E2 and specifically the Gαq-mER modulation of Tc has not been identified. However, one potential cellular mechanism is the direct action of E2 via the Gαq-mER on thermosensitive (GABAergic) neurons in the medial preoptic area of the hypothalamus (Griffin et al., 2001; Nakamura et al., 2002; Boulant, 2006). Previous studies in ovariectomized female guinea pigs have shown that medial preoptic GABAergic neurons respond to acute E2 treatment via the Gαq-mER signaling pathway to attenuate $GABA_B$ inhibitory input, leading to increased GABA neuronal activity (Wagner et al., 2001). Moreover, activation of medial preoptic GABA neurons are responsible for evoking the vasomotor responses in rodents (Nakamura and

Morrison, 2010) underlying heat dissipation responses (i.e., vasodilatation, sweating) as seen in women experiencing hot flashes.

Importantly, the coupling of Gαq-mER to downstream signaling pathways is similar to the serotonin 5HT2A/2C receptors, which when activated, lower Tc and are implicated in thermoregulation dysfunction caused by ovariectomy (Berendsen et al., 2001; Sipe et al., 2004). Selective serotonin reuptake inhibitors elevate endogenous serotonin levels and are therefore efficacious for treating hot flashes (Stearns et al., 2002) and can significantly attenuate the effects of ovariectomy on thermoregulation in rodents (Deecher et al., 2007). Moreover, both the Gαq-mER and 5HT2C receptors increase POMC neuronal excitability (Qiu et al., 2007). These similarities imply that serotonin via its Gαq-coupled receptor and E2 via Gαq-mER have similar cellular targets in the hypothalamic neurons that regulate Tc and energy homeostasis.

### 2.3. Bone remodeling and osteoporosis

Recently, STX has been found to mimic the ability of E2 to maintain bone density in the ovariectomized female guinea pigs (Roepke et al., 2010). Although E2 has direct effects on the osteoclast/osteoblast cells involved in bone remodeling (Sylvia et al., 2002; Endoh et al., 1997), E2 can reduce bone loss, a hallmark of hypo-estrogenic states, in part, by controlling the hypothalamic regulation of bone remodeling. It is known that the preautonomic paraventricular (PVN) neurons that drive sympathetic activity are involved in bone remodeling (Yadav et al., 2009; Takeda et al., 2002). These PVN neurons receive a robust μ-opioid receptor inhibitory input, presumably from POMC neurons (Wuarin and Dudek, 1990), and therefore, E2 via Gαq-mER can affect their excitability via inhibitory POMC input. Indeed, we have found that STX, like E2, preserves cancellous bone density, which supports a potential role of this hypothalamic signaling pathway in maintenance of bone density. However, STX, like E2, may also have peripheral effects (e.g., directly on osteoblasts) which cannot be ruled out at this time. Certainly, the decreased PVN output would dampen the sympathetic nervous system activity that controls bone remodeling via the adrenergic β2 receptor activity in the osteoblast cells (Takeda et al., 2002). In fact, there is an increase in bone formation and a decrease in bone reabsorption in adrenergic β2 receptor knockout mice (Elefteriou et al., 2005). Unlike in wild-type mice, gonadectomy of adrenergic β2 receptor knockout 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 such as menopause (Elefteriou et al., 2005).

## 3. Summary

It is obvious from the plethora of studies using membrane-delimited E2 ligands and the mER selective ligand STX that “genomic” actions of E2 in the brain do not require the direct nuclear targeting of estrogen receptors (ERα and ERβ). Signals that are initiated by E2 at the plasma membrane can trigger multiple intracellular signaling cascades including activation of MAPK, PI3K, and PKC pathways (Watters et al., 1997; Bi et al., 2001; Cato et al., 2002; Yang et al., 2003; Deisseroth et al., 2003) that result in the phosphorylation of hundreds of proteins that ultimate can affect cell excitability and gene transcription. E2 can

bind to the novel Gαq-mER to upregulate cAMP in hypothalamic neurons by increasing adenylyl cyclase activity within minutes (Lagrange et al., 1997). Cyclic-AMP activates PKA, which in turn phosphorylates $K^+$ channels (Zhang et al., 2010) and/or calcium channels (Zhang et al., 2009) to alter their activity. In addition, PKA can phosphorylate CREB to elicit new gene transcription (Zhou et al., 1996; Gu et al., 1996; Watters and Dorsa, 1998; Abrahám et al., 2004). Therefore, not only does membrane excitability change within a matter of seconds, but gene transcription can be activated within a relatively short time course (within tens of minutes) in neurons independent of classic estrogen receptors (ERα and ERβ) interacting with EREs. Thus, we need to elucidate the role of membrane estrogen receptors not only in hypothalamic homeostatic functions but also in higher cortical functions. By developing selective non-steroidal agonists for targeting these mERs we would be able to expand the therapeutic window for treating menopausal symptoms associated with aging without concern for the deleterious side effects of the gonadal steroids.

## Acknowledgments

The authors thank current and former members of their laboratories who contributed to the work described herein, especially Drs. Jian Qiu, Troy A. Roepke and Chunguang Zhang and Ms. Martha A. Bosch. Research reported in this publication was supported by the National Institutes Health Grants NS 38809, NS 43330 and DK 68098. The content is solely the responsibility of the authors and does not necessarily represent the official view of the National Institutes of Health.

## References

Abel TW, Rance NE. Proopiomelanocortin gene expression is decreased in the infundibular nucleus of postmenopausal women. Mol Brain Res. 1999; 69:202–208. [PubMed: 10366741]

Abraham IM, Han SK, Todman MG, Korach KS, Herbison AE. Estrogen receptor beta mediates rapid estrogen actions on gonadotropin-releasing hormone neurons in vivo. J Neurosci. 2003; 23:5771–5777. [PubMed: 12843281]

Abrahám IM, Todman MG, Korach KS, Herbison AE. Critical in vivo roles for classical estrogen receptors in rapid estrogen actions on intracellular signaling in mouse brain. Endocrinology. 2004; 145:3055–3061. [PubMed: 14976146]

Ahdieh HB, Wade GN. Effects of hysterectomy on sexual receptivity, food intake, running wheel activity, and hypothalamic estrogen and progestin receptors in rats. J Comp Physiol Psychol. 1982; 96:886–892. [PubMed: 7153386]

Appleyard SM, Hayward M, Young JI, Butler AA, Cone RD, Rubinstein M, Low MJ. A role for the endogenous opioid beta-endorphin in energy homeostasis. Endocrinology. 2003; 144:1753–1760. [PubMed: 12697680]

Asarian L, Geary N. Cyclic estradiol treatment normalizes body weight and restores physiological patterns of spontaneous feeding and sexual receptivity in ovariectomized rats. Horm Behav. 2002; 42:461–471. [PubMed: 12488112]

Berendsen HHG, Weekers AHJ, Kloosterboer HJ. Effect of tibolone and raloxifene on the tail temperature of oestrogen-deficient rats. Eur J Pharmacol. 2001; 419:47–54. [PubMed: 11348629]

Bethea CL, Hess DL, Widmann AA, Henningfeld JM. Effects of progesterone on prolactin, hypothalamic beta-endorphin, hypothalamic substance P, and midbrain serotonin in guinea pigs. Neuroendocrinology. 1995; 61:695–703. [PubMed: 7544880]

Bi R, Foy MR, Vouimba RM, Thompson RF, Baudry M. Cyclic changes in estradiol regulate synaptic plasticity through the MAP kinase pathway. Proc Natl Acad Sci USA. 2001; 98:13391–13395. [PubMed: 11687663]

Bologa CG, Revankar CM, Young SM, Edwards BS, Arterburn JB, Kiselyov AS, Parker MA, Tkachenko SE, Savchuck NP, Sklar LA, Oprea TI, Prossnitz ER. Virtual and biomolecular

screening converge on a selective agonist for GPR30. Nat Chem Biol. 2006; 2:207–212. [PubMed: 16520733]

Bondar G, Kuo J, Hamid N, Micevych P. Estradiol-induced estrogen receptor-α trafficking. J Neurosci. 2009; 29:15323–15330. [PubMed: 19955385]

Boulant JA. Neuronal basis of Hammel's model for set-point thermoregulation. J Appl Physiol. 2006; 100:1347–1354. [PubMed: 16540713]

Boulware MI, Kordasiewicz H, Mermelstein PG. Caveolin proteins are essential for distinct effects of membrane estrogen receptors in neurons. J Neurosci. 2007; 27:9941–9950. [PubMed: 17855608]

Boulware MI, Weick JP, Becklund BR, Kuo SP, Groth RD, Mermelstein PG. Estradiol activates group I and II metabotropic glutamate receptor signaling, leading to opposing influences on cAMP response element-binding protein. J Neurosci. 2005; 25:5066–5078. [PubMed: 15901789]

Brailoiu E, Dun SL, Brailoiu GC, Mizuo K, Sklar LA, Oprea TI, Prossnitz ER, Dun NJ. Distribution and characterization of estrogen receptor G protein-coupled receptor 30 in the rat central nervous system. J Endocrinol. 2007; 193:311–321. [PubMed: 17470522]

Brooks EM, Morgan AL, Pierzga JM, Wladkowski SL, O'Gorman JT, Derr JA, Kenney WL. Chronic hormone replacement therapy alters thermoregulatory and vasomotor function in postmenopausal women. J Appl Physiol. 1997; 97:477–484.

Butera PC, Czaja JA. Intracranial estradiol in ovariectomized guinea pigs: effects on ingestive behaviors and body weight. Brain Res. 1984; 322:41–48. [PubMed: 6518373]

Carrer HF, Araque A, Buño W. Estradiol regulates the slow $Ca^{2+}$-activated $K^{+}$ current in hippocampal pyramidal neurons. J Neurosci. 2003; 23:6338–6344. [PubMed: 12867518]

Cato ACB, Nestl A, Mink S. Rapid actions of steroid receptors in cellular signaling pathways. Science's STKE. 2002; 2002:re9–re21.

Clegg DJ, Brown LM, Woods SC, Benoit SC. Gonadal hormones determine sensitivity to central leptin and insulin. Diabetes. 2006; 55:978–987. [PubMed: 16567519]

Clegg DJ, Brown LM, Zigman JM, Kemp CJ, Strader AD, Benoit SC, Woods SC, Mangiaracina M, Geary N. Estradiol-dependent decrease in the orexigenic potency of ghrelin in female rats. Diabetes. 2007; 56:1051–1058. [PubMed: 17251274]

Colvin GB, Sawyer CH. Induction of running activity by intracerebral implants of estrogen in overiectomized rats. Neuroendocrinology. 1969; 4:309–320. [PubMed: 4894216]

Couse JF, Korach KS. Estrogen receptor null mice: what have we learned and where will they lead us? Endocr Rev. 1999; 20:358–417. [PubMed: 10368776]

Crowley WR, Tessel RE, O'Donohue TL, Adler BA, Kalra SP. Effects of ovarian hormones on the concentrations of immunoreactive neuropeptide Y in discrete brain regions of the female rat: correlation with serum luteinizing hormone (LH) and median eminence LH-releasing hormone. Endocrinology. 1985; 117:1151–1155. [PubMed: 3893992]

Cunningham MJ, Fang Y, Selley DE, Kelly MJ. μ-opioid agonist-stimulated [$^{35}$S]GTPgammaS binding in guinea pig hypothalamus: effects of estrogen. Brain Res. 1998; 791:341–346. [PubMed: 9593984]

Czaja JA. Sex differences in the activational effects of gonadal hormones on food intake and body weight. Physiol Behav. 1984; 33:553–558. [PubMed: 6522475]

Czaja JA, Goy RW. Ovarian hormones and food intake in female guinea pigs and rhesus monkeys. Horm Behav. 1975; 6:329–349. [PubMed: 816725]

Deecher DC, Alfinito PD, Leventhal L, Cosmi S, Johnston GH, Merchanthaler I, Winneker R. Alleviation of thermoregulatory dysfunction with the new serotonin and norepinephrine reuptake inhibitor desvenlafaxine succinate in ovariectomized rodent models. Endocrinology. 2007; 148:1376–1383. [PubMed: 17122073]

Deisseroth K, Mermelstein PG, Xia H, Tsien RW. Signaling from synapse to nucleus: the logic behind the mechanisms. Curr Opin Neurobiol. 2003; 13:354–365. [PubMed: 12850221]

Dewing P, Boulware MI, Sinchak K, Christensen A, Mermelstein PG, Micevych PE. Membrane estrogen receptor-α interactions with metabotropic glutamate receptor 1a modulate female sexual receptivity in rats. J Neurosci. 2007; 27:9294–9300. [PubMed: 17728443]

Dominguez R, Micevych P. Estradiol rapidly regulates membrane estrogen receptor levels in hypothalamic neurons. J Neurosci. 2010; 30:12589–12596. [PubMed: 20861365]

Dubal DB, Zhu H, Yu J, Rau SW, Shughrue PJ, Merchenthaler I, Kindy MS, Wise PM. Estrogen receptor alpha, not beta, is a critical link in estradiol-mediated protection against brain injury. Proc Natl Acad Sci USA. 2001; 98:1952–1957. [PubMed: 11172057]

Elefteriou F, Ahn JD, Takeda S, Starbuck M, Yang X, Liu X, Kondo H, Richards WG, Bannon TW, Noda M, Clement K, Valsse C, Karsenty G. Leptin regulation of bone resorption by the sympathetic nervous system and CART. Nature. 2005; 434:514–520. [PubMed: 15724149]

Endoh H, Sasaki H, Maruyama K, Takeyama K, Waga I, Shimizu T, Kato S, Kawashima H. Rapid activation of MAP Kinase by estrogen in the bone cell line. Biochem Biophys Res Commun. 1997; 235:99–102. [PubMed: 9196043]

Filardo EJ. Epidermal growth factor receptor (EGFR) transactivation by estrogen via the G-protein-coupled receptor, GPR30: a novel signaling pathway with potential significance for breast cancer. J Steroid Biochem Mol Biol. 2002; 80:231–238. [PubMed: 11897506]

Filardo EJ, Quinn JA, Bland KI, Frackelton ARJ. Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF. Mol Endocrinol. 2000; 14:1649–1660. [PubMed: 11043579]

Filardo EJ, Quinn JA, Frackelton AR Jr, Bland KI. Estrogen action via the G protein-coupled receptor, GPR30: Stimulation of adenylyl cyclase and cAMP-Mediated attenuation of the epidermal growth factor receptor-to-MAPK signaling axis. Mol Endocrinol. 2002; 16:70–84. [PubMed: 11773440]

Filardo EJ, Thomas P. GPR30: a seven-transmembrane- spanning estrogen receptor that triggers EGF release. Trends Endocrinol Metab. 2005; 16:362–367. [PubMed: 16125968]

Foy MR, Xu J, Xie X, Brinton RD, Thompson RF, Berger TW. 17 β-estradiol enhances NMDA receptor-mediated EPSPs and long-term potentiation. J Neurophysiol. 1999; 81:925–929. [PubMed: 10036289]

Freedman RR. Hot flashes: behavorial treatments, mechanisms, and relation to sleep. Am J Med. 2005; 118:1245–1305.

Freedman RR, Blacker CM. Estrogen raises the sweating threshold in postmenopausal women with hot flashes. Fertil Steril. 2002; 77:487–490. [PubMed: 11872200]

Freedman RR, Norton D, Woodward S, Cornelissen G. Core body temperature and circadian rhythm of hot flashes in meopausal women. J Clin Endocrinol Metab. 1995; 80:2354–2358. [PubMed: 7629229]

Funakoshi T, Yanai A, Shinoda K, Kawano MM, Mizukami Y. G protein-coupled receptor 30 is an estrogen receptor in the plasma membrane. Biochem Biophys Res Commun. 2006; 346:904–910. [PubMed: 16780796]

Furchgott RF. Pharmacological characterization of receptors: its relation to radioligand-binding studies. Fed Proc. 1978; 37:115–120. [PubMed: 203488]

Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ, Caron MG. Desensitization of G protein-coupled receptors and neuronal functions. Annu Rev Neurosci. 2004; 27:107–144. [PubMed: 15217328]

Geary N, Asarian L, Korach KS, Pfaff DW, Ogawa S. Deficits in E2-dependent control of feeding, weight gain, and cholecystokinin satiation in ER-alpha null mice. Endocrinol. 2001; 142:4751–4757.

Griffin JD, Saper CB, Boulant JA. Synaptic and morphological characteristics of temperature-sensitive and -insensitive rat hypothalamic neurons. J Physiol. 2001; 537(2):521–535. [PubMed: 11731583]

Gu G, Rojo AA, Zee MC, Yu J, Simerly RB. Hormonal regulation of CREB phosphorylation in the anteroventral periventricular nucleus. J Neurosci. 1996; 16:3035–3044. [PubMed: 8622133]

Gu Q, Korach KS, Moss RL. Rapid action of 17beta-estradiol on kainate-induced currents in hippocampal neurons lacking intracellular estrogen receptors. Endocrinology. 1999; 140:660–666.

Gu Q, Moss RL. 17beta-estradiol potentiates kainate-induced currents via activation of the cAMP cascade. J Neurosci. 1996; 16:3620–3629. [PubMed: 8642406]

Gu Q, Moss RL. Novel mechanism for non-genomic action of 17beta-oestradiol on kainate-induced currents in isolated rat CA1 hippocampal neurons. J Physiol (London). 1998; 506:745–754. [PubMed: 9503335]

Hayward MD, Pintar JE, Low MJ. Selective reward deficit in mice lacking β-endorphin and enkephalin. J Neurosci. 2002; 22:8251–8258. [PubMed: 12223579]

Hewitt SC, Deroo BJ, Hansen K, Collins J, Grissom S, Afshari C, Korach KS. Estrogen receptor-dependent genomic responses in the uterus mirror the biphasic physiological response to estrogen. Mol Endocrinol. 2003; 17:2070–2083. [PubMed: 12893882]

Huang GZ, Woolley CS. Estradiol acutely suppresses inhibition in the hippocampus through a sex-specific endocannabinoid and mGluR-dependent mechanism. Neuron. 2012; 74:801–808. [PubMed: 22681685]

Jones MEE, Thorburn AW, Britt KL, Hewitt KN, Wreford NG, Proietto J, Oz OK, Leury BJ, Robertson KM, Yao SG, Simpson ER. Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. Proc Natl Acad Sci USA. 2000; 97:12735–12740. [PubMed: 11070087]

Kelly, MJ., Rønnekleiv, OK. Rapid membrane effects of estrogen in the central nervous system. In: Pfaff, DW., editor. Hormones, Brain and Behavior. Academic Press; San Diego: 2002. p. 361-380.

Kenealy BP, Keen KL, Rønnekleiv OK, Terasawa E. STX, a novel nonsteroidal estrogenic compound, induces rapid action in primate GnRH neuronal calcium dynamics and peptide release. Endocrinology. 2011; 152:182–191.

Komm BS, Kharode YP, Bodine PVN, Harris HA, Miller CP, Lyttle CR. Bazedoxifene Acetate: a selective estrogen receptor modulator with improved selectivity. Endocrinology. 2005; 146:3999–4008. [PubMed: 15961563]

Kuo J, Hariri OR, Bondar G, Ogi J, Micevych P. Membrane estrogen receptor-α interacts with metabotropic glutamate receptor type 1a to mobilize intracellular calcium in hypothalamic astrocytes. Endocrinology. 2009; 150:1369–1376. [PubMed: 18948402]

Lagrange AH, Rønnekleiv OK, Kelly MJ. Modulation of G protein-coupled receptors by an estrogen receptor that activates protein kinase A. Mol Pharmacol. 1997; 51:605–612. [PubMed: 9106625]

Lebesgue D, Traub M, De Butte-Smith M, Chen C, Zukin RS, Kelly MJ, Etgen AM. Acute administration of non-classical estrogen receptor agonists attenuates ischemia-induced hippocampal neuron loss in middle-aged female rats. PLos One. 2010; 5:1–8.

Malyala A, Pattee P, Nagalla SR, Kelly MJ, Rønnekleiv OK. Suppression subtractive hybridization and microarray identification of estrogen regulated hypothalamic genes. Neurochem Res. 2004; 29:1189–1200. [PubMed: 15176476]

Malyala A, Zhang C, Bryant D, Kelly MJ, Rønnekleiv OK. PI3K signaling effects in hypothalamic neurons mediated by estrogen. J Comp Neurol. 2008; 506:895–911. [PubMed: 18085586]

McCaffrey TA, Czaja JA. Diverse effects of estradiol-17 beta: concurrent suppression of appetite, blood pressure and vascular reactivity in conscious, unrestrained animals. Physiol Behav. 1989; 45:649–657. [PubMed: 2756058]

Merchenthaler I, Funkhouser JM, Carver JM, Lundeen SG, Ghosh K, Winneker RC. The effect of estrogens and antiestrogens in a rat model for hot flush. Maturitas. 1998; 30:307–316. [PubMed: 9881331]

Micevych P, Dominguez R. Membrane estradiol signaling in the brain. Front Neuroendocrinol. 2009; 30:315–327. [PubMed: 19416735]

Moline ML, Broch L, Zak R, Gross V. Sleep in women across the life cycle from adulthood through menopause. Sleep Med Rev. 2003; 7:155–177. [PubMed: 12628216]

Musatov S, Chen W, Pfaff DW, Mobbs CV, Yang XJ, Clegg DJ, Kaplitt MG, Ogawa S. Silencing of estrogen receptor α in the ventromedial nucleus of hypothalamus leads to metabolic syndrome. Proc Natl Acad Sci USA. 2007; 104:2501–2506. [PubMed: 17284595]

Nakamura K, Matsumura K, Kaneko T, Kobayashi S, Katoh H, Negishi M. The rostral raphe pallidus nucleus mediates pyrogenic transmission from the preoptic area. J Neurosci. 2002; 22:4600–4610. [PubMed: 12040067]

Nakamura K, Morrison SF. A thermosensory pathway mediating heat-defense responses. Proc Natl Acad Sci USA. 2010; 107:8848–8853. [PubMed: 20421477]

Noel SD, Keen KL, Baumann DI, Filardo EJ, Terasawa E. Involvement of G-protein couple receptor 30 (GPR30) in rapid action of estrogen in primate LHRH neurons. Mol Endocrinol. 2009; 3:349–359.

Padilla SL, Carmody JS, Zeltser LM. *Pomc*-expressing progenitors give rise to antagonistic neuronal populations in hypothalamic feeding circuits. Nat Med. 2010; 16:403–405. [PubMed: 20348924]

Padilla SL, Reef D, Zeltser LM. Defining POMC neurons using transgenic reagents: impact of transient Pomc expression in diverse immature neuronal populations. Endocrinology. 2012; 153:1–13. [PubMed: 22186411]

Park CJ, Zhao Z, Glidewell-Kenney C, Lazic M, Chambon P, Krust A, Weiss J, Clegg DJ, Dunaif A, Jameson JL, Levine JE. Genetic rescue of nonclassical ERα signaling normalizes energy balance in obese Erα-null mutant mice. J Clin Invest. 2011; 121:604–612. [PubMed: 21245576]

Pedram A, Razandi M, Levin ER. Nature of functional estrogen receptors at the plasma membrane. Mol Endocrinol. 2006; 20:1996–2009. [PubMed: 16645038]

Pelletier G, Li S, Luu-The V, Labrie F. Oestrogenic regulation of pro-opiomelanocortin, neuropeptide Y and corticotrophin-releasing hormone mRNAs in mouse hypothalamus. J Neuroendocrinol. 2007; 19:426–431. [PubMed: 17388940]

Prossnitz ER, Arterburn JB, Sklar LA. GPR30: A G protein-coupled receptor for estrogen. Mol Cell Endocrinol. 2007; 265–266:138–142.

Prossnitz ER, Arterburn JB, Smith HO, Oprea TI, Sklar LA, Hathaway HJ. Estrogen signaling through the transmembrane G protein-coupled receptor GPR30. Annu Rev Physiol. 2008; 70:165–190. [PubMed: 18271749]

Qiu J, Bosch MA, Tobias SC, Grandy DK, Scanlan TS, Rønnekleiv OK, Kelly MJ. Rapid signaling of estrogen in hypothalamic neurons involves a novel G protein-coupled estrogen receptor that activates protein kinase C. J Neurosci. 2003; 23:9529–9540. [PubMed: 14573532]

Qiu J, Bosch MA, Tobias SC, Krust A, Graham S, Murphy S, Korach KS, Chambon P, Scanlan TS, Rønnekleiv OK, Kelly MJ. A G protein-coupled estrogen receptor is involved in hypothalamic control of energy homeostasis. J Neurosci. 2006; 26:5649–5655. [PubMed: 16723521]

Qiu J, Rønnekleiv OK, Kelly MJ. Modulation of hypothalamic neuronal activity through a novel G-protein coupled estrogen membrane receptor. Steroids. 2008; 73:985–991. [PubMed: 18342349]

Qiu J, Xue C, Bosch MA, Murphy JG, Fan W, Rønnekleiv OK, Kelly MJ. Serotonin 5HT2c receptor signaling in hypothalamic POMC neurons: role in energy homeostasis in females. Mol Pharm. 2007; 72:885–896.

Rapkin AJ. Vasomotor symptoms in menopause: physiologic condition and central nervous system approaches to treatment. Am J Obstet Gynecol. 2007; 196:97–106. [PubMed: 17306645]

Razandi M, Pedram A, Greene GL, Levin ER. Cell membrane and nuclear estrogen receptors (ERs) originate from a single transcript: studies of ERα and ERβ expressed in Chinese hamster ovary cells. Mol Endocrinol. 1999; 13:307–319. [PubMed: 9973260]

Roepke TA. Oestrogen modulates hypothalmic control of energy homeostasis through multiple mechanisms. J Neuroendocrinol. 2009; 21:141–150. [PubMed: 19076267]

Roepke TA, Bosch MA, Rick EA, Lee B, Wagner EJ, Seidlová-Wuttke D, Wuttke W, Scanlan TS, Rønnekleiv OK, Kelly MJ. Contribution of a membrane estrogen receptor to the estrogenic regulation of body temperature and energy homeostasis. Endocrinology. 2010; 151:4926–4937. [PubMed: 20685867]

Roepke TA, Xue C, Bosch MA, Scanlan TS, Kelly MJ, Rønnekleiv OK. Genes associated with membrane-initiated signaling of estrogen and energy homeostasis. Endocrinology. 2008; 149:6113–6124. [PubMed: 18755790]

Rønnekleiv OK, Kelly MJ. Diversity of ovarian steroid signaling in the hypothalamus. Front Neuroendocrinol. 2005; 26:65–84. [PubMed: 16009409]

Rudick CN, Woolley CS. Selective estrogen receptor modulators regulate phasic activation of hippocampal CA1 pyramidal cells by estrogen. Endocrinology. 2003; 144:179–187. [PubMed: 12488344]

Shimizu H, Ohtani K, Kato Y, Tanaka Y, Mori M. Estrogen increases hypothalamic neuropeptide Y (NPY) mRNA expression in ovariectomized obese rat. Neurosci Lett. 1996; 204:81–84. [PubMed: 8929983]

Simpkins JW, Katovich MJ, Song C. Similarities between morphine withdrawal in the rat and the menopausal hot flush. Life Sci. 1983; 32:1957–1966. [PubMed: 6835016]

Singer CA, Figueroa-Masot XA, Batchelor RH, Dorsa DM. The mitogen-activated protein kinase pathway mediates estrogen neuroprotection after glutamate toxicity in primary cortical neurons. J Neurosci. 1999; 19:2455–2463. [PubMed: 10087060]

Sipe K, Leventhal L, Burroughs K, Cosmi S, Johnston GH, Deecher DC. Serotonin 2A receptors modulate tail-skin temperature in two rodent models of estrogen deficiency-related thermoregulatory dysfunction. Brain Res. 2004; 1028:191–202. [PubMed: 15527744]

Smejkalova T, Woolley CS. Estradiol acutely potentiates hippocampal excitatory synaptic transmission through a presynaptic mechanism. J Neurosci. 2010; 30:16137–16148. [PubMed: 21123560]

Smith EP, Boyd J, Frank GR, Takahashi H, Cohen RM, Specker B, Williams TC, Lubahn DB, Korach KS. Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. N Engl J Med. 1994; 331(16):1056–1061. [PubMed: 8090165]

Stearns V, Ullmer L, Lopez JF, Smith Y, Isaacs C, Hayes DF. Hot flushes. Lancet. 2002; 360:1851–1861. [PubMed: 12480376]

Sylvia VL, Walton J, Lopez D, Dean DD, Boyan BD, Schwartz Z. 17 beta-estradiol-BSA conjugates and 17 beta-estradiol regulate growth plate chondrocytes by common membrane associated mechanisms involving PKC dependent and independent signal transduction. J Cell Biochem. 2002; 81:413–429.

Szegõ ÉM, Barabás K, Balog J, Szilágyi N, Korach KS, Juhász G, Abrahám IM. Estrogen induces estrogen receptor α-dependent cAMP response element-binding protein phosphorylation via mitogen activated protein kinase pathway in basal forebrain cholinergic neurons in vivo. J Neurosci. 2006; 26:4104–4110. [PubMed: 16611827]

Takeda S, Elefteriou F, Levasseur R, Liu X, Zhao L, Parker KL, Armstrong D, Ducy P, Karsenty G. Leptin regulates bone formation via the sympathetic nervous system. Cell. 2002; 111:305–317. [PubMed: 12419242]

Tankersley CG, Nicholas WC, Deaver DR, Mikita D, Kenney WL. Estrogen replacement in middle-aged women: thermoregulatory responses to exercise in the heat. J Appl Physiol. 1992; 73:1238–1245. [PubMed: 1447065]

Thomas P, Pang Y, Filardo EJ, Dong J. Identity of an estrogen membrane receptor coupled to a G-protein in human breast cancer cells. Endocrinology. 2005; 146:624–632. [PubMed: 15539556]

Thornton JE, Loose MD, Kelly MJ, Rønnekleiv OK. Effects of estrogen on the number of neurons expressing β-endorphin in the medial basal hypothalamus of the female guinea pig. J Comp Neurol. 1994; 341:68–77. [PubMed: 8006224]

Tobias SC, Qiu J, Kelly MJ, Scanlan TS. Synthesis and biological evaluation of SERMs with potent nongenomic estrogenic activity. ChemMedChem. 2006; 1:565–571. [PubMed: 16892393]

Toran-Allerand CD. Estrogen and the brain: beyond ER-α, ER-β and 17β-estradiol. Ann NY Acad Sci. 2005; 1052:136–144. [PubMed: 16024756]

Toran-Allerand CD. Minireview: a plethora of estrogen receptors in the brain: where will it end? Endocrinology. 2004; 145:1069–1074. [PubMed: 14670986]

Wade CB, Robinson S, Shapiro RA, Dorsa DM. Estrogen receptor (ER)alpha and ERbeta exhibit unique pharmacologic properties when coupled to activation of the mitogen-activated protein kinase pathway. Endocrinology. 2001; 142:2336–2342.

Wagner EJ, Rønnekleiv OK, Bosch MA, Kelly MJ. Estrogen biphasically modifies hypothalamic GABAergic function concomitantly with negative and positive control of luteinizing hormone release. J Neurosci. 2001; 21:2085–2093. [PubMed: 11245692]

Watters JJ, Campbell JS, Cunningham MJ, Krebs EG, Dorsa DM. Rapid membrane effects of steroids in neuroblastoma cells: effects of estrogen on mitogen activated protein kinase signalling cascade and c-fos immediate early gene transcription. Endocrinology. 1997; 138:4030–4033. [PubMed: 9275096]

Watters JJ, Dorsa DM. Transcriptional effects of estrogen on neuronal neurotensin gene expression involve cAMP/protein kinase A-dependent signaling mechanisms. J Neurosci. 1998; 18:6672–6680. [PubMed: 9712639]

Weatherill PJ, Wilson APM, Nicholson RI, Davies P, Wakeling AE. Interaction of the antioestrogen ICI 164,384 with the oestrogen receptor. J Steroid Biochem Mol Biol. 1988; 30:263–266.

Wong M, Moss RL. Long-term and short-term electrophysiological effects of estrogen on the synaptic properties of hippocampal CA1 neurons. J Neurosci. 1992; 12:3217–3225. [PubMed: 1353794]

Wuarin JP, Dudek FE. Direct effects of an opioid peptide selective for μ-receptors: intracellular recordings in the paraventricular and supraoptic nuclei of the guinea pig. Neuroscience. 1990; 36:291–298. [PubMed: 1699161]

Xu Y, Nedugadi TP, Zhu L, Sobhani N, Irani BG, Davis KE, Zhang X, Zou F, Gent LM, Hahner LD, Khan SA, Elias CF, Elmquist JK, Clegg DJ. Distinct hypothalamic neurons mediate estrogenic effects on energy homeostasis and reproduction. Cell Metab. 2011; 14:453–465. [PubMed: 21982706]

Yadav VK, Oury F, Suda N, Liu ZW, Gao XB, Confavreux C, Klemenhagen KC, Tanaka KF, Gingrich JA, Guo XE, Tecott LH, Mann JJ, Hen R, Horvath TL, Karsenty G. A serotonin-dependent mechanism explains the leptin regulation of bone mass, appetite, and energy expenditure. Cell. 2009; 138:976–989. [PubMed: 19737523]

Yang SH, Sharrocks AD, Whitmarsh AJ. Transcriptional regulation by the MAP kinase signaling cascades. Gene. 2003; 320:3–21. [PubMed: 14597384]

Yoshida K, Li X, Cano G, Lazarus M, Saper CB. Parallel preoptic pathways for thermoregulation. J Neurosci. 2009; 29:11954–11964. [PubMed: 19776281]

Zhang C, Bosch MA, Rick EA, Kelly MJ, Rønnekleiv OK. 17β-estradiol regulation of T-type calcium channels in gonadotropin-releasing hormone neurons. J Neurosci. 2009; 29:10552–10562. [PubMed: 19710308]

Zhang C, Kelly MJ, Rønnekleiv OK. 17β-estradiol rapidly increases adenosine 5′-triphosphate-sensitive potassium channel activity in gonadotropin-releasing hormone neurons via a protein kinase signaling pathway. Endocrinology. 2010; 151:4477–4484. [PubMed: 20660067]

Zhou Y, Watters JJ, Dorsa DM. Estrogen rapidly induces the phosphorylation of the cAMP response element binding protein in rat brain. Endocrinology. 1996; 137:2163–2166. [PubMed: 8612562]