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REVIEW ARTICLE

Rapid Nongenomic Effects of Oestradiol on Gonadotrophin-Releasing Hormone Neurones

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That oestradiol can have both negative- and positive-feedback actions upon the release of gonadotrophin-releasing hormone (GnRH) has been understood for decades. The vast majority of studies have investigated the effects of *in vivo* oestrogen administration. In the past decade, evidence has accumulated in many neuronal and non-neuronal systems indicating that, in addition to traditional genomic action via transcription factor receptors, steroids can also initiate effects rapidly via signalling cascades typically associated with the cell membrane. Here, we review work examining the rapid actions of oestradiol on GnRH neurones, addressing the questions of dose dependence, receptor subtypes, signalling cascades and intrinsic and synaptic properties that are rapidly modulated by this steroid.

Key words: GnRH, feedback, steroid, nonclassical, GABA, patch-clamp.

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Introduction

Gonadotrophin-releasing hormone (GnRH) neurones form the final common pathway for the central regulation of reproduction. GnRH stimulates release of the pituitary gonadotrophins, which in turn activate gonadal steroidogenesis. These steroids complete both homeostatic and nonhomeostatic feedback loops centrally to regulate GnRH release (1,2). In the male and during most of the normal female reproductive cycle, homoeostatic negative-feedback upon the frequency of GnRH release is enforced by oestradiol at low levels and, in the female, progesterone. Nonhomeostatic feedback is confined to females during the preovulatory period and occurs in response to sustained elevations of oestradiol, which generate positive-feedback to induce the preovulatory surge of GnRH release (3,4).

Our understanding of oestradiol feedback has come largely from studies using *in vivo* treatments. More recently, the availability of identified GnRH neurones has made electrophysiological approaches possible (5,6). Using *in vivo* treatment followed by acute brain slice preparation, investigators have shown changes in GnRH neurone firing pattern, neurotransmission to GnRH neurones and the function of specific ion channels in these cells in response to oestradiol (7–13). Most evidence indicates the alpha isoforms of the oestrogen receptor (ER) mediate these actions via classical binding to oestrogen response elements (14,15). Because treatments in these studies were performed *in vivo*, it is not possible to parse the mechanisms mediating the effects. In this regard, steroids including oestradiol can engage nuclear transcription factor receptors, via classical and nonclassical pathways, and subsequently alter gene expression to bring about effects (16). In addition, very rapid effects of steroids have been observed throughout the central nervous system (17,18), including in GnRH neurones (19–22) and GT1 cells (23), as well as in the periphery (24). The rapid time course of these effects suggested they did not require the macromolecular synthesis typically associated with classical oestrogen signalling.

Receptors mediating the rapid effects of oestradiol

A number of receptor subtypes have been proposed to mediate the rapid actions of oestradiol. The classical nuclear subtypes ERα and ERβ have been demonstrated to engage signalling cascades typically associated with the cell membrane (25). This can occur via lipid modifications of the ERs, such as palmitoylation, allowing association with the lipid bilayer, or by binding with caveolins. In addition to classical ERs, however, at least three putative transmembrane oestrogen receptors have been proposed to mediate rapid effects in the central nervous system: G-protein coupled receptor 30 (GPR30) (26), ER-X (27) and mER (28). GnRH neurones themselves appear to express ERβ (29) and GPR30 (30) but could potentially be influenced indirectly by all of these receptor subtypes in a rapid manner.

The rapid effects of high physiological levels of oestradiol on GnRH neurones

The main approach that has been used to distinguish rapid from classical oestrogen action is one using a short duration treatment. This approach should minimise actions via genomic pathways, allowing nongenomic actions to be revealed. One note of caution is that genomic actions may well occur more quickly than typically currently assumed; for example, promoter occupancy changes within 1 min of oestradiol exposure on the *crh* gene (31). Nonetheless, this approach has been used to demonstrate the rapid effects of oestrogens on GnRH neurones both *in vivo* and *in vitro* (21,32–37). A majority of these studies demonstrate an increase in firing rate, calcium oscillation frequency or transcription factor phosphorylation, although inhibitory effects have also been reported (21). These studies have rarely examined dose dependence or the neurobiological mechanism involved in these responses, however, necessitating further investigation.

To examine the nongenomic actions of oestradiol on GnRH neurones, we used acutely prepared brain slices from adult, short-term ovariectomised mice. The parameter of interest was first recorded under control conditions, and then native 17β-oestradiol was applied via the bath. After a 5-min wash in period, response was recorded 5–15 min after the initiation of oestradiol treatment, followed by a return to control solution and the recording of responses as they returned to baseline levels. In our initial studies, we wished to focus on effects that might be mediated directly at the GnRH neurone, and thus included blockers of ionotropic GABA and glutamate receptors in the bath solution at all times to isolate GnRH neurones from indirect effects mediated by fast synaptic transmission. Of note, this would not block effects via changes in neuromodulation.

Under these recording conditions, oestradiol induced an increase in firing rate, observable within 2–3 min of application (38) (Fig. 1A). The percentage of neurones responding and the percent increase in firing rate increased with dose (100 pM to 100 nM). Although steroids are lipophilic, drug penetration in brain slices is typically low as a result of their thickness. It is thus notable that these responses were observed at treatment levels (100 pM and 1 nM) that would be expected to produce physiological levels of oestradiol in the vicinity of the GnRH neurone based on measured circulating levels (39). Also of note, low physiological levels of oestradiol (10 pM) had no effect on the firing rate under these conditions (Fig. 1B).

To explore further the mechanisms of this effect, a series of pharmacological and electrophysiological studies were done. The excitatory effect of oestradiol was blocked by the pure classical antagonist ICI182780, and mimicked by the ERβ agonist 2,3-bis-(4-hydroxyphenyl)-propionitrile (DPN), although neither the ERα agonist propylpyrazoletriol (PPT), nor the GPR30 agonist G1 had any effect. Oestradiol or DPN also increased the excitability of GnRH neurones and, in rare cases, overt depolarisation of membrane potential was observed in response to oestradiol after action potential firing had been blocked with tetrodotoxin (38). Together these observations suggest a rapid activation of GnRH neurones

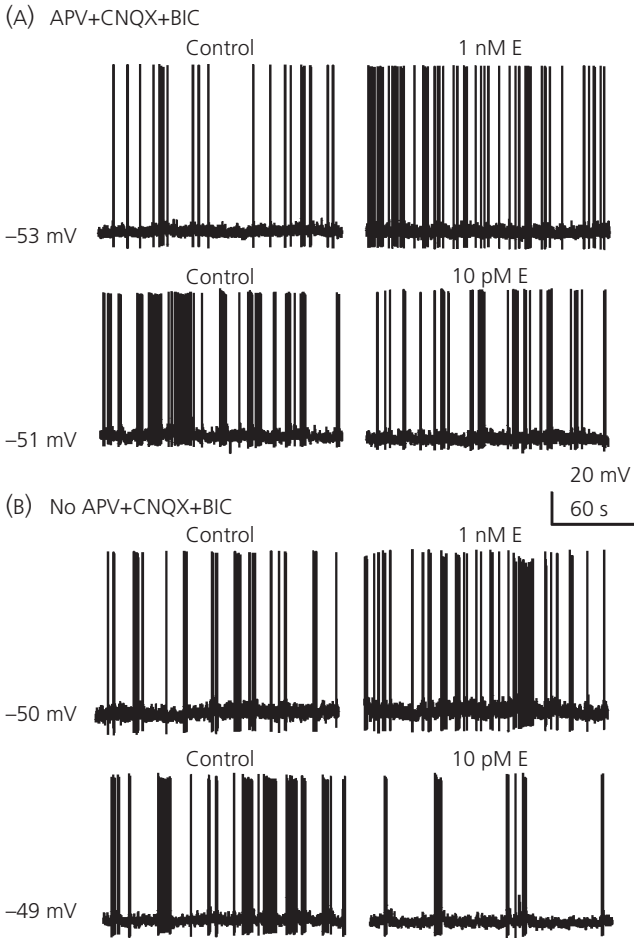

Fig. 1. Rapid effects of oestradiol on gonadotrophin-releasing hormone (GnRH) neurones. (A) In the presence of blockers of ionotropic GABA and glutamate receptors [D(-)2-amino-5-phosphonovaleric acid (APV) + 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) + BIC] GnRH neurones are excited to fire action potentials at a higher rate by 1 nM oestradiol (E), although 10 pM oestradiol has no effect. (B) Without blockers, 1 nM oestradiol (E) still increases the firing rate but now inhibition is observed with 10 pM oestradiol, presumably mediated by the upstream fast synaptic transmission network.

mediated via ERβ expressed in these cells, although as noted above, indirect effects via neuromodulation cannot be eliminated.

In vivo work had indicated that CREB was rapidly phosphorylated in GnRH neurones in an ERβ-dependent manner (37). Consistent with this, blockade of protein kinase A stopped the ability of oestradiol to increase the GnRH neurone firing rate (38). Via this kinase, and potentially others, oestradiol has been shown to rapidly alter conductance of several types of ion channels in GnRH neurones. Oestradiol rapidly increases the current underlying the slow afterdepolarisation, which is carried by sodium (40), decreases calcium-activated potassium currents that underlie the afterhyperpolarisation (38) and increases L and R type calcium channels via ERβ and GPR30, respectively (41). The above three targets change in a direction that is consistent with an increased activity of GnRH neurones and/or potentially increased neurosecretion in response to

action potential generation in the case of the high-voltage gated calcium channels. It is important to note that the story with regard to GRP30 is still evolving and species differences may well exist, with perhaps a more predominant role in the primate (30,42). A recent study also demonstrated a rapid activation of ATP-sensitive potassium channels (K_{ATP}) (32), which would tend to decrease activity of GnRH neurones. Although this latter result may appear contradictory with the excitation of GnRH neurones, it is important to note that, although the integrated response of the cell to higher doses of oestradiol appears to be excitatory, it is a net of all the changes that occur. Oestrogen modulation of K_{ATP} channels may play a critical role in response to negative energy balance because these channels can serve as energy sensors.

The rapid effects of low physiological oestradiol levels

Under the recording conditions used above, 10 pM oestradiol had no effect on the firing activity of GnRH neurones. In the intact brain, however, fast synaptic transmission is not blocked as it was in the experimental conditions used above to reduce variables. We next investigated the effects of 10 pM versus 10 nM oestradiol when fast synaptic transmission was allowed to operate within the slice (38). As in the presence of ionontropic GABA and glutamate receptors, high levels of oestradiol excited GnRH neurones. By contrast to the situation in which fast synaptic transmission was blocked, however, low (10 pM) oestradiol now inhibited GnRH neurones in a rapid manner (Fig. 1B). This effect was mimicked by the ERα agonist PPT but not the ERβ nor GPR30 agonists, suggesting a separate mechanism. Consistent with this, low-dose oestradiol had no effect on any GnRH neurone intrinsic property that was examined. This, in combination with the need for active fast synaptic transmission, suggested that this effect was mediated upstream of the GnRH neurone. Indeed, the ERα agonist PPT was found to rapidly reduce GABAergic transmission. Because activation of $GABA_A$ receptors in these cells is primarily excitatory (43–45), this would reduce synaptic drive to fire action potentials. Interestingly, the ERβ agonist DPN increased both GABAergic transmission and the postsynaptic response to GABA, both of which would support excitation in response to activation of this receptor. In other work, the alpha isoform of the oestradiol receptor was found to mediate the ability of high (100 nM) oestradiol to induce a delayed (approximately 15 min) increase in GABAergic transmission in some GnRH neurones, possibly consistent with the excitatory effects of high oestradiol (13). This suggests that, even within our current thinking of the rapid versus genomic actions of oestradiol, subdivisions of different mechanisms engaged with different time courses and requiring different receptors may exist.

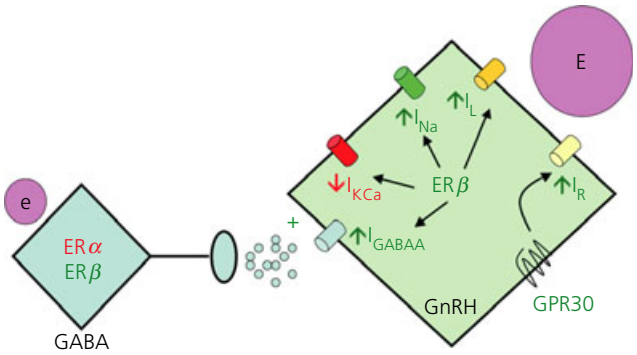

Fig. 2. Model summarising the neurobiological mechanisms engaged by oestradiol to regulate gonadotrophin-releasing hormone (GnRH) neurone function. Low physiological levels of oestradiol (e) act upstream to alter GABAergic transmission to GnRH neurones. High physiological levels of oestradiol (E) can act directly via oestrogen receptor (ER)β and perhaps GPR30. Red, inhibitory action; green, stimulatory or excitatory action.

Summary and future directions

After a long period of dispute, there is now little question that steroid hormones can have rapid effects, likely both directly on GnRH neurones and on their upstream network, via multiple mechanisms (Fig. 2). As with long-term effects, these rapid effects are dose-dependent, with higher levels of oestradiol being excitatory and lower doses inhibitory. A similar dose and receptor dependence of the action of oestradiol was also found in cultured hypothalamic GnRH neurones (46). There are several questions that remain. How does the endogenous oestradiol level affect the response to acute changes in oestradiol in the vicinity of the GnRH neurone? The vast majority of studies have utilised ovariectomised animals to remove interference from endogenous steroids; however, this is clearly not the physiological situation. In the one case in which the rapid effects of oestradiol were also studied in brain slices from oestradiol-treated mice, the ability of oestradiol to increase high-voltage activated calcium currents was similar in both animal models, suggesting that rapid changes in nongenomic oestradiol signalling are not precluded in the presence of circulating oestradiol (38). The effects on other aspects of this response are not known. How do oestradiol levels change in the vicinity of GnRH neurones? What, if any, are the physiological role of nongenomic actions of oestradiol? And finally, how are the genomic and nongenomic effects integrated *in vivo* to produce the overall response to this steroid?

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References

1 Christian CA, Moenter SM. The neurobiology of preovulatory and estradiol-induced gonadotropin-releasing hormone surges. *Endocr Rev* 2010; **31**: 544–577.
2 Docke F, Dorner G. The mechanism of the induction of ovulation by oestrogens. *J Endocrinol* 1965; **33**: 491–499.
3 Moenter SM, Caraty A, Locatelli A, Karsch FJ. Pattern of gonadotropin-releasing hormone (GnRH) secretion leading up to ovulation in the ewe: existence of a preovulatory GnRH surge. *Endocrinology* 1991; **129**: 1175–1182.
4 Clarke IJ, Thomas GB, Yao B, Cummins JT. GnRH secretion throughout the ovine estrous cycle. *Neuroendocrinology* 1987; **46**: 82–88.

5 Suter KJ, Song WJ, Sampson TL, Wuarin JP, Saunders JT, Dudek FE, Moenter SM. Genetic targeting of green fluorescent protein to gonadotropin-releasing hormone neurons: characterization of whole-cell electrophysiological properties and morphology. *Endocrinology* 2000; **141**: 412–419.

6 Spergel DJ, Ulrich D, Hanley DF, Sprengel R, Seeburg PH. GABA- and glutamate-activated channels in green fluorescent protein-tagged gonadotropin-releasing hormone neurons in transgenic mice. *J Neuroscience* 1999; **19**: 2037–2050.

7 Christian CA, Mobley JL, Moenter SM. Diurnal and estradiol-dependent changes in gonadotropin-releasing hormone neuron firing activity. *Proc Natl Acad Sci USA* 2005; **102**: 15682–15687.

8 Christian CA, Moenter SM. Estradiol induces diurnal shifts in GABA transmission to gonadotropin-releasing hormone neurons to provide a neural signal for ovulation. *J Neurosci* 2007; **27**: 1913–1921.

9 Christian CA, Moenter SM. Critical roles for fast synaptic transmission in mediating estradiol negative and positive feedback in the neural control of ovulation. *Endocrinology* 2008; **149**: 5500–5508.

10 Christian CA, Moenter SM. Vasoactive intestinal polypeptide can excite gonadotropin-releasing hormone neurons in a manner dependent on estradiol and gated by time of day. *Endocrinology* 2008; **149**: 3130–3136.

11 Christian CA, Pielecka-Fortuna J, Moenter SM. Estradiol suppresses glutamatergic transmission to gonadotropin-releasing hormone neurons in a model of negative feedback in mice. *Biol Reprod* 2009; **80**: 1128–1135.

12 Zhang C, Bosch MA, Rick EA, Kelly MJ, Ronnekleiv OK. 17{beta}-estradiol regulation of T-type calcium channels in gonadotropin-releasing hormone neurons. *J Neurosci* 2009; **29**: 10552–10562.

13 Romano N, Lee K, Abraham IM, Jasoni CL, Herbison AE. Nonclassical estrogen modulation of presynaptic gaba terminals modulates calcium dynamics in gonadotropin-releasing hormone neurons. *Endocrinology* 2008; **149**: 5335–5344.

14 Wintermantel TM, Campbell RE, Porteous R, Bock D, Grone H-J, Todman MG, Korach KS, Greiner E, Perez CA, Schutz G, Herbison AE. Definition of estrogen receptor pathway critical for estrogen positive feedback to gonadotropin-releasing hormone neurons and fertility. *Neuron* 2006; **52**: 271–280.

15 Christian CA, Glidewell-Kenney C, Jameson JL, Moenter SM. Classical estrogen receptor alpha signaling mediates negative and positive feedback on gonadotropin-releasing hormone neuron firing. *Endocrinology* 2008; **149**: 5328–5334.

16 Heldring N, Pike A, Andersson S, Matthews J, Cheng G, Hartman J, Tujague M, Ström A, Treuter E, Warner M, Gustafsson JA. Estrogen receptors: how do they signal and what are their targets? *Physiol Rev* 2007; **87**: 905–931.

17 Woolley CS. Acute effects of estrogen on neuronal physiology. *Ann Rev Pharmacol Toxicol* 2007; **47**: 657–680.

18 Kelly MJ, Kuhnt U, Wuttke W. Hyperpolarization of hypothalamic parvocellular neurons by 17 beta-estradiol and their identification through intracellular staining with procion yellow. *Exp Brain Res* 1980; **40**: 440–447.

19 Kelly MJ, Ronnekleiv OK, Ibrahim N, Lagrange AH, Wagner EJ. Estrogen modulation of K(+) channel activity in hypothalamic neurons involved in the control of the reproductive axis. *Steroids* 2002; **67**: 447–456.

20 Kelly MJ, Ronnekleiv OK, Eskay RL. Identification of estrogen-responsive LHRH neurons in the guinea pig hypothalamus. *Brain Res Bull* 1984; **12**: 399–407.

21 Lagrange AH, Ronnekleiv OK, Kelly MJ. Estradiol-17 beta and mu-opioid peptides rapidly hyperpolarize GnRH neurons: a cellular mechanism of negative feedback? *Endocrinology* 1995; **136**: 2341–2344.

22 Herbison AE. Rapid actions of oestrogen on gonadotropin-releasing hormone neurons; from fantasy to physiology? *The Journal of Physiology* 2009; **587**: 5025–5030.

23 Navarro CE, Saeed SA, Murdock C, Martinez-Fuentes AJ, Arora KK, Krsmanovic LZ, Catt KJ. Regulation of cyclic adenosine 3′,5′-monophosphate signaling and pulsatile neurosecretion by Gi-coupled plasma membrane estrogen receptors in immortalized gonadotropin-releasing hormone neurons. *Mol Endocrinol* 2003; **17**: 1792–1804.

24 Mendelsohn ME, Karas RH. Rapid progress for non-nuclear estrogen receptor signaling. *J Clin Invest* 2010; **120**: 2277–2279.

25 Levin ER. Plasma membrane estrogen receptors. *Trends Endocrinol Metab* 2009; **20**: 477–482.

26 Revankar CM, Cimino DF, Skalar LA, Arterburn JB, Prossnitz ER. A transmembrane intracellular estrogen receptor mediates rapid cell signaling. *Science* 2005; **307**: 1625–1630.

27 Toran-Allerand CD, Guan X, MacLusky NJ, Horvath TL, Diano S, Singh M, Connolly ES Jr, Nethrapalli IS, Tinnikov AA. ER-X: a novel, plasma membrane-associated, putative estrogen receptor that is regulated during development and after ischemic brain injury. *J Neurosci* 2002; **22**: 8391–8401.

28 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.

29 Hrabovszky E, Shughrue PJ, Merchenthaler I, Hajszan T, Carpenter CD, Liposits Z, Petersen SL. Detection of estrogen receptor-beta messenger ribonucleic acid and 125I-estrogen binding sites in luteinizing hormone-releasing hormone neurons of the rat brain. *Endocrinology* 2000; **141**: 3506–3509.

30 Terasawa E, Noel SD, Keen KL. Rapid action of oestrogen in luteinising hormone-releasing hormone neurones: the role of GPR30. *J Neuroendocrinol* 2009; **21**: 316–321.

31 Lalmansingh AS, Uht RM. Estradiol regulates corticotropin-releasing hormone gene (crh) expression in a rapid and phasic manner that parallels estrogen receptor-{alpha} and – recruitment to a 3′,5′-cyclic adenosine 5′-monophosphate regulatory region of the proximal crh promoter. *Endocrinology* 2008; **149**: 346–357.

32 Zhang C, Kelly MJ, Ronnekleiv OK. 17{beta}-estradiol rapidly increases KATP activity in GnRH via a protein kinase signaling pathway. *Endocrinology* 2010; **151**: 4477–4484.

33 Abe H, Terasawa E. Firing pattern and rapid modulation of activity by estrogen in primate luteinizing hormone releasing hormone-1 neurons. *Endocrinology* 2005; **146**: 4312–4320.

34 Abe H, Keen KL, Terasawa E. Rapid action of estrogens on intracellular calcium oscillations in primate LHRH-1 neurons. *Endocrinology* 2008; **149**: 5325–5327.

35 Temple JL, Laing E, Sunder A, Wray S. Direct action of estradiol on gonadotropin-releasing hormone-1 neuronal activity via a transcription-dependent mechanism. *J Neurosci* 2004; **24**: 6326–6333.

36 Abraham 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.

37 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.

38 Chu Z, Andrade J, Shupnik MA, Moenter SM. Differential regulation of gonadotropin-releasing hormone neuron activity and membrane properties by acutely applied estradiol: dependence on dose and estrogen receptor subtype. *J Neurosci* 2009; **29**: 5616–5627.

39 Nelson JF, Felicio LS, Osterburg HH, Finch CE. Differential contributions of ovarian and extraovarian factors to age-related reductions in plasma

estradiol and progesterone during the estrous cycle of C57BL/6J mice. *Endocrinology* 1992; **130**: 805–810.

40 Chu Z, Moenter SM. Physiologic regulation of a tetrodotoxin-sensitive sodium influx that mediates a slow afterdepolarization potential in gonadotropin-releasing hormone neurons: possible implications for the central regulation of fertility. *J Neurosci* 2006; **26**: 11961–11973.

41 Sun J, Chu Z, Moenter SM. Diurnal in vivo and rapid in vitro effects of estradiol on voltage-gated calcium channels in gonadotropin-releasing hormone neurons. *J Neurosci* 2010; **30**: 3912–3923.

42 Noel SD, Keen KL, Baumann DI, Filardo EJ, Terasawa E. Involvement of G protein-coupled receptor 30 (GPR30) in rapid action of estrogen in primate LHRH neurons. *Mol Endocrinol* 2009; **23**: 349–359.

43 DeFazio RA, Heger S, Ojeda SR, Moenter SM. Activation of A-type {gamma}-aminobutyric acid receptors excites gonadotropin-releasing hormone neurons. *Mol Endocrinol* 2002; **16**: 2872–2891.

44 Moenter SM, DeFazio RA. Endogenous gamma-aminobutyric acid can excite GnRH neurons. *Endocrinology* 2005; **146**: 5374–5379.

45 Herbison AE, Moenter SM. Depolarising and hyperpolarising actions of $GABA_A$ receptor activation on GnRH neurones: towards an emerging consensus. *J Neuroendocrinol* 2011; in press.

46 Hu L, Gustofson RL, Feng H, Ki Leung P, Mores N, Krsmanovic LZ, Catt KJ. Converse regulatory functions of estrogen receptor-{alpha} and -{beta} subtypes expressed in hypothalamic gonadotropin-releasing hormone neurons. *Mol Endocrinol* 2008; **22**: 2250–2259.