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17 β -Oestradiol Regulation of Gonadotrophin-Releasing Hormone Neuronal Excitability

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Abstract

17 β -Oestradiol (E₂) is essential for cyclical gonadotrophin-releasing hormone (GnRH) neuronal activity and secretion. In particular, E₂ increases the excitability of GnRH neurones during the afternoon of pro-oestrus in the rodent, which is associated with increased synthesis and secretion of GnRH. It is well established that E₂ regulates the activity of GnRH neurones through both presynaptic and postsynaptic mechanisms. E₂ significantly modulates the mRNA expression of numerous ion channels in GnRH neurones and alters the associated endogenous conductances, including potassium (K_{ATP}, A-type) currents and low-voltage T-type and high-voltage L-type calcium currents. Notably, K_{ATP} channels are critical for maintaining GnRH neurones in a hyperpolarised state for recruiting the T-type calcium channels, which are important for burst firing in GnRH neurones. In addition, there are other critical channels contributing to burst firing pattern, including the small conductance Ca²⁺-activated K⁺ channels that may be modulated by E₂. Despite these advances, the cellular mechanisms underlying the cyclical GnRH neuronal activity and GnRH release are largely unknown. Ultimately, the ensemble of both pre- and post-synaptic targets of the actions of E₂ will dictate the excitability and activity pattern of GnRH neurones.

Keywords

gonadotrophin-releasing hormone neurones; hyperpolarisation; T-type calcium channels; burst firing

Introduction

Gonadotrophin-releasing hormone (GnRH) neurosecretion and the control of the ovulatory cycle in females are modulated by gonadal steroids secreted from the ovaries. In addition, there is evidence for a role of locally (hypothalamic) synthesised steroids in the positive-feedback regulation of the luteinising hormone (LH) surge (1, 2). It has been found in a number of species, including the rat, sheep and rhesus monkey, that the preovulatory LH surge is accompanied by a surge in GnRH (3–6). The detailed relation between LH and GnRH has been most extensively evaluated in the sheep because, in this animal model, both portal and peripheral blood can be sampled simultaneously from awake freely moving animals, thus avoiding the stress of anaesthesia, restraint or the placement concerns

associated with sampling from the arcuate nucleus/median eminence region (7, 8). During the luteal phase in the sheep, GnRH is secreted as low-frequency pulses, which change to high-frequency low-amplitude pulses during the mid-follicular phase (9). In comparison, the LH surge during the late-follicular phase (proestrus) is accompanied by a high amplitude GnRH surge (4, 10). Interestingly, in the sheep but not in the macaque or rat, the GnRH surge continues long after the LH surge has terminated (4, 6, 9), indicating that the gonadotrophs desensitise to the GnRH surge input in sheep, thus shutting off LH secretion before feedback inhibition of GnRH secretion. In ovariectomised females, the GnRH and accompanying LH pulse size are increased and pulse frequency decreased to approximately one every hour. 17β -Oestradiol (E_2) treatment significantly reduces the GnRH and LH pulse size but increases pulse frequency, leading to an overall reduction in GnRH and LH secretion before induction of the GnRH and LH surge (7, 11). Collectively, these findings would indicate there are fundamental changes in GnRH neuronal firing activity during the different stages of the ovulatory cycle that are responsible for these changes. Consequently, in ovariectomised animals treated with E_2 to mimic negative- and positive-feedback regulation of GnRH, one would predict robust differences in neuronal firing activity between the different stages. However, notwithstanding recent advances on cellular experimentation, we still do not understand all of the signalling mechanism(s) by which E_2 regulates GnRH neurones. This review addresses current knowledge on E_2 regulation of mRNA expression, signalling properties and excitability in GnRH neurones, comparing changes associated with E_2 -induced negative- and positive-feedback regulation of GnRH neurones.

GnRH neuronal activity during negative- and positive-feedback

Using cell attached single-unit extracellular recording to evaluate GnRH neuronal firing activities during negative- compared to positive- feedback, it has been shown that the GnRH neuronal firing rate is low during the morning negative-feedback period and significantly higher during the evening positive-feedback period (12). As a potential mechanism, the investigators explored the spontaneous GABAergic postsynaptic currents, which were increased during positive-feedback and decreased during negative-feedback (13). This is congruent with the idea that GABA acting at GABA_A receptors is excitatory in GnRH neurones (14). The timing of the GnRH (LH) surge, and thus the increased activity of GnRH neurones at the time of the surge, is not only E_2 -dependent, but also it is entrained to a circadian input from the suprachiasmatic nucleus (SCN) (12, 15–17). The SCN expresses not only GABA and glutamate, but also vasopressin (VP) and vasoactive intestinal peptide (VIP) (18), which have received much attention as possible mediators of SCN circadian input to GnRH neurones and involvement in the timing of the GnRH surge (19–22). VP neurones within the SCN may act indirectly via kisspeptin neurones in the anteroventral periventricular nucleus (AVPV) area, but SCN VIP-projections may have a direct effect on GnRH neurones (16, 22, 23). VIP, when applied to GnRH neurones to test for alteration of firing rate in a cell attached mode, caused increased firing at surge onset in subpopulations of GnRH neurones, and had no effect on cell activity during morning negative-feedback (16). This supports the idea of a direct SCN input to GnRH neurones; however, further studies are necessary to more fully elucidate the SCN VIP functional input to GnRH neurones. Concerning the role of VP, anatomical studies support direct VP fibres from the SCN to the AVPV, and VP receptors are expressed on AVPV kisspeptin neurones (21). However, exogenous VP appears not to alter neuronal firing in the AVPV, questioning the physiological role of VP (24). Again, further experiments are needed to fully evaluate the SCN–AVPV–GnRH functional connection.

Models for GnRH neuronal firing

Studies in enhanced green fluorescent protein (EGFP)-GnRH mice have illustrated that GnRH neurones often fire in episodes of repetitive bursts (25–27). Currently, it is not known how the bursts are generated in GnRH neurones, although depolarising afterpotentials (also called afterdepolarising potentials) are proposed to be involved (27, 28). On the basis of current clamp and voltage clamp recordings in neurosecretory hypothalamic neurones, models have been developed including an ensemble of channels that appear to be critical for burst firing in parvocellular neurosecretory neurones (29–32). A recent model designed to explain short burst firing and related calcium oscillations in GnRH neurones has predicted that GnRH neurones spike spontaneously, which initiates the burst firing process by allowing an influx of a few Ca^{2+} ions. This is proposed to stimulate calcium-induced calcium release (CICR) from inositol-1,4,5-trisphosphate (IP_3) receptors, which is responsible for a large $[\text{Ca}^{2+}]$ transient. The calcium transient activates the slow afterhyperpolarisation small conductance (SK) current ($\text{sI}_{\text{AHP-SK}}$), which arrests the firing. In addition, CICR turns on a much slower K^+ channel, which is active for a much longer time and determines the interburst interval (31, 32).

On the basis of a model similar to that described and validated for thalamocortical relay neurones and hypothalamic neurosecretory neurones (29, 33–35), we had predicted that the T-type calcium current together with the hyperpolarisation-activated cation current (I_h) are essential for induction of burst firing in GnRH neurones and that calcium-dependent SK-type channels (underlying an AHP) are crucial for afterhyperpolarisation, allowing repetitive cycles of burst firing (29, 30).

E_2 regulation of low threshold spikes (LTS) and T-type channels in GnRH neurones

We became interested in LTS and burst firing after discovering that LTS were prominent in arcuate nucleus dopamine neurones from E_2 -treated females (36). Later, it was determined that T-type calcium channels were responsible for the LTS (33, 37). Three subunits of the T-type channel ($\text{Ca}_v3.1$, $\text{Ca}_v3.2$, $\text{Ca}_v3.3$) have been cloned (38, 39), with the specific gating properties of the T-type channel being dependent on the subunit composition. Thus, the $\text{Ca}_v3.1$ and $\text{Ca}_v3.2$ current profiles are more similar compared to $\text{Ca}_v3.3$, and compelling evidence suggests that these subunits are involved in short burst firing in thalamocortical relay neurones (34).

Initially, we found that there was an E_2 -induced augmentation in gene expression of a Ca^{2+} channel ($\text{Ca}_v3.1$) subunit in the arcuate nucleus after 24 h of treatment (40) and after long-term E_2 treatment (30 days) (41). The E_2 -induced increase in $\text{Ca}_v3.1$ subunit expression was accompanied by an increase in peak T-type Ca^{2+} current in arcuate neurones, including dopamine and POMC neurones (40), and in unidentified neurones in the VMH (42). On the basis of the role of T-type Ca^{2+} channels in modulating neuronal excitability (35, 43), the augmentation in the T-type Ca^{2+} current would facilitate burst firing and increase neurotransmitter release. Furthermore, the increase in $\text{Ca}_v3.1$ expression in the arcuate nucleus from E_2 treatment did not occur in oestrogen receptor (ER) α knockout mice (44), indicating that the expression is dependent on the nuclear receptor or signalling molecule(s) induced by ER α during development. Importantly, a ligand for a putative membrane ER (mER) called STX, with no binding affinity to ER α or ER β (45, 46), also significantly up-regulated the $\text{Ca}_v3.1$ subunit in the arcuate nucleus after long-term treatment (41). Collectively, this would suggest that the putative mER- and ER α -mediated transcriptional effects converge at the $\text{Ca}_v3.1$ gene.

GnRH neurones express all three of the T-type calcium channel subunits (Fig. 1) and the expression of these subunits are regulated by E_2 in a subunit-specific and diurnal manner (Fig. 2) (47, 48). Interestingly, the mRNA expression of all three subunits are increased by E_2 already in the morning (negative-feedback), and $Ca_v3.3$ remains elevated during the afternoon/evening (positive-feedback). These findings would indicate that the mRNA expression of the three Ca_v3 subunits is increased with increasing plasma E_2 concentration but, during the afternoon (at the time of lights out), a yet unidentified circadian signal shuts off the mRNA production of $Ca_v3.1$ and $Ca_v3.2$, but not $Ca_v3.3$. Of importance is the fact that T-type calcium currents can be measured in the majority of GnRH neurones (47, 49), and the $V_{1/2}$ for T-channel de-inactivation is -80.4 mV (i.e. the potential at which half of the T-type calcium channels are recruited as measured during the morning) (47).

Complementing the mRNA data, T-type current density is also increased with E_2 treatment already in the morning and remains elevated in the afternoon/evening together with increased rebound excitation (47). The role of the T-type Ca^{2+} currents, as found in other neuronal systems, is to facilitate rhythmic intracellular Ca^{2+} transients by initiating membrane depolarisation that recruits high voltage-activated (HVA) Ca^{2+} currents (50, 51). This is of functional significance because HVA Ca^{2+} currents are prevalent in GnRH neurones but they can only be recruited from a more depolarised (-40 mV) state (52); whereas, low voltage-activated T-type currents are recruited from a more hyperpolarised state (Fig. 3) (i.e. they provide the link for setting off burst firing as indicated from subunit deletion studies) (33, 47–49, 52–54). Importantly, the T-type channel subunit expression, current density and rebound excitation of GnRH neurones are significantly enhanced with a surge-inducing E_2 -treatment paradigm (47). Therefore, the E_2 -dependent increase in T-type calcium channels may serve to augment the excitability of GnRH neurones in preparation for the GnRH (LH) surge. However, further studies are needed for a more complete understanding of the role of T-type channels in GnRH neurones. Not unexpectedly, L-type calcium currents are also regulated by E_2 in GnRH neurones (52). In ovariectomised mice given an E_2 treatment to induce afternoon LH surges, the peak L-type current was reduced during the morning at the time of negative-feedback but increased during the afternoon at the time of positive-feedback. This would indicate that L-type currents may be significant contributors to the afternoon surge of GnRH (LH). However, the diurnal signal that precisely activates this current is unknown and needs to be further investigated.

Hyperpolarising input to GnRH neurones

It is well known that Kir channels are critical for maintaining excitable cells in a hyperpolarised resting state closer to E_K but, once cells are depolarised, the channels allow for the quick transition to long depolarising responses because of their inwardly rectifying properties (55). Accordingly, with greater membrane hyperpolarisation of GnRH neurones, there is an increase in the number of Na^+ spikes during rebound excitation (Fig. 3) (47). Indeed, K_{ATP} and G protein-coupled inwardly-rectifying potassium (GIRK) channels appear to be the critical Kir channels in GnRH neurones for maintaining the membrane in a relatively negative resting state (56, 57). Blocking K_{ATP} channels with the sulphonylurea tolbutamide significantly depolarises GnRH neurones, which is indicative of tonic K_{ATP} channel activity that is significantly augmented with E_2 treatment (56). We also know that GABAergic, opioid, neuropeptide Y and perhaps melanin-concentrating hormone (MCH) input via their respective $GABA_B$, μ -opioid, Y5 and MCH1 receptors activate GIRK channels to hyperpolarise GnRH neurones (57–60). This combination of inputs, leading to membrane hyperpolarisation, would allow for the recruitment of multiple excitatory conductances that are critical for generating burst firing in central nervous system neurones (29, 30, 35, 43).

Importantly, GnRH neurones express a time-dependent, hyperpolarisation-activated, cation current (I_h or pacemaker current) that contributes to rhythmic burst firing (30, 56, 58, 61). The activation of I_h by hyperpolarisation increases the spike frequency (61). In addition, the inhibition of I_h alters the pattern of burst firing and reduces the slope of recovery from the afterhyperpolarisation, supporting the idea that I_h plays an important role in GnRH neuronal excitability and contributes to burst firing.

Neuronal excitability and frequency of firing are also determined by the AHP that follows an action potential, and SK channels underlie a component of the median afterhyperpolarisation (mAHP) K^+ currents in CA1 pyramidal cells and other neurones (62). SK channel subunits are expressed in GnRH neurones (63, 64), and SK channel activation exerts a powerful influence on firing properties of GnRH neurones. However, recent findings have demonstrated at least two components of the slow I_{AHP} in GnRH neurones; one that is relatively fast and sensitive to apamin (SK) and a different slow-onset yet uncharacterised current that is sensitive to UCL2077 (31). The former regulates both intraburst and interburst neuronal firing, whereas the latter regulates only the interburst activity. Thus, multiple K^+ channels may play a key role in modulating GnRH neuronal excitability and the firing pattern during different reproductive states (31, 64, 65).

Intracellular calcium oscillation in GnRH neurones

Experiments *in vitro* using perfused hypothalamic tissue, primary hypothalamic cultures or a GT1 GnRH neuronal cell line have revealed that pulsatile GnRH release is present *in vitro* (66, 67). The spontaneous pulsatility in GnRH release from neuronal cultures is abolished in Ca^{2+} -deficient medium and is attenuated in the presence of nifedipine, an antagonist of L-type voltage-sensitive Ca^{2+} channels (67), emphasising the importance of calcium ions, calcium (L-type) channels and / or other calcium-dependent mechanisms for GnRH pulsatility.

More recently, a series of studies have shown that, in mouse hypothalamic neuronal explants and in primate nasal placode cultures, E_2 augments synchronous intracellular Ca^{2+} oscillations in developing GnRH neurones (68–70). This relatively rapid effect is observed within 10–30 min of the application of E_2 , implicating novel ERs or novel actions of classical ERs as mediators of the action of E_2 in GnRH neurones. The calcium oscillations in the presence of TTX are most likely the result of E_2 acting directly on GnRH neurones. Furthermore, E_2 conjugated to bovine serum albumin (BSA) at the C-17 position (E_2 -17 BSA) or to a large nondegradable poly(amido)amine-dendrimer macromolecule (71) mimics the effects of E_2 on intracellular calcium oscillations (68–70). However, in mouse explant experiments (68, 69), the E_2 -induced calcium oscillations are blocked by ICI 182,780 suggesting that an ER is involved. By contrast, E_2 -induced calcium oscillations in the primate placode cultures are not blocked by the antagonist ICI 182,780, and ICI 182,780 alone has no effect, suggesting that a non-ER mediated mechanism may be involved in E_2 regulation of calcium oscillations in primate GnRH neuronal cultures (70, 72). The actions of E_2 -BSA in mouse and monkey GnRH neuronal cultures are abrogated by pertussis toxin treatment, supporting a role for a G protein-coupled membrane receptor (68, 69, 72). Interestingly, GPR30 is expressed in developing primate GnRH neurones and may be responsible, in part, for the rapid actions of E_2 in these neurones (72). Calcium oscillations in GnRH neurones can also be induced presynaptically via an $ER\alpha$ -dependent release of GABA from presynaptic terminals (73). Collectively, these findings indicate that there are multiple acute actions of E_2 in GnRH neurones, some of which may involve a novel receptor and perhaps GPR30, and some that involve $ER\alpha$ and / or $ER\beta$.

To study calcium oscillations in adult GnRH neurones, slices from genetically-modified mice that express the calcium indicator ratiometric pericam in GnRH neurones have been utilised (74). With this model, dual recordings of calcium transients and electrical activity revealed that intracellular calcium transients, generated through L-type calcium channels and amplified by calcium release from intracellular stores, are synchronised with burst firing in subpopulations of GnRH neurones (31). It is, however, unknown whether E₂-treatment will induce synchronised calcium oscillations that are associated with E₂-induced burst firing of adult GnRH neurones *in situ*.

Kisspeptin excitatory input to GnRH neurones

Kisspeptin potentially depolarises hypothalamic GnRH neurones through a combination of inhibiting an inwardly rectifying potassium (Kir) conductance and activating a nonselective cationic (TRPC) conductance (75–78). To date, kisspeptin is the most potent and efficacious neuropeptide/neurotransmitter to modulate GnRH neuronal activity (26, 27, 58, 79–83). Besides activating TRPC channels in GnRH neurones, kisspeptin also attenuates a resting (barium and cesium sensitive) Kir current. The inhibition of Kir may be critical because Kir channels (e.g. K_{ATP} channels) are highly expressed in GnRH neurones and clamp the cells in a negative resting state of –63 mV (56–58). This effect of kisspeptin would also be vital for inhibiting G protein-coupled receptor-activated (μ -opioid, GABA_B and MCH) GIRK (Kir) currents which are active in GnRH neurones (57, 58, 60). Moreover, kisspeptin treatment of mature as well as developing GnRH neurones causes increased calcium oscillations and these changes, for the most part, reflect the stimulatory effect of kisspeptin on the plasma membrane via coupling of GPR54 to a phospholipase C signalling pathway (75, 76, 78, 84). Therefore, by inhibiting Kir channels along with the pronounced activation of nonselective cationic (TRPC) channels, kisspeptin depolarises GnRH neurones to threshold (approximately –45 mV) and induces sustained firing, which may be accompanied by a sustained calcium ion influx via calcium channels/TRPC channels and GnRH release during positive-feedback.

Interestingly, GnRH neurones express all of the ‘brain’ TRPC channel subunits (TRPC1, 3, 4, 5, 6, 7), with TRPC1, 4 and 5 being the most prevalent in GnRH neurones (75). It is well known that the current–voltage relationship and mechanism of regulation of TRPC channels are dependent on the channel subunit composition (85). On the basis of the current–voltage relationship, pharmacological profile and mRNA expression, TRPC1, 4, and 5 are key players in mediating the excitatory effects of kisspeptin in GnRH neurones. Traditionally, these channels are known as ‘store operated calcium channels’, although this description may be the result of poorly understood signalling mechanisms (85, 86). Therefore, current research has focused on elucidating the signalling pathway(s) by which kisspeptin activates TRPC channels, and the sources of calcium mobilisation following kisspeptin activation of GnRH neurones. Although most findings appear to indicate that the initial calcium signal comes via plasma membrane channels (75, 78, 84), there is also evidence that kisspeptin activation results in the release of calcium from intracellular stores in GnRH neurones via IP₃ receptors (76).

The precise nature by which the kisspeptin signals reach GnRH neurones is not fully understood. Kisspeptin neurones in the AVPV are positively regulated by E₂ and are considered to contribute to positive-feedback input to rodent GnRH neurones (87). The AVPV is a complex nucleus that expresses other neurones (transmitters) in addition to kisspeptin, such as dopamine, GABA and glutamate (88, 89). Although anatomical connections from the AVPV to GnRH neurones have been described in a number of studies, the functional interactions have not been elucidated until recently (90). Thus, stimulation of the AVPV and the recording of responses in GnRH neurones revealed that low stimulation

rates (< 1 Hz) induced glutamate and GABA synaptic currents in GnRH neurones, whereas higher frequency stimulation (5–10 Hz) induced delayed excitation considered to be a kisspeptin response because it was absent in GPR54 knockout animals and antagonised by a kisspeptin antagonist (90). Therefore, the AVPV kisspeptin neurones may provide a critical excitatory input to GnRH neurones.

Conclusions

Recent experimental findings have greatly increased our understanding of E₂ regulation of GnRH neurones. In particular, remarkable progress has been made subsequent to the production of EGFP-GnRH expressing mice and rats, which have allowed direct targeting of the otherwise scattered GnRH neurones. Numerous publications addressing questions concerning E₂ regulation of GnRH neuronal activity and its association with calcium signalling and GnRH secretion, coupled with a better understanding of the influence of diurnal rhythms, have advanced the field. However, challenging questions remain concerning the mechanisms by which neuronal burst firing drives pulsatile GnRH release, the integration of presynaptic and postsynaptic E₂ actions, as well as the mechanism by which rapid membrane-initiated effects of E₂ is coupled to long-term genomic effects. In addition, the mechanism of kisspeptin signalling in GnRH neurones, and the mechanism by which the circadian signals is transmitted to GnRH neurones for the induction of negative- and positive-feedback needs to be elucidated. Future studies need to address these challenging questions to allow a more complete understanding of E₂ modulation of GnRH neuronal excitability.

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Fig. 1.

Ca_v3.1, Ca_v3.2, and Ca_v3.3 mRNA expression in single gonadotrophin- releasing hormone (GnRH) neurones. (A) Representative gel illustrating the mRNA expression of Ca_v3.1, Ca_v3.2, and Ca_v3.3 subunits in harvested GnRH neurones from 17 β -oestradiol (E₂)-treated animals. The expected size of the polymerase chain reaction products are (in base pairs): for GnRH, 239 bp; for Ca_v3.1, 250 bp; for Ca_v3.2, 284 bp; for Ca_v3.3, 128 bp. Control GnRH neurone (-RT) was amplified from a harvested cell without reverse transcriptase. (A total of eight neurones were processed without RT, all of which were negative) MM, Molecular marker. (B) Summary bar graphs of the percentages of expression of Ca_v3.1, Ca_v3.2, and Ca_v3.3 in GnRH neurones from oil- or E₂-treated animals. Twelve to 20 GnRH neurones from each of four or five E₂-treated and 12–20 neurones from each of four oil-treated females were analysed, and the mean number of neurones expressing Ca_v3 subunits from each animal was used for further analysis. Bar graphs represent the mean $\pm$ SEM of percentage GnRH neurones expressing each Ca_v3 subunit/animal. Numbers of animals are indicated Reproduced with permission from Zhang *et al.* (47).

Fig. 2.

17 β -Oestradiol (E₂) regulates Cav3.1, Cav3.2 and Cav3.3 mRNA expression in gonadotrophin-releasing hormone (GnRH) neurones. (A, B) Quantitative real-time polymerase chain reaction measurements of Cav3.1, Cav3.2 and Cav3.3 mRNAs in GnRH neuronal pools (3–5 per animal) from oil- and E₂-treated mice (n = 3–6 animals per group) obtained during the morning (A) or during the afternoon (B). E₂ upregulates the mRNA expression of Cav 3.1, Cav3.2, and Cav3.3 during the morning. Cav3.3 remained high during the afternoon but Cav3.1 and Cav3.2 were down-regulated. The expression values were calculated via the $\Delta\Delta$ CT method and normalised to the mean Δ CT of the oil-treated samples. Bar graphs represent the mean $\pm$ SEM. *P < 0.05, oil versus E₂. Reproduced with permission from Zhang *et al.* (47).

Fig. 3.

17 β -Oestradiol (E_2) enhances T-type Ca^{2+} channel-induced low threshold spikes (LTS) in gonadotrophin-releasing hormone (GnRH) neurones. (A) A silent GnRH neurone was induced to fire from its resting membrane potential (-57 mV) with a depolarising current pulse. Note the regular firing (action potentials) in this representative GnRH neurone. (B, C) Representative evening recordings from GnRH neurones in E_2 - (B) and oil-treated (C) females showing hyperpolarisation-induced burst firing. There was no spontaneous firing at the resting membrane potentials; however, a hyperpolarising current pulse to -83 mV for 1 s with a quick return to their resting membrane potential induced rebound excitation with four versus one fast sodium spikes riding on the LTS in a GnRH neurone from E_2 and oil-treated females, respectively. Cells were recorded in K^+ -gluconate internal solution with 11 mM ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA) and 100 μ M 2-aminoethyl diphenylborinate (2-APB) added to the normal artificial cerebrospinal fluid to block the A-type potassium channel (91). (D, E) In another cell from an E_2 -treated female, T-type calcium channel-mediated calcium spikes (superimposed traces of rebound depolarisation) were clearly displayed in the presence of tetrodotoxin (D), and the LTS were blocked by nickel (200 μ M) (E). The bottom trace shows the current-clamp protocol for (D) and (E). Note the minor activation of I_h , supporting findings that T-type channels underlie the LTS in GnRH neurones. The LTS serves to bring the membrane potential to threshold for fast sodium spike generation, which is the most efficient mode for transmitter (neuropeptide) release not only in GnRH neurones, but also neurones throughout the central nervous system.