



# Ion Channels of Pituitary Gonadotrophs and Their Roles in Signaling and Secretion

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Gonadotrophs are basophilic cells of the anterior pituitary gland specialized to secrete gonadotropins in response to elevation in intracellular calcium concentration. These cells fire action potentials (APs) spontaneously, coupled with voltage-gated calcium influx of insufficient amplitude to trigger gonadotropin release. The spontaneous excitability of gonadotrophs reflects the expression of voltage-gated sodium, calcium, potassium, non-selective cation-conducting, and chloride channels at their plasma membrane (PM). These cells also express the hyperpolarization-activated and cyclic nucleotide-gated cation channels at the PM, as well as GABA<sub>A</sub>, nicotinic, and purinergic P2X channels gated by  $\gamma$ -aminobutyric acid (GABA), acetylcholine (ACh), and ATP, respectively. Activation of these channels leads to initiation or amplification of the pacemaking activity, facilitation of calcium influx, and activation of the exocytic pathway. Gonadotrophs also express calcium-conducting channels at the endoplasmic reticulum membranes gated by inositol trisphosphate and intracellular calcium. These channels are activated potently by hypothalamic gonadotropin-releasing hormone (GnRH) and less potently by several paracrine calcium-mobilizing agonists, including pituitary adenylate cyclase-activating peptides, endothelins, ACh, vasopressin, and oxytocin. Activation of these channels causes oscillatory calcium release and a rapid gonadotropin release, accompanied with a shift from tonic firing of single APs to periodic bursting type of electrical activity, which accounts for a sustained calcium signaling and gonadotropin secretion. This review summarizes our current understanding of ion channels as signaling molecules in gonadotrophs, the role of GnRH and paracrine agonists in their gating, and the cross talk among channels.

**Keywords:** gonadotrophs, gonadotropin-releasing hormone, voltage-gated channels, ligand-gated channels, electrical activity, calcium signaling, luteinizing hormone secretion

## INTRODUCTION

Gonadotrophs are the anterior pituitary cell lineage specialized for synthesis and release of two gonadotropins, such as follicle-stimulating hormone and luteinizing hormone (LH) (1). In addition to genes encoding beta subunits of gonadotropins, *Fshb* and *Lhb* (2, 3), gonadotrophs are defined by at least two other genes not expressed in other secretory pituitary cell types, such as gonadotropin-releasing hormone (GnRH) receptor (GnRHR) gene (*Gnrhr*) (4) and dentin matrix protein 1 gene

(5). Together with thyrotrophs, gonadotrophs express the *Cga* gene encoding the  $\alpha$  glycoprotein subunit (6). Ontogenetically, the lineage commitment is associated with the expression of the orphan nuclear receptor NR5A1, a transcriptional factor that also plays a role in the expression of gonadotroph-specific genes in the postnatal animals (7).

Gonadotrophs are neuron-like; they express numerous voltage-gated sodium ( $\text{Na}_v$ ), calcium ( $\text{Ca}_v$ ), potassium ( $\text{K}_v$ ), and chloride channels at the plasma membrane (PM), and fire action potentials (APs) spontaneously (8). These cells also express ligand-gated ion channels at PM, which activation by hypothalamic and intrapituitary ligands leads to increase in firing frequency and facilitation of  $\text{Ca}^{2+}$  influx and hormone release (9). The function of gonadotrophs is regulated by several  $\text{Ca}^{2+}$ -mobilizing receptors capable of modulating electrical activity and AP-dependent  $\text{Ca}^{2+}$  influx and hormone release (10). The main  $\text{Ca}^{2+}$ -mobilizing receptor for these cells is GnRHR, signaling through heterotrimeric  $\text{G}_{q/11}$  proteins (11), which  $\alpha$  subunit activates phospholipase C- $\beta$ 1, leading to generation of inositol-1,4,5-trisphosphate ( $\text{IP}_3$ ) and diacylglycerol (12) and release of  $\text{Ca}^{2+}$  from endoplasmic reticulum (ER) through  $\text{IP}_3$  receptor ( $\text{IP}_3\text{R}$ ) channels (9).

Here, we focus on the role of ion channels in electrical/ $\text{Ca}^{2+}$  signaling and  $\text{Ca}^{2+}$ -controlled cellular functions in gonadotrophs. We will first review the expression and roles of voltage-gated channels in spontaneous excitability and accompanied  $\text{Ca}^{2+}$  influx in these cells, followed by description of additional channels contributing to facilitation or modulation of excitability of these cells. These include the hyperpolarization-activated and cyclic nucleotide-gated (HCN) channels, acetylcholine (ACh)-gated receptor (AChR) channels,  $\gamma$ -aminobutyric acid (GABA)-gated A-type receptor ( $\text{GABA}_A\text{R}$ ) channels, and ATP-gated receptor ( $\text{P2XR}$ ) channels, all expressed at PM, and  $\text{IP}_3\text{R}$  channels expressed at ER membranes.

## SIGNALING BY VOLTAGE-GATED CHANNELS

The superfamily of voltage-gated ion channels of more than 140 members, including  $\text{Na}_v$ ,  $\text{Ca}_v$ ,  $\text{K}_v$ , and numerous less selective channels, is one of the largest groups of signal transduction proteins (13). These channels are also expressed in gonadotrophs and account for spontaneous and receptor-controlled electrical and  $\text{Ca}^{2+}$  signaling (9).

Nine members of  $\text{Na}_v$  channels are expressed in mammals, which contribute to the initiation and propagation of APs (14). The inward  $\text{Na}_v$  current has been identified in rat (15, 16), ovine (17), fish (18, 19) and mouse native (20, 21), and immortalized gonadotrophs (22, 23). **Figure 1A** shows traces of  $\text{Na}_v$  currents in cultured rat gonadotrophs. It appears that the level of  $\text{Na}_v$  channel expression is greater in these cells than in other secretory anterior pituitary types (16). Voltage-insensitive  $\text{Na}^+$  conductance is also present in all endocrine pituitary cells, including gonadotrophs (24, 25).

The  $\text{Ca}_v$  channels have a dual role in excitable cells: they generate inward currents that can initiate APs and are also critical for coupling of electrical signals on PM with physiological intracellular

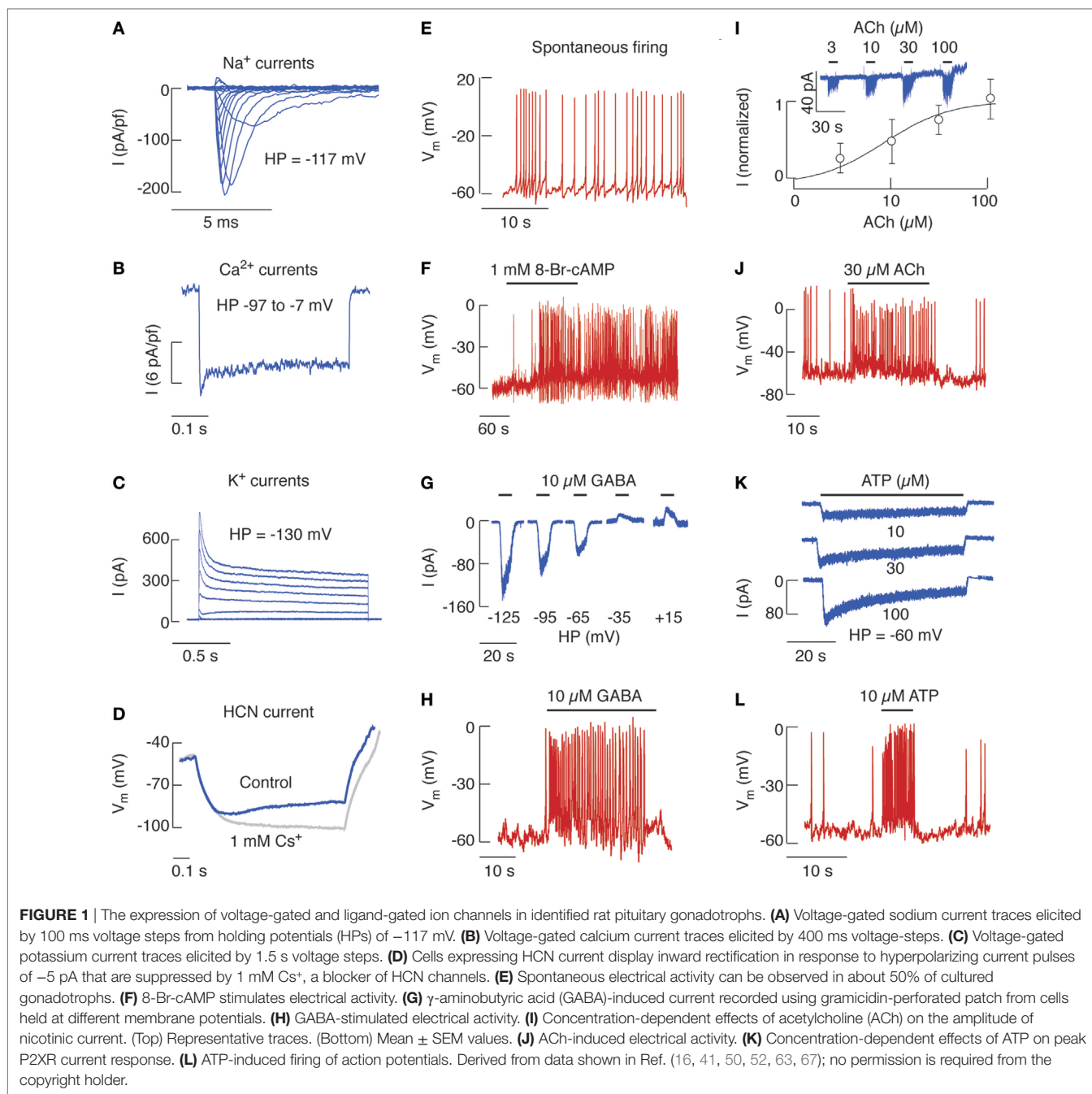
events by generating intracellular  $\text{Ca}^{2+}$  signals. There are 10 members of these channels that exhibit different electrophysiological and pharmacological properties (26). Pituitary gonadotrophs express at least inactivating T-type and non-inactivating L-type  $\text{Ca}_v$  currents, as documented in cultured cells from rat (16), mouse (21), ovine (27), fish (19), as well as in  $\alpha\text{T3-1}$  immortalized gonadotrophs (22). **Figure 1B** shows a representative trace of  $\text{Ca}_v$  current in rat gonadotrophs.

The  $\text{K}_v$  channels are composed of at least four functional classes: fast activating delayed rectifier, slow activating delayed rectifier (including M channels), A-type  $\text{K}_v$  channels, and ether-a-go-go-gene channels (28). **Figure 1C** illustrates total  $\text{K}_v$  currents in rat pituitary gonadotrophs, which are driven by several  $\text{K}_v$  channels.  $\alpha\text{T3-1}$  gonadotrophs (22) and native goldfish (19), rat (16) and ovine (29) gonadotrophs express delayed rectifiers, which expression is controlled by estrogens (29). The A-type  $\text{K}_v$  channels are also expressed in  $\alpha\text{T3-1}$  cells (22) as well as in native fish (18, 19, 30), frog (31), and rat (16, 32, 33) gonadotrophs. In rats, the expression level of these channels is much higher in gonadotrophs than somatotrophs (16). Functional M-type channels are expressed in mouse gonadotrophs and GnRH through a still uncharacterized signal cascade inhibits these channels (34). Moreover, our transcriptome study implies that a pulsatile GnRH application downregulates the expression of *Kcna2* ( $\text{K}_v$  1.2) and *Kcnh6* (ether-a-go-go), while it upregulates *Kcnk10* (outward rectifier) and  $\text{Na}^+/\text{Ca}^{2+}$  exchanger *Slc24a3*, indicating that GnRHR may indirectly be involved in regulation of cell excitability (5).

Calcium-activated  $\text{K}^+$  channels ( $\text{K}_{\text{Ca}}$ ) are composed of two families: three small-conductance  $\text{K}^+$  (SK) channels and one intermediate-conductance channel are members of the first family and the high-conductance  $\text{K}^+$  (BK) channels belong to the second family. These channels are activated by elevation in cytosolic  $\text{Ca}^{2+}$  and play a critical role in control of firing properties of excitable cells (35), including pituitary cells (36). The expression of SK channels is well documented in fish (37), rat (38, 39), mouse (40), and ovine gonadotrophs (17), and the level of their expression is dependent on estradiol (20). Whole-cell current recordings confirmed the presence of BK current in several pituitary cell types but not in gonadotrophs (16).

Gonadotrophs also express the hyperpolarization-activated and cyclic nucleotide-gated (HCN) channels (41), which are permeable to both  $\text{K}^+$  and  $\text{Na}^+$  and play a critical role in cardiac rhythmicity (42). As their name indicates, HCN channels are activated by voltage (**Figure 1D**) and cyclic nucleotides. Rat gonadotrophs and other pituitary cell types also express the cation-conducting transient receptor potential (TRP) cation-like channels (43), initially characterized by their role in *Drosophila* phototransduction (44). Mouse gonadotrophs express TRPC5 subtype of these channels, which are activated by GnRH and promote  $\text{Ca}^{2+}$  influx (45). Finally,  $\text{Ca}^{2+}$ -activated non-selective cationic currents are present in rat gonadotrophs, but the nature of these channels has not been identified (46).

The expression of voltage-gated channels in gonadotrophs makes them electrically excitable cells, i.e., capable of exhibiting regenerative and propagated APs spontaneously or in response to stimulation. In general, the membrane potential ( $V_m$ ) of



single gonadotrophs in culture is not stable but fluctuates from resting potentials of  $-60$  to  $-50$  mV due to spontaneous activity of hyperpolarizing and depolarizing channels. When the depolarization waves reach the threshold level, gonadotrophs fire tall and narrow APs (**Figure 1E**), with spiking frequency of  $\sim 0.7$  Hz, amplitude of more than 60 mV, and half-width of about 50 ms (47). Ovine gonadotrophs also fire single APs spontaneously (17). In contrast to gonadotrophs, other pituitary cell types predominantly exhibit bursting pattern of spontaneous electrical activity, i.e., periodic depolarized potentials with superimposed small-amplitude spikes (47–50).

Depolarizing currents are pacemaking currents, accounting for a gradual reduction of PM resting potential toward the threshold for AP firing, and spike depolarization currents, accounting for the upstroke of an AP. The nature of channels contributing to pacemaking depolarization in gonadotrophs is not well characterized. The ongoing work is focused on the potential role of background  $\text{Na}^+$  (24) and TRP channels (43) in this process. The cell permeable cAMP analog 8-Br-cAMP initiates AP firing in quiescent gonadotrophs (**Figure 1F**) and increases the frequency of spikes in spontaneously firing cells (41), an action consistent with the expression of HCN channels (**Figure 1D**) and/or protein

kinase A-mediated phosphorylation of some other channels in gonadotrophs (51).

The main function of  $\text{Na}_v$  channels is to depolarize cells and generate the upstroke of the AP, controlling the firing amplitude in excitable cell. In gonadotrophs, they act in conjunction with  $\text{Ca}_v$  channels to generate APs (17) or  $\text{Ca}_v$  channels are exclusively responsible for the spike depolarization (52). Simultaneous measurements of  $V_m$  and  $[\text{Ca}^{2+}]_i$  showed that the bulk  $\text{Ca}^{2+}$  levels are low (50–100 nM) in spontaneously spiking gonadotrophs, in contrast to spontaneously bursting lactotrophs, somatotrophs and  $\text{GH}_3\text{B}_6$  cells, which generate much higher (300–1,200 nM) and clearly oscillatory  $\text{Ca}^{2+}$  transients (48, 52, 53). In gonadotrophs, AP-driven  $\text{Ca}^{2+}$  influx is below the threshold needed to trigger exocytosis (52), whereas the bursting type of electrical activity in lactotrophs and somatotrophs accounts for high basal hormone secretion (48, 52). Because in intact pituitary cell lineages are organized as complex networks (54–56), further studies are needed to characterize the excitatory and secretory patterns in pituitary cells with preserved tridimensional structure.

## SIGNALING BY LIGAND-GATED RECEPTOR CHANNELS

Ligand-gated receptors channels are activated by chemical signals (ligands) rather than to changes in the  $V_m$ . These proteins are typically composed two different domains: a pore forming transmembrane domain and an extracellular domain containing the ligand binding site. There are three families of these channels: the Cys-loop family of channels activated by ACh, 5-HT, GABA, and glycine (57), glutamate-gated receptor-channels (58), and ATP-gated purinergic P2XR channels (59). Pituitary gonadotrophs express  $\text{GABA}_A\text{R}$ , nicotinic AChR, and P2X2R channels (9).

$\gamma$ -Aminobutyric acid is acting through  $\text{GABA}_A\text{R}$  and  $\text{GABA}_C\text{R}$  channels permeable to  $\text{Cl}^-$ ; in the central nervous system, GABA usually silences electrical activity and  $\text{Ca}^{2+}$  signaling (60). However, in gonadotrophs GABA and muscimol, a  $\text{GABA}_A\text{R}$  agonist, increase intracellular  $\text{Ca}^{2+}$ , suggesting that chloride-mediated depolarization activates  $\text{Ca}_v$  channels. Furthermore, the  $\text{GABA}_A\text{R}$  channel reversal potential for chloride ions is positive to the baseline  $V_m$  (Figure 1G), and the activation of these channels results in depolarization of cells and initiation of AP firing (Figure 1H) and stimulation of *Fshb* and *Lhb* expression (61) and LH release (62). The lower expression of cation/chloride transporter KCC2 in rat pituitary cells probably accounts for the depolarizing nature of  $\text{GABA}_A\text{R}$  channels in cultured gonadotrophs (63).

The binding of nicotine, ACh, or other ligands to AChR channels stimulates cation ( $\text{Na}^+$  and  $\text{K}^+$  and for some neuronal subtypes  $\text{Ca}^{2+}$  as well) influx through a channel and generally results in membrane depolarization. Seventeen subunits of nicotinic AChR have been identified and were shown to assemble into a variety of receptor subtypes (64, 65). We have shown recently the expression of  $\beta 2$ ,  $\beta 1$ ,  $\alpha 9$ , and  $\alpha 4$  mRNAs in cultured rat pituitary cells and  $\beta 2$ ,  $\alpha 4$ , and  $\alpha 1$  in immortalized L $\beta$ T2 mouse gonadotrophs. We also showed the expression of  $\beta 2$  subunit protein in gonadotrophs (50). These cells express nicotinic AChR channels capable

of generating an inward current (Figure 1I) and facilitating electrical activity (Figure 1J) and  $\text{Ca}^{2+}$  influx (not shown). We also found that GnRHR stimulation downregulates gene expression of both  $\alpha 4$  and  $\alpha 9$  subunits (5, 50), suggesting that the expression of nicotinic AChR in gonadotrophs *in vitro* compensates for the loss of GnRH stimulation.

ATP is not only an intracellular molecule but is also released by cells and acts as an extracellular ligand for P2XR family of channels, composed of three subunits, each composed of a large ectodomain, two transmembrane domains and the N- and C-terminus facing the cytoplasm (59). In intact gonadotrophs, ATP-induced extracellular  $\text{Ca}^{2+}$ -dependent rise in cytosolic  $\text{Ca}^{2+}$  (66). In voltage-clamped cells, extracellular ATP-induced non-oscillatory current composed of rapidly depolarizing, slowly desensitizing, and rapidly deactivating phases, with the peak amplitudes and the rates of current desensitization determined by ATP concentration (Figure 1K). In current-clamped gonadotrophs, ATP induces a rapid depolarization that initiated firing of APs in quiescent cells, an increase in the frequency of firing in spontaneously active cells (Figure 1L), and a transient stimulation of LH release (67). The biophysical and pharmacological investigations suggested that gonadotrophs express the P2X2R subtype of these channels (67). Consistent with this conclusion, the full size and several splice forms of P2X2 subunit were identified in pituitary gland (68).

ATP is released by GnRH-secreting GT1 cells and cultured pituitary cells and metabolized by ectonucleotidase (69). Furthermore, GnRH increases ATP release in cultured pituitary cells (66). In accordance with these observations, it has been shown that ATP is co-secreted with GnRH from the median eminence into the hypophyseal-portal vasculature in ovariectomized sheep and that gonadotrophs have intrinsic ability to metabolize ATP in the extracellular space (70). This is consistent with the autocrine actions of extracellular ATP, where this molecule amplifies GnRH-induced  $\text{Ca}^{2+}$  signaling and LH secretion by activating P2X2Rs (67, 70). Pituitary cells other than gonadotrophs also express pannexin-1 and -2 channels (71), which contribute to ATP release in the extracellular medium in cultured pituitary cells (72). Thus, ATP and its degradation products ADP and adenosine may serve as paracrine factors to provide a cross talk between cell lineages within the pituitary gland *via* P2X2R (67), P2X4R (73), G-protein-coupled P2YRs (74), and adenosine receptors (75). By physical association with P2XRs, pannexin-1 may also provide a mechanism for autocrine control of functions of pituitary cell types expressing both proteins (76).

## SIGNALING BY CHANNELS EXPRESSED IN ER MEMBRANES

Two families of structurally and functionally similar  $\text{Ca}^{2+}$  release channels, ryanodine receptors and  $\text{IP}_3\text{Rs}$ , are expressed in the ER membrane. Ryanodine receptors account for intracellular transduction and translation of PM electrical signals by  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release from ER, whereas  $\text{IP}_3\text{Rs}$  are activated by  $\text{Ca}^{2+}$ -mobilizing receptors. In non-excitabile cells, the  $\text{IP}_3\text{R}$ -induced depletion of ER- $\text{Ca}^{2+}$  stores facilitates  $\text{Ca}^{2+}$  influx

through store-operated  $\text{Ca}^{2+}$ -conducting PM channels. Two proteins, named stromal-interacting molecule and Orai, are critical for this  $\text{Ca}^{2+}$  entry pathway (77).  $\text{IP}_3$ Rs are expressed in all secretory pituitary cells as indicated by ability of numerous  $\text{Ca}^{2+}$ -mobilizing agents to trigger  $\text{Ca}^{2+}$  release from ER (9). In contrast, no conclusive evidence was presented about the expression and role of ryanodine receptors and Orai channels in gonadotrophs and other secretory pituitary cell types (36).

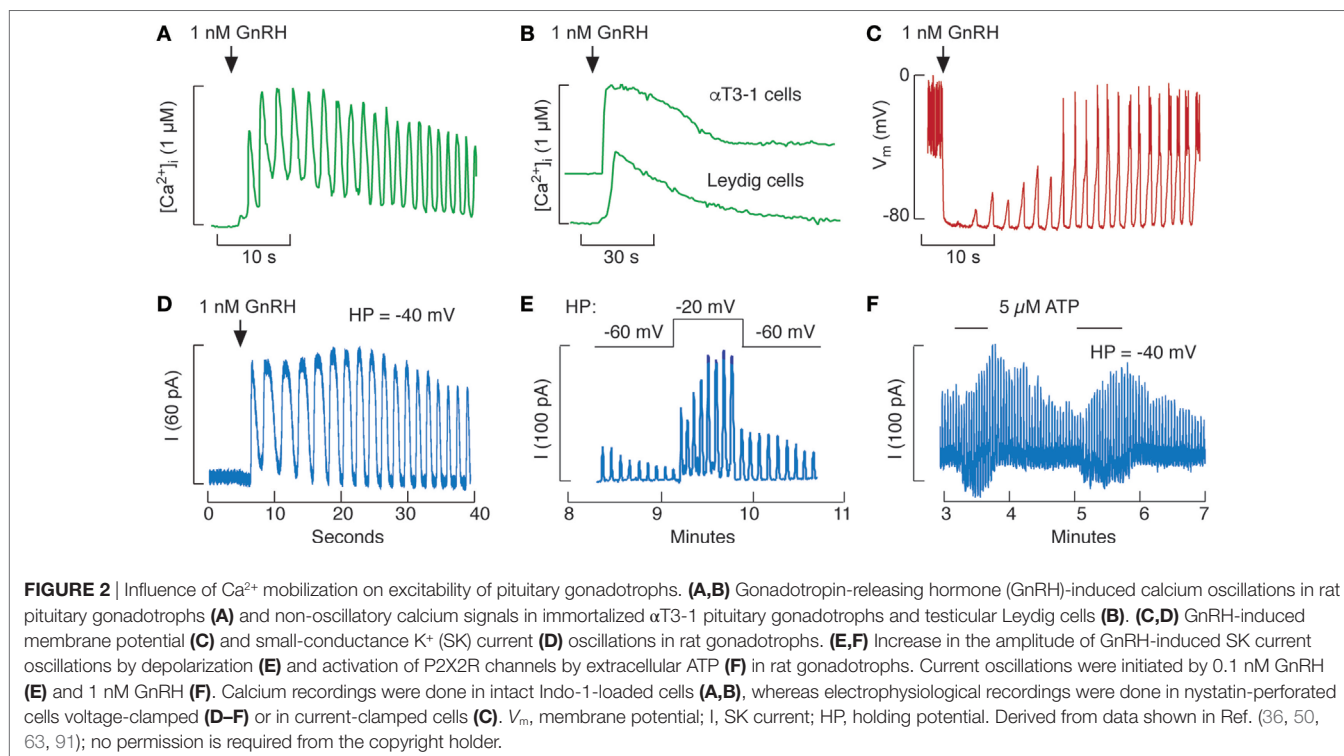
The  $\text{Ca}^{2+}$ -mobilizing pathway is operative in gonadotrophs and is activated by GnRH as well as by pituitary adenylate cyclase-activating peptides, endothelins, ACh, vasopressin and oxytocin (50, 78–80). Among pituitary cells, a unique characteristic of mammalian gonadotrophs is the oscillatory pattern of  $\text{Ca}^{2+}$  release through  $\text{IP}_3$ Rs. **Figure 2A** illustrated GnRH-induced  $\text{Ca}^{2+}$  oscillations. In contrast,  $\alpha\text{T3-1}$  (**Figure 2B**) and  $\text{L}\beta\text{T2}$  gonadotrophs (not shown) release  $\text{Ca}^{2+}$  in a non-oscillatory manner when stimulated with GnRH (81, 82). GnRH-induced calcium signaling is also non-oscillatory in fish pituitary cells (83) as well as in rat Leydig cells (**Figure 2B**) (84). In rat gonadotrophs, the frequency of  $\text{Ca}^{2+}$  oscillations is determined by GnRH concentration and varies between 3 and 20 pulses per minute (85, 86). In neonatal rat gonadotrophs, GnRH-induced, but not  $\text{IP}_3$ -stimulated,  $\text{Ca}^{2+}$  oscillations are inhibited by melatonin (87–90).

Gonadotropin-releasing hormone-induced  $\text{Ca}^{2+}$  oscillations have profound effects on electrical activity of these cells. In current-clamped gonadotrophs, GnRH-induced a transient hyperpolarization, followed by a bursting pattern episode of tall electrical spikes (**Figure 2C**). When the membrane was voltage-clamped, GnRH-induced current oscillations were observed (**Figure 2D**) (91, 92). Patterns of  $\text{Ca}^{2+}$  and current oscillations are

highly comparable in the same cell and current oscillations coincide with transient hyperpolarization of PM. It is well established that  $\text{Ca}^{2+}$ -activated SK channels account for coupling from the ER to PM in rat gonadotrophs (16, 38, 46, 93), whereas BK channels may also contribute to such coupling in mice gonadotrophs (40). In non-oscillatory  $\alpha\text{T3-1}$  gonadotrophs, GnRH stimulates L-type  $\text{Ca}^{2+}$  channels, leading to protein kinase C-dependent ERK activation (94), a process that requires dynamin GTP-ase activity (95).

The physiological relevance of bursting electrical activity in GnRH-stimulated gonadotrophs has been shown in voltage-clamped cells. By controlling the holding potential (HP) of the cell, this procedure provides a way to control the  $\text{Ca}^{2+}$  influx rate. In hyperpolarized cells with silent  $\text{Ca}_v$  channels, GnRH-induced current oscillations persist for about 5 min, reflecting a gradual depletion of the ER  $\text{Ca}^{2+}$  content. However, when the HP was more depolarized, many  $\text{Ca}_v$  channels are open and GnRH-induced current oscillations last much longer (**Figure 2E**), indicating that voltage-gated  $\text{Ca}^{2+}$  influx sustains signaling (91). Facilitation of  $\text{Ca}^{2+}$  influx through P2X2R channels also increases amplitudes of sustained GnRH-stimulated current oscillations (**Figure 2F**), a finding consistent with effect of ATP on GnRH-induced  $V_m$  oscillations and LH release (67).

The gating properties of  $\text{IP}_3$ R channels in gonadotrophs were not studied directly, and our understanding of kinetics of opening and closing is based on analysis of GnRH/ $\text{IP}_3$ -induced  $\text{Ca}^{2+}$ /current oscillations.  $\text{IP}_3$  is needed to initiate the ER-dependent  $\text{Ca}^{2+}$  signaling, oscillations in intracellular  $\text{IP}_3$  are not required to generate oscillatory  $\text{Ca}^{2+}$  release as documented by injection of non-metabolizable  $\text{IP}_3$  analogs, and the concentration of  $\text{IP}_3$



underlines the frequency of spiking (96). Furthermore, cytosolic  $\text{Ca}^{2+}$  influences  $\text{IP}_3$ -dependent  $\text{Ca}^{2+}$  release in these cells bidirectionally, stimulatory at lower concentrations and inhibitory at higher concentrations. The rapid stimulatory effect of  $\text{Ca}^{2+}$  on  $\text{IP}_3$ -dependent  $\text{Ca}^{2+}$  release is shown by phase resetting of GnRH-induced oscillations by a brief pulse of voltage-gated  $\text{Ca}^{2+}$  entry (97). The inhibitory effect of high  $\text{Ca}^{2+}$  concentrations on GnRH-induced  $\text{Ca}^{2+}$  oscillations was also shown (98).

## INTERCELLULAR SIGNALING BY GAP JUNCTION CHANNELS

Secretory cells are not randomly spread throughout the pituitary gland but represent very organized three-dimensional network structures critical for the proper cell-type function (54, 99). Tridimensional imaging also suggested that pituitary gonadotrophs form a homotypic network (55). These and other pituitary cells express connexin-43 (100). In general, coupling of cells through connexin gap junctions provides a pathway for the passage of ions, metabolites, small molecules, and second messengers from cell to cell, without exposure to the extracellular environment (101, 102). However, the roles of connexins in synchronization of gonadotroph activity in intact tissue have not been systematically investigated.

## CONCLUSION

This short review clearly indicates the complexity in expression and role of PM and ER channels in gonadotrophs. Various

voltage-gated and related channels provide a background pathway for spontaneous firing of APs and  $\text{Ca}^{2+}$  signaling. In contrast to other secretory pituitary cells, spontaneous electrical activity is not coupled to exocytosis, i.e.,  $\text{Ca}^{2+}$  signals generated by APs are subthreshold. However, the excitability of gonadotrophs is facilitated by activation of nicotinic AChRs,  $\text{GABA}_A$ R, and  $\text{P2X}_2$ R, and the accompanied  $\text{Ca}^{2+}$  signals can trigger gonadotropin secretion. Activation of GnRH and other  $\text{Ca}^{2+}$ -mobilizing receptors in gonadotrophs leads to  $\text{Ca}^{2+}$  release from ER through  $\text{IP}_3$ R channels coupled with a rapid LH secretion, and switch in the pattern of firing of APs from tonic single spiking to periodic plateau bursting, the latter being essential for sustained  $\text{Ca}^{2+}$  signaling and LH secretion. Further studies are needed to detail the role of ion channels in intracellular signaling cascade, gene expression,  $\text{Ca}^{2+}$  secretion coupling, and mechanism of synchronous activation of gonadotrophs in intact tissue.

## AUTHOR CONTRIBUTIONS

All the authors participated in writing; SS prepared figures.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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