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Molecular Mechanisms of Pituitary Endocrine Cell Calcium Handling

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Abstract

Endocrine pituitary cells express numerous voltage-gated Na^+, Ca^{2+}, K^+, and Cl^- channels and several ligand-gated channels, and they fire action potentials spontaneously. Depending on the cell type, this electrical activity can generate localized or global Ca^{2+} signals, the latter reaching the threshold for stimulus-secretion coupling. These cells also express numerous G-protein-coupled receptors, which can stimulate or silence electrical activity and Ca^{2+} influx through voltage-gated Ca^{2+} channels and hormone release. Receptors positively coupled to the adenylyl cyclase signaling pathway stimulate electrical activity with cAMP, which activates hyperpolarization-activated cyclic nucleotide-regulated channels directly, or by cAMP-dependent kinase-mediated phosphorylation of K^+, Na^+, Ca^{2+}, and/or non-selective cation-conducting channels. Receptors that are negatively coupled to adenylyl cyclase signaling pathways inhibit spontaneous electrical activity and accompanied Ca^{2+} transients predominantly through the activation of inwardly rectifying K^+ channels and the inhibition of voltage-gated Ca^{2+} channels. The Ca^{2+}-mobilizing receptors activate inositol trisphosphate-gated Ca^{2+} channels in the endoplasmic reticulum, leading to Ca^{2+} release in an oscillatory or non-oscillatory manner, depending on the cell type. This Ca^{2+} release causes a cell type-specific modulation of electrical activity and intracellular Ca^{2+} handling.

Keywords

action potential; calcium oscillations; cAMP; G protein-coupled receptors; IP_3 receptor channels; protein kinase A; voltage-gated calcium channels

1. Introduction

The pituitary gland is composed of two embryonically, anatomically, and functionally distinct entities: the neurohypophysis and the adenohypophysis. The neurohypophysis includes the posterior pituitary lobe, whereas the adenohypophysis includes the intermediate and anterior pituitary lobes. The intermediate lobe is populated more than 95% by melanotrophs. These cells are electrically excitable cells, and spontaneous electrical activity is sufficient to trigger the release of pro-opiomelanocortin-derived peptides. Melanotrophs express GPCRs (G-protein-coupled receptors) activated by DA (dopamine), GABA (γ-aminobutyric acid), prostaglandin E_2, and serotonin (5-hydroxytryptamine; 5-HT) [1].

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Corticotrophs are the other pro-opiomelanocortin-producing cell type derived from the intermediate pituitary. These cells are scattered throughout the anterior lobe in adult animals and make up 10-15% of anterior pituitary cells. The main regulation of these cells is mediated by CRH (corticotropin-releasing hormone), which is secreted by paraventricular neurons that project to the median eminence and release CRH into the hypophysial portal system. In addition to CRH and the CRH family of peptides (urocortin 1-3), AVP (arginine vasopressin) acts in synergy with CRH to potentiate hormone release [2].

Lactotrophs are a non-homogenous group of endocrine cells that account for 10-25% of cells in the anterior lobe. Spontaneous electrical activity in these cells is also sufficient to trigger prolactin secretion. The predominant hypothalamic influence is inhibitory rather than stimulatory and is mediated by dopamine D_2 receptors. These cells also express endothelin-activated ET_A receptors, which transiently stimulate hormone release and then sustained inhibition. In contrast, TRH (thyrotropin-releasing hormone), angiotensin II, oxytocin, ATP, acetylcholine, and 5-HT stimulate prolactin release [3]. The sister cells, somatotrophs, are the most common cells in the anterior pituitary. They represent up to 50% of all cells and are localized predominantly to the lateral portions of the anterior lobe. The function of these cells is controlled by two hypothalamic neuropeptides, GHRH (growth hormone-releasing hormone), which stimulates growth hormone release, and SST (somatostatin), which inhibits growth hormone release. GHRH is secreted by neurons in the arcuate nucleus of the hypothalamus, whereas SST is secreted by neurons in the periventricular nucleus of the hypothalamus. These cells also express receptors for ghrelin, PACAP (pituitary adenylate cyclase-activating peptide), and endothelins (ETs) [4].

Thyrotrophs and gonadotrophs represent the third group of endocrine pituitary cells that express the 92 amino acid α-glucoprotein hormone α-subunit, which is needed for the formation of follicle-stimulating hormone, luteinizing hormone, and thyroid-stimulating hormone (as well as chorionic gonadotropin) heterodimers with hormone-specific beta subunits. Thyrotrophs represent less than 10% of cells in the gland and are regionally localized within the anteriomedial and lateral portions of the gland. Hypothalamic control of these cells is mediated by TRH acting as an agonist for TRH receptors. The thyrotroph function is also controlled by numerous autocrine and paracrine factors [5]. Gonadotrophs constitute about 10-15% of the anterior pituitary cell population and are localized throughout the anterior lobe, frequently adjoining lactotrophs. The decapeptide GnRH (gonadotropin-releasing hormone) is the main agonist for these cells and is secreted in a pulsatile manner by neurons that are dispersed within the mediobasal hypothalamus and preoptic areas. In addition to GnRH receptors, gonadotrophs also express functional receptors for PACAP, AVP, and substance P, which contribute to Ca^{2+} signaling and gonadotropin synthesis and secretion [6].

Here, we summarize our findings on the expression, signaling functions and GPCR-mediated regulation of the plasma membrane and ER (endoplasmic reticulum) ion channels in endocrine pituitary cells. For a more extensive review of this topic, see the following reviews [7-9] and the accompanying articles in this issue by Chan et al., Chang et al., Tse et al., and Zorec et al. For a review of the three-dimensional organization of the pituitary gland and cell-to-cell communication, see the article by Mollard et al. in this issue. For a review of cyclic nucleotide signaling in pituitary cells, see Antoni et al.

2. Spontaneous Electrical Activity and Calcium Signaling

The membrane potential of isolated pituitary cells *in vitro* is not stable; rather, it oscillates between resting potentials of −60 to −50 mV, reflecting a balance between the activities of depolarizing and hyperpolarizing channels. When membrane potential oscillations reach a

threshold level, cells generate APs (action potentials). Pituitary cells fire APs independently of external stimuli, a phenomenon termed spontaneous electrical activity. Each AP is composed of a slow depolarizing phase, a rapid depolarizing phase or spiking depolarization, and a rapid or delayed repolarizing phase. Initially, it was believed that only lactotrophs and GH cells are excitable [9]. It later became obvious that other secretory pituitary cell types also fire APs spontaneously and/or in response to hypothalamic neurohormones: melanotrophs [10], corticotrophs [11, 12], somatotrophs [13], gonadotrophs [14], thyrotrophs [15]. Firing of APs causes transient elevation in $[Ca^{2+}]_i$ (intracellular Ca^{2+} concentration) as it well documented in gonadotrophs, lactotrophs, somatotrophs [16] and immortalized pituitary cells [12, 17]. However, not all cells fire APs and the frequency of firing vary from cell to cell. Furthermore, other investigators found that spontaneous APs or Ca^{2+} transients were rarely detected in corticotrophs [18] and male gonadotrophs [19], which could indicate that cultural and/or recording conditions also influence firing.

2.1. Patterns of Electrical Activity

Two types of APs can be observed in pituitary cells (Fig. 1). In rat gonadotrophs, the APs are tall and narrow, with amplitudes of more than 60 mV (from initiation to peak), half-widths of less than 50 ms, and spiking frequencies that are typically ~0.7 Hz [20]. Ovine gonadotrophs also fire single APs spontaneously [21], as do rat thyrotrophs [15]. The pattern of activity in lactotrophs and somatotrophs can be similar to that in gonadotrophs, with large and narrow spikes [22, 23]. More often, however, a bursting pattern is produced, consisting of periodic depolarized potentials with superimposed small-amplitude spikes [13, 20, 22]. The bursts have a much longer duration (several seconds) than gonadotroph APs, and the burst frequency is significantly lower (~0.3 Hz). The membrane potential rarely goes above −10 mV during a plateau burst, and the spikes are quite small [20]. Corticotrophs also exhibit both spontaneous large-amplitude spiking and plateau bursting [11, 24], as do melanotrophs [10] and GH cell lines [17, 25].

2.2. Channels Contributing to Resting Membrane Potential

Members of the K_{ir} (inwardly rectifying K^+) family of channels contribute to the regulation of resting membrane potentials in excitable cells. There are 15 members of this family of channels, and they can be divided into three groups based on the type of regulation. The majority of channel subtypes are “classical” K_{ir} channels that are controlled by intracellular messengers (K_{ir}1, 2, 4, 5, and 7). K_{ir}3 channels are regulated by G-proteins, and K_{ir}6 channels are regulated by intracellular ATP. The transcripts for the majority of these channels have been identified in GH_3 cells [26]. K_{ir}-like channels also contribute to the control of resting membrane potential in lactotrophs, somatotrophs, corticotrophs and GH_3 cells [24, 27-29], but the nature of these channels has not been identified. This could be mediated by “classical” K_{ir} 1 and 2 channels, but also by ERG (ether-a-go-go-related gene) channels, which mRNA transcripts are also expressed in GH mammosomatotrophs and native rat lactotrophs [30]. Blockade of ERG channels by E-4031 causes depolarization of the membrane potential of about 5 mV, facilitating the release of prolactin [31]. ERG currents are also expressed in MMQ lactotrophs, and their blockade facilitates AP firing and prolactin secretion [32]. Functional ERG current was also identified in mouse gonadotrophs [33]. Human prolactin-secreting tumors also express ERG and they are functionally coupled to hormone secretion [34]. Recently, contribution of TREK-1 (TWIK-related) channels in control of resting membrane potential in corticotrophs was also identified [35].

The resting membrane potential of −50 to −60 mV in pituitary cells suggests that, in addition to resting K^+ conductance, there is also a depolarizing conductance due to other ions (Fig. 1). When extracellular Na^+ is substituted with large organic cations, the resting membrane potential rapidly reaches approximately −85 mV. This value is close to the

equilibrium potential for K^+, suggesting that a Na^+-conducting channel has constitutive activity. This prominent hyperpolarization of the plasma membrane in the absence of bath Na^+ abolishes spontaneous firing of APs in lactotrophs, somatotrophs, gonadotrophs, and GH_3 and GC cell lines. The addition of TTX (tetrodotoxin) in micromolar concentrations to inhibit all voltage-gated Na^+ (Na_v) channels does not mimic the effect of the removal of bath Na^+ on the resting membrane potential. These and other data indicate that the constitutive activity of TTX-insensitive Na^+-conducting channels, termed background Na^+ channels, contributes to the control of resting membrane potential and may account for the pacemaking depolarization [23, 25, 36]. The nature of these channels is unclear.

2.3. Channels Involved in Spike Depolarization

All pituitary cells express TTX-sensitive Na_v channels, which in neurons are critical for the development of the depolarizing phase of APs [9]. In a fraction of ovine gonadotrophs [21] and bovine lactotrophs [37], Na_v channels are also responsible for AP generation. Furthermore, TTX-sensitive Na_v channels may contribute to the firing of APs and the accompanied VGCI in frog and rat melanotrophs [38]. In the majority of rat anterior pituitary cells *in vitro*, these channels do not contribute to the spike depolarization because they are inactivated at the resting membrane potential. However, in hyperpolarized cells, which *in vivo* occurs under tonic influence by a GPCR coupled to the $G_{i/o}$ signaling pathway, Na_v channels could play an important role in the transition from the quiescent to the firing mode [9].

In excitable cells, Ca_v channels also contribute to spike depolarization. Electrophysiologically, Ca_v channels are high (L, N, P/Q, and R type) or low (T-type) voltage-activated channels, which are distinguished by their single-channel conductance, pharmacology, and metabolic regulation [9]. The functional expression of both high and low voltage-activated Ca_v currents is well documented in cultured gonadotrophs [39] and in genetically labeled mouse gonadotrophs [40]. These currents are also present in somatotrophs and lactotrophs [39], GH cells [25] and other normal and immortalized pituitary cell types [9]. *In vitro*, the removal of extracellular Ca^{2+} and the addition of Ca_v channel blockers abolishes electrical activity in the majority of endocrine pituitary cells without affecting the resting membrane potential, indicating that these channels are critical for spiking depolarization but not for resting membrane potential [13, 16]. In contrast, T-type Ca_v channels contribute to slow depolarization in pituitary cells [41].

2.4. Mechanisms for Bursting and Repolarization

The simplest explanation why some cell fire tall AP spiking, while others fire plateau-bursting type of APs could be that there is a cell-specific expression of channels, leading to different patterns of spiking. One type of channels that is larger in somatotrophs and lactotrophs than in gonadotrophs is the BK type of Ca^{2+}-controlled K^+ (K_{Ca}) channels. These channels activate rapidly upon membrane depolarization, most likely due to colocalization of the BK channels with Ca_v channels [13, 20, 39]. The BK current acts in conjunction with the delayed rectifying K^+ current to repolarize the cell membrane during the downstroke of an AP. There is some evidence that BK channels are also a key element in the production of bursting, and that their greater expression in somatotrophs/lactotrophs is responsible for the different activity patterns of these cells vs. gonadotrophs [20]. The proximity of BK channels to Ca_v channels could be the major factor that determines differential (plateau-bursting vs. repolarization) role of BK channels among endocrine pituitary cells [42].

2.5. Voltage-Gated Ca^{2+} Influx

The high-voltage-activated Ca_v channels in pituitary cells not only give rise to APs in the same way as Na_v channels, but they also provide an effective pathway for Ca^{2+} influx during transient depolarization (termed voltage-gated Ca^{2+} influx or VGCI). In cells not exhibiting firing of APs, a plateau depolarization also results in Ca^{2+} entry via Ca_v channels, but in a non-oscillatory manner [18]. In other excitable cells, Ca^{2+} influx is coupled to Ca^{2+} release from intracellular stores through the calcium-induced calcium release process, which involves ryanodine receptor channels expressed in the ER membrane. It is unlikely, however, that Ca^{2+}-induced Ca^{2+} release contributes to the generation of Ca^{2+} signaling in mammalian lactotrophs and somatotrophs [43, 44]. In frog melanotrophs, the rise in Ca^{2+} occurs in a stepwise manner [45], and the generation of Ca^{2+} transients is abolished in cells that have the ER Ca^{2+} pump blocked [46]. It appears that in these cells, spontaneous VGCI is coupled to Ca^{2+}-induced Ca^{2+}-release, presumably through IP_3Rs (inositol trisphosphate receptor channels) [47].

Numerous studies have indicated that patterns of spontaneous electrical activity in different cell types largely impact intracellular Ca^{2+} dynamics and overall Ca^{2+} levels. Simultaneous measurements of membrane potential and $[Ca^{2+}]_i$ have shown that the bulk Ca^{2+} levels are low in spontaneously spiking gonadotrophs (20 nM to 70 nM) but much higher (300 nM to 1.2 μM) and clearly oscillatory in spontaneously bursting lactotrophs and somatotrophs [13, 16], corticotrophs [12] and immortalized pituitary cells [17, 25, 27]. Rhythmic bursts of Ca^{2+} transients have also been observed in acute anterior pituitary slices [48].

The difference in the patterns of Ca^{2+} transients between cells firing single APs and those exhibiting plateau bursting is reflected in the spatial distribution of Ca^{2+} within the cell (Fig. 1B). Both types of APs depolarize the membrane sufficiently to activate the various types of Ca_v channels that are expressed in pituitary cells [39, 49]. However, Ca_v channels are open only briefly during the short time that the pituitary cells are firing a single AP, and the elevated Ca^{2+} concentration is localized to nanodomains that form at the inner mouths of open channels. With longer durations and smaller amplitudes of bursts, channels stay open longer and significant Ca^{2+} entry occurs, resulting in individual Ca^{2+} nanodomains overlapping and producing a global signal that can be easily resolved with fluorescent Ca^{2+} dyes [44]. Thus, the Ca^{2+} influx summed over time is much greater during bursting than during large-amplitude spiking [42].

3. Stimulation of VGCI by GPCRs

cAMP is a ubiquitous intracellular messenger that is generated by the adenylyl cyclase family of enzymes, which regulates numerous cellular responses, including electrical activity and VGCI. Adenylyl cyclase produces cAMP in the absence of hormonal stimulation; this basal activity is markedly enhanced upon the binding of G_s. In corticotrophs, the G_s-signaling pathway is triggered by CRH and in somatotrophs by GHRH. The G_s-coupled VIP/PACAP receptors operate in somatotrophs, melanotrophs, lactotrophs, and folliculostellate cells. Some eicosanoids may also signal through this pathway in pituitary cells [9]. In general, the activation of these G_s-linked GPCRs causes plasma membrane depolarization, leading to increased electrical activity and facilitated VGCI. The type of Ca^{2+} response that is typically obtained through this pathway is a plateau elevation of $[Ca^{2+}]_i$ or an increase in the frequency and/or amplitude of Ca^{2+} transients. cAMP can modulate channel activities directly by activating HCN (hyperpolarization-activated and cyclic nucleotide-regulated) channels or indirectly through PKA-mediated phosphorylation of several plasma membrane channels (Fig. 2).

3.1. Direct Effects of cAMP on VGCI

Structurally, HCN channels belong to the superfamily of K_v channels. HCN channels, however, are functionally distinct from the six other transmembrane domain K^+ channels; their activation does not dampen excitation but increases the firing of APs. This paradoxical role for channels that structurally belong to the K^+-channel family comes from their permeability properties; HCN channels are non-selective cation channels that are weak K^+-selective channels [9]. RT-PCR and electrophysiological experiments have confirmed the presence of HCN in AtT-20 cells [50], GH_3 cells [51, 52], melanotrophs [53], somatotrophs [52], and lactotrophs [54]. More recent findings indicate that these channels are also expressed in gonadotrophs and thyrotrophs [15]. The biophysical and pharmacological properties of this current are similar to those that have been described of the HCN channels in neuronal and cardiac cells. These properties include the sensitivity to both ZD7288 and Cs^+ [50-52]. It appears that the basal cAMP production in the majority of pituitary cells *in vitro* is high enough to partially or fully activate the these channels, explaining the relatively weak effect of the activation of adenylyl cyclase on HCN gating [51]. A decrease in the AP frequency in spontaneously firing cells with inhibited HCN has also been frequently observed, indicating that these channels contribute to slow depolarization. Blocking these channels, however, does not abolish spontaneous electrical activity, indicating that other channels also contribute to slow depolarization [15]. Because basal cAMP production is down-regulated *in vivo* by several hypothalamic and intrapituitary factors, it is reasonable to suggest that an increase in adenylyl cyclase activity under physiological conditions should lead to the activation of HCN channels and firing of APs (Fig. 2).

3.2. Role of cAMP-Dependent Kinase in VGCI

PKAs (cAMP-dependent kinases) are present in all eukaryotic cells and function as the major mediators of the cAMP response. In the absence of cAMP, each PKA is an inactive, asymmetric tetramer containing two regulatory and two catalytic subunits that bind to each other with high affinity. The binding of cAMP to the regulatory subunit alters its affinity for the catalytic subunits by four orders of magnitude, leading to its dissociation into a dimer of regulatory subunits and two active monomeric catalytic subunits. The catalytic subunit-mediated phosphorylation of several plasma membrane channels increases the excitability of pituitary cells and thereby increases VGCI (Fig. 2).

PKA and Hyperpolarizing Currents—As discussed above, “classical” K_{ir} channels could contribute to the regulation of the resting membrane potential. In general, PKA-dependent phosphorylation of these channels silences them, leading to cell depolarization and enhanced excitability. It is possible that GHRH decreases the intrinsic activity of a K_{ir} channel in somatotrophs [13]. In cortocotrophs, CRH also changes the resting membrane potential and the rate of the pacemaking depolarization, causing an increase in the firing rate of spontaneously active cells and causing quiescent cells to become active [11, 12, 55]. It appears that the slow membrane depolarization is mostly caused by a reduction in the background K^+ conductance mediated by a member of the K_{ir} channel family [24]. The inhibition of K_{ir} and the associated depolarization and increase in spike frequency last up to 15 minutes after the end of stimulation, suggesting that the phosphorylation of K_{ir} channels could account for this memory. However, inhibition of K_{ir} channels does not account for all effects of CRH on electrical activity [24]. Consistent with this, in these cells the presence of background hyperpolarizing current was also observed at positive potentials [18] and this current appears to be generated by TREK-1 channels and inhibited in a PKA-dependent manner [35].

PKA and Depolarizing Currents—The main effect of G_s-coupled receptors on the electrical activity in somatotrophs appears to be the facilitation of inward depolarizing

currents; the phosphorylation of several channels accounts for this effect. GHRH and the synthesized growth-hormone-releasing peptide GHRP-2 increase L- and T-type Ca^{2+} conductance in ovine somatotrophs and human adenoma GH cells [56-59]. In contrast, the effect of GHRH on VGCI in rat somatotrophs depends on bath Na^+, presumably because it enters into cells through the TTX-insensitive Na_v5 and/or Na_v8-9 channels [60]. A TTX-insensitive Na^+ current that is upregulated by PKA phosphorylation is also present in rat somatotrophs [61]. Chen's group also reported a stimulatory effect of GHRH on TTX-resistant Na_v channels in somatotrophs from GH-green fluorescent protein transgenic mice, but they suggested that protein kinase C rather than PKA mediates the action of GHRH [62]. Electrophysiological experiments have also revealed that PACAP stimulation of melanotrophs causes an inward non-selective cation current that depolarizes cells and stimulates VGCI [63]. Recently, we provided evidence to support the hypothesis that TRPC channels could account for inward non-selective cation current and that their phosphorylation by PKA facilitates VGCI in rat somatotrophs and other pituitary subtypes [64].

4. Inhibition of VGCI by GPCRs

GPCRs linked to the $G_{i/o/z}$-signaling pathways also operate in endocrine pituitary cells [9]. Their activation leads to the inhibition of electrical activity and hormone secretion. SST and DA are two major hypothalamic factors that inhibit pituitary hormone secretion via $G_{i/o/z}$-coupled receptors. Pituitary cells also express several other GPCRs linked to this signaling pathway, including receptors activated by adenosine, ET-1, GABA, melatonin, neuropeptide Y and 5-HT. The inhibition of AC activity by $G_{i/o/z}\alpha$ is one of the mechanisms by which spontaneous electrical activity and VGCI are inhibited. The $\beta\gamma$ dimer of these G-proteins also prominently affects electrical activity and Ca^{2+} signaling in a cAMP/PKA-independent manner by altering the gating of K_{ir} and Ca_v channels (Fig. 2).

4.1. G protein-regulated K^+ channels

The $K_{ir}3$ channels that are regulated by G-proteins are present in endocrine pituitary cells and have a well-established role in regulating electrical activity [9]. In somatotrophs, SST inhibits spontaneous and GHRH-stimulated electrical activity, VGCI, and hormone secretion. This inhibition reflects hyperpolarization of the plasma membrane [13, 65]. The effects of SST on $K_{ir}3$ channels and electrical activity are blocked by pertussis toxin, an inhibitor of the $G_{i/o}$ signaling pathways [66]. In lactotrophs and melanotrophs, DA also hyperpolarizes the membrane and suppresses APs and bursts, explaining the decrease in VGCI [67, 68]. The effects of DA on VGCI are sensitive to pertussis toxin, but they persist in cells with elevated cAMP [67, 69]. Furthermore, the activation of voltage-independent K^+ channels by DA can be observed in excised outside-out patch [70], demonstrating that no second messenger is required. This result suggests that the coupling between the G-protein and K_{ir} channels is mediated by the $\beta\gamma$ dimer [69]. Similarly, the activation of $GABA_B$ receptors in pituitary cells induces late hyperpolarization, the facilitation of $K_{ir}3$ channels, and the inhibition of AC activity [71]. In somatotrophs and lactotrophs, ETs activate the Ca^{2+}-mobilization pathway, but the stimulatory effect of ET is followed by an inhibition of VGCI and hormone secretion below basal levels [72]. This inhibition reflects increase a Cs^+-sensitive K_{ir} current in both cell types [28, 73].

4.2. G-Protein-Regulated Ca_v Channels

There is also evidence that SST inhibits Ca_v channels in somatotrophs [74] and AtT-20 cells [75]. Whereas GHRH-stimulated and PKA-mediated phosphorylation facilitates Ca_v currents in somatotrophs, SST inhibits these channels in a cAMP/PKA-independent manner [74]. It appears that the L-type Ca_v channels are negatively coupled to SST receptors [76].

DA was also reported to inhibit Ca_v channels in rat lactotrophs [77] and melanotrophs from neonatal rats [78, 79]. DA application was shown to inhibit Ca_v currents after a short (1-10 min) and a prolonged (over 24 h) application in GH_4C_1 cells transfected with D_2 receptors. Porcine pituitary melanotrophs also express $5\text{-}HT^{1A}$ and $5\text{-}HT^{1C}$ receptors; their activation inhibits L-type Ca_v channels [80]. The inhibition of L-type and Q-type Ca_v channels by 5-HT also occurs in rat melanotrophs [81]. In both cell types, the inhibition of Ca_v currents is abolished by pertussis toxin, indicating that 5-HT receptors are coupled to the $G_{i/o}$ signaling pathway. Adenosine inhibits electrical activity-driven Ca^{2+} transients in GH cell lines [82] and in frog melanotrophs [83], presumably reflecting an inhibition of Ca_v channels. Neuropeptide Y also inhibits spontaneous electrical activity and Ca_v currents in these cells [84]. Similarly, the activation of $GABA_B$ receptors in pituitary cells induces attenuation of Ca_v currents [71].

5. Calcium-Mobilizing Receptors and Ca^{2+} Signaling

Ca^{2+}-mobilizing GPCRs are expressed in all endocrine pituitary cells. In gonadotrophs, Ca^{2+} mobilization is triggered not only by GnRH, the main agonist for these cells, but also ETs, PACAP, substance P, and AVP/oxytocin. In thyrotrophs, the $G_{q/11}$ signaling pathway is activated by TRH, the main agonist for these cells, and ETs. Lactotrophs express Ca^{2+}-mobilizing receptors activated by acetylcholine, angiotensin II, ATP, ETs, oxytocin, 5-HT, substance P, TRH and galanin. Mammalian melanotrophs express muscarinic receptors, and frog melanotrophs express Ca^{2+}-mobilizing receptors for TRH and neuropeptide Y, in addition to muscarinic receptors. In corticotrophs, the Ca^{2+}-mobilizing pathway is activated by AVP, norepinephrine and potentially 5-HT. Somatotrophs express Ca^{2+}-mobilizing ghrelin and ET_A receptors [9]. In general, the activated receptors trigger phosphoinositide hydrolysis and the production of IP_3 and DAG. IP_3 binds to IP_3Rs expressed in the ER membrane, causing the release of Ca^{2+} (Fig. 3).

5.1. Pattern of Ca^{2+} Release from the ER

The ER is the primary storehouse for Ca^{2+} in most cells, including pituitary cells. The ER's resting Ca^{2+} concentration ($[Ca^{2+}]_{ER}$) is a few hundred micromolar, in contrast to the resting level of $[Ca^{2+}]_i$, which is ~100 nM. The high $[Ca^{2+}]_{ER}$ is maintained by SERCA pumps. Ca^{2+} effluxes from the ER through passive leakage and through IP_3Rs and/or RyRs [9]. Because of the large concentration difference, the activation of IP_3Rs by a $G_{q/11}$ agonist results in a large and sudden increase in $[Ca^{2+}]_i$. Following this initial Ca^{2+} pulse, one of two behaviors can be observed in pituitary cells, depending on the cell type and, in some cases, the agonist. In lactotrophs, somatotrophs, thyrotrophs and cells from the GH cell lines, the pulse is typically followed by a slow decline to a plateau in $[Ca^{2+}]_i$, although some cells may only a have pulse or a plateau [72]. This non-oscillatory pattern of Ca^{2+} response is termed biphasic or pulse-decay-plateau. In mammalian gonadotrophs, the pulse is typically followed by large baseline $[Ca^{2+}]_i$ oscillations [85, 86] (Fig. 3, bottom). Fish gonadotrophs also show an oscillatory Ca^{2+} response to the application of GnRH [87], as do corticotrophs in response to norepinephrine application [88].

5.2. Mechanism of Biphasic Ca^{2+} Response

The biphasic Ca^{2+} response requires only that the IP_3Rs open and remain open during agonist application. The initial rapid increase in $[Ca^{2+}]_i$ is followed by a slow decline, reflecting the removal of Ca^{2+} from the cell by plasma membrane ATPase pumps and Na^+-dependent Ca^{2+} efflux and reuptake of Ca^{2+} by SERCA pump and mitochondria. The decline in $[Ca^{2+}]_i$ is mirrored by a decline in $[Ca^{2+}]_{ER}$, although $[Ca^{2+}]_{ER}$ is much larger. As $[Ca^{2+}]_{ER}$ declines to a sufficiently low level, a voltage-gated Ca^{2+} entry pathway is activated, bringing additional Ca^{2+} into the cell and producing an elevated plateau or

fluctuations in $[Ca^{2+}]_i$ that are evident near the end of the agonist application [9]. In cells bathed in Ca^{2+}-deficient medium or blocked VGCI, $[Ca^{2+}]_i$ drops to basal levels within a few minutes, indicating that sustained Ca^{2+} signaling by Ca^{2+} mobilizing GPCRs is critically dependent on Ca^{2+} influx. This phenomenon is well illustrated in TRH-stimulated lactotrophs bathed in Ca^{2+}-deficient medium and by lactotrophs and somatotrophs stimulated with ETs in the presence of Ca^{2+} [72, 89].

5.3. Mechanism of Oscillatory Ca^{2+} Release

Unlike AP-driven Ca^{2+} oscillations, those induced by GnRH in gonadotrophs or norepinephrine in corticotrophs persist for 3-15 min when the Ca^{2+} bath is removed, as do cells that are bathed in the presence of Ca^{2+} but clamped at potentials that silence Ca^{2+} influx through Ca_v channels [88, 90]. Thus, oscillation is intrinsic to the Ca^{2+}-handling properties of the cell. These two cell types differ in their oscillatory Ca^{2+} mobilization mechanisms. In gonadotrophs, the frequency of Ca^{2+} oscillations varies from 5 to 20 pulses per minute [86, 91, 92], whereas norepinephrine generates Ca^{2+} oscillations in corticotrophs that have a frequency of about one per minute [88]. In gonadotrophs, oscillations in IP_3 are not required to generate oscillatory Ca^{2+} release, as demonstrated by the injection of non-metabolized IP_3 analogs, and the concentration of IP_3 underlies the frequency of Ca^{2+} spiking [92]. The $[Ca^{2+}]_i$ influences the IP_3-dependent Ca^{2+} release in these cells. The rapid stimulatory effect of Ca^{2+} on IP_3-depenent Ca^{2+} release in gonadotrophs is nicely demonstrated by the phase resetting of GnRH-induced oscillations by a brief pulse of Ca^{2+} entry [93]. The inhibitory effect of high $[Ca^{2+}]_i$ on GnRH-induced Ca^{2+} oscillations has also been demonstrated [94]. Thus, in contrast to cells that exhibit continuous opening of IP_3Rs, resulting in biphasic Ca^{2+} signals, IP_3Rs in gonadotrophs undergo periodic activation and inhibition mediated by cytosolic Ca^{2+} (Fig. 3). The mechanism responsible for IP_3-dependent oscillations in corticotrophs is unknown.

5.4. Contribution of Mitochondria and SERCA Pumps to IP_3-Induced Ca^{2+} Release

In general, Ca^{2+} is transported into mitochondria through Ca^{2+} uniporters, which are powered by the membrane potential across the inner membrane. Calcium is transported out of mitochondria primarily by Na^+/Ca^{2+} exchangers. In corticotrophs, the rate of Ca^{2+} clearance after depolarization-induced Ca^{2+} influx is dramatically slowed by mitochondrial uncouplers or inhibitors of the mitochondrial uniporter. This inhibition enhances the exocytotic response [95]. In oscillating gonadotrophs, Ca^{2+} released from the ER is partly taken up again by the ER and partly pumped into other intracellular compartments or out of the cell [96]. In these cells, collapsing the mitochondrial inner membrane potential with the protonophore carbonyl cyanide m-chlorophenylhydrazone inhibits Ca^{2+} uptake by mitochondria and slows or inhibits GnRH-induced $[Ca^{2+}]_i$ oscillations [97, 98]. In non-oscillating GnRH-stimulated gonadotrophs, the removal of Ca^{2+} from cells in a Na^+-dependent manner dominates over reuptake of Ca^{2+} by mitochondria [99]. In contrast, the inhibition of SERCA pumps causes a transition from an oscillatory to non-oscillatory mode of Ca^{2+} release in GnRH-stimulated gonadotrophs, indicating that the reuptake of Ca^{2+} by the ER is essential for periodic activation of IP_3R by $[Ca^{2+}]_i$ [100, 101].

5.5. Dependence of IP_3-Mediated Ca^{2+} Signaling on VGCI

The Ca^{2+} response to a $G_{q/11}$-activating agonist impacts the plasma membrane potential. In cells that respond with biphasic Ca^{2+} signals, the rapid rise in $[Ca^{2+}]_i$ activates the apamin-sensitive SK-type K_{Ca} channels in the plasma membrane. The K_{Ca} current hyperpolarizes the membrane, terminating any spontaneous electrical activity that was present prior to agonist application [72, 102]. The rapid hyperpolarization phase is followed by a sustained depolarization phase due to the modulation of a still unidentified current, presumably the downregulation of an M/ERG channel [30, 103, 104], and/or the stimulation of a TRPC

current [64]. This depolarization activates Ca_v channels, further depolarizing the cell and initiating single spiking or bursting, depending on the cell type [72]. Such electrical activity would then be reflected in the $[Ca^{2+}]_i$ time course as small oscillations on top of the plateau and would contribute to the plateau (Fig. 3).

In gonadotrophs, coupling between the ER and the plasma membrane is also mediated through the apamin-sensitive SK type K_{Ca} channels [85, 86], but the integration of VGCI into IP_3-dependent Ca^{2+} oscillations is more complex. In cells at a hyperpolarized potential, few Ca_v channels are open, and the Ca^{2+} current is small, whereas at depolarized holding potentials, many Ca_v channels are open, and thus, there is a larger Ca^{2+} current. In addition to demonstrating that GnRH-induced Ca^{2+} oscillations persist in the absence of membrane potential oscillations, these studies have shown that the oscillations die out if the holding potential is not sufficiently depolarized, due to gradual depletion of the ER. These experiments also showed that no patterned electrical activity is required to keep the ER-Ca^{2+} store replenished [90, 105].

Stimulated gonadotrophs do produce electrical bursting, however, due to bidirectional interactions between the plasma membrane and the ER [42, 106]. Ion channels in the plasma membrane bring Ca^{2+} into the cell during each spike, which replenishes the ER and thereby enables coupling between the membrane and the ER. The key feature is the antiphasic pattern of electrical activity and Ca^{2+} spikes (Fig. 3) due to the inhibitory action of each Ca^{2+} spike on the plasma membrane mediated by the SK current. The electrical spiking resumes once $[Ca^{2+}]_i$ returns to a low level following the Ca^{2+} spike. Thus, the Ca^{2+} oscillator periodically interrupts the plasma membrane oscillator, producing a bursting pattern of electrical activity. Recent investigation also revealed that inhibition of ERG channel by GnRH receptors facilitates the depolarizing phase, promoting Ca^{2+} influx [33]. The electrical activity and secretion are out of phase; the former serves to refill the ER, which provides the periodic Ca^{2+} pulse that is needed to evoke secretion [107].

6. Concluding Remarks

Endocrine pituitary cells express numerous Na_v, Ca_v, K_v, K_{Ca}, and K_{ir} channels, as well as cation-conducting HCN and TRPC channels, and generate APs spontaneously and in response to depolarization of the cell membrane. Physiologically, electrically driven Ca^{2+} signals resemble neuronal cell signaling, which requires high Ca^{2+} in the extracellular medium and APs as a driving force for Ca^{2+} influx. In cells that fire single APs, Ca_v channels are open for a short amount of time, and the elevated Ca^{2+} concentration is localized to nanodomains that form at the inner mouth of open channels. Cells that exhibit bursting activity generate oscillatory global Ca^{2+} signals of sufficient amplitude to trigger exocytosis (Fig. 1). The activation of G_s-coupled receptors initiates the firing of APs in quiescent cells and increases the frequency of firing or duration of bursting in spontaneously active cells in a cAMP-dependent manner. Both direct and indirect (through PKA) action of cAMP accounts for the enhanced electrical activity and accompanying VGCI (Fig. 2). This signaling pathway plays a major role in somatotrophs and corticotrophs, operated by GHRH and CRH receptors, respectively. In contrast, activation of the $G_{i/o}$ signaling pathway inhibits electrical activity and the accompanying VGCI by stimulating K_{ir} channels and/or inhibiting Ca_v channels in a cAMP/PKA-independent manner (Fig. 2). Of the $G_{i/o}$-coupled receptors that are expressed in pituitary cells, D_2 receptors play a major role in cellular Ca^{2+} homeostasis in melanotrophs and lactotrophs and SST receptors in somatotrophs.

All pituitary cells have an additional system to control intracellular Ca^{2+}, which is composed of the IP_3Rs in the ER membrane. Endocrine pituitary cells express at least 15 subtypes of $G_{q/11}$-coupled GPCRs and several receptor tyrosine kinases, whose activation leads to the

mobilization of intracellular Ca^{2+} in an IP_3-dependent manner (Fig. 3). In melanotrophs, somatotrophs, and lactotrophs, Ca^{2+} mobilization provides only a transient source of non-oscillatory $[Ca^{2+}]_i$ elevation due to the continuous opening of IP_3Rs in the presence of agonist; Ca^{2+} influx through Ca_v channels is critical for sustaining Ca^{2+} signaling. Gonadotrophs have the most sophisticated Ca^{2+} mobilization pathway; they release Ca^{2+} in an oscillatory manner in response to the activation of any of the Ca^{2+}-mobilizing receptors that are expressed in these cells, with the frequency of spiking being determined by the IP_3 concentration. In these cells, oscillations in $[Ca^{2+}]_i$ are generated by the periodic activation of IP_3Rs during continuous stimulation of Ca^{2+}-mobilizing receptors due to bidirectional actions of cytosolic Ca^{2+} on the IP_3-dependent gating of these channels. Intracellular Ca^{2+} is redistributed between the ER and mitochondria, providing a relatively long-lasting spike when cells are bathed in Ca^{2+}-deficient medium. In this way, gonadotrophs resemble skeletal muscle cells, relying on Ca^{2+} mobilization for a prolonged period of time and with VGCI controlling the “excitability” of the ER membrane during receptor activation.

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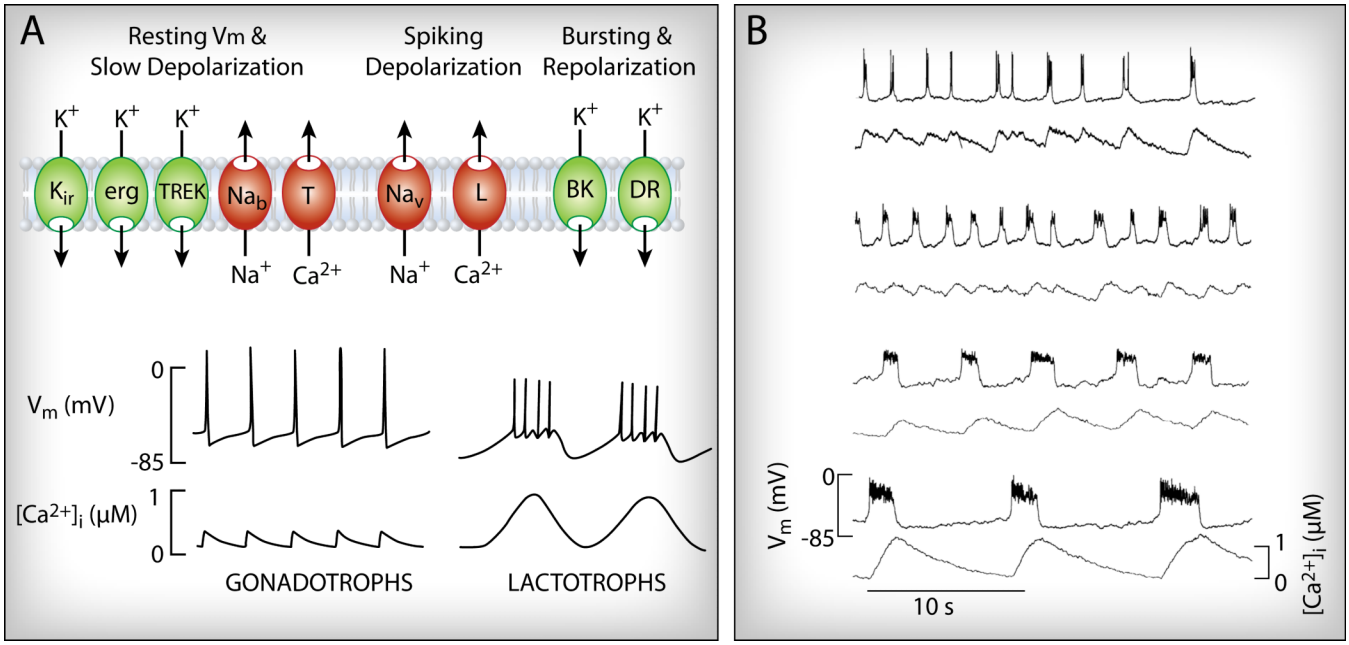

Fig. 1.

Spontaneous electrical activity and Ca^{2+} signaling in pituitary cells. (A) The resting membrane potential (V_m) and slow depolarization in endocrine pituitary cells are determined by several channels, including classic inward rectifier K^+ (K_{ir}), ether-a-go-go-related gene (ERG) and TWIK-related (TREK-1) K^+-conducting channels, still unidentified Na^+-conducting background channels (Na_b), and T-type Ca^{2+} channels. Once the threshold for action potential firing is reached, L-type Ca^{2+} channels alone or together with voltage-gated Na^+ (Na_v) channels generate spiking depolarization. Depending on the cell type, spiking depolarization is followed by a rapid and sharp repolarization (bottom left) or plateau bursting type of electrical activity (bottom right). Interactions between delayed rectifier (DR) and Ca^{2+}-activated BK-type K^+ channels appear to play a major role in determining the pattern of electrical activity. (B) Influence of variable durations of bursting activity on the Ca^{2+} signaling pattern in pituitary cells: experimental records.

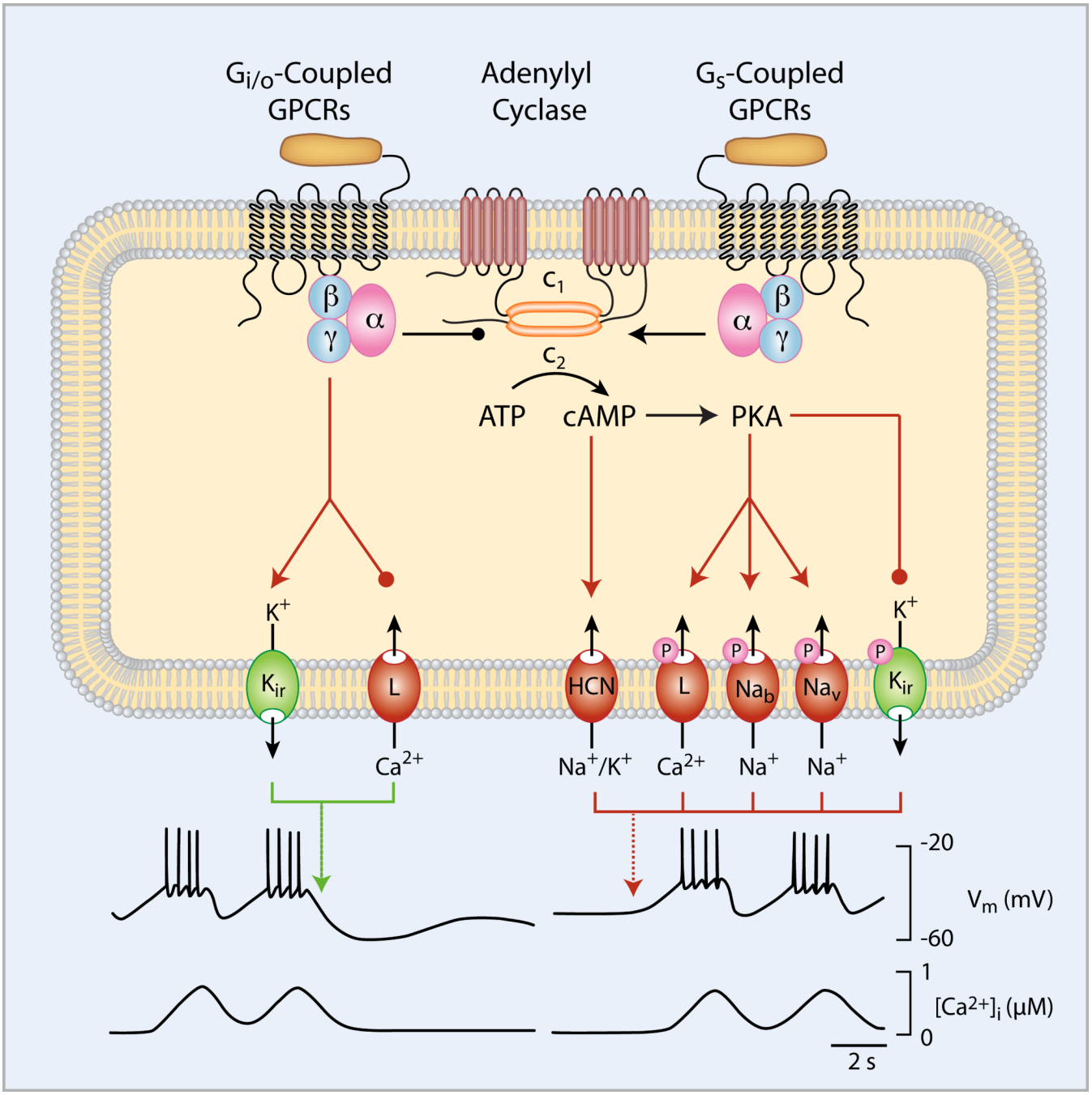

Fig. 2.

Role of adenylyl cyclase-coupled GPCRs on plasma membrane channel activity in pituitary cells. The $G_{i/o}$-coupled receptors, including dopamine D_2 and somatostatin receptors, inhibit spontaneous electrical activity and Ca^{2+} signaling predominantly through the activation of $K_{ir}3$ channels and inhibition of L-type Ca^{2+} channels; both are mediated by βγ dimers of heterotrimeric G proteins. The G_s-coupled receptors, including CRH and GHRH receptors, stimulate electrical activity and Ca^{2+} signaling in quiescent cells and increase the frequency of firing in spontaneously active cells by the cAMP-dependent stimulation of hyperpolarization-activated (HCN) channels or by protein kinase A (PKA)-mediated

phosphorylation of several plasma membrane channels; this process presumably occurs in a cell type-specific manner.

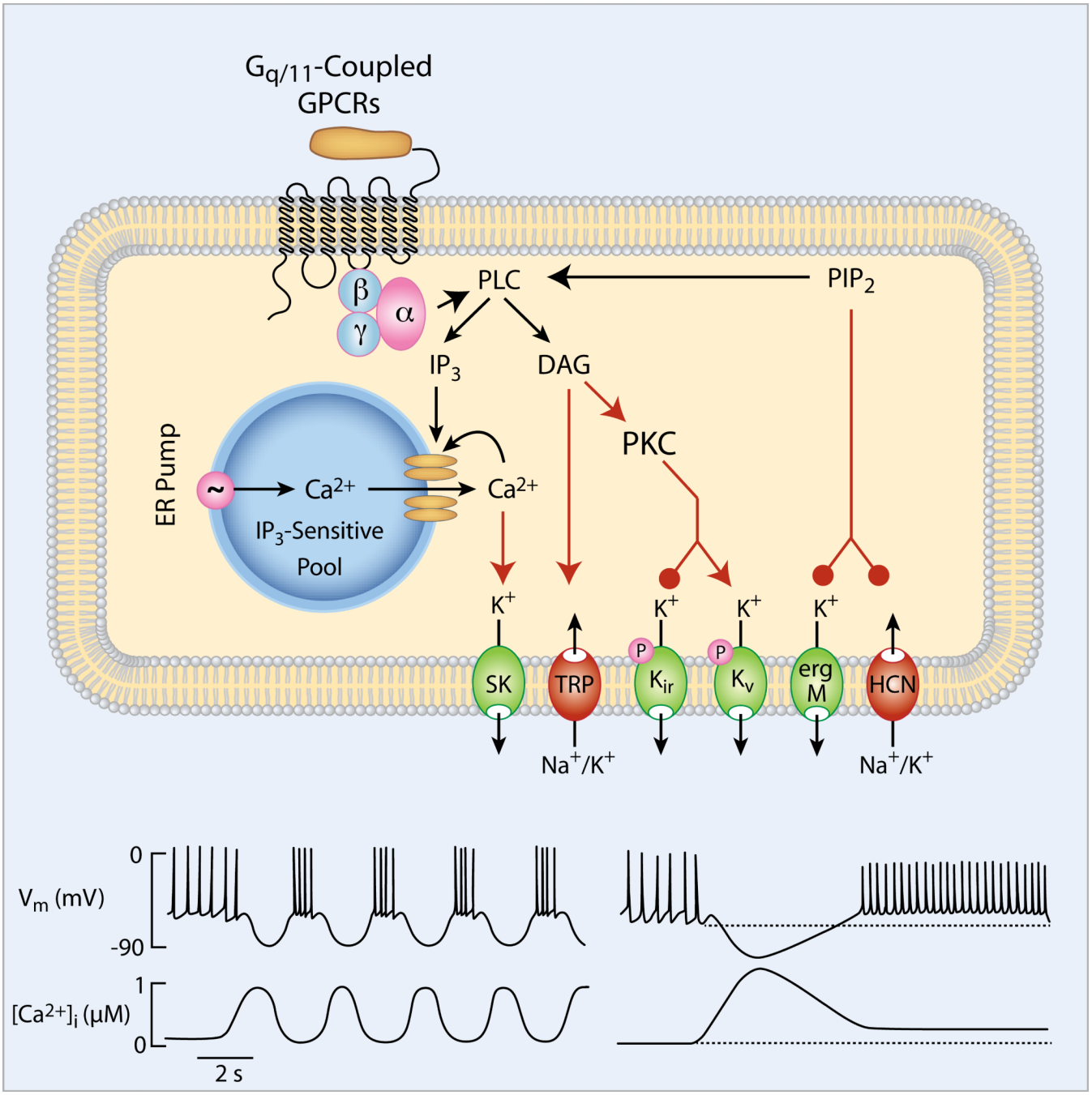

Fig. 3.

Role of Ca^{2+}-mobilizing receptors on channel activity and Ca^{2+} signaling in pituitary cells. The $G_{q/11}$-coupled receptors stimulate phospholipase C (PLC), leading to the depletion of phosphatidyl inositol-bisphosphate (PIP_2) and the formation of two intracellular messengers: inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 binds to IP_3 receptors in the endoplasmic reticulum (ER), causing two types of Ca^{2+} signaling: baseline Ca^{2+} oscillations (bottom left) or biphasic Ca^{2+} signaling (bottom right). In gonadotrophs, the generation of baseline Ca^{2+} oscillations reflects periodic activation of IP_3 receptors mediated by IP_3 and Ca^{2+}, whereas the generation of a biphasic response reflects the tonic activation of IP_3 receptors. Due to the activation of SK-type Ca^{2+}-activated K^+ channels, spontaneous

electrical activity in pituitary cells is also affected, generating a bursting type of electrical activity in gonadotrophs and sustained plateau firing in non-oscillating cells. DAG may stimulate transient receptor potential (TRP) channels or may alter the gating properties of K_{ir} and voltage-gated K^+ (K_v) channels through protein kinase C (PKC)-dependent phosphorylation. Depletion of PIP_2 may also influence ERG and M type K^+ channels and HCN channels. TRP, K_{ir}, ERG and/or M channels could play an important role in sustaining plasma membrane depolarization in non-oscillating cells.