Published in final edited form as:

J Neuroendocrinol. 2012 September ; 24(9): 1183–1200. doi:10.1111/j.1365-2826.2012.02335.x.

Dependence of the Excitability of Pituitary Cells on Cyclic Nucleotides

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Abstract

Cyclic 3',5'-adenosine monophosphate and cyclic 3',5'-guanosine monophosphate are intracellular (second) messengers that are produced from the nucleotide triphosphates by a family of enzymes consisting of adenylyl and guanylyl cyclases. These enzymes are involved in a broad array of signal transduction pathways mediated by the cyclic nucleotide monophosphates and their kinases, which control multiple aspects of cell function through the phosphorylation of protein substrates. Here, we review the findings and working hypotheses on the role of the cyclic nucleotides and their kinases in the control of electrical activity of the endocrine pituitary cells and the plasma membrane channels involved in this process.

Keywords

cAMP; cGMP; protein kinase A; protein kinase G; voltage-gated channels

Introduction

Pituitary cells express several subtypes of adenylyl cyclases (ACs), including the calcium-inhibitable forms of these enzymes (1, 2), which generate cyclic 3',5'-adenosine monophosphate (cAMP). These cells also express the particulate guanylyl cyclase (pGC) and soluble guanylyl cyclase (sGC), responsible for synthesis of cyclic 3',5'-guanosine monophosphate (cGMP) (3). AC activity is controlled by numerous hypothalamic and intrapituitary agonists acting on G-protein-coupled receptors (GPCRs). Cyclic nucleotide production is stimulated by G_s -coupled growth hormone-releasing hormone (GHRH), corticotropin-releasing hormone (CRH), vasoactive intestinal polypeptide (VIP)/pituitary adenylyl cyclase-activating peptide (PACAP) receptors and others. Conversely, it is inhibited by adenosine, dopamine, endothelin, *gamma*-aminobutyric acid, melatonin, neuropeptide Y, serotonin and somatostatin receptors (4). Pituitary cells also express several phosphodiesterases (5, 6), and multidrug resistance proteins operate as cyclic nucleotide transporters (7, 8). Cyclic nucleotides play numerous important roles in the control of pituitary cell functions, including control of the exocytotic pathway downstream of the voltage-gated calcium influx (VGCI) (9–11), and the transcriptional pathway (12–15), as well as the mitogenic pathway (16).

Endocrine pituitary cells express numerous voltage-gated Na^+ , Ca^{2+} , K^+ , and Cl^- channels and several ligand-gated channels. *In vitro*, they are silent or fire action potentials (APs) 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 (4, 17). The excitability of pituitary cells and accompanied voltage-gated calcium influx (VGCI) and hormone release are also influenced by cyclic nucleotides. G_s -coupled GPCRs stimulate electrical activity in pituitary cells, whereas $G_{i/o}$ -coupled receptors inhibit it (4). Here, we summarise the current knowledge on the role of cyclic nucleotides in the control of pituitary cell excitability and VGCI.

Spontaneous Electrical Activity and Calcium Signalling

In vitro, isolated pituitary cells generate APs independently of external stimuli, a phenomenon termed spontaneous electrical activity. Initially, firing of APs was observed in lactotrophs and GH cells (4). It later became obvious that other secretory pituitary cell types also fire APs: melanotrophs (18), corticotrophs (19–21), somatotrophs (22), gonadotrophs (23), thyrotrophs (24). Firing of APs causes transient elevation in intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) and this was well documented in gonadotrophs, lactotrophs, somatotrophs (25) and immortalized pituitary cells (20, 26). However, not all pituitary cells fire APs; for example, fish pituitary cells are quiescent (27). *In vivo*, pituitary cells are organized as a large-scale network (28, 29). The existence of such network, however, does not argue against the relevance of electrical activity and VGCI in rapid hormone release, but indicates the complexity in coordination of calcium signaling and influence of other factors in this process (30, 31).

Forskolin-stimulated Electrical Activity and Calcium Signalling

Forskolin is a labdane diterpene found in the roots of the plant *Plectranthus barbatus* (*Coleus forskohlii*). It is commonly used to raise levels of cAMP by directly activating ACs. In secretory anterior pituitary cells, forskolin stimulates electrical activity and VGCI in quiescent cells, and it increases the frequency of APs in spontaneously firing cells. Figure 1A shows the effects of forskolin on the electrical activity in gonadotrophs and lactotrophs. The inhibition of phosphodiesterases also increases the electrical activity of somatotrophs (22). The stimulatory effect of forskolin on Ca^{2+} influx through voltage-gated calcium (Ca_v) channels was observed in $\alpha\text{T3-1}$ immortalised gonadotrophs (32). Forskolin also increases the Ca^{2+} influx in somatotrophs (33–35), corticotrophs (36), lactotrophs (2), and gonadotrophs (35). Figure 1B illustrates the stimulatory effect of forskolin on VGCI in lactotrophs, somatotrophs, gonadotrophs and GH₃ cells. Thus, the facilitatory role of forskolin on electrical activity and VGCI is common feature of endocrine pituitary cells.

Agonist-stimulated Electrical Activity and Calcium Signalling

CRH is the main regulator of ACTH release in normal and immortalised corticotrophs. It acts on CRF-R₁ receptors coupled to the G_s signalling pathway, leading to the stimulation of cAMP production (37, 38). One of the main functions of CRH in corticotrophs is to depolarise the plasma membrane and facilitate VGCI influx. Both patterns of electrical activity, including steady depolarisation and depolarisation accompanied with firing of APs, were observed (19–21, 36, 39). The GHRH receptor is expressed in somatotrophs and is coupled to the G_s signalling pathway. In rats, there are two splice forms of this receptor showing similar sensitivity to GHRH, but only the short receptor isoform stimulates cAMP production (40). Like CRH in corticotrophs, GHRH facilitates electrical activity and Ca^{2+} influx through the L-type Ca_v channels of the silent or already active somatotrophs (22, 41–47). An increase in electrical activity after GHRH application is also observed in pituitary slices (48). Pituitary cells also express PAC₁ and VPAC₂ receptors. Gonadotrophs express the PAC₁ receptor, linked to the phospholipase C signalling pathway, and somatotrophs, lactotrophs and melanotrophs express VPAC₂ receptors, coupled to the G_s signalling pathway (49, 50). In $\alpha\text{T3-1}$ cells, PACAP also stimulates cAMP production and facilitates

extracellular Ca^{2+} influx through dihydropyridine-sensitive Ca_v channels (51, 52). PACAP also stimulates cAMP production and α -MSH release from melanotrophs and ACTH release from AtT-20 cells (53). In the melanotrophs, PACAP stimulates electrical activity and Ca^{2+} influx through L-type Ca_v channels (54). In somatotrophs, PACAP also stimulates the Ca^{2+} influx through Ca_v channels (55, 56). Furthermore, PACAP causes extracellular Ca^{2+} influx in lactotrophs (57). In GH_3 cells, VIP evokes a modest VGCI via an increase in cAMP (58), and both VIP and PACAP increase cAMP levels in GH_4C_1 cells (59).

Role of cAMP and cGMP in Electrical Activity and Calcium Signalling

There is consensus that the stimulatory effect of forskolin and G_s -coupled receptors on the electrical activity of secretory anterior pituitary cells is mimicked by the application of cAMP. Figure 2A shows the depolarising effects of 8-bromoadenosine-3',5'-cyclic monophosphate (8-Br-cAMP), a cell-permeable cAMP analogue, in the thyrotrophs. The application of 8-Br-cAMP also facilitates the firing of APs in lactotrophs (2) and gonadotrophs (24). In somatotrophs, the stimulation of GH release by 8-Br-cAMP depends on Ca^{2+} influx (60, 61). In melanotrophs, 8-Br-cAMP stimulates Ca^{2+} oscillations that are dependent on Ca^{2+} influx (62). In corticotrophs, 8-(4-chlorophenylthio)adenosine-3',5'-cyclic monophosphate (8-CTP-cAMP), another cell permeable cAMP analogue, was shown to depolarise the cell membrane and facilitate Ca^{2+} influx (36).

Earlier work with somatotrophs suggested that 8-bromoguanosine-3',5'-cyclic monophosphate (8-Br-cGMP) facilitates GH release (63–65), which could indicate that $[\text{Ca}^{2+}]_i$ is elevated. Consistent with this hypothesis, the application of dibutyryl cGMP was suggested to stimulate VGCI in GH_3 cells (66). However, others observed that dibutyryl-cGMP causes a progressive reduction in the frequency and amplitude of the spontaneous AP-driven $[\text{Ca}^{2+}]_i$ oscillations in these cells (67). Furthermore, 8-Br-cGMP-stimulated GH release also caused a small reduction in $[\text{Ca}^{2+}]_i$ (65), suggesting that cGMP stimulates GH release in a Ca^{2+} -independent manner. The application of nitric oxide (NO) donors to GH_3 cells also causes an inhibition of VGCI (68).

Recently, we systematically investigated the effects of 8-Br-cGMP on electrical activity in a primary culture of pituitary cells (24). These experiments revealed a consistent inhibitory effect of 8-Br-cGMP on electrical activity in all endocrine cell types. Figs. 2B and C illustrate the hyperpolarising action of 8-Br-cGMP, which slows the AP frequency in the spontaneously firing thyrotrophs and hyperpolarises the cell membrane in both firing and quiescent cells. These observations are consistent with the inhibitory effect of the NO signalling pathway on hormone release by cultured pituitary cells (69–71). Thus, it is reasonable to conclude that cAMP and cGMP have opposite effects on the excitability of pituitary cells and the accompanied VGCI; specifically, cGMP hyperpolarises the cell membrane, whereas cAMP depolarises cell membranes.

The cyclic nucleotides can facilitate electrical activity and VGCI directly by activating cyclic nucleotide-gated (CNG) and/or hyperpolarization-activated cyclic nucleotide-regulated (HCN) channels (72), or indirectly through their kinases that phosphorylate several channels, including the inwardly rectifying K^+ (K_{ir}), Ca_v , voltage-gated Na^+ (Na_v) and non-selective cation channels (73). The cyclic nucleotides could also inhibit the electrical activity and Ca^{2+} influx by activating Ca^{2+} -controlled K^+ channels (74). In the following sections, we will discuss in detail the direct and indirect effects of cyclic nucleotides on the plasma membrane channel functions in the endocrine pituitary cells.

Cyclic Nucleotide-gated Channels

In vertebrates, there are six CNG subunits, termed CNGA1, CNGA2, CNGA3, CNGA4, CNGB1, and CNGB3, and several splicing forms of primary transcripts have been identified. The CNGA1-3 subunits can form homomeric channels in the heterologous expression systems, and other subunits can co-assemble to form functional heteromeric channels. These channels are expressed in olfactory neurons and the outer segments of rod and cone photoreceptors, where they play a critical role in sensory transduction. Photoreceptors have a strong preference for cGMP, whereas the olfactory channel is almost equally sensitive to both ligands. The channels are permeable to Na^+ , K^+ , and Ca^{2+} , but not to Cl^- or other anions. The low levels of mRNA transcripts for these channels are also found in the brain, testes, kidneys, and heart (72). The mRNA transcripts for the rod CNG were also detected in rat pituitary cells by RT-PCR analysis (41) and RNA blot hybridisation (75). The zebrafish-specific CNGA5 mRNA and protein transcripts are also expressed in the pituitary cells (76). Further studies are required to clarify their expression at the protein level and their potential role in electrical activity and Ca^{2+} signalling in pituitary cells.

Hyperpolarisation-activated Cyclic Nucleotide-regulated Channels

Structure and function

In mammals, the HCN channel family is comprised of four subunit isoforms, encoded by the four genes, HCN1-4. Each subunit forms functional homomeric channels, which are tetramers; however, the native channels are probably organised as heterotetramers. The HCN channels are activated by hyperpolarisation beyond -60 mV, thereby generating a current termed I_h (h stands for hyperpolarisation), which does not inactivate, and these channels conduct both sodium and potassium. In the cells expressing these channels, their activation leads to a slow depolarisation, which is an action consistent with their equilibrium potential of about -30 mV. Their voltage-sensitivity is modulated by cAMP. HCN channels serve the following three principal functions in excitable cells: i) they contribute to the resting potential, ii) they generate or contribute to the pacemaker depolarisation that controls the rhythmic activity in spontaneously firing cells, and iii) they compensate for inhibitory postsynaptic potentials. A small fraction of HCN channels are tonically activated at rest, and this subset produces the former two functions of these channels (77).

Expression in pituitary cells

HCN channels were first identified in cardiac sinoatrial node cells, and subsequently they have been found in a variety of peripheral and central neurons. Qualitative RT-PCR analysis suggests that AtT-20 cells express mRNA transcripts for HCN1 (78). GH₃ cells express the mRNA transcripts for HCN2, HCN3, and HCN4, but not for HCN1 (79). Quantitative RT-PCR analysis showed higher levels of expression of mRNA transcripts for HCN2 and HCN3 subunits and lower expression of HCN1 and HCN4 subunits in rat anterior pituitary cells (Fig. 3A). Electrophysiological experiments confirmed the presence of a hyperpolarisation-activated current in GH₃ cells (79, 80), AtT-20 cells (78), melanotrophs (81), somatotrophs (80), lactotrophs (82), thyrotrophs, and gonadotrophs (24). The biophysical properties of this current (including activation of this current by hyperpolarising voltages, the time course of activation, a lack of inactivation during sustained recording, a rapid deactivation, and the activation curve obtained by tail current analysis) are consistent with the expression of the I_h current in pituitary cells. The pharmacological profile of this current (e.g., its reversible inhibition by 1 mM Cs^+ , the low and irreversible inhibition by ZD7288, and the sensitivity to tramadol, a synthetic analgesic used to relieve pain) also supports the functional expression of HCN channels in endocrine pituitary cells (24, 78–80, 83). Figure 3 shows the inhibition of the I_h current by 1 mM Cs^+ in lactotrophs (B), somatotrophs (C), and

gonadotrophs (G), and with 10 μ M ZD7288 in thyrotrophs (D). Although the current density was relatively low in both normal and immortalised pituitary cells, such a current may still profoundly influence the resting membrane potential, because the input resistance of these cells is usually > 5 G Ω . In gonadotrophs, the voltage response to a hyperpolarising current pulse showed a slowly developing inward rectification, which was blocked by extracellular Cs^+ and was absent in cells lacking I_h . This could indicate that I_h currents in pituitary cells contribute to the control of the resting membrane potential in the range where other channels can act as pacemakers (24). I_h may also limit the excessive hyperpolarisation in response to hyperpolarising stimuli (78–80). Furthermore, we observed a decrease in the AP frequency in the spontaneously firing gonadotrophs with inhibited I_h , indicating that these channels contribute to the pacemaking (Fig. 3H) in a manner comparable to that observed in the gonadotropin-releasing hormone (GnRH) neurons (84). The abolition of spontaneous electrical activity was not observed in any of these experiments, further indicating the role of other channels in slow depolarisation.

cAMP-dependent facilitation

The HCN channels in AtT-20 pituitary cells are weakly modulated by forskolin (78). The cultured anterior pituitary cells also exhibit a weak facilitation by forskolin and 8-Br-cAMP (24). Figure 3DE shows a current facilitation by 8-Br-cAMP, in thyrotrophs. In GH₃ cells, the forskolin-induced changes in the properties of the I_h current were not observed (79, 80). However, in both normal and immortalised GH₃ pituitary cells, the inhibition of the basal cAMP production significantly attenuated the I_h current, which fully recovered with the application of 8-Br-cAMP (24, 79). Figure 3F illustrates the effects of MDL12330A, an inhibitor of ACs, on the amplitude of the I_h current in thyrotrophs. In AtT-20 cells, the current is also robustly inhibited by a cAMP antagonist (78). These results suggest that, in pituitary cells, *in vitro* HCN channels are tonically activated by the basal AC activity. Because basal cAMP production is down-regulated *in vivo* by several hypothalamic and intrapituitary factors, including dopamine and somatostatin (4), this suggests that the facilitation of AC activity, under physiological conditions, could lead to the activation of HCN channels and the firing of APs. This hypothesis should be addressed in the future. The activity of these channels in pituitary cells is not only determined by the status of AC activity, but it is also affected by GPCR signalling through the phospholipase C pathway. As documented for the GnRH receptor, this family of receptors inhibits HCN channels (Fig. 3I), thereby reflecting the depletion of phosphatidylinositol 4,5 bisphosphate (24), which is required for normal HCN function (85, 86).

PKA/PKG-dependent effects of cyclic nucleotides

Pharmacological approaches

To identify the indirect effects of cAMP and cGMP on electrical activity, cAMP-dependent protein kinase (PKA) and cGMP-dependent protein kinase (PKG) inhibitors are frequently used. Among the PKA inhibitors, the most commonly used is N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonylamide dihydrochloride (H-89), which inhibits both types of PKA (I and II). *In vitro*, the residual PKA activity is about 2% of baseline levels in the presence of 10 μ M H-89. It is important to emphasise that this compound also inhibits S6K1 (100%), ROCK-II (100%), MSK1 (97%), PKBa (83%), AMPK (81%), CHK1 (79%), SGK (75%), and PHK (49%) (87). The 8-Br-cAMP-induced Ca^{2+} oscillations in melanotrophs that are dependent on Ca^{2+} influx are abolished by H-89 (62). The forskolin-stimulated Ca^{2+} influx in gonadotrophs, lactotrophs, somatotrophs and α T3-1 gonadotrophs is also inhibited by 10 μ M H-89 (32, 35); these observations are consistent with earlier studies with somatotrophs (42, 88). Figure 4B shows the lack of response to a forskolin application in pituitary cells bathed in a medium containing 10 μ M

H-89. The CRH and 8-CPT-cAMP-induced Ca^{2+} influx in corticotrophs is blocked by adenosine cyclic 3',5'-(Rp)-phosphothioate (Rp-cAMPS), another PKA inhibitor (36). However, some effects of CRH were resistant to the PKA inhibitor H-89, raising the possibility that CRH might act through an additional G-protein pathway (39, 89). Our experiments with Rp-cAMPS also revealed that spontaneous electrical activity in the pituitary cells was not affected by the inhibition of PKA, but that the 8-Br-cAMP-stimulated increase in the frequency of APs was abolished in the presence of this compound (Fig. 4C). In melanotrophs, PACAP stimulates a Ca^{2+} influx through L-type Ca_v channels, which is inhibited by a blockade of PKA (54). In somatotrophs, the PACAP-stimulated Ca^{2+} influx through Ca_v channels is also blocked by the PKA antagonists (90).

PKA animal models

To more directly investigate the role of PKA in the regulation of these channels, we recently used the following two mouse models with altered PKA signalling: *Prkar1a*^{+/-} and *Prkar1a*^{+/-} *Prkaca*^{+/-} (91, 92). We expected that *Prkar1a* haploinsufficiency would be accompanied by an elevated PKA activity in the pituitary cells, because the down-regulation of the regulatory subunit type 1A leads to endocrine and other tumours (93). This, in turn, should induce a loss of forskolin's stimulatory action on electrical activity and Ca^{2+} signalling. In contrast, we expected that the basal and stimulated PKA activity be normalised in cells from *Prkar1a*^{+/-} *Prkaca*^{+/-} mice, and that forskolin's stimulatory action on electrical activity and Ca^{2+} influx would not be affected. Consistent with these predictions, we found that basal and forskolin-stimulated cAMP production is significantly reduced in pituitary cells from *Prkar1a*^{+/-} mice. We further showed that these cells expressed AC5/6, whose phosphorylation, by PKA, causes an inhibition of the enzyme activity. This observation indirectly suggests that *Prkar1a* haploinsufficiency is accompanied by an elevated PKA activity; this hypothesis was confirmed by the direct measurement of PKA activity. The *Prkar1a* haploinsufficiency also caused the loss of stimulatory action of the forskolin on Ca^{2+} influx. In contrast to *Prkar1a*^{+/-} mice, basal and stimulated PKA activity was normalised in cells from *Prkar1a*^{+/-} *Prkaca*^{+/-} mice, and the stimulatory action of forskolin on Ca^{2+} influx was restored (35).

Calcium-controlled Potassium Channels as Effectors for cGMP

Structure and function

Calcium-controlled K^+ channels (K_{Ca}) are composed of two families. One family of these channels includes three small-conductance (SK) channels ($\text{K}_{\text{Ca}2.1}$, 2.2 and 2.3) and one intermediate-conductance (IK) channel ($\text{K}_{\text{Ca}3.1}$) (94, 95). The high-conductance K^+ (BK) channels represent the other family of K_{Ca} channels, only distantly related to SK and IK channels. The BK channels are composed of four pore-forming $\text{K}_{\text{Ca}1.1}$ (Slo1) α subunits that share the same six transmembrane topology of the voltage-gated K^+ channels; however, they also contain an additional transmembrane segment at the N-terminus. In contrast to the small-conductance channels, the BK channels form macromolecular complexes with Ca_v channels and establish a prototypic Ca^{2+} nanodomain. This provides an effective mechanism for the control of the activity of these channels by Ca^{2+} influx through Ca_v channels. The co-localisation of BK and Ca_v channels thereby facilitates the spike repolarisation and influences the frequency of AP-driven $[\text{Ca}^{2+}]_i$ transients by slowing the pacemaker depolarisation. Both actions result in the reduction of VGCI. The activation of these channels may also remove the steady inactivation of Na_v and Ca_v channels, which may stimulate or enhance AP generation in some cells. BK channels may also play a role in the generation of the bursting type of electrical activity in pituitary cells (96, 97).

Expression in pituitary cells

Whole-cell current recordings showed the presence of the BK current in normal somatotrophs and lactotrophs (98), as well as in the GH₃ and AtT-20 pituitary cell lines (99–102). The activation of PKC inhibits these channels, which could account for the sustained excitability of the pituitary cells during the prolonged activation of G_{q/11}-coupled GPCRs (103). Single channel recordings also showed that the BK channels are expressed in melanotrophs (104) and lactotrophs (105). The *mslo* transcripts that encode the pore-forming α -subunit of the BK channels are robustly expressed in the AtT-20 cells, and the native channels are not functionally coupled to the β -subunits (106).

PKG-dependent facilitation

In numerous cell types, it is well established that cGMP activates the BK channels (107–111). The α subunit of BK channels is directly phosphorylated by PKG, causing the activation of these channels (74). Multiple PKG-dependent serine phosphorylation sites have been identified (112, 113). In the posterior pituitary, the NO enhances K_{Ca} activity by stimulating sGC and PKG (114), whereas the role of the cGMP-PKG signalling pathway in BK channel activation in the endocrine anterior pituitary cells has not been studied.

Inwardly Rectifying Potassium Channels

Structure and function

The term “inward rectifier” describes the activation of inward current under hyperpolarisation, leading to K⁺ influx, with almost no K⁺ efflux under depolarisation. Because of these unusual activation properties, these channels are also known as anomalous rectifiers. Although the amplitude of the outward currents following through these channels is limited, they profoundly influence the resting membrane potential. Among voltage-gated channels, the structures of K_{ir} channels and bacterial K⁺ channels, known as KcsA, are the most simple. The α subunit of these channels is a tetramer, each monomer of which contains two transmembrane segments, connected with a re-entry P loop. The K_{ir} channels are expressed in numerous tissues, including the brain, heart, kidney, endocrine cells, ears, and retina. They participate in the control of the resting potentials and are closed by strong depolarisations. There are 15 members of this family of channels that are divided into three groups, based on their regulation patterns. Some of these channels are constitutively active at rest, leading to K⁺ leaking from cells, whereas the activity of others is influenced by certain modulators, such as G proteins and nucleotides. The long cytoplasmic pore of these channels plays a critical role for inward rectification, and it provides the structural basis for the modulation of gating by G proteins and phosphatidylinositol 4,5 biphosphate (115).

Expression and role in pituitary cells

RT-PCR analysis showed the presence of K_{ir} 3.1, 3.2, and 3.4 mRNA transcripts in the rat pituitary cells (116), and K_{ir}3.1–3.4 mRNA transcripts in GH₃/B₆ mammosomatotrophs (117). The presence of K_{ir}3.1 and 3.2 proteins in AtT-20 cells was also confirmed by western blot analysis (118). RT-PCR analysis also revealed the presence of the K_{ir} 1.1, 2.2, 4.1, 6.1, and 6.2 mRNA transcripts in the GH₃/B₆ cells (117). Electrophysiological studies confirmed the functional expression of G protein-regulated K_{ir} channels in rat pituitary lactotrophs, where they are activated by dopamine (119) and endothelin (120). In somatotrophs, they are activated by somatostatin (121) and ETs (122), whereas the AtT-20 corticotrophs are activated by somatostatin and muscarinic receptors (118, 123–125). The G protein-dependent activation of the K_{ir} currents in AtT-20 cells and human GH-secreting adenoma cells was demonstrated by the down regulation of their expression by antisense oligonucleotides (126, 127). In the GH₃ cells, the constitutively active K_{ir} channels were also identified and found to play an important role in the maintenance of the resting

membrane potential (128, 129). The presence of the ATP-sensitive K^+ channels in the pituitary cells has also been reported (130).

PKA-dependent inhibition

A key element in the control of spontaneous firing of APs in the pituitary cells, including corticotrophs, is the control of the resting membrane potential and slow membrane depolarisation, called the pacemaking depolarisation. The CRH changes the resting membrane potential and the rate of the slow depolarisation, leading to an increase in the firing rate of spontaneously active cells and causing silent cells to become active (19, 20, 39). The slow membrane depolarisation is caused, in large part, by a reduction in a background K^+ conductance, mediated by a member of the K_{ir} channel family (36, 131). The inhibition of K_{ir} , including its associated depolarisation and increases in spike frequency, lasts up to 15 minutes after the removal of CRH from the physiological solution. This relationship suggests that phosphorylation of K_{ir} channels could account for this memory (131). These effects of CRH and their time courses are mimicked by the application of forskolin and by membrane-permeable analogues of cAMP (36, 131). No CRH-induced depolarisation is observed in the presence of intracellular Rp-cAMPS, a blocker of PKA (36), thereby confirming that the effects of CRH on the pacemaking depolarisation are mediated through the cAMP activation of PKA. However, some effects of CRH were resistant to the PKA inhibitor H-89, raising the possibility that the CRH might act through an additional G-protein pathway (39, 89). It is possible that the GHRH decreases the intrinsic activity of a K_{ir} channel in somatotrophs, just as CRF does in corticotrophs. This was shown in experiments that blocked these channels in spontaneously firing cells and model simulations (22). In ovine somatotrophs, GH-releasing peptide-2 reduces the K_{ir} current via the PKA-dependent signalling pathway (132). K_{ir} channels are also inhibited by the activation of the thyrotropin-releasing hormone (TRH) receptor, presumably through their cross-coupling to the G_s signalling pathway (133). The TRH also inhibits K_{ir} channels in lactotrophs from lactating rats (134).

The K_{2P} Channel TREK-1

Depolarisation alone is not sufficient to induce spiking in quiescent cell (39), and the firing frequency of cells depolarised by CRH is higher than that for corticotrophs depolarised to the same voltage level by blocking the K_{ir} current. This indicates that a component of the depolarisation might be mediated by the reduction of another type of background K^+ conductance, or by the facilitation of an inward current (131). Among the K^+ conducting channels, two members of the two pore-forming domain channels, TASK-1 (KCNK3) and TASK-3 (KCNK9), are constitutively open at rest, whereas TREK-1 (KCNK2) requires physical and chemical stimulation to open (135). At the present time, there are no pharmacological agents available that are selective for these channels (136). Based on the sensitivity of a background K^+ current on fluoxetine, chlorpromazine, extracellular acidification, and arachidonic acid application, it has been recently suggested that TREK-1 channels are important in setting the resting potential in mouse corticotrophs. Furthermore, both CRH and 8-CTP-cAMP inhibited this current (137). In the presence of fluoxetine, the CRH-induced depolarisation was significantly reduced, but not abolished. This finding could be explained by an incomplete inhibition of TREK-1 channels, or the contribution of K_{ir} channels in CRH action. Further work should be focused on the molecular identification of TREK-1 channels in corticotrophs and other pituitary cells, and on a clarification of the role of PKA in the action of cAMP on activity of these channels.

Voltage-gated Calcium Channels

Structure and function

Electrophysiologically, Ca_v channels are separated into two groups. The first group of channels is known as the high-voltage activated Ca_v channels, because these channels require moderate to strong membrane depolarisations to open. Among this group, biophysical and pharmacological studies have identified the L-, N-, P/Q-, and R-type Ca^{2+} channels, which are distinguished by their single-channel conductance, pharmacology, and metabolic regulation. The second group is known as the low-voltage activated Ca_v channels, because they require less depolarisation for their activation and their subsequent inactivation, as compared to the high-voltage activated channels. Furthermore, a strong membrane hyperpolarisation is required to bring them out of their steady inactivation. Because of their gating properties, these channels are often referred to as the transient or T-type Ca_v channels. The purification of Ca^{2+} channels has identified the following five subunits: a pore forming large α_1 subunit, and four smaller ancillary subunits, including α_2 , β , γ , and δ . In addition to the voltage sensor, the gating machinery, and the channel pore, the α_1 subunit also contains most of the known sites of channel regulation provided by the intracellular messengers, drugs, and toxins. These sites include G $\beta\gamma$ domains and multiple PKA phosphorylation sites (138). The Ca_v channels serve two major functions in cells: electrogenic and regulatory. The major function of the T-type Ca_v channels is electrogenic; at the resting potential, these channels depolarise cells to the threshold level for a Na^+ or Ca^{2+} spike. In contrast, the high-voltage activated channels will inactivate incompletely and help to keep the cells depolarised for a prolonged period. In some neurons and many neuroendocrine cells, these channels give rise to APs in the same way as Na_v channels, although typically with slower kinetics and lower amplitudes. In other neurons, Ca_v channels shape the Na^+ -dependent APs. Such APs increase in $[\text{Ca}^{2+}]_i$ of a sufficient amplitude to trigger the Ca^{2+} -dependent processes (138, 139). In cells that are not firing AP, depolarisation also facilitates Ca^{2+} influx through Ca_v channels. The regulatory function of these channels is based on the Ca^{2+} influx during the transient depolarisation, which acts as an intracellular messenger, controlling a variety of cellular functions.

Expression in pituitary cells

Progress has been made in the identification of Ca_v - α subunit transcripts that are present in pituitary cells. The $\text{Ca}_v3.1$ and $\text{Ca}_v3.3$ mRNAs were detected in GH₃ cells exhibiting a prominent T-type Ca^{2+} current (140). Several pore forming subunits of Ca_v channels are also present in GH₃/B₆ pituitary cells, and these subunits account for the formation of T-type ($\text{Ca}_v3.1$), L-type ($\text{Ca}_v1.1$, 1.2, and 1.3), and P/Q ($\text{Ca}_v2.1$) type currents. The mRNA transcripts for the β_1 , β_2 , and β_3 Ca_v subunits were also detected in these cells (141). Immunocytochemical analyses confirmed the expression of the $\text{Ca}_v1.2$, 1.3, 2.2, and 3.1- α subunits in mouse anterior pituitary cells (142). The functional expression of both the inactivating and non-inactivating Ca_v currents is well documented in rat (98, 143–145), ovine (146), and fish (147) gonadotrophs, as well as in genetically-labelled mouse gonadotrophs (148) and $\alpha\text{T}-3$ immortalised mouse gonadotrophs (149). These currents are also present in the rat somatotrophs and lactotrophs (98, 150), fish somatotrophs (151), frog melanotrophs (152), and in the immortalized GH cells (141, 153, 154). In the GH₃ cells, there are multiple conductance levels of the L-type Ca_v channels (155), and estrogens stimulate the expression of these T-type channels (156). The properties of the inactivating Ca_v channels are consistent with the expression of the T-type Ca^{2+} channels. In contrast, the non-inactivating Ca^{2+} current in the pituitary cells is mediated by the dihydropyridine-sensitive and -insensitive Ca_v channels (144, 145, 153, 157).

Role in pituitary cells

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 depolarisations, called VGCI. The patterns of the spontaneous electrical activity in different cell types (e.g., single spiking vs. plateau bursting) largely impact intracellular Ca^{2+} dynamics and overall Ca^{2+} levels. In single spiking gonadotrophs, the bulk Ca^{2+} levels are 20 nM to 70 nM, whereas in spontaneously bursting lactotrophs and somatotrophs, the levels are much higher, are variable between 300 nM to 1.2 μM , and are clearly oscillatory (22, 25). Corticotrophs (20) and immortalised pituitary cells (26, 128, 153) also exhibit plateau bursting type electrical activity and high amplitude Ca^{2+} transients. Both types of APs depolarise the membrane sufficiently to activate the various types of Ca_v channels that are expressed in pituitary cells. However, the majority of the Ca^{2+} influx occurs through dihydropyridine-sensitive L-type Ca_v channels (98, 157). In cells not exhibiting firing of APs, a plateau depolarisation also results in Ca^{2+} entry via Ca_v channels, but in a non-oscillatory manner (36). The T-type channels may also contribute to the slow depolarisation between spikes (158).

PKA-dependent facilitation

In somatotrophs, GHRH depolarises the plasma membrane and facilitates Ca^{2+} influx through Ca_v channels (42, 159). GHRH was also suggested to increase the L- and T-type Ca^{2+} conductance in rat and ovine somatotrophs (42, 160). The L- and T-type Ca_v channel currents are also expressed in the human GH-secreting pituitary adenomas, and both GHRH and 8-Br-cAMP increase the amplitude of both currents. The stimulatory action of GHRH on the L- and T-type currents is abolished in the cells with inhibited PKA, indicating a role of PKA in this action (161). The synthesised GH releasing peptide, GH releasing peptide-2, also depolarises the plasma membrane of the ovine somatotrophs, and this agonist enhances the L- and T-type Ca_v conductance (162). Other synthesised GH releasing peptides also depolarise the somatotrophic membrane (163). In corticotrophs, the Ca_v channels can be activated with a sufficiently strong pacemaking depolarisation, and their opening produces the upstroke of the AP spike and a robust Ca^{2+} influx. It appears that the main channel involved is the L-type Ca_v channel, but P-type Ca_v channels also play a role in the regulation of spike frequency. Indeed, another high-voltage activated and toxin-resistant Ca_v channel may do so as well (19). In these cells, the rapid PKA-mediated phosphorylation of L-type Ca_v channels has not been studied and should not be excluded. Corticotrophs also express T-type Ca_v channels, but their role in CRH action has also not been studied. These results are consistent with the finding that the L-type Ca^{2+} channels are rapidly stimulated by the PKA-dependent phosphorylation of their α subunits (138). Because PKA does not phosphorylate the T-type channel (139), the indirect action of PKA probably accounts for effects on those channels. Furthermore, cAMP stimulates the expression of the L-type Ca_v channels in AtT-20 cells at the mRNA and protein levels, presumably through PKA (164). Ghrelin and GH releasing peptide-6 also upregulate L-type Ca_v channels after a long-term exposure in GH cell lines (165), indicating a role of cAMP/PKA in the transcriptional regulation of Ca_v channels.

Voltage-gated Sodium Channels

Structure and function

Mammals express nine genes for the Na^+ channel α subunit, termed $\text{Na}_v1.1$ - $\text{Na}_v1.9$, and the closely related Na^+ channel-like proteins (Na_x) share approximately 50% of their structural similarity with Na_v1 channels. The α subunit contains four homologous domains, each consisting of a six transmembrane domain and a re-entry P loop between 5S and 6S. The P loop contains the tetrodotoxin (TTX) binding site, a voltage gate and sensor, and the sites for

phosphorylation by the protein kinases on the intracellular surface. Na_v 1.5, 1.8 and 1.9 are insensitive to TTX in a nanomolar concentration range. Four auxiliary subunits have been identified so far, termed Na_vβ₁, Na_vβ₂, Na_vβ₃, and Na_vβ₄. They belong to a single family of proteins, which interact with different α subunits and alter their physiological properties. The main function of Na_v channels is to depolarise cells and to generate the upstroke of the AP, thereby controlling the firing amplitude in excitable cells, including nerve, muscle, and neuroendocrine cell types. In some cells, these channels are solely responsible for the rapid and regenerative upstroke of an AP. In others, they act in conjunction with Ca_v channels to depolarise cells. The Na_v channels are also expressed in non-excitable cells at a lower level, where their physiological role is unclear (166).

Expression and role in pituitary cells

The expression of TTX-sensitive and -insensitive Na_v channels has been extensively studied in endocrine pituitary cells. Rat melanotrophs express the mRNA transcripts of seven α subunits, including the TTX-insensitive Na_v1.8 and 1.9 subunit mRNAs, and the β₁ and β₂ auxiliary subunit mRNAs (167). The mRNA transcripts for the α-subunit of Na_v1.1, Na_v1.2, Na_v1.3, and Na_v1.6, as well as the β₁ and β₃-subunits of Na⁺ channels, are present in GH₃ cells (168). The expression of the Na_v1.7-α subunit in the rat anterior pituitary was confirmed by *in situ* hybridisation and immunohistochemistry (169). Somatotrophs from GH-green fluorescent protein transgenic mice express mRNA transcripts for Na_v1.5, 1.8, and 1.9, as well as the TTX-sensitive and TTX-resistant Na⁺ currents (170). Electrophysiological experiments revealed that both freshly dispersed and cultured rat melanotrophs express functional channels that are composed of TTX-sensitive and TTX-resistant components (167, 171). These channels are also identified in goldfish somatotrophs (151) and *Xenopus laevis* melanotrophs (172, 173). The single cell Ca²⁺ measurements further indicated the presence of functional Na_v channels in frog melanotrophs (172, 173). The TTX-sensitive current has also been identified in rat (98, 143, 145), mouse (174), ovine (175, 176) and fish (147, 177) gonadotrophs, as well as in αT3-1 mouse gonadotrophs (149, 178). Rat lactotrophs (179, 180), somatotrophs (98), corticotrophs (143), and GH₃ cells (181), as well as fish lactotrophs (182), also express Na_v channels. In a fraction of ovine gonadotrophs (175) and bovine lactotrophs (183), the Na_v channels are also responsible for AP generation. TTX-sensitive Na_v channels may contribute to the firing of APs and the accompanied VGCI in frog and rat melanotrophs (172). However, in the majority of rat anterior pituitary cells *in vitro*, these channels do not contribute to the spike depolarisation because they are inactivated at the resting membrane potential. In hyperpolarised cells, which is tonically maintained *in vivo* by a GPCR coupled to the G_{i/o} signalling pathway, Na_v channels could play an important role in the transition from the quiescent to the firing modes (4).

PKA-dependent facilitation

Several investigations have suggested that the Na⁺-conducting channels are responsible for the facilitation of electrical activity and Ca²⁺ influx in the pituitary somatotrophs, by GHRH receptors. Kato and colleagues showed that GHRH-stimulated hormone secretion by somatotrophs was suppressed in cells bathed in a Na⁺-deficient medium (88). The same group also showed that GHRH depolarised somatotrophs, which was greatly suppressed by the removal of the bath Na⁺ (184). Others also showed the dependence of GHRH-induced Ca²⁺ influx on the depolarising Na⁺ conductance (42, 44). It has been reported that GHRH increases the total Na_v current in somatotrophs, which was blocked by low levels of TTX (185). Others reported that GHRH facilitates a TTX-insensitive Na_v current in cultured somatotrophs from GH-green fluorescent protein transgenic mice, but in a cAMP-independent and PKC-dependent manner (170). The relevance of Na⁺ conductance in the action of VIP-PACAP has also been suggested (186).

Non-selective Cation Channels

Contribution to the resting membrane potential

The resting membrane potential of -50 to -60 mV in the pituitary cells suggests that, in addition to the leaking K^+ conductance, there is also a depolarising conductance due to other ions. The resting membrane potential rapidly reaches approximately -85 mV when extracellular Na^+ , but not Ca^{2+} , is removed. This value is close to the equilibrium potential for K^+ , suggesting that a Na^+ -conducting channel has a constitutive activity, e.g. the Na^+ -conducting channel acts as a leak channel. Regarding the inhibition of all Na_v channels, the addition of TTX in micromolar concentrations does not mimic the effect of the removal of bath Na^+ on the resting membrane potential. This leak current is termed the background Na^+ current (8, 21, 153, 180). Two other reports have suggested the presence of Ca^{2+} -activated, non-selective cationic currents in GH₃ cells (187) and gonadotrophs (188).

Structure and function

The nature of these channels is unclear at the present time. In general, the majority of transient receptor potential (TRP) channels are relatively non-selective cation-conducting channels. These channels were initially found in *Drosophila*, in which they contribute to phototransduction. Six protein families comprise the mammalian TRP superfamily: the “canonical” receptors (TRPCs), the vanilloid receptors (TRPVs), the melastatin receptors (TRPMs), the polysistins (TRPPs), the mucolpins (TRPMLs), and the ankyril transmembrane protein 1 (TRPA1). These channels resemble K_v channels in their overall structures. However, they show a limited conservation of the S4 positive charges and P loop sequences. The assembly of the channel subunits as homo- and heterotetramers results in the formation of cation-selective channels (189). The TRPM3 channel mRNA transcripts are present in pituitary cells (190), as well as in unidentified member(s) of the TRPC family of channels that are activated by phospholipase C (191). The mRNA transcripts for TRPC1, TRPC3, TRPC5, and TRPC7 have also been identified in human pituitary cells (192). Our quantitative RT-PCR analysis revealed that the mRNA transcripts for TRPC channels are expressed in the anterior pituitary cells in the following order: TRPC1 $\gg$ TRPC6 $>$ TRPC4 $>$ TRPC5 $>$ TRPC3. Our pharmacological studies further indicate that the cation-conducting TRPC channels are good candidates for the background Na^+ conductance; spontaneous electrical activity was abolished by the application of three blockers of these channels, including SKF96365, 2-APB, and Gd^{3+} (35).

PKA-dependent facilitation

The relevance of cation channels in the GHRH action observed in human GH-secreting adenoma cells was originally introduced by Takei et al (161). A subsequent study by the same group revealed that hGHRH stimulates Na^+ conductance, with the reversal potential of -20 to 0 mV. The channel was also permeable to Li^+ and K^+ , but not to TMA^+ . A similar nonselective cation current was activated by 8-Br-cAMP and forskolin, and the activation of the hGHRH-induced current was inhibited by PKA inhibitors, including (R)-p-adenosine 3', 5'-cyclic monophosphate, H-89, and PKA inhibitor peptide PKI-(5–24). These findings indicate that the hGHRH-induced current was activated by PKA. A cholera toxin pretreatment eliminated the hGHRH-induced current, suggesting that G is involved in the activation of this current (193). Our recent study indicated that forskolin was unable to facilitate Ca^{2+} influx in cells bathed in a medium containing SKF96365 and 2-APB, which are blockers of the TRPC channels. A high level of extracellular Mg^{2+} , achieved with a blocker of variable channels, including the non-selective cationic channels (194), also inhibits the cAMP-dependent current in pituitary cells. We also observed that forskolin, 8-Br-cAMP, and GHRH stimulated an inward non-selective current, with larger amplitude at more negative potentials. The reversal potential of this current is consistent with the

operation of a non-selective cation channel. The channel was inhibited by H89, further suggesting a role for PKA in the activation of these channels. Finally, in cells bathed in a medium containing NMDG⁺ instead of Na⁺, forskolin was unable to stimulate Ca²⁺ influx. This result indicates that the other cations present in the bath medium were unable to fully substitute Na⁺ in re-establishing the resting membrane potential, and in evoking the spontaneous and cAMP-PKA-stimulated Ca²⁺ influx (35).

Summary and Perspectives

Pituitary cells express several subtypes of transmembrane ACs, and their basal and receptor-stimulated activity provides effective pathways for the generation of cAMP, whereas the expression and role of soluble AC has not been studied in this gland. Pituitary cells also express pGCs and sGC, which generate cGMP in an agonistic and NO-dependent manner, respectively. As summarised in Fig. 5, these messengers play important roles in the plasma membrane channel physiology, by altering the patterns of spontaneous electrical activity and the accompanying VGCI. cAMP and cGMP directly affect the gating of the CNG and HCN channels, and they indirectly affect the gating of BK-K_{Ca}, L-type Ca_v, Na_v, K_{ir} and voltage-insensitive Na⁺-conducting channels, through PKA and PKG-dependent phosphorylation. Although these channels are expressed in all pituitary cell types, this pattern does preclude the possibility that their modulation by the cyclic nucleotides may occur in a cell-type and/or receptor-type-specific manner.

In general, cAMP and cGMP could have a cooperative effect on membrane depolarisation and the facilitation of VGCI, by acting as co-agonists at CNG and HCN channels. However, in endocrine pituitary cells, these two intracellular messengers appear to have opposite roles in the control of endocrine pituitary cell excitability. Specifically, cAMP depolarises the cell membrane, leading to the facilitation of AP firing and VGCI, whereas cGMP hyperpolarises the cell membrane, causing the cessation of electrical activity in spontaneously firing cells. The hyperpolarising nature of cGMP could be explained by the expression and operation of BK type K_{Ca} channels in endocrine pituitary cells, whereas the stimulatory action of this messenger on hormone release could be independent of the electrical status of cells. cAMP facilitates the excitability of pituitary cells directly by activating HCN channels, which are expressed in all endocrine pituitary cell types, through PKA-dependent phosphorylation of several plasma membrane channels. It is interesting that the basal AC activity of cultured pituitary cells is of a sufficient amplitude to integrate HCN channels into electrical activity. *In vivo*, the removal of the dopaminergic and somatostatinergic inhibition of AC could also play a role in the integration of these channels in electrical activity. This hypothesis should be tested by studying effects of the withdrawal of dopamine and/or somatostatin on the status of the HCN channels in lactotrophs, melanotrophs and somatotrophs, *in vitro*. The mechanisms and physiological significance of GnRH-induced inhibition of the HCN channels in gonadotrophs should also be addressed.

The major action of cAMP on the excitability of pituitary cells appears to occur through a PKA-mediated phosphorylation of plasma membrane channels. The enlargement of the L-type Ca_v current, by GHRH in somatotrophs, is consistent with experiments with recombinant channels phosphorylated by PKA. Future work with this cell type should be focused on the changes in the pattern of electrical activity caused by the phosphorylation of these channels, because in somatotrophs, lactotrophs and probably corticotrophs, L-type Ca_v and BK channel interplay is important in the formation of the plateau bursting type of electrical activity. All other pituitary cell types also express L-type Ca_v channels, but at the present time, it is not clear whether their gating is enhanced by receptors coupled to AC signalling pathway. Solid evidence also exists for the relevance of Na⁺-conducting channels in PKA-mediated facilitation of electrical activity in pituitary cells. It is reasonable to

suggest that the TTX-resistant, rather than TTX-sensitive, Na⁺ channels play a major role in PKA (and/or PKC) phosphorylation of these channels. Earlier investigations and our recent data indicate that the non-selective cation channels, presumably the TRPC family of these channels, could also contribute to cAMP-PKA-dependent facilitation of electrical activity. When considered with findings regarding the role of the K_{ir} and TREK-1 channels in the PKA-mediated facilitation of electrical activity, these findings suggest the presence of multiple channel types for the PKA-dependent facilitation of pituitary cell excitability (Fig. 5).

Acknowledgments

The authors are thankful to all past and present members of their laboratories who have contributed to the understanding of the role of cyclic nucleotides and pituitary cells. This work is supported by the National Institute of Health, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development intramural grants.

Abbreviations

AC	adenylyl cyclase
ACTH	adrenocorticotropin hormone
AP	action potential
BK	high conductance
[Ca²⁺]_i	intracellular calcium concentration
cAMP	Ca _v , voltage-gated calcium
cyclic 3'	5'-adenosine monophosphate
cGMP	3'5'-guanosine monophosphate
CNG	cyclic nucleotide-gated
CRH	corticotropin-releasing hormone
GH	growth hormone
GHRH	GH releasing hormone
GnRH	gonadotropin-releasing hormone
GPCR	G protein-coupled receptors
H89	N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonylamide dihydrochloride
HCN	hyperpolarization-activated cyclic nucleotide-regulated
K_{Ca}	calcium-controlled K ⁺ channels
K_{ir}	inwardly rectifying potassium
Na_v	voltage-gated sodium
NO	nitric oxide
PACAP	pituitary adenylyl cyclase-activating peptide
PAC1	PACAP-preferring
PKA	cAMP-dependent protein kinase
PKC	protein kinase C

PKG	cGMP-dependent protein kinase
pGC	particulate guanylyl cyclase
Rp-cAMPS	3',5'-(Rp)-phosphothioate
sGC	soluble guanylyl cyclase
TASK1, TASK3, and TEK-1	members of potassium leak channels with two-P-domain subunits
TRH	thyrotropin-releasing hormone
TRP	transient receptor potential
TTX	tetrodotoxin
VIP	vasoactive intestinal polypeptide
VPAC₂	PACAP/VIP-shared
VGCI	voltage-gated calcium influx

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Box 1

Cyclic 3',5'-adenosine monophosphate (cAMP) was the first historically identified intracellular messenger in 1957. cAMP is used in practically all types of organisms, from prokaryotes to mammals. It is synthesised from ATP by a family of enzymes consisting of transmembrane and soluble adenylyl cyclases (ACs). Molecular cloning has confirmed that several structurally related plasma membrane-bound ACs are expressed in the animal cells, and in mammals, these enzymes are encoded by nine different genes. ACs contain two hydrophobic domains, each composed of six transmembrane helices, and two cytoplasmic catalytic domains. All of the characterised isoforms of ACs produce cAMP in the absence of hormonal stimulation, and such basal activity is markedly enhanced upon the binding of G_s and is reduced upon the binding of G_i , G_o , and G_z alpha subunits. The $\beta\gamma$ dimers of G_i and G_o proteins also contribute to the control of AC2, AC4, and AC7 activity. The $G_{q/11}$ -coupled calcium-mobilising receptors modulate AC activity indirectly through the Ca^{2+} and protein kinase C (PKC) signalling pathways. Cytosolic Ca^{2+} exhibits direct inhibitory effects on the AC5 and AC6 isoforms and an indirect effect on AC9 (through the Ser/Thr protein phosphatase calcineurin) and AC3 (through Ca^{2+} -calmodulin kinase II). Ca^{2+} also stimulates AC1 and AC8, via calmodulin. PKC activates the AC2, AC4, AC5, and AC7 isoforms. In contrast, cAMP-dependent protein kinase (PKA) inhibits the AC5 and AC6 isoforms, providing a form of negative feedback to the cAMP formation. Forskolin, a hypotensive agent, activates all isoforms except AC9, whereas several 3'-adenosine nucleotide analogues bind to the activated pyrophosphate complexes of ACs, thereby inhibiting the enzyme activity (195, 196). In 1999, a soluble AC was also identified, but their expression and potential roles in the pituitary gland have not been studied (197, 198).

Two families of enzymes, the particulate guanylyl cyclase (pGC) and soluble guanylyl cyclase (sGC), synthesise cyclic 3',5'-guanosine monophosphate (cGMP) from GTP using a mechanism that is stereochemically analogous to that of the ACs. pGC contains an extracellular peptide receptor domain and an intracellular catalytic domain, connected by a single transmembrane domain. The active enzyme is now generally thought to be a homodimer of approximately 120 kDa subunits, and the dimerisation is mediated by the intracellular domain. Seven mammalian pGSs and a sea urchin enzyme have been identified and sequenced. Three of these have known peptide ligands (GC-A, atrial natriuretic peptide; GC-B, brain natriuretic peptide; and GC-C, the heat stable enterotoxin of *Escherichia coli* and endogenous intestinal peptide guanylin) (199–201). Based on cDNA cloning, four sGC subunits have been identified and termed $\alpha 1$, $\alpha 2$, $\beta 1$, and $\beta 2$. In the absence of nitric oxide (NO), sGC-derived cGMP production is negligible, and the three enzymes of NO synthases, including endothelial, neuronal and inducible NO, supply cells with NO by catalyzing the oxidation of L-arginine to L-citrulline and NO (202). Many $G_{q/11}$ and G_s protein-coupled receptors also stimulate cGMP production in a Ca^{2+} - and protein kinase-dependent manner (203).

As a family, ACs, pGCs, and sGCs are involved in a broad array of signal transduction pathways, mediated by the cyclic nucleotide monophosphates, including vision, blood pressure regulation, and responses to numerous hormones, neurotransmitters, and NO. Experimentally, the actions of cAMP and cGMP are mimicked by cell permeable analogues. Most of the physiologic actions of cAMP are mediated by PKA, which controls multiple aspects of cell function through the phosphorylation of protein substrates. cAMP also directly regulates certain guanine nucleotide exchange factors, the hyperpolarisation-activated cyclic nucleotide-regulated (HCN) cation channels, and the cyclic nucleotide-gated (CNG) channels. In contrast to cAMP, the intracellular messenger functions of cGMP are more restricted, and these functions are indirectly

mediated through protein phosphorylation by cGMP-dependent protein kinase (PKG) and directly mediated by the activation of HCN and CNG channels (204).

PKAs are present in all eukaryotic cells. In the absence of cAMP, PKA is an inactive, asymmetric tetramer containing 2 regulatory and 2 catalytic subunits, which bind to each other with high affinity. The binding of cAMP to the regulatory subunit alters its affinity for the catalytic subunits, leading to the dissociation of the regulatory subunits and 2 active monomeric catalytic subunits. There are two categories of regulatory subunits, and several isoforms of each regulatory subunit are cloned and expressed in most cells. Each regulatory subunit has 2 high-affinity binding sites for cAMP per monomer, localised at the C-terminus, which differ in their structure and affinity for various analogues of cAMP. The catalytic PKA subunits are also encoded by at least three genes, giving rise to the α , β , and γ isoforms. The α -isoform appears to be expressed constitutively in most cells, whereas the expression of the β -isoform is tissue-specific. Because each regulatory subunit can associate with any of the catalytic subunits, a wide variety of AC holoenzymes can exist in various tissues (16, 205).

The marked amino acid sequence homologies between the PKAs and PKGs, as well as the predicted similarities in their structures, suggest that the 2 enzymes have evolved from an ancestral phosphotransferase. However, the PKGs have a more limited distribution in mammalian tissues than PKA. In high concentrations, they are present in cerebellar Purkinje cells, platelets, and intestinal epithelial cells. In other tissues, PKG is at a 10–100-fold lower concentration than PKA. In mammals, there are two forms of this enzyme. Type I kinase is a soluble protein, consisting of two spliced forms, and it mediates the effects of natriuretic peptides and NO in cardiovascular cells. On the other hand, type II kinase is a membrane-associated enzyme that transduces signals from the *Escherichia coli* heat-stable enterotoxin STa, and from the endogenous intestinal peptide, guanylin. PKGs operate as dimers, although each monomer seems to be self-sufficient in its regulatory and catalytic properties (206, 207).

Fig. 1.

Effects of forskolin on the electrical activity and VGCI in pituitary cells. A, Forskolin initiates electrical activity in quiescent cells and increases the frequency of APs in spontaneously firing pituitary cells. B, Forskolin-stimulated calcium transients in cultured pituitary cells. The addition of a medium was used as a control (left panel - arrows). S, somatotrophs; G, gonadotrophs; L, lactotrophs; U, unidentified cells. C, Forskolin-stimulated calcium influx in GH₃ cells. Horizontal bars (A) and gray areas (B) indicate the duration of forskolin stimulation. Derived from (2, 35).

Fig. 2.

Opposite effects of cAMP and cGMP on the excitability of pituitary thyrotrophs. A, Depolarising effect of cAMP, leading to the initiation of electrical activity in quiescent cells. B and C, Hyperpolarising effects of cGMP in spontaneously active (B) and quiescent (C) thyrotrophs. Derived from (24).

Fig. 3.

Expression of HCN channels in pituitary cells. **A**, Quantitative RT-PCR analysis of the HCN mRNA transcript expression in cultured anterior pituitary cells. **B** and **C**, Electrophysiological and pharmacological characterisation of HCN channels in lactotrophs (**B**) and somatotrophs (**C**). **D** – **F**, Characterisation of the I_h current in rat pituitary thyrotrophs. **D**) Inhibition of the I_h current by ZD7288. **E**, Stimulation of I_h by 8-Br-cAMP. **F**, Inhibition of I_h by MDL12230A, an AC inhibitor. **G**–**I**, Properties of the HCN channels in pituitary gonadotrophs. **G**) Blockade of the I_h current by the addition of 1 mM Cs^+ to the extracellular solution. **H**, Effects of I_h blockade on the frequency of spontaneous firing of APs in gonadotrophs. **I**, Inhibition of I_h by GnRH. In **B**, **C**, **D**, **E**, **F**, **G** and **I**, representative traces of the whole-cell current response to a hyperpolarizing voltage step to -120 mV from a holding potential of -40 mV are shown. Derived from (24).

Fig. 4.

Effects of HCN channel and PKA inhibitors on the forskolin-stimulated calcium influx in anterior pituitary cells. **A**, The stimulation of Ca^{2+} influx by forskolin in cells with the HCN channels, inhibited by pretreatment with ZD7288, which was applied for 10 minutes prior to recording. **B**, The lack of an effect of forskolin on Ca^{2+} influx after treatment of cells with 10 μ M H89, a PKA inhibitor. Notice the change in the pattern of calcium signalling after addition of H89 (two top traces). **C** and **D**, PKA inhibitor Rp-cAMPs does not alter the pattern of spontaneous firing of APs (**C**), but it does abolish the 8-Br-cAMP-induced increase in the firing frequency (**D**). Derived from (35).

Fig. 5.

Schematic representation of the expression and role of cyclic nucleotide signalling pathway in control of electrical activity in pituitary cells. BK, big calcium-activated potassium channels; CNG, cyclic nucleotide-gated channels; GPCR, G protein-coupled receptors; HCN, hyperpolarisation-activated nonselective cation channels; K_{ir}, inward rectifier potassium channels; L, dihydropyridine-sensitive voltage-gated calcium channels; Na_b, background Na⁺-conducting channels; Na_v, TTX-resistant voltage-gated Na⁺ channels; PKA, protein kinase A; PKG, protein kinase G; pGc, particulate guanylyl cyclase; sGC, soluble guanylyl cyclase.