Control of anterior pituitary cell excitability by calcium-activated potassium channels

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Abstract

In anterior pituitary endocrine cells, large (BK), small (SK) and intermediate (IK) conductance calcium activated potassium channels are key determinants in shaping cellular excitability in a cell type- and context- specific manner. Indeed, these channels are targeted by multiple signaling pathways that stimulate or inhibit cellular excitability. BK channels can, paradoxically, both promote electrical bursting as well as terminate bursting and spiking dependent upon intrinsic BK channel properties and proximity to voltage gated calcium channels in somatotrophs, lactotrophs and corticotrophs. In contrast, SK channels are predominantly activated by calcium released from intracellular IP3-sensitive calcium stores and mediate membrane hyperpolarization in cells including gonadotrophs and corticotrophs. IK channels are predominantly expressed in corticotrophs where they limit membrane excitability. A major challenge for the future is to determine the cell-type specific molecular composition of calcium-activated potassium channels and how they control anterior pituitary hormone secretion as well as other calcium-dependent processes.

Keywords: Kcnma1, Kcnn1-4, ion channel, secretion, signalling

1. Introduction

Since the seminal work demonstrating that endocrine cells of the anterior pituitary gland generate calcium-dependent action potentials more than 40 years ago (Kidokoro, 1975) our understanding of how this electrical activity is shaped and the role of different patterns of excitability in coordinating hormone secretion in pituitary cells has undergone a dramatic transformation (Mason et al., 1988; Mollard and Schlegel, 1996; Ozawa and Sand, 1986; Stojilkovic, 2006; Stojilkovic et al., 2010). Important in this context is that endocrine pituitary cells exploit an eclectic array of ion channels, signaling pathways and mechanisms to control patterns of excitability in a cell specific manner dependent upon the ionic makeup of endocrine pituitary cell types. Moreover, while anterior pituitary cells exploit actions potentials they are not simply neurons or skeletal muscles in disguise – the classical

electrically excitable cells. Rather, interrogation of anterior pituitary endocrine excitability has provided seminal insights into the mechanisms of ion channel regulation and endocrine stimulus-secretion coupling and how unique and diverse ionic mechanisms can be exploited by excitable cells to allow context- and cell- specific regulation.

Nowhere is the extraordinary diversity of mechanism, properties and physiological function of pituitary cell excitability been more evident than in studies that have revealed novel, and often paradoxical, roles of the family of calcium-activated potassium channels. This channel family, that comprises three different gene families whose members have distinct properties (Figure 1), regulation and function (Kaczmarek et al., 2017), are activated by elevations in intracellular free calcium resulting from influx of calcium from the extracellular space or resulting from release from intracellular calcium stores (Figure 2). Conceptually, activation of a calcium-activated potassium channel and subsequent efflux of potassium ions might simply be assumed to result in membrane repolarization and hyperpolarization and thus dampen endocrine pituitary excitability. While this is the 'classical' case in many systems, what is now evident, is that calcium-activated potassium channels play very diverse and surprising roles that provide mechanisms to allow cell-specific and context specific control of anterior pituitary endocrine cell excitability.

In this review, the aim is to highlight key features of the family of calcium activated potassium channels and focus on work in primary anterior pituitary endocrine cells that have provided new insights into the eclectic and important role of this channel family.

2. A brief primer on calcium-activated potassium channels

Since the initial discovery that intracellular free calcium can activate potassium selective conductances in red blood cells almost 60 years ago (Gardos, 1958) three distinct families of calcium-activated potassium channels have been identified (Figure 1) that differ in their properties, mode of regulation by calcium, tissue expression, pharmacology and physiological function (Kaczmarek et al., 2017). Importantly, dysfunction of calcium-activated potassium channels in humans and animals is associated with an eclectic array of diseases ranging from cancers to neurological, metabolic, endocrine and vascular disorders (for reviews see Adelman et al., 2012; Christophersen and Wulff, 2015; Contreras et al., 2013; Köhler et al., 2016; Latorre et al., 2017; Stocker, 2004). All three families have been reported to control anterior pituitary cell excitability however, their functional role displays considerable cell and context specificity and the extent to which their dysfunction in the pituitary may contribute to human and animal disease is largely unexplored.

2.1 Large conductance calcium- and voltage- activated potassium (BK) channels (KCa1.1; Kcnma1, BK, maxiK, Slo1)

Large conductance calcium- and voltage- activated channels represent the prototypical calcium-activated potassium channel family, but as their name suggests have several rather unique properties (Contreras et al., 2013; Latorre et al., 2017).

Firstly, they are the only potassium selective ion channel that is activated by both elevation in intracellular free calcium as well as membrane depolarisation thus providing a mechanism to integrate two of the most powerful signaling mechanisms in excitable cells. In general, these channels can be considered as rather poor voltage-activated channels (the S4 domain has only a few positively charged amino acids compared to prototypical voltage-gated channels) whose voltage dependence is shifted to more negative potentials as calcium rises. This results from a complex allosteric mechanism. This involves multiple calcium binding domains in the regulator of potassium conductance (RCK) domains, in the very large intracellular C-terminus, forming a 'gating ring' that moves in response to calcium and controls movement of the transmembrane domains of the pore domain, via a short cytoplasmic linker.

Secondly, the channels have a very large unitary conductance (~ 250pS in equimolar K^+ gradients) a value 1-2 orders of magnitude higher than most typical potassium and other cation channels. This is important as relative few channels need to be active (or open probability of a single channel does not need to be very high) to have significant effects on K^+ flux and thus membrane potential, especially in endocrine pituitary cells that have a high resistance.

Thirdly, although the pore-forming α-subunits of these channels are encoded by a single gene (Kcnma1), and assemble as tetramers, the core properties of BK channels can by dramatically, and dynamically regulated by a wide range of mechanisms. This includes: i) extensive alternative pre-mRNA splicing of the α-subunit, that can dramatically change channel kinetics, calcium-sensitivity and regulation by diverse signaling pathways (Fodor and Aldrich, 2009; Shipston and Tian, 2016); ii) assembly with a family of regulatory β- and γ-subunits that modify the core properties of the α-subunit (Li and Yan, 2016; Torres et al., 2014; Zhang and Yan, 2014); iii) regulation by a diverse array of post-translational modifications including protein phosphorylation and lipidation (Shipston, 2014; Shipston and Tian, 2016) as well as iv) being targets for a wide array of other signalling molecules including magnesium, heme, ethanol and fatty acids (Dopico et al., 2014; Torres et al., 2014). Importantly all these control mechanisms can dramatically modify BK channel surface

expression, calcium- and voltage-sensitivity, activation and deactivation kinetics as well as pharmacology. For example, two different alternatively spliced variants of the α-subunit (ZERO and STREX) whose expression is dynamically regulated in the anterior pituitary (Lai and McCobb, 2006) have different kinetics, sensitivity to calcium as well as opposite regulation by PKA-dependent phosphorylation (Chen et al., 2005; Tian et al., 2001a). In addition, the anterior pituitary expresses several regulatory subunits, including: i) the β2-subunit that confers fast inactivation to BK channels and ii) the β4-subunit that has complex effects on calcium sensitivity and channel kinetics and also makes channels largely resistant to the two most commonly used inhibitory toxins used experimentally iberiotoxin (IbTx) and charybdotoxin (ChTx)) (Li and Yan, 2016; Torres et al., 2014; Zhang and Yan, 2014). In this regard, paxilline, when used at low-micromolar concentrations, is currently the best pharmacological inhibitor to use for inhibiting all BK channels. In addition, a number of BK channel activators, such as NS11021, are reported that display some specificity although several activators are non-selective and also depend on the molecular make-up of endogenous BK channels (Bentzen et al., 2014).

Although multiple mechanisms exist to determine distinct BK channel properties in anterior pituitary cells we know surprisingly little about the precise molecular make up of BK channels in specific cell types. Thus, as discussed below (section 4.1), anterior pituitary cells express BK channels at different levels and an emerging theme is that distinct populations of BK channels may exist in the same cell – some closely coupled to Ca^{2+} influx through voltage gated calcium channels and thus activated very rapidly, while other BK channels are more distant from voltage gated calcium channels and respond to more global elevations in calcium (Figure 2A).

2.2 Small conductance calcium-activated potassium (SK) channels (KCa2 family; Kcnn1-3)

The small conductance calcium-activated potassium channel family comprises three paralogous gene members that encode potassium selective channels. SK channels are activated by calcium binding to calmodulin (Figure 2) that is constitutively assembled with the pore-forming subunits in the tetrameric channel (Adelman et al., 2012; Stocker, 2004). Although cloned human SK1 subunits form functional homotetrameric channels mouse or rat SK1 do not, rather they can form functional heterotetrameric channels with SK2.

SK channels are typically more sensitive to elevations in intracellular free calcium (in the submicromolar range) compared to BK channels and become saturated at low micromolar concentrations. The channels have a relatively small conductance (~10-20pS when recorded

in equimolar potassium) but are voltage-insensitive as they lack a prototypical voltage sensing S4 segment even though they retain a transmembrane architecture that resembles typical voltage gated potassium channels. All three members of the SK family are selectively blocked by the neurotoxin apamin, that is a major component of bee venom, with SK2 being the most sensitive (IC_{50} is ~ 40 pM). SK channels are also activated by a range of molecules that increase the apparent calcium sensitivity including 1-ethyl-2-benzimidazolinone (1-EBIO), 6,7-dichloro-1*H*- indole-2,3-dione 3-oxime (NS309) as well as the structurally distinct trisubstituted pyrimidine *N*-cyclohexyl-*N*-[2-(3,5-dimethyl-pyrazol-1-yl)-6-methyl-4-pyrimidinamine] (CyPPA) (Christophersen and Wulff, 2015). These channels are also potently regulated by reversible phosphorylation (Adelman et al., 2012; Stocker, 2004) that plays an important role in neuromodulation in neurones. However, as discussed in section 4.2 below, in several endocrine anterior pituitary cells an important emerging feature is that these channels are typically closely coupled to GPCRs that activate the IP3/DAG pathways and are potently regulated by Ca^{2+} release from intracellular Ca^{2+} stores via IP3 receptors, rather than by voltage dependent calcium influx (Figure 2B).

2.3 Intermediate calcium-activated potassium (IK) channels (KCa3.1; Kcnn4, SK4)

Although closely related to the KCa2 family, including regulation by calcium binding to constitutively channel-associated calmodulin, only one member of the KCa3 family exists (Kaczmarek et al., 2017). This turns out to be the channel underlying the Gardos channel in red blood cells but is also widely expressed in many non-excitable tissues including endothelial and immune cells (Christophersen and Wulff, 2015; Köhler et al., 2016). These channels have a unitary conductance intermediate to that of BK and SK channels (~ 40pS) but with a calcium sensitivity in the sub-micromolar range as for SK channels, leading to their common name of intermediate conductance (IK) calcium-activated potassium channels. Although many of the activators of SK channels (e.g. EBIO) also activate IK channels, specific inhibitors of IK channels are distinct. IK channels are insensitive to apamin but are potently inhibited by low micromolar concentrations of TRAM-34 (Christophersen and Wulff, 2015; Wulff et al., 2000). IK channels are also inhibited by charybdotoxin and clotrimazole (as are BK channels) but are insensitive to the selective BK channel inhibitor, paxilline. As discussed below, expression of IK channels in the anterior pituitary appears to be rather restricted, in accordance with their predominant expression in non-excitable cells in a range of tissues.

3. Some general considerations on anterior pituitary cell type, models, sex and drugs

Before discussing the role of calcium-activated potassium channels in the anterior pituitary, it is prescient to consider some general issues that are important for interrogating and understanding the control of anterior pituitary cell excitability.

The anterior pituitary gland contains 5 secretory endocrine cell types that represent a fundamental link between the brain and periphery by controlling the release of an array of physiologically important effector hormones. These include: growth hormone (GH from somatotrophs), prolactin (PRL from lactotrophs), follicle-stimulating hormone and luteinizing hormone (FSH and LH from gonadotrophs), thyroid stimulating hormone (TSH from thyrotrophs) and adrenocorticotrophin hormone (ACTH from corticotrophs). In turn these hormones control a diverse array of physiological systems to control body functions as diverse as growth, maternal behavior, reproduction and the response to stress.

Historically, real-time analysis of native endocrine anterior pituitary cell excitability has been somewhat limited and thus much of the pioneering work on anterior pituitary cell excitability and stimulus-secretion coupling was elucidated with a variety of immortalized clonal cell lines that retain some, but not all, properties of native cells (Ooi et al., 2004). Indeed, several differences in excitability and regulation between clonal cell lines and native cells are well known. For example, variants of the mouse corticotroph cell line AtT20 lack receptors for the major secretagogue AVP, but it is perhaps not surprising to find such differences when these lines were derived from pituitary tumours derived from mice exposed to an atomic bomb! In general, isolation of distinct pituitary cell types has remained a challenge and the vast majority of work has been undertaken on primary dispersed cells in short term culture with cellular identification determined by responses to hypothalamic secretagogues. A number of recent studies have begun to exploit mouse models in which specific cell types are genetically labelled with fluorescent proteins – either as transgenes or using viral transduction with fluorescent protein expression driven by cell specific promoters (Duncan et al., 2015; 2016; Fletcher et al., 2017; Liang et al., 2011; Romanò et al., 2017; Zemkova et al., 2016). These data have begun to provide valuable insights into basal/spontaneous activity in the absence of secretagogue and allow a clearer examination of heterogeneity of responsiveness among pituitary cell populations.

However, there are also several broader considerations to take into account when analyzing anterior pituitary cell excitability, that are further highlighted in section 4, including:

i) it is now well established that most endocrine cell types in the anterior pituitary are not ‘randomly’ distributed but form both anatomical and function networks including homo- and hetero- cellular communication via gap junctions (Budry et

al., 2011; Le Tissier et al., 2017; 2012). While isolated cells provide excellent models for understanding cell autonomous functions the network properties of pituitary endocrine cells are likely very important determinants of hormone output from the gland – akin to emergent properties from neural networks.

ii) a variety of morphological and functional evidence suggests that 'sub-populations' of each pituitary cell type may exist and different techniques/analysis may select subsets of cell types potentially biasing the interpretation of excitability (Le Tissier et al., 2017; 2012). Whether these are true 'sub-populations' or rather it supports evidence that while individual cells show deterministic responses to the same stimulus the population may vary considerably depending on the individual molecular/ionic makeup of each cell (Romanò et al., 2017). This of course may be advantageous to allow endocrine cell types to respond across a wide dynamic range to the wide variety of physiological challenges they face.

iii) as most studies are performed in short term isolation/culture how cell responses change under specific cellular conditions or in the absence of hypothalamic drive or peripheral feedback needs to be considered. This is particularly relevant as BK and SK channel expression, as well as channel regulation by diverse signaling pathways, may be determined by steroid hormones (see section 4).

iv) the vast majority of studies of native mouse or rat pituitary cells have typically used mixed age and sex preparations of cells assuming that male and female endocrine pituitary cells are identical. However, increasing evidence suggests there are sexually dimorphic differences in intrinsic control of excitability (e.g see Duncan et al., 2015; Fletcher et al., 2017; Liang et al., 2011; Stojilkovic et al., 2010) in addition to the effects of sex steroids on channel expression and regulation.

v) the method used to monitor excitability is critical. Most anterior pituitary cells are relatively small, and of high resistance, thus their cellular compartments can be easily disrupted using conventional whole-cell patch clamp electrophysiological analysis. In this regard, while conventional whole cell recordings provide important insights and allow dialysis of the intracellular contents spontaneous activity can be disrupted in many pituitary cells (Duncan et al., 2015; Fletcher et al., 2017; Liang et al., 2011; Stojilkovic et al., 2010) – in particular when considering exogenous buffering of calcium. This can be alleviated using perforated patch recordings that often also facilitates analysis of channel regulation by GPCR signaling cascades but again are not without caveats as the typical antibiotics used (nystatin and amphotericin) of course still allow movement of monovalent ions so careful consideration of internal Cl^-, Na^+ and K^+

concentrations are required. In addition, while providing high temporal resolution and manipulation of excitability, patch clamp recordings are low throughput, only sampling a fraction of the cell population. This is often overcome using calcium imaging to measure excitability but calcium responses are not a simple surrogate of electrical excitability. For example, intracellular calcium elevation is in phase with membrane hyperpolarization in GnRH-stimulated gonadotrophs but in phase with excitability in CRH-stimulated corticotrophs (see sections 4.1 & 4.2).

vi) a major conceptual gap is how the relatively fast biophysical measures of electrical excitability, calcium elevation and biophysical measures of secretion (e.g capacitance that are measured on the millisecond to second timescale) correspond to most assays of actual hormone secretion that are typically measured over minutes to hours. This is particularly prevalent when undertaking pharmacological studies of basal and evoked hormone secretion and correlating back to changes in excitability (see sections 4.1 & 4.2).

vii) Finally, in most anterior pituitary cell types we really do not know the molecular make-up of calcium activated potassium (or most other) channels that are expressed and most studies rely on use of pharmacological tools. Relatively few studies, have addressed the contributions of specific channel subunits using knockout or knockdown studies to compliment pharmacological assays. Moreover, several studies predict that anterior pituitary cells express different functional populations of the same calcium-activated potassium channel – this can be very difficult to interrogate with current pharmacological and/or genetic tools. In this regard, the recent exploitation of predictive mathematical models and dynamic clamp approaches in patch clamp electrophysiology assays allow interrogation of channel currents with predicted properties.

These are clearly challenges for the field that need to be addressed in the future. However, it is already clear that calcium-activated potassium channels play unique and diverse roles in controlling anterior pituitary cell excitability.

4. Calcium-activated potassium channels and control of anterior pituitary excitability

4.1 BK channels and dual roles in both promoting and reducing cellular excitability

The pore forming α-subunit of the BK channel encoded by the Kcnma1 gene is widely expressed in mouse anterior pituitary at both the mRNA and protein level and electrophysiological recordings from primary rat and mouse pituitary cells reveal BK currents at both whole cell and single channel level. Importantly, splice variants of Kcnma1 are also

robustly expressed in the anterior pituitary, including the stress regulated exon variant (STREX) whose expression is dynamically regulated during development and in response to changes in steroid hormone status and confers distinct properties on BK channels (Chen et al., 2005; Lai and McCobb, 2006; Tian et al., 2001a; Xie and McCobb, 1998). Moreover, β2 and β4 regulatory subunits are also robustly expressed although their cell specific expression is not well established. Fast inactivating BK currents, that are indicative of functional β2 subunit expression, have been reported in some pituitary cell types. Thus, while BK channel subunits are robustly expressed in native pituitary cells the molecular composition of BK channels in specific cell types is very poorly understood. In contrast, we have a better idea of splice variant and β-subunit expression in clonal pituitary cell lines (Erxleben et al., 2002; Shipston et al., 1999) however, whether these reflect native cells is not clear and as highlighted below significant differences in the functional role of BK channels can exist between clonal and native primary cells.

Whole cell current density of BK currents varies widely between different pituitary cell types. For example, primary rat somatotrophs and lactotrophs have large whole cell BK currents whereas in mouse and rat corticotrophs and gonadotrophs (Duncan et al., 2015; Liang et al., 2011; Van Goor et al., 2001a; 2001b; Waring and Turgeon, 2009) outward current carried by BK channels is relatively modest (~ 20%) even though single channel BK currents can be readily determined in isolated membrane patches. Based on the canonical model of BK channels generally limiting cellular excitability it thus might be predicted that spontaneous Ca^{2+}-dependent action potentials in corticotrophs and gonadotrophs would be considerably longer than in somatotrophs and lactotrophs as BK channels would not be expected to play a major repolarizing/hyperpolarizing function under basal conditions in corticotrophs and gonadotrophs. However, the opposite is largely true with gonadotrophs and corticotrophs typically displaying spontaneous action potentials of 20-100 ms duration, measured at potentials just above threshold, whereas lactotrophs and somatotrophs display complex spontaneous bursting behavior with typically 100-500 ms duration (Duncan et al., 2015; Liang et al., 2011; Van Goor et al., 2001b; 2001a; Waring and Turgeon, 2009; Zemkova et al., 2016). In both lactotrophs and somatotrophs short (< 25ms) depolarisations, that promote voltage gated calcium influx, activate BK currents suggesting that the long duration depolarisations in these cells is not due to an inability of BK channels to be activated by Ca^{2+} influx during a spontaneous action potential (Van Goor et al., 2001a).

4.1.1 *Paradoxical role of BK channels in promoting bursting behavior*

Rather, these data revealed an 'apparent' paradoxical role of BK channel activity to in fact promote cellular depolarisation and spontaneous excitability in somatotrophs and lactotrophs (Van Goor et al., 2001a). Intriguingly, a similar mechanism has also been proposed in corticotrophs that are stimulated by the CRH/cAMP pathway in which excitability transitions from largely single spikes to bursting behaviour (Duncan et al., 2015; Fletcher et al., 2017). In both rat somatotrophs and mouse corticotrophs this paradoxical role of BK channels has been elegantly revealed using a combination of experiment using patch clamp electrophysiology, mathematical modelling and exploitation of the models to test model predictions in native cells using dynamic clamp to add or subtract BK-like currents as we largely lack specific genetic or pharmacological tools to interrogate endogenous BK channels with different properties (Duncan et al., 2016; Tabak et al., 2011).

At the core of the paradoxical mechanism of BK channels promoting cellular excitability, initially described in a proportion of somatotrophs, is the hypothesis that some BK channels are co-localised with voltage gated calcium channels and these BK channels (termed BK_{near}) activate very rapidly upon membrane depolarization (Figure 2A). This model considers BK channel activation to be a purely voltage-dependent process as BK channels are typically located adjacent to L-type Ca^{2+} channels (Van Goor et al., 2001b; 2001a). As Ca^{2+} concentrations in micro domains reach equilibrium within microseconds the gating of BK-near is essentially driven by membrane potential (Tabak et al., 2011). The model also predicts that BK channels (BK_{far}) located distant from VGCC, and thus activated by a global rise in Ca^{2+}, are important for terminating the burst (Figure 2A).

Several lines of evidence support this model in somatotrophs (Van Goor et al., 2001a). Firstly, in rat somatotrophs the BK channel inhibitors paxilline and IbTx convert spontaneous bursting to single spike activity, whereas apamin has no effect. Secondly, chelation of intracellular calcium with the fast calcium buffer BAPTA, that is able to chelate calcium in the Ca^{2+} nanodomain at the mouth of an open Ca^{2+} channel, reduces BK channel activation and switches spontaneous bursting to single spike action potentials. Thirdly, depolarization of somatotrophs with GHRH or KCl only increased frequency of bursting and cellular depoalrisation rather than switching spontaneous bursting to spiking. Finally, development of a mathematical model of somatotroph bursting (Van Goor et al., 2001a), revealed a potential mechanism and properties of the BK current required for spontaneous bursting that was tested in the GH4C1 somatotroph cell line using dynamic clamp (Tabak et al., 2011). In the model, addition of a hyperpolarizing voltage –independent current simply reduces spike frequency and promotes hyperpolarization. However, addition of a hyperpolarisaing voltage-dependent BK-like current to a spiking cell converts it to a "pseudo plateau bursting' mode

with burst frequency and spike amplitude decreasing as more fast BK current is introduced. The proposed mechanism is that fast activation of the BK current limits the spike depolarization and thus attenuates activation of other voltage-dependent potassium channels that would normally promote repolarization. As such in “pseudo plateau bursting’ the membrane potential then oscillates around a depolarised state so that the spike rides on a depolarizing plateau before a slow BK current (BK_{far}) activates due to a rise in global Ca^{2+} and results in burst termination (Tabak et al., 2011; Teka et al., 2011; Van Goor et al., 2001a). Testing of the model predictions are clearly difficult as both the properties of the proposed different BK channel population as well as their activity are hard to control experimentally. However, using dynamic clamp in the lacto-somatotroph cell line (GH4C1) showed spontaneously bursting cells could be switched to spiking using the BK channel blocker IbTx which then could be reversed by adding back an artificial fast-activating BK conductance (Tabak et al., 2011). BK channel activation has to be sufficiently fast (< 10 ms) to promote bursting otherwise BK current in fact inhibits bursting (Vo et al., 2014).

An intriguing variation of this paradoxical role has also been determined in native mouse corticotrophs from experiments, mathematical modelling and dynamic clamp where CRH-evoked bursting is promoted by BK channels (Duncan et al., 2016; 2015; Fletcher et al., 2017). Spontaneous corticotroph excitability is predominantly characterised by single spike events < 100 ms in duration (Figure 3A). However, inhibition of BK channels by paxilline, or genetic deletion of BK channels, in corticotrophs has no significant effect on spontaneous spiking behaviour. In male corticotrophs activation of the CRH/cAMP pathway can robustly switch spiking to “pseudo plateau bursting” behavior (Figure 3A) (Duncan et al., 2015). Two independent mathematical models support a role for BK channels to promote CRH-dependent bursting in corticotrophs (Duncan et al., 2015; Fletcher et al., 2017). In this case, the model predicts that CRH changes the properties of the BK channels (BK_{near}, those closest to voltage gated calcium channels) in particular by speeding their activation, simulated by decreasing the time constant of activation from 20 to 4 ms. Thus, the model predicts that the properties of BK channels closest to VGCC in corticotrophs may be different from those in somatotrophs in the basal state but can be ‘converted’ to fast activating BK channels in the presence of CRH. Indeed, using dynamic clamp, introduction of a fast activating BK-like current induces bursting in mouse corticotrophs from BK channel knockout mice or in wild-type corticotrophs treated with paxilline. Conversely, subtraction of a fast BK-like current from CRH- stimulated wild-type corticotrophs switches CRH-evoked bursting to spiking (Duncan et al., 2016; 2015).

How does bursting activity correlate with Ca^{2+} signaling and hormone secretion? It is clear in those systems studied that bursting provides a powerful mechanism to drive voltage-gated calcium influx and thus the assumption is that bursting favours hormone secretion. The relatively high basal secretion rates of native lactotrophs and somatotrophs that display robust spontaneous bursting, compared to cortcotrophs and gonadotrophs, would support such a link. Clearly secretagogue evoked bursting, as seen in corticotrophs, results in effective hormone secretion. However, the assumption that bursting is most favourable for pituitary hormone secretion needs to be directly tested experimentally. Recent elegant modelling and dynamic clamp studies support that while bursting can be more effective for hormone secretion than continuous spiking this can also be achieved by other patterns of excitability that are dependent on the ionic configuration of individual cell types (Tagliavini et al., 2016).

Intriguingly, additional support for bursting also being important for effective secretion emerges from studies of glucocorticoid feedback inhibition of CRH-evoked bursting in corticotrophs (Duncan et al., 2016). In corticotrophs, CRH evoked secretion is potently inhibited by circulating glucocorticoids with different mechanisms across distinct time domains. Exposure of male mouse corticotrophs to corticosterone for 30-60 mins prevents subsequent CRH-evoked transition to bursting. The inhibitory effect of corticosterone appears, at least in part, to result from an inability of CRH to modify BK channels (as described above) as injection of a fast BK-like current into CORT treated corticotrophs can restore CRH-evoked bursting (Duncan et al., 2016). Several potential mechanisms could explain how corticosterone occludes CRH-evoked bursting through control of a fast activating BK current. In endocrine cells glucocorticoids have been reported to have direct effects on BK channel activity mediated through accessory β-subunits (King et al., 2006; Lovell et al., 2004), switching of BK channel splice variant or β-subunit expression (Lai and McCobb, 2006; 2002) or control of BK channel phosphorylation and properties through control of protein phosphatase activity (Shipston et al., 1996; Tian et al., 2001b; 1998). In the time scale (within 30 mins to 2hrs), and at the concentrations of glucocorticoid used in these studies, the latter represents a plausible working hypothesis but the molecular mechanisms and make-up of BK channels needs to be established in native corticotrophs. Steroid control of BK channel activity is also functionally important in other pituitary cells. For example, exposure of mouse gonadotrophs to oestrogen increases the peak BK current. In oestrogen treated gonadotrophs, blockade of BK channels decreases GnRH-stimulated LH secretion however BK channel blockade has no significant effect on evoked secretion, in the absence of oestrogen (Waring and Turgeon, 2009).

4.1.2 Classical repolarization role of BK channels in anterior pituitary cells

As discussed above, in both the somatotroph and corticotroph models a 'classical' role for BK channels is also proposed to promote termination of a burst. In this case BK channels that are located more distant from voltage gated calcium channels (termed BK_{far}) are activated by global elevations in intracellular Ca^{2+} (Duncan et al., 2015; Tsaneva-Atanasova et al., 2007; Van Goor et al., 2001a) and promote membrane repolarization (Figures 2A & 3A). Furthermore, in a wide range of clonal anterior pituitary cells lines BK channels have been proposed to play this 'classical' role in controlling membrane repolarization. For example, in spiking GH3 and GH4C1 somatotroph cell lines BK channels play a classical role in controlling action potential repolarization and mediating afterhyperpolarisation and in some bursting GH3 cells inhibition of BK channels with paxilline does not switch bursting to spiking (Miranda et al., 2003) supporting that BK channels are important for promoting but not essential for bursting (Duncan et al., 2016; 2015; Fletcher et al., 2017). Moreover, in clonal GH4C1 cells and mouse AtT20 corticotrophs, BK channels are potently regulated by receptor-mediated signaling pathways that control protein kinases and phosphatases. In these cell lines, signaling pathways that stimulate excitability and hormone secretion typically inhibit BK channel activity whereas inhibitory pathways stimulate BK channel activity (Shipston et al., 1996; Shipston and Armstrong, 1996; White et al., 1993; 1991). Whether this classical role is more a feature of clonally derived cells (that generally express large BK currents) or simply reflects that multiple mechanisms can be used by cells to control cell excitability dependent on the molecular make-up and properties/localization remains to be determined. For example, in cells that undergo bursting BK channels may play a dual role: the paradoxical role of one BK population to promote bursting and another population performing the 'classical' role by terminating a burst. In contrast, in spiking cells BK channels may only play the 'classical' role and it will be of interest if the molecular composition of BK channels in these different cell types is distinct. This would allow tremendous diversity and context specificity for how different signaling pathways could control cellular excitability.

4.1.3 Do BK channels control hormone secretion in native anterior pituitary cells?

The fact that BK channels can exert opposite effects on excitability – by both promoting bursting as well as terminating bursting or spiking, their role in controlling pituitary hormone secretion is likely to be complex. Indeed, this dual role also would allow multiple mechanisms for cellular signaling pathways activated by circulating hormones to exert cell specific control of cellular excitability by targeting these opposing mechanisms. Currently we do not know the precise molecular identity of functional BK channels that may represent BK

channels that promote bursting versus BK channels that terminate bursting. In large part this is because genetic or pharmacological tools that would allow us to discriminate these populations are not currently available. Indeed, currently available tools target both mechanisms – loss of BK channel activity would thus be predicted to decrease hormone secretion in systems where BK-dependent promotion of bursting predominates whereas it would be predicted to increase secretion in systems where the burst/spike termination mechanism predominates. However, although many studies have proposed a role for BK channels in controlling hormone secretion in native (or clonal) anterior pituitary cells, largely based on evidence of BK channel regulation by signaling pathways or effects on action potential/calcium waveforms, few studies have in fact attempted to measure hormone secretion in native cells when BK channel function is modified.

In female mice with a global deletion of BK channel α-subunits (Kcnma1) restraint stress-induced (but not basal) secretion of ACTH is reduced and the mice thus show a hyporesponsiveness to acute stress (Brunton et al., 2007). While, at face value, this might support a role for BK channels in promoting bursting in corticotrophs global deletion also results in reduced hypothalamic drive as well as decreasing the total ACTH stored in corticotrophs. In fact, in dispersed female BK knockout corticotrophs, 10 nM CRH (30 min) had little effect on absolute ACTH secretion however, when corrected for ACTH content they released proportionally more stored ACTH in response to 10 nM CRH than their wild-type counterparts. However, female mouse corticotrophs also display an attenuated CRH-evoked bursting response compared to male mouse corticotrophs with CRH-evoked excitability largely driven by an increase in spiking (Duncan et al., 2015; Liang et al., 2011). Similarly in the AtT20 D16:16 cell line pharmacological blockade of BK channels also promotes CRH-evoked ACTH secretion in accordance with a predominant role of BK channels in controlling repolarization in these clonal corticotrophs (Shipston et al., 1996). However, in dispersed native male corticotrophs, loss of BK channel function results in reduced CRH-evoked ACTH secretion (Shipston, unpublished data) in accordance with the greater dependence on CRH-evoked bursting (Duncan et al., 2016; 2015) in these cells. In contrast, in dispersed male rat corticotrophs inhibition of BK channels with charybdotoxin had little effect on cAMP-evoked ACTH secretion although the effect of cAMP on excitability was not tested in this system (Lim et al., 1998). Systematic evaluation of the specific role of BK channels in control of hormone secretion (using specific inhibitors or genetic tools) from other native anterior pituitary cell hormone secretion has not been performed to date.

In all these assays of hormone secretion, hormone release is measured over tens of minutes with continuous exposure to secretagogue. As highlighted in section 4.2 below and section 3

above a very significant challenge for the field is linking single cell biophysical assays of excitability and vesicle fusion (measured in ms to few minutes range) with typical measures of hormone secretion in *in vitro* population studies or *in vivo*. As such, whether such differences in control of hormone secretion results from the relative contribution of BK channels to promotion of bursting or termination of bursting/spiking in these different systems, or perhaps reflects the different timescales over which these distinct assays of excitability and hormone release are performed remains to be elucidated

4.2 SK channels and coupling to intracellular calcium release

In total adult mouse anterior pituitary, Kcnn1-3 mRNA is expressed with Kcnn2 being the major transcript although single cell RT-PCR does reveal differences in specific cell types – for example while Kcnn2 mRNA is robustly expressed in corticotrophs Kcnn3mRNA expression is much lower (Liang et al., 2011). In mouse, immunohistochemical detection of SK2 co-localises with gonadotrophs as well as other (unidentified) cell types, and the apamin sensitivity of SK currents correlates with SK2 being the major SK channel in gonadotrophs (Waring and Turgeon, 2009). This also supports evidence for apamin-sensitive potassium conductances in many anterior pituitary cell types including in rat and mouse gonadotrophs, corticotrophs, thyrotrophs and somatotrophs (Stojilkovic et al., 2010).

In most primary rat pituitary cells voltage-gated calcium influx does not activate SK channels (Van Goor et al., 2001b) rather, a common theme in several anterior pituitary cell types is that apamin-sensitive SK channels are activated by release of calcium from intracellular inositol trisphosphate (IP3)-sensitive calcium stores as a result of stimulation of G-protein coupled receptors (GPCRs). In general, two distinct modes of calcium elevation and subsequent SK channel activation are initiated, dependent upon the GPCR activated as discussed below.

4.2.1 SK channels and oscillations in intracellular calcium and membrane hyperpolarization

In both male and female gonadotrophs, activation of GnRH receptors stimulates IP3 production resulting in oscillations of intracellular calcium elevation (Figure 3B) (Kukuljan et al., 1992; A. Tse et al., 1993). The frequency and transient nature of these oscillations are GnRH concentration dependent – for example at sub-nanomolar GnRH oscillation frequency increases with concentration whereas in the 10 -100 nM range a more biphasic (initial transient followed by sustained or oscillatory response) calcium elevation is observed (e.g. see Li et al., 1994; Stojilkovic et al., 1993). Release of Ca^{2+} from IP3-sensitive intracellular stores results in activation of SK channels causing transient membrane hyperpolarization followed by periods of increased depolarisation and action potential firing (Kukuljan et al.,

1992; Tse et al., 1993). In gonadotrophs, activation of SK channels saturates above elevation of intracellular calcium > 1uM and thus the pattern and frequency of membrane potential oscillations are GnRH concentration dependent. However, the calcium oscillations *per se* do not require oscillations in IP3 levels (Stojilkovic et al., 1993). The periods of hyperpolarization are associated with cessation of action potential firing but are followed by periods of action potential generation (Figure 3B). The action potential generation likely results from two main mechanisms: i) the reduction in hyperpolarizing SK current during the nadir of the ER-calcium oscillation and ii) the release of voltage gated sodium and calcium channels from inactivation upon membrane depolarization. In male rat gonadotrophs, cell membrane capacitance assays reveal that intracellular calcium release from IP3-sensitive stores stimulates exocytosis much more efficiently than global elevations of calcium suggesting that subplasmalemmal IP3-sensitive stores are close to sites of peptidergic vesicle fusion and that the rate of rise of calcium at these sites is critical (Tse et al., 1997). Thus, elevation of calcium and hormone secretion are, at first sight paradoxically, in phase with the period of membrane hyperpolarization. In this model, the membrane depolarization and action potential generation following hyperpolarization is required for calcium influx to replenish the intracellular calcium stores, rather than calcium influx driving exocytosis. Indeed, secretion can be maintained for several minutes in the absence of extracellular calcium (Stojilkovic, 2006).

However, are SK channels essential for controlling secretion or is their activation simply a passive consequence of the elevation of intracellular free calcium from IP3 sensitive stores? In mouse gonadotrophs inhibition of SK channels with apamin results in *enhanced* GnRH-induced (1nM) LH secretion measured over 30 mins in dispersed cell populations (Waring & Turgeon, 2009). How can enhanced GnRH-induced LH secretion be explained upon inhibition of SK channels? Two complimentary mechanisms are likely to be involved – the relative contributions of these mechanisms will be dependent on the requirement of recovery of voltage gated sodium and calcium channels from inactivation. The simplest mechanism to explain enhanced secretion would be that during GnRH-induced ER-calcium oscillations blockade of SK channels with apamin during the peak of the ER-calcium oscillation promotes calcium influx and thus predicted to enhance secretion. However, if a primary role of SK channels is to drive hyperpolarization, to allow recovery of voltage gated sodium and calcium channels to allow Ca^{2+} influx to replenish the stores, it might be predicted that blockade of SK channels should gradually reduce agonist-induced secretion as the intracellular stores are depleted. However, even if calcium channel recovery from inactivation is important, a plausible explanation of enhanced GnRH-evoked LH secretion upon SK blockade may be due to the different phases of calcium-dependent exocytosis and

mobilization of vesicles to the plasma membrane. The local IP3-sensitive calcium release may allow exocytosis of vesicles closely associated with the plasma membrane and capacitance measurements suggest that the majority of this secretion is over within a few minutes (Tse et al., 1997). However, for sustained secretion *recruitment* of vesicles to the plasma membrane may require calcium influx through voltage gated calcium channels. Thus, inhibition of SK channels in the long term (minutes) may enhance calcium influx and promote recruitment of vesicles to the plasma membrane that would be available for exocytosis upon a new cycle of IP3-mediated calcium release from intracellular stores in the presence of GnRH.

Clearly, the relative contribution of these different mechanisms requires formal testing but does reveal the importance of understanding the different timescales over which biophysical measures of electrical excitability, calcium signaling and hormone secretion are typically measured. Furthermore, the relative contribution of each mechanism may vary between individual cells. Indeed, heterogeneity in gonadotroph responsiveness to GnRH is reported with potentially distinct mechanisms operating in different 'subpopulations' that combined determine the population level secretory response. In this regard, it is not known if SK channel knockout mice have defects in GnRH-induced secretion from gonadotrophs although both male and female SK2 null mice are reported to have reduced fertility (Bond et al., 2004).

4.2.2 Non-oscillatory role of SK channels

Similar agonist-induced oscillations of SK-current driven by IP3-dependent intracellular calcium oscillations have also been reported in male rat corticotrophs. However, this is GPCR dependent in rat corticotrophs. α-adrenergic receptor agonists induce oscillations in calcium and SK current (Tse and Tse, 1998) whereas AVP receptor stimulation (with supraphysiological AVP concentrations) increases intracellular calcium in a biphasic pattern. The biphasic pattern includes an initial transient release from IP3-sensitive intracellular stores followed by a delayed plateau resulting from calcium influx (Corcuff et al., 1993; Tse and Lee, 1998). Supraphysiological levels (100nM) of AVP results in activation of apamin sensitive SK current and membrane hyperpolarization during both the transient and plateau phase of calcium elevation in whole cell patch clamped rat corticotrophs (Tse and Lee, 1998). However, in metabolically intact murine corticotrophs physiological (low nanomolar) concentrations of AVP (Duncan et al., 2015; Fletcher et al., 2017) induce robust increases in action potential frequency without significant membrane hyperpolarization. Although murine corticotrophs express Kcnn1-3 mRNA and small apamin sensitive currents (Liang et al., 2011) SK channels appear to play little role in either spontaneous activity of murine

corticotrophs or AVP-mediated excitability at physiological AVP concentrations. Thus, whether SK channels play a more predominant role in rat corticotrophs, or are only activated at supraphysiological AVP, levels remains to be clarified.

TRH also activates SK channels in clonal GH4C1 cells most likely via an IP3-dependnet mechanism involving intracellular calcium release. However, in the GH3 somatotroph cell line voltage gated calcium influx does activate SK currents following high frequency action potentials or following prolongation of action potential duration. In GH3 cells apamin-sensitive SK currents contribute to the after spike hyperpolarisation and subsequent decay of the current contributes to subsequent pacemaker depolarisation (Kwiecien et al., 1998; Lang and Ritchie, 1990a). In support of a role of SK channels in GH3 cells, apamin increases spontaneous action potential frequency without affecting action potential duration (Lang and Ritchie, 1990a) and apamin induces prolactin secretion from acutely dispersed rat anterior pituitary cells (Wang et al., 1994).

4.3 IK channels and control of excitability

In contrast to the robust expression of Kcnn1-3 mRNA in mouse pituitary the expression of Kcnn4 mRNA is at least an order of magnitude lower. Indeed, IK immunoreactivity is predominantly (~ 85%) co-expressed with ACTH-immunoreactive cells of the anterior pituitary gland and Kcnn4 mRNA can be detected in corticotrophs at the single cell level (Liang et al., 2011). This suggests that expression of IK channel expression is largely confined to corticotrophs, at least in mice. In female mouse corticotrophs, a significant component of the outward potassium current is blocked by the specific (when used at low micromolar concentrations) IK inhibitor TRAM-34 (Duncan et al., 2015; Liang et al., 2011). The same current is blocked by clotrimazole, and clotrimazole–sensitive currents have been reported in both rat GH3 (Wu et al., 1999) and GH4C1 cells (Mørk et al., 2005). However, as clotrimazole blocks a number of other potassium channels (including BK channels) (Wulff et al., 2000; 2007) whether IK currents are prominent in other pituitary cell types is not well established. Functionally, in female, but not male (Duncan et al., 2015; Liang et al., 2011), mouse corticotrophs, TRAM-34 alone resulted in an almost five fold increase in spontaneous action potential frequency and a transition towards ‘pseudo plateau bursting’ that was not associated with a significant depolarization of resting membrane potential (Liang et al., 2011). This suggests two important functions for IK channels in female corticotrophs. Firstly, that IK channels normally limit electrical bursting behavior in female corticotrophs and thus must be active under ‘basal’ conditions of spontaneous (‘single spike action potential’) electrical activity. This would imply that IK channels are activated under ‘basal’ conditions by the small influx of calcium as a result of the < 100 ms action potentials rather than being

active at resting membrane potential. Secondly, this would suggest IK channels contribute to limiting basal ACTH release in female corticotrophs and would predict that inhibition of IK channels would lead to increased basal and secretagogue-evoked ACTH secretion. Indeed, in dispersed mouse pituitary cultures TRAM-34 significantly enhanced both basal and CRH/AVP-evoked ACTH secretion and female mice with a global deletion of Kcnn4 show an enhanced ACTH secretion in response to acute restraint stress (Liang et al., 2011). However, in the global Kcnn4 knockout, changes in hypothalamic drive also likely contribute to the stress hyperesponsiveness (Liang et al., 2011). Interestingly, this represents another clear demonstration of different functions of distinct types of calcium-activated K channels in the pituitary as the Kcnn4 (IK) knockout displays hyperesponsiveness to stress whereas the Kcnma1 (BK) channel knockout shows a reduced response to acute restraint stress (Brunton et al., 2007). Moreover, it will be of considerable interest to establish whether IK channels play a role in corticotroph physiology in other species, including man.

In the rat somatotroph cell line GH4C1 Kcnn4 mRNA is detectable and 10μM clotrimazole is reported to reduce the amplitude of transient outward current as well as reduce the duration of the outward current elicited by 100 nM TRH (Mørk et al., 2005). In contrast, although apamin and IbTx also reduced the amplitude of the outward potassium current apamin or IbTx had no significant effect on the duration of the first phase of the TRH-evoked outward current. A TRH-activated intermediate conductance (~35pS) calcium activated K channel has also been observed in the related GH3 cell line (Lang and Ritchie, 1990b) however whether IK channels are expressed and play a function role in primary somatotrophs is not known.

5. Conclusions & perspectives

The family of calcium-activated potassium channels clearly play an important and diverse role in controlling electrical excitability of anterior pituitary endocrine cells. Importantly, these roles are both cell- and context- specific with calcium-activated channels playing some unexpected roles beyond their ‘classical’ contribution to calcium-dependent repolarization and decrease in membrane excitability observed in many other excitable cells. However, while we are starting to understand some of the physiological functions of BK, SK and IK channels in anterior pituitary endocrine function many outstanding questions remain that represent a major challenge for the field including:

i) What is the molecular composition and functional properties of native calcium-activated potassium channels in specific endocrine cell types and how dynamic is their expression, regulation and properties?

ii) Are different calcium activated potassium channel subpopulations differentially localised and coupled to distinct sources of calcium, and/or signaling cascades, in anterior pituitary cells as predicted from functional experiments and modelling? Moreover, what mechanisms determine the spatial localization of BK and SK channels and whether they functionally couple to voltage gated calcium channels or IP3-sensitive calcium stores respectively?

iii) How does the function and properties of calcium-activated potassium channels integrate with other ionic mechanisms to control cellular excitability? These channels do not exist, or function, in isolation to the other array of ion channels that determine how anterior pituitary cell excitability is controlled.

iv) To what extent do BK, SK and IK channels control basal and secretagogue evoked hormone secretion in different anterior pituitary cell types and how does this relate to their control of electrical excitability and calcium signaling? A major challenge here is to reconcile the often fundamentally different timescales over which excitability, calcium signals and hormone release are currently assayed.

v) Moreover, as calcium activated potassium channels shape calcium signals to what extent do these channels control other calcium-dependent processes in endocrine anterior pituitary cells, such as gene transcription?

vi) Mutations in, or dysregulation of, BK, SK and IK channels are reported in humans and animals leading to a wide variety of diseases ranging from cancers to neurological, metabolic, endocrine and vascular disorders (for reviews see Adelman et al., 2012; Christophersen and Wulff, 2015; Contreras et al., 2013; Köhler et al., 2016; Latorre et al., 2017; Stocker, 2004). However, the extent to which dysregulation of calcium-activated potassium channel function affects anterior pituitary function and the diverse physiological processes the effector hormones control is essentially unknown. To what extent may targeting calcium activated potassium channels in the anterior pituitary be useful for disease diagnostics or treatment?

We have come a long way since the first demonstration of electrical excitability and subsequent unique insights into how all three families of calcium-activated potassium channels can control the electrical excitability of anterior pituitary cells. However, physiologists and neuroendocrinologists have major challenges and opportunities ahead to unravel the properties, regulation and function of this family of potassium channels in anterior pituitary endocrine cells. One thing is certain, these important cells are sure to reveal several surprises into the diverse functional role and regulation of calcium activated potassium channels that will be of major interest to the broader scientific community.

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Figure Legends

Figure 1: Properties of calcium-activated potassium channels

The pore forming α-subunit of large conductance voltage and calcium activated potassium (**BK**) channels are encoded by the Kcnma1 gene that undergoes extensive pre-mRNA splicing. The α-subunit has 7 transmembrane (TM) domains including S4 that constituents the core of the voltage sensor, an additional N-terminal TM (S0) resulting in an extracellular N-terminus, the pore loo between S5 and S6 and a large C-terminus composed of two regulator of potassium conductance domains (RCK) that include sites required for calcium binding. The α-subunit assembles as a tetramer to form functional channels with the core properties of the channel also modified by accessory transmembrane β- and γ- subunits. Small (**SK**) and intermediate (**IK**) calcium-activated potassium channels have 6 transmembrane domains and assemble as tetramers but lack an intrinsic voltage sensor. The pore-forming subunit of SK channels is encoded by three genes Kcnn1-3 whereas IK channels have a single gene member encoded by Kcnn4. Calcium sensitivity of SK and IK channels is conferred by calmodulin (Cam) that is constitutively bound to the channel. Selective inhibitors for each family are indicated. * note: BK channels that assemble with the β4 accessory subunit are largely insensitive to IbTX whereas paxilline inhibits all known BK channel assemblies.

Figure 2: Differential spatial localization of BK and SK channels with extracellular and intracellular sources of calcium.

A) In lactotrophs, somatotrophs and corticotrophs distinct BK channel populations are proposed with BK channels (BK_{near}) closest to voltage gated calcium channels (VGCC) and hence exposed to rapid and high increases in intracellular calcium upon VGCC activation and implicated in promoting “pseudo plateau bursting’ behavior (see section 4.1). In contrast, BK channels further away (BK_{far}) from VGCC respond to global elevations in intracellular calcium and contribute to membrane repolarisation that terminate burst activity (see Figure 3A).
B) In several pituitary cell types, including gonadotrophs and corticotrophs, SK channels couple to calcium release from intracellular inositol trisphosphate (IP3)-sensitive stores from sub-plasmalemmal endoplasmic reticulum (ER) compartments, rather than VGCC. In these systems G-protein coupled receptors (GPCR), that stimulate IP3 synthesis, activate SK channels to promote membrane hyperpolarization (see Figure 3B).

Figure 3: Role of BK and SK channels in controlling membrane excitability

A) Distinct BK channel populations exert opposite effects on membrane excitability in corticotrophs. In mouse corticotrophs spontaneous electrical activity is largely characterized by single spike action potentials of duration < 100ms. Upon exposure to physiological (low nanomolar) concentrations of corticotrophin releasing hormone (CRH) this spiking activity transitions to 'pseudo plateau bursting' that is proposed to promote hormone secretion from corticotrophs. In this model, fast activation of BK_{near} facilitates transition to 'pseudo plateau bursting' (see section 4.1) whereas the slower activation of BK_{far} terminates bursting activity as global calcium rises. A similar mechanism is proposed for the spontaneous BK-dependent 'pseudo plateau bursting' observed in unstimulated lactotrophs and somatotrophs. Panel traces adapted from (Duncan et al., 2016).

B) In gonadotrophs spontaneous activity is characterised by single, short duration (< 100ms) action potentials. Exposure to gonadotrophin releasing hormone (GnRH) stimulates production of IP3 to promote calcium release from intracellular calcium stores that activates SK channels. Activation of SK channels results in membrane hyperpolarization that relieves voltage gated sodium channels and calcium channels from inactivation so that the membrane potential oscillates between periods of hyperpolarization and subsequent depolarization with generation of action potentials. In this system, the majority of secretion is thought to be driven by intracellular calcium release during the hyperpolarization phase with the subsequent depolarization required to refill the intracellular calcium stores. Panel traces adapted from (Stojilkovic, 2006).

In A) and B) the shaded bar indicates membrane potential between -50 and + 10 mV. Note the difference in timescale between traces in A) and B)

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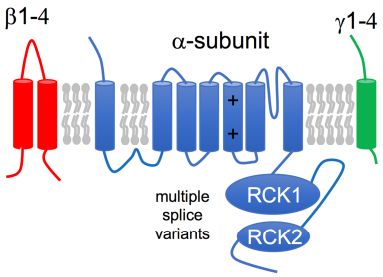
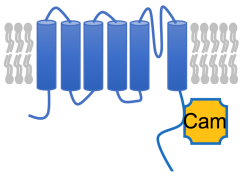
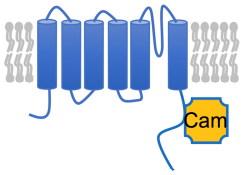
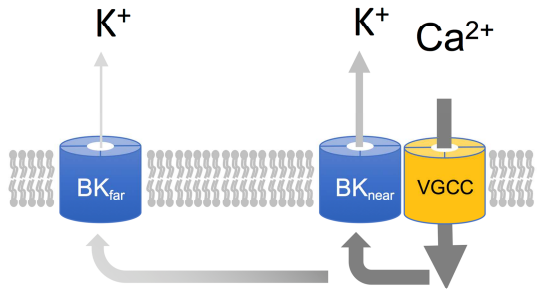
	BK channel (Kcnma1, KCa1.1)	SK channels (Kcnn1-3, KCa2 family)	IK channel (Kcnn4, KCa3.1)
			
Voltage	sensitive	insensitive	insensitive
$[Ca^{2+}]_i$	EC_{50} variable <1μM to >10μM	< 0.5 μM	< 0.5 μM
Selective inhibitors	iberiotoxin (IbTx)*, paxilline	apamin (sensitivity SK2>SK3>SK1)	TRAM-34

A



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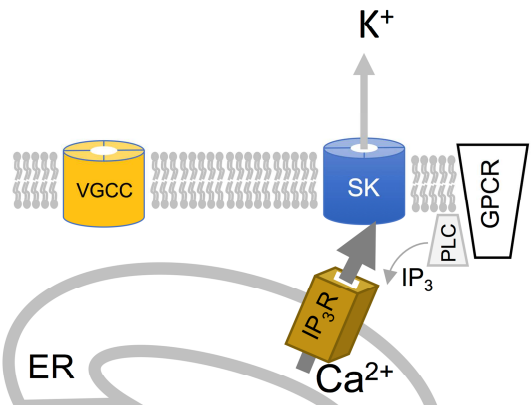

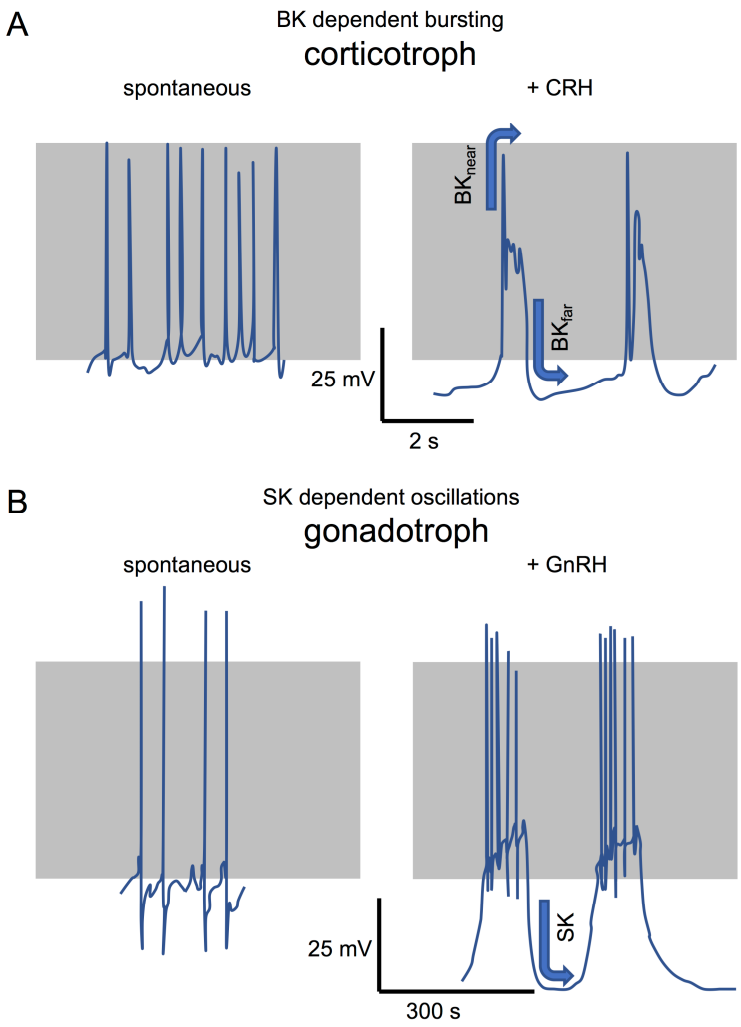

Highlights

- Anterior pituitary cells are electrically excitable
- Ca^{2+}-activated K^+ channels shape the pattern of cell type specific excitability
- Ca^{2+}-activated K^+ channels promote or reduce excitability depending on cell context
- Pattern of excitability determines pituitary hormone secretion & physiology