Mechanism of Spontaneous and Receptor-controlled Electrical Activity in Pituitary Somatotrophs: Experiments and Theory

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Date: 4/2/2007
ABSTRACT

Cultured pituitary somatotrophs release growth hormone in response to spontaneous Ca^{2+} entry through voltage-gated calcium channels (VGCCs), which is governed by plateau-bursting electrical activity and is regulated by several neurohormones, including GH-releasing hormone (GHRH) and somatostatin. Here we combine experiments and theory to clarify the mechanisms underlying spontaneous and receptor-controlled electrical activity. Experiments support a role of a Na^{+}-conducting and tetrodotoxin-insensitive channel in controlling spontaneous and GHRH-stimulated pacemaking, the latter in a cAMP-dependent manner; an opposing role of spontaneously active inwardly rectifying K^{+} (K_{ir}) channels and G-protein-regulated K_{ir} channels in somatostatin-mediated inhibition of pacemaking; as well as a role of VGCCs in spiking and BK-type Ca^{2+}-activated K^{+} channels in plateau bursting. The mathematical model is compatible with a wide variety of experimental data involving pharmacology and extracellular ion substitution and supports the importance of constitutively active tetrodotoxin-insensitive Na^{+} and K_{ir} channels in maintaining spontaneous pacemaking in pituitary somatotrophs. The model also suggests that these channels are involved in the up- and down-regulation of electrical activity by GHRH and somatostatin. In the model, the plateau bursting is controlled by two functional populations of BK channels, characterized by distance from the VGCCs. The rapid activation of the proximal BK channels is critical for the establishment of the plateau, whereas slow recruitment of the distal BK channels terminates the plateau.
INTRODUCTION

In the absence of hypothalamic factors in vitro, the membrane potential ($V_m$) of secretory anterior pituitary cells in culture is not stable, but oscillates from the baseline potential of about –60 mV. When $V_m$ oscillations reach the threshold level, pituitary cells fire action potentials (APs). This is a common feature of all secretory anterior pituitary cell types and is observed in 15 to 80% of cells, depending on the cell type and preparation (Kwiecien and Hammond 1998; Stojilkovic and Catt 1992). The majority of cultured somatotrophs and lactotrophs frequently exhibit broader $V_m$ oscillations, in the form of a depolarizing plateau with bursts of APs superimposed. These spikes are small in amplitude and usually do not reach 0 mV (Kwiecien et al. 1997; Sims et al. 1991; Van Goor et al. 2001a). Such complex plateau bursting activity is periodic and results in an oscillatory increase in intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) of sufficient amplitude to maintain high and steady growth hormone (GH) release (Holl et al. 1988; Van Goor et al. 2001b). A fraction of somatotrophs and lactotrophs may also generate single APs that are sharp and short in duration, and reverse polarization of $V_m$. These are termed axonal type APs (Chen et al. 1990a; Gonzalez-Iglesias et al. 2006a). Consistent with this, GH immortalized pituitary lacto-somatotrophs can exhibit both axonal APs and the plateau-bursting pattern of firing (Giraldez et al. 2002; Kwiecien et al. 1998; Schlegel et al. 1987). In contrast, cultured gonadotrophs exclusively exhibit axonal type APs that are associated with small amplitude Ca$^{2+}$ transients and low basal gonadotropin release (Van Goor et al. 2001b). Corticotrophs also fire axonal type of APs spontaneously (Kuryshev et al. 1997).

In this study, we focus on pituitary somatotrophs. These cells express numerous voltage-gated and ligand-gated channels, including tetrodotoxin (TTX)-sensitive (Chen et al. 1990a; Mason and Rawlings 1988; Van Goor et al. 2001c), and -insensitive (Kato and Sakuma 1997;
Naumov et al. 1994) Na⁺ channels, voltage-gated Ca²⁺ channels (VGCCs) (Chen et al. 1990a; Kwiecien et al. 1997; Lewis et al. 1988; Van Goor et al. 2001c), transient, long-lasting, and calcium-dependent K⁺ channels (Mason and Rawlings 1988; Van Goor et al. 2001c), inwardly rectifying K⁺ (Kᵢᵣ) channels (Sims et al. 1991; Tomic et al. 1999b), and ATP-gated P₂X₂ receptor-channels (Koshimizu et al. 1998). The depolarizing spiking phase of APs in somatotrophs is probably mediated by L-type VGCCs (Van Goor et al. 2001b), and a role for TTX-sensitive channels in rapid depolarization has also been suggested for cells exhibiting axonal type firing (Chen et al. 1990a). It has been proposed that in somatotrophs BK-type Ca²⁺-controlled K⁺ channels play a paradoxical role in the initiation of plateaus bursting (Van Goor et al. 2001a), whereas the channels contributing to plateau repolarization have not been identified.

The spontaneous bursting activity in somatotrophs is bidirectionally controlled by hypothalamic neurohormones. GH-releasing hormone (GHRH) stimulates firing of APs in quiescent somatotrophs (Kwiecien et al. 1997) and increases the frequency of AP-driven Ca²⁺ transients in spontaneously active cells (Holl et al. 1988; Tomic et al. 1999a). In contrast, somatostatin (SRIF) inhibits spontaneous electrical activity, the associated Ca²⁺ transients, and basal GH release (Lussier et al. 1991; Patel and Srikant 1986; Sims et al. 1991). Activation of GHRH receptors in pituitary cells leads to stimulation of adenylyl cyclase activity, whereas SRIF inhibits basal activity of this enzyme (Bilezikjian and Vale 1983). GHRH stimulates and SRIF inhibits TTX-insensitive Na⁺ current in a cAMP/protein kinase A-dependent manner (Kato and Sakuma 1997). Potential roles of VGCCs and voltage-gated K⁺ channels in GHRH actions have also been suggested (Chen and Clarke 1995; Xu et al. 1999), as well as a role of Kᵢᵣ channels (Sims et al. 1991), and L-type Ca²⁺ channels (Kato 1995) in SRIF actions. In immortalized pituitary cells, it has been suggested that SRIF inhibits electrical activity through stimulation of
BK channel activity by protein dephosphorylation (White et al. 1991), activation of $K_{ir}$ channels (Takano et al. 1997) and/or delayed rectifying $K^+$ channels (Mollard et al. 1988; Yamashita et al. 1987), and inhibition of VGCCs (Lewis et al. 1986; Luini et al. 1986; Mollard et al. 1988).

Here we studied spontaneous and receptor-controlled electrical activity in somatotrophs both experimentally and theoretically. Specifically, we addressed the hypotheses that TTX-insensitive Na$^+$ channels play a role both in sustaining spontaneous firing of APs and the associated voltage-gated Ca$^{2+}$ influx and in mediating the modulation of pacemaking by GHRH and SRIF. We also studied the relevance of $K_{ir}$ channels in spontaneous and receptor-controlled electrical activity, as well as the importance of the expression pattern of VGCCs and BK channels in the plasma membrane in plateau-bursting and repolarization phases of APs. The complex pattern of spontaneous electrical activity and Ca$^{2+}$ transients could be successfully mimicked by the somatotroph model. These results suggest that the multiple control of pacemaking by cAMP/protein kinase A, Ca$^{2+}$ and G proteins, provides an effective mechanism for up- and down-regulation of electrical activity and voltage-gated Ca$^{2+}$ influx.
MATERIALS AND METHODS

Cell Cultures and Treatments

Experiments were performed on anterior pituitary cells from normal postpubertal female Sprague Dawley rats obtained from Taconic Farm (Germantown, NY). Pituitary cells were dispersed as described previously (Koshimizu et al. 2000), and cultured as mixed cells or enriched lactotrophs, somatotrophs, and gonadotrophs in medium 199 containing Earle’s salts (Sodium bicarbonate (2.2g/L), HEPES (25mM), Heat-inactivated horse serum (10%), Penicillin (100U/ml), Streptomycin (100ug/ml); Gibco-Invitrogen Corp., Carlsbad, CA). A two-stage Percoll discontinuous density gradient procedure (Koshimizu et al. 2000) was used to obtain an enriched somatotroph population. These cells were further identified by their cell-type specific morphologies and by their responses to GHRH and SRIF.

Electrophysiological measurements

Current- and voltage-clamp recordings were performed at room temperature using an Axopatch 200 B patch-clamp amplifier (Axon Instruments, Foster City, CA) and were low-pass filtered at 2 kHz. $V_m$ was measured using the perforated-patch recording technique (Rae et al. 1991). Briefly, an amphotericin B (Sigma, St. Louis, MO) stock solution (60 mg/ml) was prepared in DMSO and stored for up to 1 week at −20 °C. Just prior to use, the stock solution was diluted in pipette solution and sonicated for 30 s to yield a final amphotericin B concentration of 240 µg/ml. Patch electrodes used for perforated-patch recordings were fabricated from borosilicate glass (1.5 mm o.d.: World Precision Instruments, Sarasota, FL) using a Flaming Brown horizontal puller (P-87; Sutter Instruments, Novato, CA). Electrodes were heat polished to a final tip resistance of 3-6 MΩ and then coated with Sylgard (Dow Corning Corporation, Midland, MI) to reduce pipette capacitance. Pipette tips were briefly
immersed in amphotericin B-free solution and then backfilled with the amphotericin B-containing solution. A series resistance of $< 15 \, \text{M}\Omega$ was reached 10 min following the formation of a gigaohm seal (seal resistance $> 5 \, \text{G}\Omega$) and remained stable for up to 1 h. Pulse generation, data acquisition and analysis were done with a PC equipped with a Digidata 1200 A/D interface in conjunction with Clampex 8 (Axon Instruments). For recording $V_m$, the extracellular medium contained (in mM): 120 NaCl, 2 CaCl$_2$, 2 MgCl$_2$, 4.7 KCl, 0.7 MgSO$_4$, 10 Glucose and 10 HEPES (pH adjusted to 7.4 with NaOH) and the pipette solution contained (in mM): 50 KCl, 90 K$^+$-aspartate, 1 MgCl$_2$, and 10 HEPES (pH adjusted to 7.2 with KOH). The bath contained $< 500 \, \mu\text{l}$ of saline and was continuously perifused at a rate of 2 ml/min using a gravity-driven perfusion system.

**Simultaneous recording of $[\text{Ca}^{2+}]_i$ and $V_m$**

Pituitary cells were incubated for 15 min at 37 °C in phenol red-free medium 199 containing Hanks’ salts, 20 mM sodium bicarbonate, 20 mM HEPES and 0.5 µM indo-1 AM (Molecular Probes, Eugene, OR). The $V_m$ was recorded as described above and bulk $[\text{Ca}^{2+}]_i$ was simultaneously monitored using a Nikon photon counter system as previously described (Van Goor et al. 2001c) The membrane potential and bulk $[\text{Ca}^{2+}]_i$ were captured simultaneously at rate of 5 kHz using a PC equipped with a Digidata 1200 A/D interface in conjunction with Clampex 8 (Axon Instruments). The $[\text{Ca}^{2+}]_i$ was calibrated in vivo according to (Kao 1994) and the values for $R_{\text{min}}$, $R_{\text{max}}$, $S_{f,480}/S_{b,480}$ and $K_d$ were determined to be 0.75, 3.40, 2.45 and 230 nM, respectively.

**Mathematical model**

A schematic diagram of the somatotroph model cell is presented in Fig. 1 and parameter values used in the simulations are given in Table 1 and in the figure legends. In our model, there
are two different populations of BK channels: one in close proximity to the VGCCs (I_{BK_{NEAR}}) and the other located far from the VGCCs (I_{BK_{FAR}}). We also incorporate a non-selective predominantly Na\(^+\)-conducting channel, which is assumed to be regulated by the cAMP signaling pathway (I_{NS,Na}). This current incorporates the TTX–insensitive Na\(^+\) current that has been detected in pituitary somatotrophs (Kato and Sakuma 1997; Kwiecien and Hammond 1998; Van Goor et al. 2001c). The TTX-sensitive Na\(^+\) channels (Van Goor et al. 2001c) are not included in the model because they inactivate rapidly in the physiological range of V_m. Indeed, we show here that TTX has no effect on electrical activity (Fig. 6B).

The model consists of four ordinary differential equations for the rate of change of the membrane potential, V_m; the delayed-rectifier activation gating variable n_{dr}; the [Ca\(^{2+}\)]_i denoted by c; and the concentration of Ca\(^{2+}\) in the endoplasmic reticulum (ER), denoted by c_{ER}. A third Ca\(^{2+}\) compartment comprised of the micro-domains of the voltage-gated Ca\(^{2+}\) channels (CaDOM) controls the activity of a sub-group of BK channels proximal to the Ca\(^{2+}\) channels and is determined algebraically by assuming it to be proportional to the Ca\(^{2+}\) current.

The equation for V_m is given by:

\[
C_m \frac{dV_m}{dt} = -I_{\text{ionic}},
\]

where \(C_m\) is the membrane capacitance and \(I_{\text{ionic}}\) is the sum of the ionic currents, given by:

\[
I_{\text{ionic}} = I_{Ca_L} + I_{Ca_T} + I_{NS,Na} + I_{K_{dr}} + I_{K_{ir}} + I_{BK_{TOT}} + I_{\text{Noise}}.
\]

\(I_{Ca_L}\) and \(I_{Ca_T}\) are the L- and T-type Ca\(^{2+}\) currents, respectively, and \(I_{K_{dr}}\) and \(I_{K_{ir}}\) are the delayed-rectifier and inward-rectifier currents, respectively. \(I_{\text{Noise}}\) represents the effect of noise.

We use the standard Hodgkin-Huxley formalism to model

\[
I_{Ca_L}(V_m) = g_{Ca_L} m_{Ca_L}^3 (V_m - V_{Ca}),
\]

where \(g_{Ca_L}\) is the channel conductance \(m_{Ca_L}\) is the steady-state
activation function and $V_{Ca}$ is the reversal potential for Ca$^{2+}$. Similar equations govern the other voltage-dependent currents:

$$I_{CaT} (V_m) = g_{CaT} m_{CaT}^2 h_{CaT} (V_m - V_{Ca}),$$

$$I_{Kdr} (V_m, n_{dr}) = g_{Kdr} n_{dr} (V_m - V_K),$$

$$I_{Kdr} (V_m) = g_{Kdr} K_{ir} (V_m - V_{K}),$$

$$I_{BKNEAR} (V_m, CaDOM) = g_{BKNEAR} b_{KNEAR} (V_m, CaDOM)(V_m - V_K),$$

$$I_{BK FAR} (V_m, c) = g_{BK FAR} b_{KFAK}^2 (V_m, c)(V_m - V_K),$$

$$I_{NS,Na} = g_{NS,Na} (V_m - V_{NS,Na}),$$

$$I_{BKTOT} = I_{BKNEAR} + I_{BK FAR}$$

where $m_{CaT}$, $K_{ir}$, $b_{KNEAR}$, and $b_{KFAK}$ are the steady-state activation functions for the respective currents, and $h_{CaT}$ is the steady-state inactivation function for the T-type Ca$^{2+}$ current. The variable $n_{dr}$ is the delayed-rectifier activation, gating variable, which obeys the following equation:

$$(2) \quad \frac{dn_{dr}}{dt} = \frac{n_{dr,0} - n_{dr}}{\tau_{n_{dr}}}.$$  

The steady-state functions are:

$$m_{CaL} (V_m) = \frac{1}{1 + \exp \left(\frac{V_m - V_{mL}}{k_{mL}}\right)},$$

$$m_{CaT} (V_m) = \frac{1}{1 + \exp \left(\frac{V_m - V_{mT}}{k_{mT}}\right)},$$

$$h_{CaT} (V_m) = \frac{1}{1 + \exp \left(\frac{V_m - V_{hT}}{k_{hT}}\right)}.$$
\[ n_{dr} (V_m) = \frac{1}{1 + \exp\left(-\frac{(V_m - V_{n_{dr}})}{k_{n_{dr}}} \right)} \]

\[ K_{ir} (V_m) = \frac{\alpha_{ir}}{\alpha_{ir} + \beta_{ir}} \]

\[ b_{K_{NEAR}} (V_m, Ca_{DOM}) = \frac{1}{1 + \exp\left(-\frac{(V_m - V_{BK_{NEAR}}(Ca_{DOM}))}{k_{BK}} \right)} \]

\[ b_{K_{FAR}} (V_m, c) = \frac{1}{1 + \exp\left(-\frac{(V_m - V_{BK_{FAR}}(c))}{k_{BK}} \right)} \]

where

\[ \alpha_{ir} = \frac{0.1}{1 + \exp[0.06(V_m - V_{K_{ir}} - 200)]} \]

\[ \beta_{ir} = \frac{3\exp[0.0002(V_m - V_{K_{ir}} - 100)] + \exp[0.1(V_m - V_{K_{ir}} - 10)]}{1 + \exp[-0.5(V_m - V_{K_{ir}})]} \]

\[ V_{BK_{NEAR}} (Ca_{DOM}) = V_{BK_0} - k_{shift} \ln \frac{Ca_{DOM}}{k_{Ca_{BK}}} \]

\[ V_{BK_{FAR}} (c) = V_{BK_0} - k_{shift} \ln \frac{c}{k_{Ca_{BK}}} \]

\[ Ca_{DOM} = -A(I_{Ca_{l}} (V_m) + I_{Ca_{v}} (V_m)) \]

The steady-state activation function used in modeling the \( I_{K_{ir}} \) is taken from (Priebe and Beuckelmann 1998). BK channels are gated to open by both micromolar Ca\(^{2+}\) concentration and voltage. In the model the steady-state activation functions for the near and far populations of BK channel are sigmoidal functions of voltage, and shift left as the Ca\(^{2+}\) concentration increases in the VGCC micro-domains (Ca_{DOM}) and in the cytosol (c). The sensitivity of BK channels to Ca\(^{2+}\) comes through \( V_{BK_{NEAR}} (Ca_{DOM}) \) and \( V_{BK_{FAR}} (c) \), as modeled previously (Chay 1986).

The current \( I_{\text{Noise}} \), which represents the effect of channel noise, is given by
\( I_{\text{Noise}} = \sigma_N \omega \)

where \( \sigma_N \) is the noise amplitude and \( \omega \) is a Wiener variable.

We include two functional calcium compartments in the model, the cytosol and the ER. We also assume a microscopic shell beneath the plasma membrane in close proximity to the Ca\(^{2+}\) channels where Ca\(^{2+}\) concentration (Ca\(_{\text{DOM}}\)) accumulates rapidly when channels open and disappears rapidly when channels close (Sherman et al. 1990). Thus, the equations governing Ca\(^{2+}\) handling are:

\[
\frac{dc}{dt} = f_{\text{cyt}} \left[ \beta (-\alpha (I_{\text{Ca_L}} + I_{\text{Ca_T}}) - J_{\text{PMCA}}) + \frac{1}{V_{\text{cyt}}} (p_{\text{ER}} (c_{\text{ER}} - c) - J_{\text{SERCA}}) \right]
\]

\[
\frac{dc_{\text{ER}}}{dt} = f_{\text{ER}} \left[ -p_{\text{ER}} (c_{\text{ER}} - c) + J_{\text{SERCA}} \right]
\]

where \( f_{\text{cyt}} \) and \( f_{\text{ER}} = f_{\text{cyt}} \) are the fractions of free Ca\(^{2+}\) in the ER and cytosolic compartments; \( V_{\text{cyt}} \) and \( V_{\text{ER}} = 0.1 V_{\text{cyt}} \) are the volumes of the cytosol and the ER; \( \beta \) is the ratio of the cell surface area \( (A_{\text{cell}} = \pi d_{\text{cell}}^2) \) to the cell volume \( (V_{\text{cell}} = \frac{\pi d_{\text{cell}}^3}{6}) \); \( d_{\text{cell}} \) is the diameter of the cell. Multiplication of the Ca\(^{2+}\) currents by the product of \( \beta \) and \( \alpha = [2FA_{\text{cell}}]^{-1} \), where \( F \) is Faraday’s constant, converts the currents to fluxes. The individual Ca\(^{2+}\) fluxes are: influx via the currents \( I_{\text{Ca_T}} \) and \( I_{\text{Ca_L}} \); efflux via the plasma membrane Ca\(^{2+}\)-ATPase \( (J_{\text{PMCA}}) \); release of Ca\(^{2+}\) from the ER via passive leak \( (p_{\text{ER}}) \) proportional to the concentration difference between the cytosol and the ER; and uptake via the SERCA pumps \( (J_{\text{SERCA}}) \). Equations for the plasma membrane Ca\(^{2+}\)-ATPase and SERCA pump are:

\[
J_{\text{PMCA}} = V_{\text{PMCA}} \frac{c^2}{c^2 + K_{\text{PMCA}}^2},
\]

\[
J_{\text{SERCA}} = k_{\text{SERCA}} c,
\]
where $V_{PMCA}$ is the maximal pump rate and $K_{PMCA}$ is the half-maximal activation concentration for the Ca$^{2+}$-ATPase. We model the plasma membrane calcium pump by a Hill equation with Hill coefficient of 2 and, for simplicity, use a linear function for the SERCA pump.
RESULTS

Characterization of spontaneous pacemaking in somatotrophs

To evaluate the potential influence of the intrapipette content on the pattern of electrical activity, we initially recorded Ca\textsuperscript{2+} transients prior to (Fig. 2A, top-left) and 5 min after establishing the whole-cell or perforated cell-attached mode (Fig. 2A, top-right). In the whole-cell recording mode, the majority of cells either was quiescent or exhibited spontaneous firing of APs that was rapidly abolished independently of the intrapipette Cl\textsuperscript{-} concentration (in the range 5 to 50 mM) and the presence or absence of ATP regenerating solutions. In perforated cell-attached patch mode, about 75% of the cells exhibited spontaneous long-lasting firing of APs, which is consistent with data in the literature (Kwiecien and Hammond 1998; Stojilkovic et al. 2005). The patterns of Ca\textsuperscript{2+} signals prior to and after establishment of perforated patch were most comparable when the intrapipette concentration of Cl\textsuperscript{-} was 50 mM (Fig. 2A, bottom). This finding is also in accordance with the depolarizing effects of GABA receptor-channels in pituitary cells (Virmani et al. 1990). Under such experimental conditions, we observed variations in the pattern of electrical activity among somatotrophs. Specifically, the period of plateau bursting in pituitary somatotrophs varied from about 2 to 10 seconds with longer active phase duration associated with the slower bursting (Fig. 2B-E, top traces). The amplitude of the parallel Ca\textsuperscript{2+} oscillations was higher for the slower bursting in $V_m$ (Fig. 2B-E, bottom traces). All other experiments were done with the same intrapipette solution using the perforated cell-attached mode, and $V_m$ and [Ca\textsuperscript{2+}]\textsubscript{i} were measured simultaneously.

Modeling of pacemaking and plateau bursting in pituitary somatotrophs

In the model two currents play a critical role in controlling pacemaking, namely a non-selective predominantly Na\textsuperscript{+}-conducting current and a $K_{ir}$ current. Consistent with the hypothesis
of (Kwiecien and Hammond 1998), the non-selective Na\(^+\) current drives \(V_m\) to the threshold potential for L-type Ca\(^{2+}\)-channel activation. The mean value of this current during spontaneous bursting is approximately \(-2\) pA, which agrees well with the value of \(-1.86\pm0.33\) pA reported in Kato and Sakuma (1997). As in other cell types, in pituitary somatotrophs \(K_{ir}\) current is essential for maintaining the resting \(V_m\) and for hyperpolarization-induced \(K^+\) influx (Chen et al. 1990b; Xu et al. 2002). In the model simulations presented in Fig. 3, the mean of \(K_{ir}\) current is approximately \(3\) pA, in agreement with our experimental measurements (not shown).

There is experimental evidence that somatotrophs exhibit high expression levels of BK channels (Van Goor et al. 2001c). Experimental and theoretical results also suggest that rapid activation/deactivation of these channels is very important for the generation of plateau bursting in pituitary somatotrophs (Van Goor et al. 2001a). In the model proposed in that study, the plateau is established by the interaction between L-type Ca\(^{2+}\) channels, BK channels, and delayed rectifying \(K^+\) channels, whereas the plateau is terminated by slow activation of SK channels. Here, we retain the idea that BK channels proximal to Ca\(^{2+}\) channels establish the plateau but hypothesize that a second group of BK distant from Ca\(^{2+}\) channels, rather than SK channels, terminates the plateau (Fig. 1). The near BK channels activate rapidly and strongly because \([Ca^{2+}]_i\) in a micro-domain rapidly reaches equilibrium at a high level, whereas the far BK channels activate slowly and weakly because bulk cytosolic Ca\(^{2+}\) is slow and at a lower concentration. This approach is motivated by experimental observations that the SK blocker apamin had little effect on Ca\(^{2+}\)-activated \(K^+\) currents (Van Goor et al. 2001c) or electrical activity (Van Goor and Stojilkovic, unpublished observations) in somatotrophs. It is important to note that the two groups of BK channels are assumed to have the same intrinsic properties and differ only in their location; this is a strong constraint as it removes a degree of freedom that was
available in the earlier model. A similar functional separation between near and far BK channels was proposed by (Prakriya and Lingle 2000) to explain the differential effects of EGTA and BAPTA on BK currents in chromaffin cells.

Typical bursting electrical activity generated by the somatotroph model is shown in Fig. 3A. The crucial role of the near BK channels is indicated in Fig. 3C, where we plot the actual conductance \( g_{BK_{NEAR}} \) of these channels during spontaneous bursting. The very fast activation of the near BK channels caused by the increased concentration of \( Ca^{2+} \) in the micro-domain is necessary to establish the plateau potential. In contrast, the far BK channels activate more slowly and are responsible for termination of the plateau bursting. In Fig. 3B, we plot the actual conductance \( g_{BK_{FAR}} \) of the far BK channels, which depends on \([Ca^{2+}]_i\) and hence builds up gradually, reflecting a progressive increase in \([Ca^{2+}]_i\) (Fig. 3D). In the model, the \([Ca^{2+}]_i\) in the micro-domain is more than three times as high as the global \([Ca^{2+}]_i\).

In addition the size of the total (near BK + far BK) current agrees well with the experimental data of (Van Goor et al. 2001a), Figs. 10A, B): In the absence of any BK block (Figs. 3 and 4C) the total BK current oscillates between 0 and 25 pA, and after the initial spike drops to around 5 pA.

Modulation of plateau bursting by BK channel block

We were able to simulate the different patterns of firing shown in Fig. 2 merely by partially blocking the total BK current, proportionally for the near and far BK channels (Fig. 4A-C). Starting with a set of parameter values corresponding to comparatively slow bursting and \( Ca^{2+} \) oscillations (approximately 8 sec; Fig. 4C) 15% (Fig. 4B) and 30% (Fig. 4A) suppression of the total BK current reproduced the observed reduction of the burst period and the active phase duration as well as the decreased amplitude of the associated \( Ca^{2+} \) oscillations. The model
simulations also agree with the experimental finding of (Van Goor et al. 2001a) that blocking BK channels leads to larger spikes, shorter active phase duration and lower [Ca^{2+}]_i. Thus, the extent of BK channel expression in pituitary somatotrophs may account for the different patterns of plateau bursting.

An important point to note is that the somatotroph model differs from the standard plateau burster (or square-wave burster) models, such as that of (Chay and Keizer 1983), in which blocking Ca^{2+}-dependent K^+ channels prolongs the plateau active phase duration and increases the level of [Ca^{2+}]_i. Although, as in Chay-Keizer, the Ca^{2+}-dependent K^+ channels are responsible for termination of the active phase, in the somatotroph model inhibition of the total BK current has the opposite effect. The difference lies in the fact that in the model, the BK channels play two roles: the far BK channels respond to slow changes in calcium and are responsible for burst termination, but the near BK channels respond to rapid changes in calcium and, as described in detail in (Van Goor et al. 2001a), are responsible for establishing and maintaining the plateau. Indeed, in the model, blocking the far channels alone does prolong the plateaus as expected, and blocking the near channels alone shortens the plateau more dramatically.

This property of the somatotroph model is illustrated by the bifurcation diagram using the slow variable [Ca^{2+}]_i as a bifurcation parameter (Fig. 5). The burst trajectory is superimposed on the diagram to confirm that the analysis done with [Ca^{2+}]_i fixed does predict approximately the behavior of the full system when [Ca^{2+}]_i is dynamic. As in the Chay-Keizer model, the active phase of a burst begins when the lower branch of steady states ends in a fold bifurcation. Biophysically, the voltage crosses the threshold, represented in the diagram by the dashed branch of saddle-point steady states. The rapid, nearly vertical rise of V_m is due to activation of L- and
T-type Ca\(^{2+}\) channels, which initiates spiking and causes [Ca\(^{2+}\)]\(_i\) to rise once the nullcline for c, labeled dc/dt = 0 in the figure, is crossed. Unlike Chay-Keizer, however, the spikes are not stable oscillations, but rather transient oscillations that damp down to an upper steady state. The damping is slow because the attraction of the upper steady state is weak and the rise of [Ca\(^{2+}\)]\(_i\) is not very slow, which limits the time available to fully reach the steady state. Furthermore, the attraction weakens as [Ca\(^{2+}\)]\(_i\) increases and the system approaches a Hopf bifurcation, at which the upper steady state becomes unstable. Before this point is even reached, the orbit has started spiraling away from the upper steady state and falls back to the silent phase when it crosses a separating surface and leaves the basin of attraction. In the terminology of (Izhikevich 2000), this is called a fold sub-Hopf burst mechanism, although the point where the trajectory falls may lie before or after the Hopf bifurcation, depending on parameters and the strength of attraction of the upper state (compare Fig. 5 to Izhikevich’s Fig. 103). From a modeling point of view, these features of the mathematical structure lead to many differences in behavior from classical square-wave bursters. Further analysis of these differences will be presented elsewhere (J. V. Stern, H. M. Osinga, A. LeBeau, and A. Sherman, manuscript in review), but here we use the bifurcation analysis only to explain the effect of BK blockade noted above.

Fig. 5 shows the bifurcation diagrams and trajectories for the cases of no BK block (panel A) and 15% BK block (panel B). In the latter case, the Z-shaped curve of steady states is stretched and shifted to the right, reflecting the fact that with fewer far BK channels, a greater rise in [Ca\(^{2+}\)]\(_i\) is required to generate enough outward current to eliminate the upper excited state. If this were the only change, the burst period would be become longer, but the reduction of the near BK channel conductance reduces the stability of the upper state, resulting in larger amplitude of the transient spikes. Consequently, the solution spirals away from the upper steady
state more rapidly and the active phase ends sooner. The latter effect more than compensates for the fact that the upper steady state remains stable for larger values of $[Ca^{2+}]_i$.

**Dependence of spontaneous pacemaking on extracellular cations**

To test the dependence of spontaneous electrical bursting and $[Ca^{2+}]_i$ oscillations on the external $Na^+$ concentration, this cation was transiently substituted with N-methyl-D-glucamine. As shown in Fig. 6A, removal of $Na^+$ reversibly hyperpolarized the membrane potential and suppressed $Ca^{2+}$ oscillations, followed by reduction in the level of $[Ca^{2+}]_i$ to near steady-state levels. In contrast, the blockade by TTX of voltage-dependent $Na^+$ channels, which are expressed in these cells (Van Goor et al. 2001c), was ineffective (Fig. 6B). Similar experimental observations have been reported previously (Kato et al. 1992; Kwiecien et al. 1997; Tomic et al. 1999a). Our model therefore incorporates a non-selective, $Na^+$-carrying current termed $I_{NS,Na}$ but not the TTX-sensitive $Na^+$ current (Fig. 1). We simulated both abolition of electrical activity and hyperpolarization by blocking only $I_{NS,Na}$ (Fig. 6C).

In further experiments, we focused on regulation of pacemaking by divalent cations. Like removal of extracellular $Na^+$, perfusion of cells with $Ca^{2+}$-deficient/Mg$^{2+}$-containing medium reversibly abolished bursting and accompanying $[Ca^{2+}]_i$ oscillations. In contrast to removal of $Na^+$, there was a slight depolarization of $V_m$ during perfusion with $Ca^{2+}$-deficient medium (Fig. 7A). On the other hand, raising either extracellular $Ca^{2+}$ or $Mg^{2+}$ to 10 mM reversibly abolished $V_m$ and $[Ca^{2+}]_i$ oscillations in spontaneously active cells (Fig. 8A and B). The current $I_{NS,Na}$ in the model is therefore assumed to be modulated by divalent cations. Increasing the conductance $g_{NS,Na}$ combined with setting $g_{Ca_L} = 0$ and $g_{Ca_T} = 0$, was sufficient to achieve depolarization of $V_m$ in a $Ca^{2+}$-deficient environment (Fig. 7D), whereas experiments with elevation in divalent cation concentrations were simulated by decreasing $g_{NS,Na}$ (Fig. 8C).
The data and simulations together support the significance of a constitutively active, Na\(^+\)-
carrying cation channels in supporting spontaneous electrical activity in somatotrophs. The
nature of these channels is unknown at the present time and the possible candidates are listed in
Discussion. Below we also addressed the hypothesis that these channels are modulated by the
cAMP signaling pathway.

The participation of Ca\(^{2+}\) influx through both L- and T-type VGCCs in spontaneous
electrical activity and [Ca\(^{2+}\)]\(_i\) oscillations was assayed by application of Ni\(^{2+}\), T-type Ca\(^{2+}\)
channel blocker, and nifedipine, which blocks L-type VGCCs (Fig. 7B and C, left panels). We
simulated these experiments in Figs. 7E, F by setting \(g_{CaT} = 0\) and \(g_{CaL} = 0\), completely blocking
the T- and L-type Ca\(^{2+}\) currents, respectively. Note that there is no change in the baseline
potential in the presence of Ni\(^{2+}\) and nifedipine in both experiments and simulation, in contrast to
the hyperpolarization caused by the removal of extracellular Na\(^+\) (Fig. 6A). These results support
the hypothesis that a depolarizing Na\(^+\) current is necessary for driving the \(V_m\) of somatotrophs to
the threshold potential for activation of VGCCs.

Note also that in the case of Ni\(^{2+}\) application, electrical activity although suppressed, is
not completely abolished as in the case of nifedipine application. We were able to simulate this
experimental observation (Fig. 7E) only if we included white noise. Thus it may be that in the
cells as well noise triggers occasional spikes although \(V_m\) is below threshold for sustained
spiking. The computations also indicate that L-type Ca\(^{2+}\) channels are involved to a greater
extent than T-type Ca\(^{2+}\) channels in the upstroke phase of the bursting electrical activity in
pituitary somatotrophs.

We next explored the contributions of K\(^+\) currents by adding extracellular Cs\(^+\) at a
concentration (5 mM) that blocks K\(_{ir}\) channels (Lee et al. 2003), which increased the frequency
of spontaneous APs and baseline potential in somatotrophs (Fig. 9A). That concentration of Cs\(^+\) also blocks the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel, which mediates \(I_h\), but this current has been shown not to participate in control of pacemaking in pituitary cells (Gonzalez-Iglesias et al. 2006b; Kretschmannova et al. 2006; Simasko and Sankaranarayanan 1997). The observed effects of Cs\(^+\) are more consistent with blockade of K\(_{ir}\) channels, which are expressed in somatotrophs (Tomic et al. 1999b; Xu et al. 2002) and are incorporated in our model (Fig. 1). We were able to simulate effects of extracellular Cs\(^+\) (Fig. 9B) by merely decreasing \(g_{K_u}\). The experiments and simulations together suggest that K\(_{ir}\) channels contribute to the control of spontaneous pacemaking by opposing the depolarizing effects of TTX-insensitive Na\(^+\) channels.

**Hormonal regulation of plateau bursting**

Figure 10A shows a typical example of the effect of GHRH application on spontaneous electrical activity and Ca\(^{2+}\) transients in somatotrophs. The action of GHRH was mimicked by application of 3-isobutyl-1-methylxanthine (IBMX), a non-selective phosphodiesterase inhibitor (Fig. 10B). The striking similarity between the effects of GHRH and IBMX strongly suggests that a cyclic nucleotide–dependent pathway is involved in the mechanism of action of GHRH. In the model, the increase in the frequency and the levels of [Ca\(^{2+}\)]\(_i\) was achieved by increasing the conductance \((g_{NS,Na})\) of the non selective cation current and decreasing the conductance of K\(_{ir}\) channels. In the simulations shown in Fig. 10C, the changes in \(g_{NS,Na}\) and \(g_{K_u}\) led to a depolarization that was amplified by increased activation of VGCCs, resulting in a significant rise in [Ca\(^{2+}\)]\(_i\). These theoretical results suggest that modest changes in the small sub-threshold TTX-insensitive Na\(^+\) and inward rectifier K\(^+\) currents have sufficient leverage to mediate the stimulatory effect of GHRH on electrical activity and Ca\(^{2+}\) signaling in somatotrophs.
In contrast to GHRH, SRIF inhibits spontaneous $V_m$ and $Ca^{2+}$ oscillations. Figure 11A shows a typical response to the application of SRIF in spontaneously active somatotrophs. In the model, the action of SRIF was simulated by decreasing $g_{NS,Na}$ and increasing $g_{K_{ir}}$ as suggested by the experimental data. We simulated these experiments in Figure 11B assuming about 60% inhibition of the TTX-insensitive Na$^+$ conductance and 120% enhancement of $K_{ir}$ conductance. As addressed in the Discussion, in vivo two different $K_{ir}$ channels probably contribute to the control of pacemaking, spontaneously active and protein kinase A-regulated, which contribute to the control of spontaneous electrical activity, and SRIF-activated and G protein-regulated $K_{ir}$ channels. In the model, for simplicity a single family of $K_{ir}$ channels was used and was sufficient to mimic effects of Cs$^+$ and SRIF on the firing pattern and $Ca^{2+}$ transients.
DISCUSSION

We have integrated experimental and theoretical approaches in order to study the spontaneous and receptor-controlled electrical activity and Ca\textsuperscript{2+} oscillations in pituitary somatotrophs. Our experimental data provide novel information relevant for understanding the mechanism of spontaneous electrical activity and Ca\textsuperscript{2+} signaling in these cells. We showed here that dialysis of the cytosol in whole-cell recording leads to abolition of spontaneous electrical firing of APs, indicating that intracellular factors are critical for pacemaking in these cells. We also showed that TTX-insensitive Na\textsuperscript{+} conductance is essential for spontaneous firing of APs, and that the constitutive activity of these channels is balanced by spontaneously active K\text{ir} channels. Our results are consistent with the hypothesis that TTX-insensitive Na\textsuperscript{+} channels and spontaneously active K\text{ir} channels are regulated by the cAMP signaling pathway. This in turn provides a mechanism for up- and down-regulation of electrical activity by GHRH and SRIF, respectively, through the opposite modulation of AC activity. SRIF also stimulates K\text{ir} channels in a G-protein-dependent manner. Finally, our results support the relevance of BK-type K\textsuperscript{+} channels in plateau-bursting and termination of firing, depending on localized and global [Ca\textsuperscript{2+}].

Earlier studies showed that removal of external Na\textsuperscript{+} induced an immediate block of spontaneous spikes and $V_m$ hyperpolarization in immortalized somatotrophs (Kwiecien et al. 1998) and somato-lactotrophs (Simasko 1994), as well as in cultured lactotrophs (Sankaranarayanan and Simasko 1996). Here we showed that a Na\textsuperscript{+}-carrying current is present in cultured somatotrophs and that it is not blocked by TTX. We characterized this current in spontaneously firing somatotrophs and its relevance for activation of voltage-gated Ca\textsuperscript{2+} influx. The model confirmed that removal of a small inward current is sufficient to abolish firing and Ca\textsuperscript{2+} influx.
Our experimental and theoretical results further showed that removal of Ca$^{2+}$ led to abolition of firing of APs and spontaneous Ca$^{2+}$ transients, a finding consistent with the role of VGCCs in the generation of spontaneous electrical activity. In accordance with this conclusion, both Ni$^{2+}$ (in concentrations that predominantly inhibit T-type VGCCs) and nifedipine (in concentrations that specifically block L-type Ca$^{2+}$ channels) also inhibited spontaneous electrical activity. However, removal of Ca$^{2+}$ also depolarized cells, in contrast to the addition of Ni$^{2+}$ and nifedipine.

Although suppression of electrical activity in Ca$^{2+}$-deficient medium is not surprising and can be attributed to cessation of Ca$^{2+}$ entry, the depolarizing effect of external Ca$^{2+}$ removal is not straightforward. It was not related to the lack of shielding effects by divalent cations (Hille 1992), because in these experiments Mg$^{2+}$ was present in the extracellular medium. We further showed that Ca$^{2+}$ and Mg$^{2+}$ in high concentrations inhibited pacemaking by hypepolarizing the cells. High Ca$^{2+}$-induced hyperpolarization of cells resembled that induced by removal of extracellular Na$^+$, suggesting that Ca$^{2+}$ inhibits the constitutively active Na$^+$ conductance. The model reproduces well the effects of these cations on spontaneous electrical activity in pituitary somatotrophs. It also agrees with the experimental data presented here and previously concerning the contribution of T- and L-type voltage-gated calcium channels to bursting. Taken together, these results support a hypothesis that depolarizing Na$^+$ current is necessary for driving the $V_m$ of somatotrophs to the threshold potential for activation of VGCCs and that extracellular Ca$^{2+}$ inhibits this current.

The identity of the Na$^+$ current is yet undetermined. Among cation channels, the conductance of cyclic nucleotide-gated channels is enhanced by removal of extracellular Ca$^{2+}$ and inhibited by increase in either Ca$^{2+}$ or Mg$^{2+}$ extracellular concentrations above physiological
levels. These channels are activated by both cAMP and cGMP (Kaupp and Seifert 2002). In agreement with this hypothesis, mRNA transcripts for the rod type of cyclic-nucleotide-gated channels were identified in pituitary cells and purified somatotrophs (Tomic et al. 1999a). Furthermore, here we showed that inhibition of phosphodiesterases by IBMX led to facilitation of pacemaking in somatotrophs. This treatment elevates both cAMP and cGMP concentrations in pituitary cells, reflecting intrinsic activity of adenylyl cyclase and soluble guanylyl cyclase (Kostic et al. 2002). In our hands, GHRH also stimulated pacemaking, and it is well established that this agonist enhances cAMP and cGMP production (Kostic et al. 2004; Lussier et al. 1991; Tomic et al. 1999a), which in turn activates TTX-insensitive cation channels that predominantly conduct Na⁺ (Kato et al. 1992; Kato and Sakuma 1997; Lussier et al. 1991). Forskolin, an activator of adenylyl cyclase, also stimulates firing of action potentials in pituitary cells (Gonzalez-Iglesias et al. 2006a). Finally, there is experimental evidence that SRIF, which inhibits basal AC activity (Petrucci et al. 2000), partially suppresses the GHRH-induced Na⁺ current (Kato and Sakuma 1997).

However, in lactotrophs spontaneous firing is not abolished in cells with blocked basal adenylyl cyclase activity, suggesting that these channels are constitutively active (Gonzalez-Iglesias et al. 2006a). Stimulation and inhibition of basal soluble guanylyl cyclase activity as well as application of cGMP permeable analogs are also ineffective in modulation of spontaneous firing of APs in lactotrophs (Gonzalez-Iglesias et al. 2006a, Andric et al. 2003). These results argue against the hypothesis that cGMP is critical for spontaneous pacemaking in pituitary cells and thus against the hypothesis that the TTX-insensitive Na⁺ current is mediated by a cyclic nucleotide gated channel.
In contrast to cyclic nucleotide-gated channels, HCN channels are regulated predominantly by cAMP. These channels are permeable to both Na\(^+\) and K\(^+\), with a reversal potential around \(-30\) mV, and thus, upon their activation cells depolarize (Frere et al. 2004). The reversal potential for TTX-insensitive Na\(^+\) conductance in GH\(_3\) immortalized pituitary cells and lactotrophs is also around \(-30\) mV (Sankaranarayanan and Simasko 1996; Simasko 1994), but HCN channels have been shown to be not essential for spontaneous pacemaking in those cells (Gonzalez-Iglesias et al. 2006b; Kretschmannova et al. 2006; Simasko and Sankaranarayanan 1997). Ongoing experiments also argue against a role of HCN channels in spontaneous firing of APs in somatotrophs (Kretschmannova and Stojilkovic, unpublished information). These results suggest that the cAMP signaling pathway modulates pacemaking in somatotrophs indirectly, through protein kinase A phosphorylation of an as yet unidentified TTX-insensitive Na\(^+\) current, as proposed several years ago for GHRH-stimulated pacemaking activity in somatotrophs (Naumov et al. 1994).

Our results indicate that Kir channels also contribute to the control of spontaneous pacemaking in somatotrophs. In other cell types, the role of constitutively active Kir2.0 channels is well established, and activation of the adenyl cyclase signaling pathway leads to inhibition of these channels through protein kinase A-dependent phosphorylation (Stanfield et al. 2002). These channels are expressed in pituitary cells (Wulfsen et al. 2000). Effects of extracellular Cs\(^+\) on pacemaking probably reflect inhibition of such constitutively active channels, leading to an increase in the frequency of spiking. In contrast, Kir3.0 channels are silent in resting cells and are activated by numerous G protein-coupled receptors (Stanfield et al. 2002). Several lines of evidence also support the hypothesis that SRIF stops spontaneous and GHRH-evoked oscillations by hyperpolarizing somatotrophs mainly by activating Kir channels (Chen et al.
Hyperpolarization-induced closure of VGCCs then leads to a decrease in Ca$^{2+}$ influx and basal GH release (Kwiecien and Hammond 1998; Lussier et al. 1991).

The model confirms that $K_{ir}$ current could modulate spontaneous and receptor-controlled electrical activity. Lacking quantitative information on the $K_{ir}$ subtypes, we did not distinguish between spontaneously active, AC-dependent $K_{ir}$ current and G protein-regulated $K_{ir}$ current in the model, but rather simulated the experimental observations by decreasing the size of spontaneously activated $K_{ir}$ current in the case of GHRH regulation and increasing it in the case of SRIF application (Fig. 11).

Because the cAMP-dependent Na$^+$ and $K_{ir}$ currents would have reciprocal effects, it is difficult to determine their relative importance for the effects of GHRH and SRIF. As reduction of basal cAMP does not eliminate spontaneous spiking, one might infer that SRIF acts mainly by activating the G-protein $K_{ir}3.0$ channels rather than inhibiting the Na$^+$ current, which would have to overcome the recovery of activity of $K_{ir}2.0$ due to the reduction of cAMP. Simulations with the model (not shown) confirm that whereas activation of $K_{ir}$ alone is sufficient to terminate bursting, a rather large increase, more than five-fold, might be needed to achieve hyperpolarizations as deep as those in Fig. 10. Indeed, the model indicates that small reductions in $g_{NS,Na}$ can have more influence than larger increases in $g_{K_u}$ when $V_m$ is near $V_K$. Enhancement of electrical activity by GHRH may similarly involve both activation of inward and inhibition of outward current. Further quantitative data will be needed to sort out the contributions of these channels.

The plateau bursting type of electrical activity in somatotrophs has previously been reported by others and us (Sims et al. 1991; Van Goor et al. 2001a). Using the model, we showed
that BK channels have appropriate properties to play two key roles in driving these oscillations. We considered two groups of BK channels, which differ only in their distances from the VGCCs. The near BK channels were assumed to be located in close proximity to the VGCCs and their rapid activation by high micro-domain $[\text{Ca}^{2+}]_i$ initiates the burst plateau. The far BK channels, in contrast, respond to the slow rise in bulk cytosolic $\text{Ca}^{2+}$ and terminate the plateau. Although the extent of activation of the far BK channels in the model ($< 2\%$) is much less than that of the near BK channels (about 50%), there are many more of them, such that they make comparable contributions to the total BK conductance (Fig. 3B, C). The dominance of the near BK channels, on the other hand, explains why the net effect of increasing the combined BK channel activity is to increase the active phase duration and the cytosolic $\text{Ca}^{2+}$ level. This feature is adequate to account for the natural variability of period we observed, although variation in other currents may contribute as well.

The above observations further suggest that BK channels are not a likely target of GHRH – neither increasing nor decreasing their activity has the right properties to account for the gross depolarization and increased frequency induced by that agonist. Kv channels, which have similar but opposite effects to BK on the burst pattern (not shown), are similarly not a good candidate for hormonal modulation. The interplay of the near and far BK channels, which allows their activation both to prolong and to terminate the plateaus, provides a compact explanation for a complex set of experimental observations. This is also of broader theoretical interest because the effects of blocking $\text{Ca}^{2+}$-activated $\text{K}^+$ channels in the somatotroph model are opposite to those in classical plateau bursting models, such as Chay-Keizer.
ACKNOWLEDGMENTS

This work was supported by the Intramural Research Program of the NIDDK and NICHD, NIH. F. V. G. current address: Vertex Pharmaceuticals, San Diego, CA 92121, USA. The work was aided by prior unpublished studies of somatotroph-inspired models by Julie Stern, Andrew LeBeau, and Peter Roper in the Laboratory of Biological Modeling.
REFERENCES


FIGURE LEGENDS

FIG. 1. Schematic diagram of the somatotroph model indicating the currents included in the model and their main functions.

FIG. 2. Simultaneous measurements of $V_m$ and $[Ca^{2+}]_i$ oscillations in spontaneously firing somatotrophs. A: Patterns of spontaneous $[Ca^{2+}]_i$ transients in a somatotroph before (left) and after (right) establishment of perforated patch (see Materials and Methods). B-E, left panels: Typical patterns of spontaneous electrical activity and associated $Ca^{2+}$ transients in somatotrophs. B-E, right panels: Expanded time scales, showing selected APs labeled with asterisks on left panels.

FIG. 3. Model simulations for the parameter values given in Table 1 and $N = 0$. A: Typical voltage ($V_m$) solutions. B: Actual conductance ($g_{BK, FAR} b_{K, FAR}(V_m, c)$) of the far BK channels. C: Actual conductance ($g_{BK, NEAR} b_{K, NEAR}(V_m, Ca_{DOM})$) of the near BK channels. D: Typical $[Ca^{2+}]_i$ ($c$) solutions.

FIG. 4. Model simulations of $V_m$ and $[Ca^{2+}]_i$ oscillations in spontaneously firing somatotrophs. Reduction in the period of electrical activity and $Ca^{2+}$ transients was achieved by reducing the BK-type $Ca^{2+}$-activated $K^+$ conductance, both near and far, by 30% (A) and 15% (B).

FIG. 5. The trajectory of a typical bursting solution of the full system (1) – (4) for the parameter values given in Table 1 superimposed on the bifurcation diagram of the fast subsystem (1) – (2) using $[Ca^{2+}]_i$ ($c$) as a bifurcation parameter. A: 0% BK channels block. B: 15% BK channels block.
block. Calcium nullcline \( (dc/dt = 0) \) is also included in the figure. HB – Hopf Bifurcation point. Dashed lines denote instability.

FIG. 6. Characterization of the role of Na\(^+\) conductance on spontaneous firing and Ca\(^{2+}\) transients: experiments (A and B) and model simulation (C). A: Hyperpolarization of cells and abolition of spontaneous firing and Ca\(^{2+}\) transients by removal of extracellular Na\(^+\). Horizontal bar indicates time during which cells were perifused with equimolar concentrations of NMDG. B: Tetrodotoxin (TTX)-insensitivity of spontaneous pacemaking. C: Simulation of effects of removal of Na\(^+\) by blockade of cAMP-dependent channel conductivity \((g_{NS,Na} = 0.001)\).

FIG. 7. Characterization of the roles of Ca\(^{2+}\) conductance in spontaneous firing and Ca\(^{2+}\) transients: experiments (A-C) and model simulations (D-F). A and D: Depolarization of cells and abolition of spontaneous firing and Ca\(^{2+}\) transients induced by removal of extracellular Ca\(^{2+}\). Simulation was achieved by blockade of VGCCs \((g_{Ca_T} = g_{Ca_L} = 0)\) and increasing the conductance of cAMP-dependent cation channels \((g_{NS,Na} = 0.1445)\). B and E: A decrease in the firing frequency induced by application of Ni\(^{2+}\) at a concentration that blocks T-type VGCCs \((g_{Ca_T} = 0)\). C and F: Abolition of electrical activity by nifedipine, a specific blocker of L-type VGCCs \((g_{Ca_L} = 0)\). Note lack of change in baseline potential in B, C, E, and F. In the simulations shown in D – F \(g_{BK_{NEAR}} = 0.495, g_{BK_{FAR}} = 9, \sigma_N = 0.0055\).

FIG. 8. Inhibitory effects of divalent cations on spontaneous firing and Ca\(^{2+}\) transients: experiments (A and B) and model simulation (C). A and B: Blockade of spontaneous pacemaking and Ca\(^{2+}\) transients by raising extracellular Ca\(^{2+}\) to 10 mM (Mg\(^{2+}\) remaining at 2 mM) (A) or by
raising extracellular Mg$^{2+}$ to 10 mM (Ca$^{2+}$ remaining at 2 mM) \((B)\). \(C\): Simulation of effects of high extracellular Ca$^{2+}$ or Mg$^{2+}$, achieved by inhibition of TTX-insensitive Na$^+$ current \((g_{\text{NS,Na}} = 0.01245, N = 0.005)\).

Fig. 9. Effects of extracellular Cs$^+$ on spontaneous electrical activity and Ca$^{2+}$ transients. \(A\): Representative experimental record. \(B\): Simulation achieved by blockade of spontaneously active K$_{ir}$ channels \((g_{K_{ir}} = 15.25, g_{\text{BK,NEAR}} = 0.495, g_{\text{BK,FAR}} = 9)\).

FIG. 10. Effects of elevated cAMP levels on the pattern of electrical activity and Ca$^{2+}$ transients in spontaneously firing somatotrophs: experiments (\(A\) and \(B\)) and model simulation (\(C\)). \(A\) and \(B\), \textit{left}: GHRH (\(A\)) and IBMX (\(B\))-induced increase in pacemaking activity – representative trace of simultaneous \(V_m\) and Ca$^{2+}$ measurement. \(A\) and \(B\), \textit{right}: mean values for baseline \(V_m\) and burst frequency prior and during GHRH (\(A\)) and IBMX (\(B\)) application \((n = 5, * P < 0.05)\). \(C\): Simulation of effects of GHRH and IBMX is achieved by an increase in conductivity of cAMP-dependent cation channels and decrease in the conductivity of K$_{ir}$ channels \((g_{\text{NS,Na}} = 0.1275, g_{K_{ir}} = 15.65)\).

FIG. 11: Inhibition of spontaneous electrical activity and Ca$^{2+}$ transients by somatostatin (SRIF). \(A\): Representative experimental trace. \(B\): Model simulation, achieved by inhibition of cAMP-dependent cation current and activation of K$_{ir}$ current \((g_{\text{NS,Na}} = 0.05, g_{K_{ir}} = 35)\).
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Table 1
Figure 1
Figure 2
A

0% BK channels block

$V_m$ (mV)

$[Ca^{2+}]_i$ (μM)

HB

dc/dt = 0
B

15% BK channels block

\[ V_m (\text{mV}) \]

\[ [\text{Ca}^{2+}]_i \text{ M} \]

HB

dc/dt = 0