The inward rectifier in a model of corticotroph electrical activity

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Abstract

Pituitary corticotroph cells generate repetitive action potentials and associated Ca^{2+} transients in response to the agonist corticotropin releasing hormone (CRH). There is indirect evidence suggesting that the agonist, by way of complex intracellular mechanisms, modulates the voltage sensitivity of the L-type Ca^{2+} channels embedded in the plasma membrane. We have previously constructed a Hodgkin–Huxley type model of this process, which indicated that an increase in the L-type Ca^{2+} current is sufficient to generate repetitive action potentials [LeBeau et al. (1997). Biophysical Journal 73, 1263–1275]. The agonist is also believed to inhibit an inwardly rectifying K^+ current. In this paper we investigate a role of the inwardly rectifying K^+ current in the action potential firing frequency and membrane excitability. We have found that a CRH-induced inhibition of the inwardly rectifying K^+ current increases the action potential firing frequency and membrane excitability. This structural alteration to the model along with parametric changes bring the model firing frequency in line with experimental data. We also show that the model exhibits experimentally observed bursting behaviour, where the depolarization spike is followed by small oscillations in the membrane potential.

Introduction

CRH is one of the major regulatory hormones linked with the neuroendocrine response to stress (Rivier and Vale, 1983; Gibbs, 1985; Jones and Gillham, 1988). Secreted from the paraventricular nucleus of the hypothalamus, CRH travels through the hypothalmo-pituitary portal system to the anterior pituitary (Merchenthaler et al., 1984; Plotsky et al.,

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1985; Whitnall et al., 1985; Plotsky and Sawchenko, 1987), stimulating the secretion of adrenocorticotropic hormone (ACTH) and other biologically significant hormones from the corticotroph cell population (Antoni, 1986; Rivier and Plotsky, 1986; Jones and Gillham, 1988). Secreted ACTH then initiates the release of adrenal glucocorticoids, which help the body reduce the metabolic demands of stress. These glucocorticoids also provide a negative feedback mechanism, inhibiting the secretory process at the pituitary and hypothalamus (Bilezikjian and Vale, 1983; Keller-Wood and Dallman, 1984; Widmaier and Dallman, 1984).

The intracellular mechanisms underlying the control of ACTH secretion in response to CRH have been only partially characterised. However CRH is known to activate the adenosine 3',5'-cyclic monophosphate (cAMP) dependent protein kinase A (PKA) pathway (Labrie et al., 1982; Aguilera et al., 1983; Reisine et al., 1986; Kuryshev et al., 1995a). The phosphorylation targets for PKA in corticotrophs have not been identified, however PKA is known to phosphorylate L-type voltage dependent Ca^{2+} channels (Mundiña-Weilenmann et al., 1991; Hille, 1992; Sculptoreanu et al., 1993). CRH also induces a membrane depolarization (Mollard et al., 1987), which is associated with the generation of action potentials in quiescent corticotrophs, and increases action potential frequency in spontaneously active corticotrophs (Guérineau et al., 1991; Kuryshev et al., 1996). Associated with the action potentials are Ca^{2+} transients, predominately, if not totally, arising due to Ca^{2+} influx via L-type Ca^{2+} channels (Kuryshev et al., 1996). H-89, an inhibitor of PKA, significantly attenuates CRH-induced action potentials (Kuryshev et al., 1995a), demonstrating a major role for PKA in mediating the changes in electrical excitability and Ca^{2+}-mobilising actions of CRH. There is thus indirect evidence supporting the hypothesis of a CRH-induced PKA-dependent phosphorylation of the L-type Ca^{2+} channels (LeBeau et al., 1997), resulting in action potentials, and Ca^{2+} transients.

The model introduced in LeBeau et al. (1997) is central to our discussion; we henceforth denote it by (I). We found that an increase in the L-type current was sufficient to generate repetitive action potentials from a resting state in the model. The increase in the L-type current could be elicited by either a shift in the voltage dependence of the current to more negative potentials, or by an increase in the conductance. However the model action potential frequency was much higher than observed experimentally. We address this issue in this paper where we obtain action potential frequencies more in line with the experimental data.

CRH is also believed to inhibit K^+ currents that help maintain a negative resting membrane potential (Mollard et al., 1987; Kuryshev et al., 1995a; Kuryshev et al., 1996; Kuryshev et al., 1997; Lee and Tse, 1997). In rat corticotrophs a Ba^{2+}-sensitive inwardly rectifying K^+ channel has been identified. Inhibition of this inwardly rectifying K^+ channel by CRH is believed to contribute to the membrane depolarization and increase in firing frequency (Kuryshev et al., 1997). In this paper we investigate a role of the inwardly rectifying K^+ current in the action potential firing frequency and membrane excitability.

Some corticotrophs exhibit complex action potential kinetics where the depolarization spike is followed by small oscillations in the membrane potential (Kuryshev et al., 1996; Kuryshev et al., 1997; Adler et al., 1983). This complex phenomena is also displayed in

Parameter	Definition	Value	Source
c_{m}	Cell membrane capacitance	7 pF	(I)
$\mathrm{d}_{\mathrm{cell}}$	Cell diameter	15 μm	(I)
V_{cell}	Cell volume	1.77 pL	$1/6\pi\mathrm{d}_{\mathrm{cell}}{}^3$
V_{c}	Cytosolic volume	0.85 V_{cell}	(Alberts et al., 1983)
A_{cell}	Cell surface area	707 $\mu\mathrm{m}^2$	$\pi\mathrm{d}_{\mathrm{cell}}{}^2$
f_{cyt}	Cytosolic Ca^{2+} buffering factor	0.005	This paper
α	Ca^{2+} current to flux density conversion factor	0.0074 $\mu\mathrm{M}\cdot\mu\mathrm{m}\cdot\mathrm{ms}^{-1}\cdot\mathrm{pA}^{-1}$	$1/(z_{\mathrm{Ca}}FA_{\mathrm{cell}})$
β	Ratio of cell surface area to cytosolic volume	0.4 μm^{-1}	$A_{\mathrm{cell}}/V_{\mathrm{c}}$
ν_{p}	Maximum plasma membrane Ca^{2+}-ATPase flux	0.025 $\mu\mathrm{M}\cdot\mu\mathrm{m}\cdot\mathrm{ms}^{-1}$	This paper
K_{p}	$[Ca^{2+}]_{\mathrm{i}}$ for half maximal pump activity	0.08 μM	(I)
$V_{\mathrm{m_L}}$	L-type Ca^{2+} channel midpoint factor	-12 mV	(I)
I_{max}	Inward rectifier reversal potential	-71.5 mV	This paper
$g_{\mathrm{K-IR}}$	Inward rectifier conductance	0.3 nS	This paper
K_{c}	$I_{\mathrm{K-Ca}}$ half maximal $[Ca^{2+}]_{\mathrm{i}}$	0.5 μM	This paper
k_{s}	$I_{\mathrm{K-IR}}$ smoothing factor	6.5	This paper

Table 1: Table of relevant model parameters.

the model. We investigate this model bursting and the underlying mechanisms.

The model

The original model

The original model (I) is of Hodgkin–Huxley form (Hodgkin and Huxley, 1952), and consists of six coupled ordinary differential equations. The model description is similar to the models of Li et al. (1995, 1997), which investigated electrical activity in pituitary gonadotroph cells. For the corticotroph model the potential difference (V) across the plasma membrane satisfies

$$c_{\mathrm{m}}\frac{dV}{dt} = -(I_{\mathrm{Ca-L}} + I_{\mathrm{Ca-T}} + I_{\mathrm{K-DR}} + I_{\mathrm{K-Ca}} + I_{\mathrm{Leak}}), \tag{1}$$

where c_{m} is the cell surface membrane capacitance (see Table 1 for parameter values). Four ionic currents are included in the model: 1) a high-voltage threshold dihydropyridine-sensitive L-type Ca^{2+} current ($I_{\mathrm{Ca-L}}$), responsible for most of the inward Ca^{2+} current during an action potential; 2) a low-voltage threshold rapidly inactivating T-type voltage-sensitive Ca^{2+} current ($I_{\mathrm{Ca-T}}$); 3) a voltage-sensitive K^+ current ($I_{\mathrm{K-DR}}$), predominantly responsible for the action potential repolarization; and 4) a Ca^{2+}-activated K^+ current ($I_{\mathrm{K-Ca}}$). The remaining leak current (I_{Leak}) represents all other ionic current contributions not specifically described. We have previously described the construction of these model ionic currents from electrophysiological measurements in corticotrophs (I).

There is evidence suggesting the existence of other channel types in corticotrophs which include: 1) TTX-sensitive Na^+ channels (Kuryshev et al., 1996); 2) P-type Ca^{2+} channels, contributing to the regulation of firing frequency (Kuryshev et al., 1995b; Kuryshev et al., 1996); and 3) a nonselective cation current (Takano et al., 1996). However these channels are not well characterised in corticotrophs and our desire is to investigate the basic

mechanisms of action potential generation and calcium signalling with as simple a model as possible. Thus we do not include a description of these other channels in the model presented here.

The inward rectifier current

It has been identified recently that a significant ionic current not explicitly included in the original model of CRH-induced corticotroph action potentials is a highly selective inwardly rectifying K^+ current (I_{K-IR}) (Kuryshev et al., 1997). This current contributes to the maintenance of the membrane resting potential. The application of CRH is believed to inhibit this inwardly rectifying current contributing to the CRH-induced depolarization and altering the frequency of the action potentials. This inhibition is believed to occur after some delay. The current through this channel is appropriately described by

$$I_{K-IR}(V) = \begin{cases} g_{K-IR}(V - V_{K-IR}), & \text{if } V \leq V_{K-IR} \\ I_{max}(1 - \exp((V_{K-IR} - V)/k_s)), & \text{if } V > V_{K-IR} \end{cases} \quad (2)$$

where g_{K-IR} is the channel conductance for $V < V_{K-IR}$, I_{max} is the maximal channel current, and k_s determines the rate of inward rectification. As this channel exhibits quick activation kinetics and minimal inactivation (Kuryshev et al., 1997), we do not include an extra differential equation for an activation or inactivation gating variable. The I–V curve for I_{K-IR} is shown in Fig.1 (—). This representation is constructed from data in

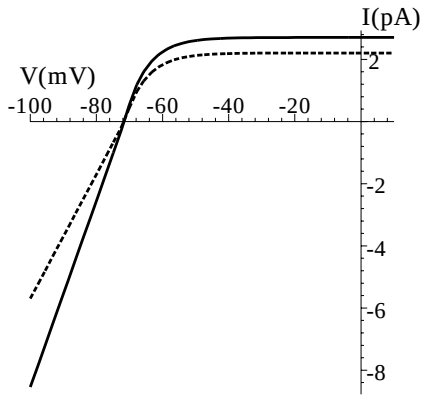

Figure 1: Model current-voltage curve for the inwardly rectifying K^+ current (I_{K-IR}) (—). CRH is believed to inhibit I_{K-IR} by about 25% (– – –).

Kuryshev et al. (1997), Lee and Tse (1997), and Hille (1992). It should be noted some of the experimental data was obtained for $[K^+]_o$ up to 50 mM, and hence were adjusted to the model value of $[K^+]_o = 5.6$ mM. The voltage dependence of the channel gating depends on $[K^+]_o$, shifting with the quantity $RT \log [K^+]_o$. CRH is believed to inhibit I_{K-IR} by about 25% (Kuryshev et al., 1997). Hence to mimic the CRH induced inhibition we accordingly decrease I_{max} from 2.7 pA to 2.2 pA and g_{K-IR} from 0.3 nS to 0.2 nS respectively (Fig.1 (– – –)). This inhibition is believed to occur 45 s after the application of CRH, a time chosen

to correspond with experimental observations (Kuryshev et al., 1997; Ritchie et al., 1996; Lee and Tse, 1997).

In the original model (I), the electrical effects of all ionic currents not explicitly included were lumped together into the leak current I_{Leak}. In place of this leak current we now insert our description of the inward rectifier (Eq. 2). It follows that the plasma membrane potential difference is now given by the differential equation

$$c_{\mathrm{m}}\frac{dV}{dt} = -(I_{\mathrm{Ca-L}} + I_{\mathrm{Ca-T}} + I_{\mathrm{K-DR}} + I_{\mathrm{K-Ca}} + I_{\mathrm{K-IR}}). \tag{3}$$

By comparing Eq. 1 and Eq. 3 it can be seen that the simple ohmic I_{Leak} current has been replaced by $I_{\mathrm{K-IR}}$.

The model equations

We model the cell as a spherical body, bounded by a plasma membrane containing Ca^{2+}-ATPase pumps and various ionic currents as outlined above. A schematic diagram of the various ionic transport processes is shown in Fig.2. Ca^{2+} influx through voltage-sensitive

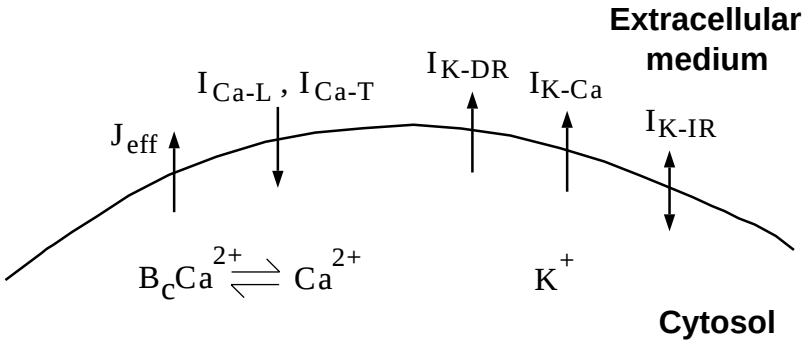

Figure 2: Schematic diagram of the ionic pathways included in the model. Bold arrows indicate the various channels and pumps. Within the cytosol significant portions of Ca^{2+} are bound to buffers B_{c}. Five ionic currents are included in the model: an L-type voltage-sensitive Ca^{2+} current $I_{\mathrm{Ca-L}}$, a fast inactivating T-type voltage-sensitive Ca^{2+} current $I_{\mathrm{Ca-T}}$, a voltage-sensitive K^+ current $I_{\mathrm{K-DR}}$, a non-voltage sensitive Ca^{2+}-activated K^+ current $I_{\mathrm{K-Ca}}$, and an inwardly rectifying K^+ current $I_{\mathrm{K-IR}}$. Also indicated is the plasma membrane Ca^{2+}-ATPase pumps, J_{eff}.

Ca^{2+} channels (J_{in}), and efflux via the plasma membrane Ca^{2+}-ATPase pump (J_{eff}) are given by

$$J_{\mathrm{in}} = -\alpha(I_{\mathrm{Ca-L}} + I_{\mathrm{Ca-T}}), \tag{4}$$

$$J_{\mathrm{eff}} = \frac{\nu_{\mathrm{p}}[\mathrm{Ca}^{2+}]_{\mathrm{i}}^{\,2}}{[\mathrm{Ca}^{2+}]_{\mathrm{i}}^{\,2} + K_{\mathrm{p}}^{\,2}}, \tag{5}$$

respectively, where α converts a Ca^{2+} ionic current into a Ca^{2+} flux density, ν_p is the maximum pump rate, and K_p is the $[Ca^{2+}]_i$ for which the pump is half-maximally activated (see Table 1). The differential equation for $[Ca^{2+}]_i$ is then given by

$$\frac{d[Ca^{2+}]_i}{dt} = f_{cyt}\beta(J_{in} - J_{eff}), \tag{6}$$

where β is the ratio of cell surface area to cytosolic volume, relating ionic fluxes in the plasma membrane to the rate of intracellular concentration accumulation.

Intracellular Ca^{2+} is bound to Ca^{2+} buffers (signified by B_cCa^{2+} in Fig.2), each with as yet undetermined binding kinetics, hence our model of Ca^{2+} buffering is simple. In the cytosol, approximately 99% of Ca^{2+} is bound to buffers (Neher and Augustine, 1992; Tse et al., 1994). Cytosolic Ca^{2+} is also transported into the endoplasmic reticulum (ER), an intracellular organelle that sequesters cytosolic Ca^{2+}. The buffering of Ca^{2+} in the ER is believed to be greater than that in the cytosol, with the ER filling approximately 15% of the cell volume (Alberts et al., 1983, p320). To account for the combined buffering properties of the cytosol and the ER, the fraction of unbuffered intracellular Ca^{2+} is $f_{cyt} = 0.005$. (See Table 1)

We have previously investigated a model including the ER with more complicated Ca^{2+} dynamics (Shorten et al., 1999). We concluded from this investigation that the I_{K-Ca} efflux current used in the original model (I) was too sensitive to $[Ca^{2+}]_i$, and hence we decrease the channel sensitivity by increasing the half-maximal $[Ca^{2+}]_i$ (K_c) from 0.4 μM to 0.5 μM.

Numerical Methods

The system of ordinary differential equations (Eq. 3 and Eq. 6 along with the equations associated with the channel activation gating variables (I)) were solved using a stiff system solver in the numerical package XPPAUT(3.0) *.

Results

Model behaviour

CRH has been shown to activate the cAMP secondary messenger system (Aguilera et al., 1983). Experimentally, application of cAMP has been shown to increase the whole-cell Ca^{2+} current in corticotroph tumour cells (Luini et al., 1985). Such an increase in the current can be generated either by an increase in the macroscopic conductance or by a shift in the voltage-sensitivity of the L-type Ca^{2+} current to more hyperpolarised potentials, the latter effect having experimental support from other cell-types (Nargeot et al., 1983; Mundiña-Weilenmann et al., 1991; Sculptoreanu et al., 1993). In the model, both effects lead to the generation of repetitive action potentials. At present, it would seem that neither the currently available experimental data, nor the model, can resolve which, if any,

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of these mechanisms genuinely underlies action potential generation in response to CRH. Previously we arbitrarily chose to use a shift in the voltage-dependence of the L-type Ca^{2+} current but noted that an increase in the macroscopic conductance always produced very similar results (I). We continue with the same choice in the following analysis. The model parameter that controls the voltage-sensitivity of the L-type current is V_{m_L} (as described in (I)). Analysis of experimental data suggested a control V_{m_L} value of -12 mV under rest conditions, and we typically used a negative shift of 6–8 mV to generate action potentials (I).

One well documented experimental observation in corticotrophs is a small CRH-induced depolarization, which apparently occurs independently of the firing of action potentials (Mollard et al., 1987; Kuryshev et al., 1995a; Kuryshev et al., 1996). These reports indicate that the depolarization is due to a reduction in a K^+ current. A component of this current is a recently identified inward rectifier (Kuryshev et al., 1997) that is active at rest and inhibited by CRH. This inhibition, which is believed to occur after about a 45 s delay, contributes to the membrane depolarization and increases the firing frequency (Kuryshev et al., 1997). Although no clear role for this effect has been determined, it could result in an increase in membrane excitability. In this paper we investigate the role of this inward rectifier channel in the model dynamics.

Action potential frequency

The model action potential period in (I) was approximately 0.5–1s, an order of magnitude smaller than typical experimental data (See Fig.3). We now investigate this to bring the model firing frequency in line with experimental data.

The period of oscillations in the model is highly dependent on the rate of clearance of Ca^{2+} from the cytosol (due to the gradual removal of the hyperpolarizing influence of the Ca^{2+}-activated K^+ current). The model $[Ca^{2+}]_i$ decrease in (I) is much faster than that observed experimentally (Guérineau et al., 1991). To reduce the rate that $[Ca^{2+}]_i$ decreases, we can either reduce the rate at which Ca^{2+} is pumped out of the cytosol, or increase the Ca^{2+} storage capacity of the cytosol by altering the buffering coefficient. The buffering is well established and less amenable to change, hence because the rate of Ca^{2+} removal from the cytosol is less well characterised we decrease the maximum Ca^{2+}-ATPase pump rate ν_p from 0.04 $\mu M \cdot \mu m \cdot ms^{-1}$ to 0.025 $\mu M \cdot \mu m \cdot ms^{-1}$. With these new components introduced into the model we now compare its behaviour with the original model (I), and some experimental data.

In Fig.4 are the model action potentials and $[Ca^{2+}]_i$ transients associated with the application of CRH. These action potentials are initiated after 10 s by firstly left-shifting the voltage sensitivity of the L-type Ca^{2+} current, V_{m_L}, from -12 mV to -20 mV. Secondly, after a 45 s delay, the inhibition of the I_{K-IR} current is mimicked by decreasing I_{max} from 2.7 pA to 2.2 pA and g_{K-IR} from 0.3 nS to 0.2 nS. The period of oscillation is now much slower than in (I) and more consistent with experimentally observed activity (Guérineau et al., 1991; Kuryshev et al., 1996). Note also that 45 s after the application of CRH the action potential firing frequency increases by about 50%. This is consistent with the

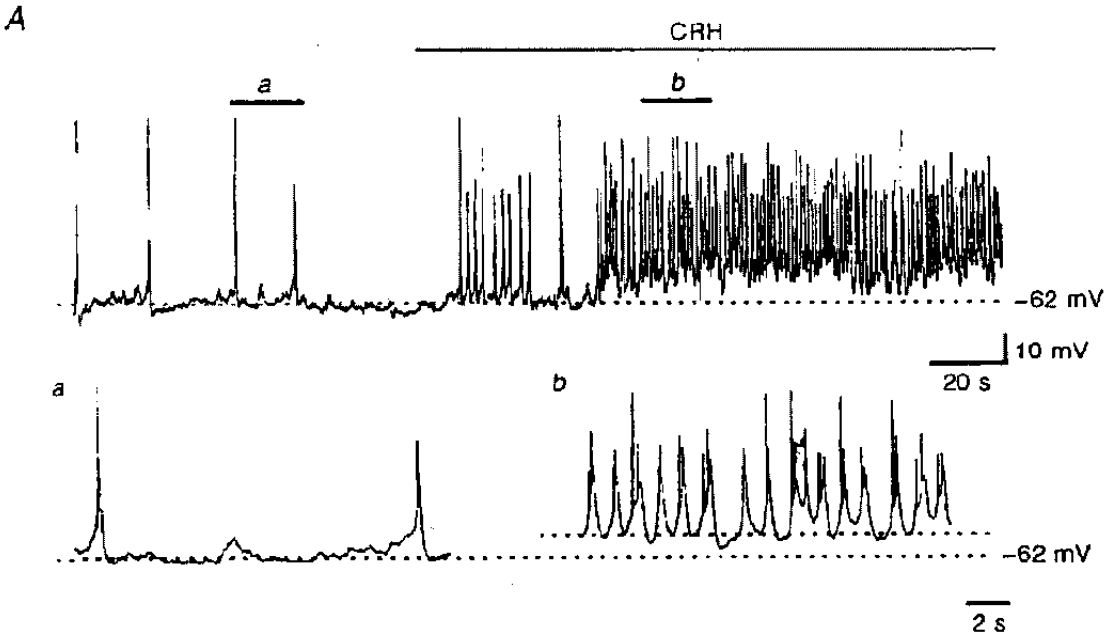

Figure 3: Experimental data showing action potentials and $[Ca^{2+}]_i$ transients in response to CRH. Data reprinted without permission from Kuryshev97. The period of oscillation significantly decreases 45s after the application of CRH. This change in period is also associated with a 10 mV depolarization. Bursting type behaviour occurs in the expanded trace b.

experimental data in Fig.3.

As in the original model (I) the action potentials display typical experimentally observed features such as a rapid upstroke, a rapid downstroke which overshoots the resting potential, and a slow ramping hyperpolarization leading to the firing of the next action potential. The model $[Ca^{2+}]_i$ profiles display kinetic features similar to the experimental data (Guérineau et al., 1991), such as a rapid rising phase, and a slower falling phase where $[Ca^{2+}]_i$ falls most of the way back to its basal value before the next action potential.

The model indicates that the inhibition of I_{K-IR} alone is not enough to generate action potentials. This situation is modeled by setting $V_{m_L} = -12$ mV, $I_{max} = 2.2$ mV and $g_{K-IR} = 0.2$ nS. In this case the inhibition of I_{K-IR} causes a membrane depolarization of 2 mV. This scenario may possibly explain some of the experimental data (Lee and Tse, 1997) where application of CRH causes a membrane depolarization of about 10 mV, but not action potentials. This inhibition also implies that a smaller left shift in the voltage sensitivity of the L-type Ca^{2+} current is required to generate action potentials. Thus the inhibition of I_{K-IR} increases membrane excitability, but is not obligatory for action potential generation.

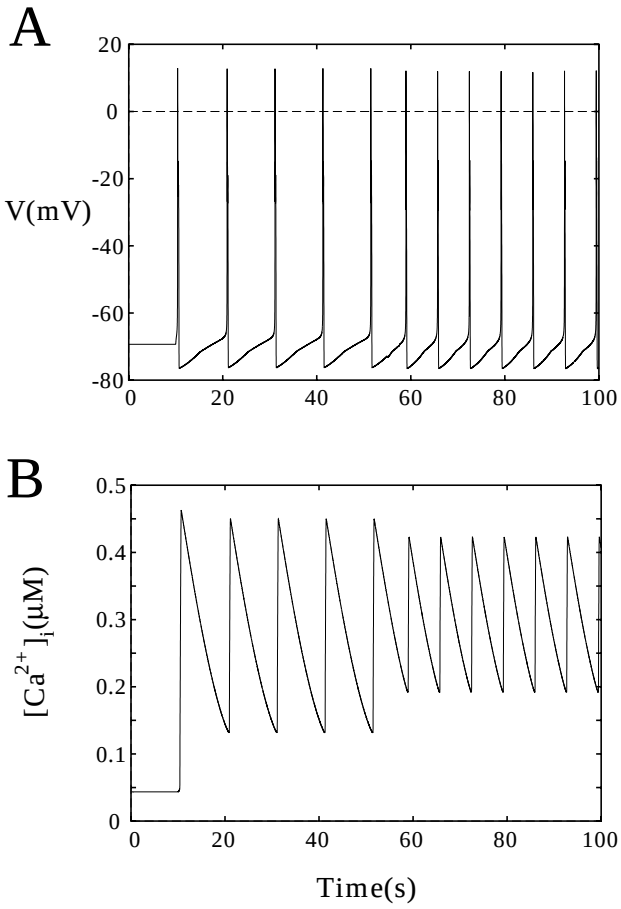

Figure 4: Model action potentials and calcium transients associated with the application of CRH. (A) The action potentials are initiated by firstly left-shifting the voltage sensitivity of the L-type Ca^{2+} current. Secondly, after a 45 s delay, inhibition of the inwardly rectifying K^+ current (I_{K-IR}) causes the action potential firing frequency to double. This is consistent with the experimental data in Fig.3. (B) Each action potential is associated with a single $[Ca^{2+}]_i$ transient.

Bursting

An interesting feature of the model is that of bursting. This behaviour is consistent with experimental observations (Kuryshev et al., 1996; Kuryshev et al., 1997; Adler et al., 1983). The model action potentials are shown over a much shorter time scale in Fig.5 compared to Fig.4. The initial depolarization spike is followed by two smaller oscillations in the membrane potential which compares well with the experimental trace in Fig.3. From the quiescent state, both V and $[Ca^{2+}]_i$ start to increase. V soon peaks just above 10 mV, and then begins to repolarize as $[Ca^{2+}]_i$ increases further. Note that $[Ca^{2+}]_i$ increases considerably during the repolarization phase, because although the L-type Ca^{2+} current is being shut off as V falls, the driving force for Ca^{2+} is increasing. These post-spike

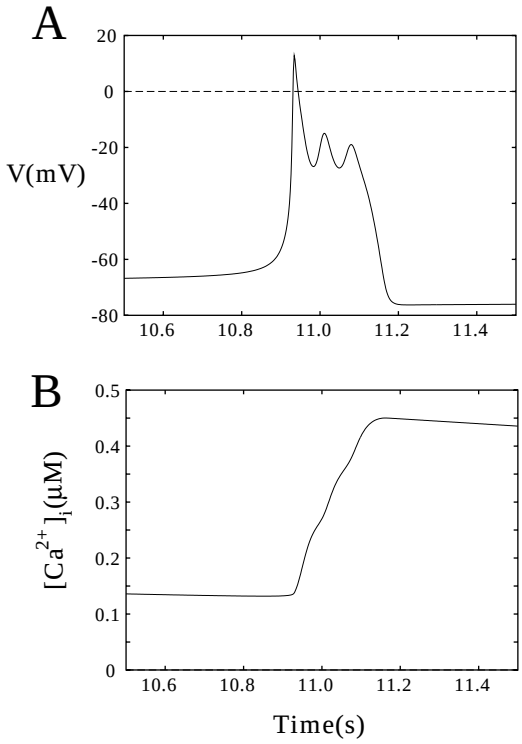

Figure 5: (A) CRH induced model action potentials shown over a much shorter time scale. The initial depolarization spike is followed by two smaller oscillations in the membrane potential which compares well with the experimental trace in Fig.3. (B) The bursting ends when $[Ca^{2+}]_i$ is sufficiently high to activate the Ca^{2+}-activated K^+ current allowing a repolarization of the membrane potential.

oscillations occur while $[Ca^{2+}]_i$ is still increasing, and terminate when $[Ca^{2+}]_i$ is sufficiently high to activate the I_{K-Ca} current allowing a repolarization of the membrane potential. In the model the I_{K-DR} and I_{K-IR} currents together cannot repolarize the membrane potential. The added influence of the I_{K-Ca} current is necessary. However because the rise in $[Ca^{2+}]_i$ is slower than the depolarization in the membrane potential there is a delay in the activation of the I_{K-Ca} current allowing the observed bursting behaviour. When V reaches its minimum value of -76 mV, the rise in $[Ca^{2+}]_i$ is complete, and V slowly ramps up as $[Ca^{2+}]_i$ falls. This represents the interspike interval. When V reaches about -65 mV, the next spike is initiated. Although it cannot be resolved in Fig.4, after the initial spike the following spikes display the same bursting behaviour.

Bursting behaviour in a model of corticotroph electrical activity has previously been analysed from a more mathematical viewpoint (LeBeau et al., 1998). However significant parameter changes were made in that work to generate the different aspects of the bursting phenomena. The current model of the bursting is more physiological with the period of oscillation, the parameter values and $[Ca^{2+}]_i$ transient levels more consistent with the experimental data.

The model bursting pattern depends on the delicate balance between inward and outward currents. It is therefore difficult to isolate the current responsible for the bursting pattern. However model simulations suggest that the duration of the bursting is due to the gradual increase in $[Ca^{2+}]_i$ and the related I_{K-Ca} current. Traditional action potentials with a much faster rehyperpolarization can be obtained from the model by suitably increasing or decreasing the K^+ or Ca^{2+} currents respectively (not shown).

The model resting state is slightly different from that in the original model (I); at equilibrium the $[Ca^{2+}]_i$ is 0.04 μM (compared with 0.1 μM in (I)) and the plasma membrane potential difference V is -69 mV (compared with -55 mV in (I)). These resting model values are slightly lower than what is generally found in corticotrophs. However the model only includes the major ionic currents behind action potential generation, with no formal descriptions of the many other smaller uncharacterised ionic currents (some of which are described in the original model section above). These smaller currents, although less significant in action potential generation will play a more crucial role in setting the resting membrane potential.

Therefore these results suggest that an integrated response to CRH (i.e., activation of the L-type Ca^{2+} current and the inhibition of the inward rectifier) is important in the CRH induced plasma membrane electrophysiology.

Summary

To investigate mechanisms by which CRH induces membrane electrical activity, we have extended a model of corticotroph electrical activity (I) by including a representation of a recently identified inwardly rectifying K^+ current. We tested the potential role of a CRH-induced reduction of an inward rectifier K^+ current, an effect observed experimentally by Kuryshev et al., 1997. We found that this reduction increased the action potential firing frequency and membrane excitability, but was not obligatory for action potential generation. These results support the hypothesis that a delayed inhibition of an inwardly rectifying K^+ current is responsible for an observed delayed increase in action potential firing frequency after the application of CRH. However the inhibition of the inwardly rectifying K^+ current did not produce an experimentally observed 10 mV depolarization. Possibly the CRH-induced depolarization may involve the modulation of other channels, or the absence of smaller uncharacterised ionic currents from the model, which are less significant in action potential generation, may eliminate the possibility of this depolarization.

The previous model action potential firing frequency was an order of magnitude smaller than typical experimental data. Our modified model has firing frequency more consistent with experimental observations.

Some corticotrophs exhibit bursting action potential kinetics where the depolarization spike is followed by small oscillations in the membrane potential (Kuryshev et al., 1996; Kuryshev et al., 1997; Adler et al., 1983). We investigated model bursting behaviour and the underlying mechanisms. Our model produced results similar to those observed experimentally.

Using this model we have found that an increase in the L-type Ca^{2+} current is sufficient to initiate repetitive action potentials in an all-or-none manner from a previous quiescent model state as observed experimentally. It must be emphasized that our hypothesis of action potential generation via a PKA-induced enhancement of the L-type Ca^{2+} current remains valid.

CRH may also affect other ion channels through PKA or some other CRH-receptor-coupled G-protein activated kinase. Possibilities identified include the acceleration in the activation kinetics of a hyperpolarization-activated K^+/Na^+ current (Ritchie et al., 1996) (which may increase the spike frequency), the inhibition of a K^+ current active at depolarized potentials (which could create a longer action potential allowing greater Ca^{2+} entry) and the inhibition of a BK-type Ca^{2+}-activated K^+ current (Shipston et al., 1996). It is therefore possible that the observed bursting behaviour or membrane depolarization depends on ionic currents or CRH-induced effects not explicitly included in the model.

The present study represents a step in a project to construct a model describing the major aspects of the regulation of ACTH secretion in response to CRH and other ACTH regulators. Many of the intricate details surrounding Ca^{2+} signaling and the CRH-induced exocytotic pathway remain to be resolved.

Acknowledgments

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