Generation of Action Potentials in a Mathematical Model of Corticotrophs

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ABSTRACT Corticotropin-releasing hormone (CRH) is an important regulator of adrenocorticotropin (ACTH) secretion from pituitary corticotroph cells. The intracellular signaling system that underlies this process involves modulation of voltage-sensitive Ca^{2+} channel activity, which leads to the generation of Ca^{2+} action potentials and influx of Ca^{2+}. However, the mechanisms by which Ca^{2+} channel activity is modulated in corticotrophs are not currently known. We investigated this process in a Hodgkin-Huxley-type mathematical model of corticotroph plasma membrane electrical responses. We found that an increase in the L-type Ca^{2+} current was sufficient to generate action potentials from a previously resting state of the model. The increase in the L-type current could be elicited by either a shift in the voltage dependence of the current toward more negative potentials, or by an increase in the conductance of the current. Although either of these mechanisms is potentially responsible for the generation of action potentials, previous experimental evidence favors the former mechanism, with the magnitude of the shift required being consistent with the experimental findings. The model also shows that the T-type Ca^{2+} current plays a role in setting the excitability of the plasma membrane, but does not appear to contribute in a dynamic manner to action potential generation. Inhibition of a K^+ conductance that is active at rest also affects the excitability of the plasma membrane.

INTRODUCTION

Corticotropin-releasing hormone (CRH) is a major agonist of adrenocorticotropin (ACTH) secretion from anterior pituitary corticotroph cells (Antoni, 1986; Jones and Gillham, 1988; Alexander et al., 1994). CRH induces ACTH secretion through the activation of the cyclic adenosine monophosphate (cAMP) second messenger system (Aguilera et al., 1983), which results in the activation of protein kinase A (PKA) (see Alberts et al., 1994). Although there appear to be a number of phosphorylation targets for PKA in corticotrophs, both in the plasma membrane and cytosolic fractions (Liu, 1994), these have not yet been identified. The ACTH secretory response to CRH also requires the influx of Ca^{2+} from outside the cell, which occurs primarily, if not exclusively, through voltage-sensitive Ca^{2+} channels (VSCCs) (Abou-Samra et al., 1987; Won and Orth, 1990; Guérineau et al., 1991; Kuryshev et al., 1995a,b).

Corticotrophs generate spontaneous and CRH-induced Ca^{2+} action potentials, with each action potential generating a Ca^{2+} transient (Fig. 1) (Guérineau et al., 1991; Kuryshev et al., 1995b, 1996a). Although corticotrophs express a number of different VSCCs, including T, L, P, and at least one unidentified Ca^{2+} channel type (Marchetti et al., 1987; Guérineau et al., 1991; Kuryshev et al., 1995a; Loechner et al., 1996), L-VSCCs appear to be the major channel type that underlies Ca^{2+} action potential generation (Kuryshev et al., 1996a). H-89, a PKA inhibitor, substantially attenuates CRH-induced action potentials and intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) responses (Kuryshev et al., 1995b), suggesting that PKA is an important regulator of Ca^{2+} influx via VSCCs. Consistent with this hypothesis is the finding that L-VSCCs are targets for phosphorylation by PKA and other kinases (Kaczmarek, 1988; Hille, 1992; McDonald et al., 1994). Therefore, these findings suggest a mechanism whereby PKA, activated after exposure to CRH, phosphorylates L-VSCCs and promotes Ca^{2+} action potential generation with consequent Ca^{2+} influx. The concomitant rise in $[Ca^{2+}]_i$ then activates exocytotic pathways, resulting in the secretion of ACTH.

Clearly, PKA-induced action potential activity is of fundamental importance in this secretory pathway. However, the mechanism by which PKA activates L-VSCC is currently unknown, and represents a major gap in the scheme for the intracellular regulation of ACTH, described above. Investigation of this aspect is therefore important in establishing the complete pathway for the regulation of ACTH secretion.

In this paper we investigate PKA regulation of L-VSCC activity using a mathematical model of corticotroph plasma membrane electrical responses. The model includes explicit descriptions of four plasma membrane ionic channels, which allows investigation of the role and actions of each channel type in corticotroph electrical responses.

THE MODEL

The model is a Hodgkin-Huxley-type formalism (Hodgkin and Huxley, 1952), consisting of six coupled ordinary differential equations. The model includes specific descrip-

Received for publication 29 October 1996 and in final form 5 June 1997.
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0006-3495/97/09/1263/13 $2.00

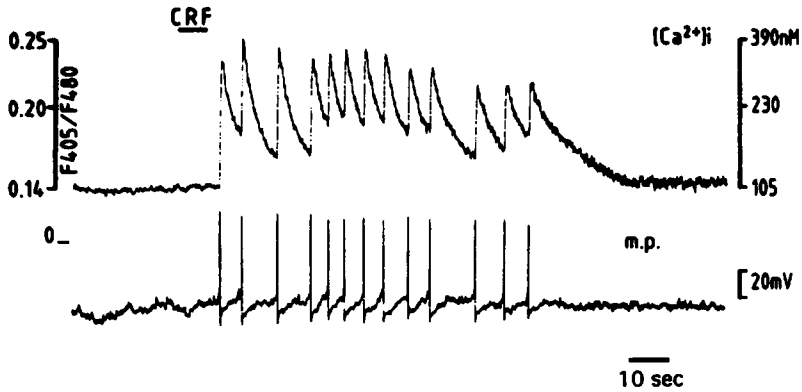

FIGURE 1 Corticotroph experimental data showing a train of action potentials and associated $[Ca^{2+}]_i$ transients in response to the application of CRH (= CRF), as indicated by the horizontal bar. Data are reprinted with permission from Guérineau, N., J.-B. Corcuff, A. Tabarin, and P. Mollard. (1991). Spontaneous and corticotrophin-releasing factor-induced cytosolic calcium transients in corticotrophs. Endocrinology 129(1):409–420. ©The Endocrine Society.

tions of L- and T-type voltage-sensitive Ca^{2+} currents, delayed-rectifier (K-DR) and Ca^{2+}-activated (K-Ca) K^+ currents, and a leakage current that represents the remainder of the plasma membrane ionic currents.

Although similar to a number of models for membrane electrical activity, the corticotroph model most closely resembles the model of Li et al. (1995), which describes spontaneous electrical activity in pituitary gonadotroph cells. Using data from electrophysiological investigations on corticotroph cells, we have determined, as much as possible, model parameter values specific to corticotrophs. Although gonadotrophs and corticotrophs utilize a number of similar processes in generating their respective cellular responses, the behavior of the cells in response to their respective agonists (primarily gonadotropin-releasing hormone (GnRH) in gonadotrophs) differs in many respects (Stojilković and Catt, 1992). In determining parameter values for corticotrophs, we found that several were similar to those for the "gonadotroph model" (Li et al., 1995), whereas others were quite different. As this paper shows, the behaviors of the corticotroph and gonadotroph models are also quite different in some respects.

In this paper we investigate the possible effects of CRH on plasma membrane electrical activity. We are not concerned at present with the dynamics of the $[Ca^{2+}]_i$. This is a complex matter, which we investigate in a subsequent paper. However, the membrane electrophysiology and $[Ca^{2+}]_i$ responses are integrally linked, and intracellular Ca^{2+} cannot be excluded, even though the focus is the electrical responses. Therefore, in this report, we use the simple Ca^{2+} handling mechanisms from the gonadotroph model and treat $[Ca^{2+}]_i$ as spatially uniform, as in the "lumped cytoplasm" version of the gonadotroph model (Li et al., 1995).

Model equations

The equation for the membrane potential (V) is given by

$$c_m \frac{dV}{dt} = -(I_{Ca-L} + I_{Ca-T} + I_{K-DR} + I_{K-Ca} + I_{Leak}) \quad (1)$$

where c_m is the cell-surface membrane capacitance. The voltage-dependent ionic currents (I_{Ca-L}, I_{Ca-T}, and I_{K-DR}) are each described by the product of their macroscopic conductances, one or more voltage-dependent activation/inactivation gating variables (which represent the fraction of current flow for a given membrane potential), and a driving force that is specific for each ion.

I_{Ca-L} represents the ionic current through the L-type Ca^{2+} channel, and is given by

$$I_{Ca-L} = g_{Ca-L} m_L^2 \phi_{Ca} \quad (2)$$

where g_{Ca-L} is the conductance; m_L is the activation gating variable, which is raised to the second power (Li et al., 1995); and ϕ_{Ca} is the driving force for Ca^{2+} given by the Goldman-Hodgkin-Katz (GHK) current equation (see Hille, 1992):

$$\phi_{Ca}(V) = V \frac{C - C_e e^{-[z_{Ca}FV/(RT)]}}{1 - e^{-[z_{Ca}FV/(RT)]}}, \quad (3)$$

where C is $[Ca^{2+}]_i$ and C_e is the extracellular Ca^{2+} concentration, which is taken to be constant. F is Faraday's constant, R is the gas constant, T is the absolute temperature, and z_{Ca} is the valence of Ca^{2+}. The driving force for K^+, ϕ_K, is defined similarly, with appropriate changes to the ionic concentrations (both of which are considered to remain constant) and valence (see Table 1).

We do not include inactivation of the L-type current, because inactivation in corticotrophs (Guérineau et al., 1991; Kuryshev et al., 1995a), as in other cells (Hille, 1992), is a slow process compared to the duration of an action potential. Thus negligible inactivation would be expected to occur during each action potential.

I_{Ca-T}, the fast inactivating or T-type Ca^{2+} channel, is defined similarly to the L-type current:

$$I_{Ca-T} = g_{Ca-T} m_T^2 h_T \phi_{Ca} \quad (4)$$

except that an inactivation gating variable (h_T) is included.

I_{K-DR}, the voltage-sensitive K^+ current, is given by

$$I_{K-DR} = g_{K-DR} n \phi_K, \quad (5)$$

TABLE 1 Parameter values for the corticotroph model

Parameter	Definition	Value	Ref/Source*
d_{cell}	Cell diameter	15 μm	This paper, 1
A_{cell}	Cell surface area	700 μm^2	πd_{cell}^2
V_{cell}	Cell volume	1.77 pL	$\frac{1}{6}\pi d_{cell}^3$
c_m	Cell surface capacitance	7 pF	1 $\mu F \cdot cm^{-2} \cdot A_{cell}$
f	Ca^{2+} buffering factor	0.01	2, 3
α	$1/(z_{Ca}FA_{cell})$	7.4 $\mu M \cdot \mu m \cdot s^{-1} \cdot pA^{-1}$	
β	A_{cell}/V_{cell}	0.4 μm^{-1}	
C_e	Extracellular $[Ca^{2+}]$	2 mM	4
K_e	Extracellular $[K^+]$	5.6 mM	4
K_i	Intracellular $[K^+]$	140 mM	5
g_{Ca-L}	L-VSCC conductance	9 nS $\cdot$ mM^{-1}	4
g_{Ca-T}	T-VSCC conductance	10 nS $\cdot$ mM^{-1}	4
g_{K-DR}	K-DR conductance	0.1 nS $\cdot$ mM^{-1}	1
g_{K-Ca}	K-Ca conductance	0.09 nS $\cdot$ mM^{-1}	1
K_C	$[Ca^{2+}]_i$ for half-maximum K-Ca activation	0.4 μM	This paper
g_L	Leak conductance	0.3 nS	This paper
V_{m_L}	Midpoint L-VSCC activation	−12 mV	4, 6
k_{m_L}	Slope factor, L-VSCC activation	12 mV	4, 6
V_{m_T}	Midpoint T-VSCC activation	−30 mV	4, 7
k_{m_T}	Slope factor, T-VSCC activation	10.5 mV	4, 7
V_{n_T}	Midpoint T-VSCC inactivation	−57 mV	4, 7
k_{h_T}	Slope factor, T-VSCC inactivation	5 mV	4, 7
V_n	Midpoint K-DR activation	−20 mV	1, 7
k_n	Slope factor, K-DR activation	4.5 mV	1, 7
V_τ	Midpoint of time factor	−60 mV	5
k_τ	Slope factor for time factor	22 mV	5
V_L	Leak current reversal potential	−67 mV	This paper
$\bar{\tau}_{m_L}$	L-VSCC activation time constant	27 ms	6
$\bar{\tau}_{m_T}$	T-VSCC activation time constant	10 ms	5
τ_{h_T}	T-VSCC inactivation time constant	15 ms	5
τ_n	K-DR activation time constant	20 ms	This paper
ν_p	Ca-ATPase maximum flux density	40 $\mu M \cdot \mu m \cdot s^{-1}$	This paper
K_p	$[Ca^{2+}]_i$ for half-maximum pump activity	0.08 μM	This paper
τ	Ca^{2+} exchange time constant	500 ms	5
C_{eq}	Equilibrium $[Ca^{2+}]_i$	0.1 μM	This paper, 5

*Code for the references: 1, Mollard et al. (1987); 2, Neher and Augustine (1992); 3, Tse et al. (1994); 4, Guérineau et al. (1991); 5, Li et al. (1995); 6, Kuryshev et al. (1995a); 7, Marchetti et al. (1987).

in which the activation variable, n, appears linearly (Li et al., 1995), and ϕ_K is the GHK expression for K^+.

Corticotrophs express both large conductance (BK-type) (Wong et al., 1982; Shipston et al., 1996) and small conductance, apamin-sensitive (SK-type) (Corcuff et al., 1993) Ca^{2+}-activated K^+ conductances. These channels differ in their voltage sensitivity (BK channels are sensitive to the membrane potential, whereas SK channels are insensitive) and in their respective sensitivities to $[Ca^{2+}]_i$, with the SK channel being ~100-fold more sensitive than the BK channel (see Hille, 1992). Neither of the channels has been particularly well defined in corticotrophs, and so we do not include specific descriptions of each channel type. Furthermore, as we are using a spatially uniform $[Ca^{2+}]_i$, the Ca^{2+} sensitivity of the model channel will not be the same as that seen in the cells. Therefore, we include a single Ca^{2+}-activated K^+ current that does not display voltage-sensitivity:

$$I_{K-Ca} = g_{K-Ca} \frac{C^4}{C^4 + K_C^4} \phi_K \quad (6)$$

where K_C is the half-maximally activating $[Ca^{2+}]_i$. The inclusion of both a voltage-sensitive and a Ca^{2+}-sensitive K^+ current means that we cover the two major mechanisms by which K^+ conductances are activated during action potentials, even though some features of these channels and several other channel types are not explicitly included.

There is evidence that voltage-sensitive Na^+ channels are also present in some, but not all, corticotrophs (Corcuff et al., 1993; Kuryshev et al., 1996a). However, Na^+ channels do not appear to play a major role in corticotroph Ca^{2+} responses (Kuryshev et al., 1996a) or ACTH secretion (Halili-Manabat et al., 1995). Thus it appears that although they may have subtle modulatory effects, Na^+ channels are not crucial to action potential generation in corticotrophs (the focus of this particular investigation), and so we do not include a Na^+ current in our model. There is evidence that other Ca^{2+} channel types are present in corticotrophs (Corcuff et al., 1993), including P-type and another, as yet uncharacterized voltage-sensitive current (Kuryshev et al., 1995a). However, these currents are not well defined at present, and it is not yet clear what roles they might perform. L-type VSCCs appear to be the principal component of both action potential generation (Kuryshev et al., 1996a) and secretion (Artalejo et al., 1994; Loechner et al., 1996),

and so, at this stage, we do not include Ca^{2+} currents other than T- and L-type channels. In future, as we attempt to resolve finer details of action potential regulation, we will consider including specific descriptions of some of the other ionic currents. However, adding more channels would increase the complexity of the model, and we feel that this factor outweighs the potential benefits of including the other currents at this stage of the investigation.

I_{Leak}, the leakage current, is given by

$$I_{Leak} = g_L(V - V_L) \tag{7}$$

which reflects the electrical effects of all of the ionic currents not explicitly included in the model. The reversal potential of the leak current (V_L) has a value of -67 mV, i.e., close to the theoretical Nernst equilibrium potential for K^+, reflecting the fact that K^+ conductances are the major component of the remaining membrane current.

The activation gating variables are given by

$$\tau_x \frac{dx}{dt} = x_\infty - x \tag{8}$$

where x stands for m_L, m_T, h_T, and n. The equation describes how each activation variable relaxes to its (voltage-dependent) steady-state value, x_∞, following a particular time course (τ_x). The steady states, x_∞, for m_L, m_T, and n are sigmoidal relationships given by

$$x_\infty = \frac{1}{1 + e^{-[(V - V_x)/k_x]}} \tag{9}$$

which gives values between 0 (no activation) and 1 (fully activated). V_x is the voltage at which the current is half-maximally activated (i.e., $x_\infty = 0.5$), and k_x represents the steepness of the curve, with a smaller value reflecting a steeper slope.

h_{T_∞}, the steady-state inactivation of the T-type channel, is a reverse sigmoidal function:

$$h_{T_\infty} = \frac{1}{1 + e^{[(V - V_h)/k_h]}} \tag{10}$$

The time constants for m_L and m_T are given by

$$\tau_x(V) = \frac{\bar{\tau}_x}{e^{[(V - V_\tau)/k_\tau]} + 2e^{-[(2(V - V_\tau))/k_\tau]}} \tag{11}$$

(Li et al., 1995), which gives a slightly skewed bell-shaped relationship, typical of voltage-sensitive channels (Hille, 1992).

The time constants for h_T and n are voltage independent (Li et al., 1995).

At this stage we are interested only in the effects of Ca^{2+} at the plasma membrane, so we assume that the $[Ca^{2+}]_i$ is spatially uniform and instantly mixed. Thus the equation for the $[Ca^{2+}]_i$ is given by

$$\frac{dC}{dt} = J_{exch} + f\beta(J_{in} - J_{eff}). \tag{12}$$

J_{exch} represents exchange (primarily uptake) of Ca^{2+} with intracellular sequestering organelles, principally the endoplasmic reticulum (ER), and is given by

$$J_{exch} = \frac{C_{eq} - C}{\tau}, \tag{13}$$

in which the $[Ca^{2+}]_i$ relaxes to an equilibrium value C_{eq}, with a time factor τ. This form has the advantage that a separate equation for the Ca^{2+} concentration in the ER is not required. However, this form has the disadvantage that it treats the ER as an infinite sink for Ca^{2+}. Clearly this is not the case; during a Ca^{2+} transient some of the Ca^{2+} that enters the cell will be sequestered by the ER and will then have to be released by the ER and removed from the cell to maintain cellular Ca^{2+} homeostasis. This process is not incorporated in the model, and it is not clear how significant this effect might be. Maintenance of cellular Ca^{2+} balance is a complex process that is not considered in detail here. Our current focus is the membrane electrical processes, and we consider cellular Ca^{2+} in more detail in a subsequent paper.

The factor f in Eq. 12 represents the Ca^{2+} buffering capacity of the cytoplasm (Neher and Augustine, 1992; Tse et al., 1994), and β represents the ratio of the cell surface area to the cell volume.

Influx of Ca^{2+} via L- and T-type VSCCs is given by

$$J_{in} = -\alpha(I_{Ca-L} + I_{Ca-T}) \tag{14}$$

where α incorporates the valence of Ca^{2+}, Faraday's constant, and the cell surface area (see Table 1).

Removal of Ca^{2+} from the cell via a Ca-ATPase pump is described by

$$J_{eff} = \nu_P \frac{C^2}{C^2 + K_P^2}, \tag{15}$$

where ν_P is the maximum rate of pumping and K_P is the $[Ca^{2+}]_i$ at which the pump is half-maximally activated. Ca^{2+} pump characteristics are not well characterized in corticotroph cells, and so to aid in determining values for the pump parameters, we used the criterion that $J_{in} \approx J_{eff}$ at rest.

Determination of parameter values

To determine the parameter values for the model, we used, as much as possible, data from studies on corticotrophs, particularly Mollard et al. (1987), Marchetti et al. (1987), Guérineau et al. (1991), and Kuryshev et al. (1995a). From our analyses of these data, we found that some aspects of corticotroph electrophysiology, such as the relative magnitudes of the individual ionic currents, differ quite substantially both between and within individual reports, whereas other aspects, such as the voltage sensitivity of the Ca^{2+} currents, were very similar. However, where comparisons were possible, the general electrophysiological and $[Ca^{2+}]_i$

responses of corticotrophs, described in these reports, are similar. Table 1 gives the standard values of the model parameters.

Numerical methods

The system of equations (Eq. 1, the four versions of Eq. 8, and Eq. 12) was solved using the XPPAUT 2.0 software written by Bard Ermentrout (e-mail: bard@mthbard.math.pitt.edu). Bifurcation diagrams were produced using the implementation of AUTO (Doedel, 1981) included in XPPAUT.

RESULTS

Validation of the model

Before investigating the effects of CRH on corticotroph membrane electrical responses, it is necessary to verify that the model is able to simulate electrophysiological responses that have been observed in corticotrophs. At least some aspects of these responses (for example, the magnitudes of the individual ionic currents) differ between, and even within, individual reports (for example, see Marchetti et al., 1987). Therefore it would be unreasonable to expect the model to accurately reproduce all previously observed experimental results. However, the model should be able to reproduce general features of corticotroph electrophysiology.

The voltage-clamp technique, used now in conjunction with the patch-clamp technique, has for some time been the cornerstone of electrophysiological investigation. Therefore, to confirm that the model behaves in a manner similar to that of experimental preparations, we simulated voltage-clamp experiments. This was done by removing the membrane potential (V) as a variable, and including it instead as a parameter that could be held constant, as desired, and then changed instantaneously to a new constant value.

Using the voltage-clamp configuration of the model, we simulated experimental protocols from investigations of corticotroph electrophysiology, i.e., the papers that we had used to obtain model parameter values (see Table 1). We tested the effects of stepping from various holding potentials and/or using voltage steps of varying magnitude. To simulate experimental protocols such as the addition of specific ion channel blockers, the relevant currents were abolished by setting their conductance values to zero. Specific examples of experiments we simulated include figure 3 *B* of Mollard et al. (1987) and figure 2 *B* of Guérineau et al. (1991). We found that in many respects, the model behaved very similarly to the experimental responses (not shown). There were some minor differences between the experimental data and model simulations, but analysis of these suggested that they were due to simplifications of the model, compared with the biological system, and were not unreasonable. Therefore, under voltage-clamp conditions at least, the model appears to be a good representation of corticotroph electrophysiology.

Model behavior under resting conditions

With the parameter set given in Table 1, the model has a single steady-state solution that corresponds to a resting membrane potential of approximately −54 mV and a resting $[Ca^{2+}]_i$ of just over 100 nM. No oscillatory solution exists, at least within the physiological range of the variables. In experimental cell preparations, resting corticotrophs (i.e., in the absence of agonists), like resting gonadotrophs (Li et al., 1995), exhibit both quiescent and spontaneously active states (Guérineau et al., 1991; Corcuff et al., 1993; Kuryshev et al., 1995b). It is not clear whether these two states represent separate subpopulations of cells (i.e., spontaneously active cells are always spontaneously active and likewise for quiescent cells) or variant behavior from a single population, where all cells may exhibit both behaviors. At this stage, the model behavior corresponds to quiescent corticotrophs and does not represent spontaneously active cells. This situation is different from the gonadotroph model, in which both a stable steady-state and a stable oscillatory solution coexisted, and therefore both resting behaviors can be exhibited without any changes in the model parameters (Li et al., 1995). At this stage we do not attempt to suggest a mechanism for the generation of spontaneous electrical activity in corticotrophs. Instead we consider secretagogue-induced electrical activity, and reconsider spontaneous activity later (see the Discussion).

CRH-induced generation of action potentials

How does CRH induce the increase in membrane electrical activity and concomitant Ca^{2+} influx seen in experiments on corticotrophs in culture (Guérineau et al., 1991; Kuryshev et al., 1995b, 1996a)? CRH-induced electrical activity has been shown to be dependent on activation of the cAMP second messenger system (Luini et al., 1985; Kuryshev et al., 1996a)—specifically, the activation of cAMP-dependent protein kinase (PKA) is required (Kuryshev et al., 1995b). Because modulation of L-type Ca^{2+} channels by phosphorylation has now been widely demonstrated (see Kaczmarek, 1988; Hille, 1992; McDonald et al., 1994), there is strong but indirect evidence supporting a CRH-induced, PKA-mediated facilitation of L-type Ca^{2+} channels in corticotrophs.

The evidence for PKA-activated Ca^{2+} influx is convincing but indirect, and so does not suggest any specific mechanisms by which facilitation of the current occurs. Thus a causal link between PKA activation and enhanced L-type channel activity is yet to be firmly established. The major goal of the work described here is to use our model to investigate whether direct modulation of L-VSCC activity by PKA can generate action potentials in corticotrophs and, if so, how this might occur.

One effect of PKA activation on the macroscopic kinetics of L-type Ca^{2+} currents that has been observed in several reports is a shift to more negative potentials of the voltage dependence of activation of the current (Nargeot et al.,

1983; Mundiña-Weilenmann et al., 1991; Sculptoreanu et al., 1993a,b; Yuan and Bers, 1995). Shifts of 6–10 mV have typically been observed. Luini et al. (1985) investigated the effects of PKA activation on Ca^{2+} currents of ACTH-secreting AtT-20 cells, a mouse corticotrophic tumor cell line. They found that the cAMP analog 8Br-cAMP caused a 35% increase in the peak of the voltage-dependent Ca^{2+} current (the individual Ca^{2+} currents were not separated). However, the authors state that neither the threshold for activation nor the voltage at maximum current amplitude was changed, suggesting that a shift in the voltage dependence of the L-type Ca^{2+} current did not occur. From an initial assessment of their data (Fig. 2 *A*), this conclusion seems entirely reasonable. However, close inspection of the figure suggests that the voltage of peak current may be shifted leftward by a few millivolts. A small effect such as this would probably be too small to resolve in terms of statistical significance, and so the authors' conclusions remain perfectly valid. However, this does not mean that a small shift in the voltage sensitivity of the L-type current, if indeed it were to occur, would not have a significant effect on the electrical activity of the cell.

On the other hand, there may have been no shift in the voltage sensitivity of the L-type current, and the increase in the peak Ca^{2+} current observed by Luini et al. (1985) could be caused by an increase in the macroscopic conductance of the L-type current. Such an increase could arise through an increase in the number of functional channels and/or in the proportion of channels available to be activated (McDonald et al., 1994).

Therefore, we tested whether either a shift to the left in the voltage dependence, or an increase in the conductance of the L-type Ca^{2+} current was consistent with the data from Luini et al. (1985). We used the voltage-clamp configuration of our model to simulate this experiment and followed the protocol described by Luini et al. (1985). A holding potential of −80 mV was used, and the conductances for both K^+ currents (g_{K-DR} and g_{K-Ca}) and the leak current (g_L) were set to zero, to leave just the Ca^{2+} currents active. We stepped to test potentials between −70 and +70 mV and recorded the peak inward current. The model parameter that controls the voltage sensitivity of the L-type Ca^{2+} current is V_{m_L}, the voltage at which the L-type current is half-maximally activated. From experimental data a value of −12 mV was derived for this parameter (Table 1), which represents the control situation in the absence of CRH (or other PKA-activating agents). We tested the effects of making V_{m_L} more negative to simulate the proposed leftward shift in the voltage sensitivity induced by PKA. On the other hand, to simulate an increase in the conductance of the L-type current, g_{Ca-L} was increased.

The current-voltage curves obtained in the simulated experiment are shown in Fig. 2, *B* and *C*. Fig. 2 *B* shows the effects of left-shifting V_{m_L} from −12 mV to −16, −20, and −24 mV. Two features are immediately obvious: 1) shifts of up to 12 mV (to −24 mV) had only a very subtle effect on the voltage at which the threshold or peak of the simu-

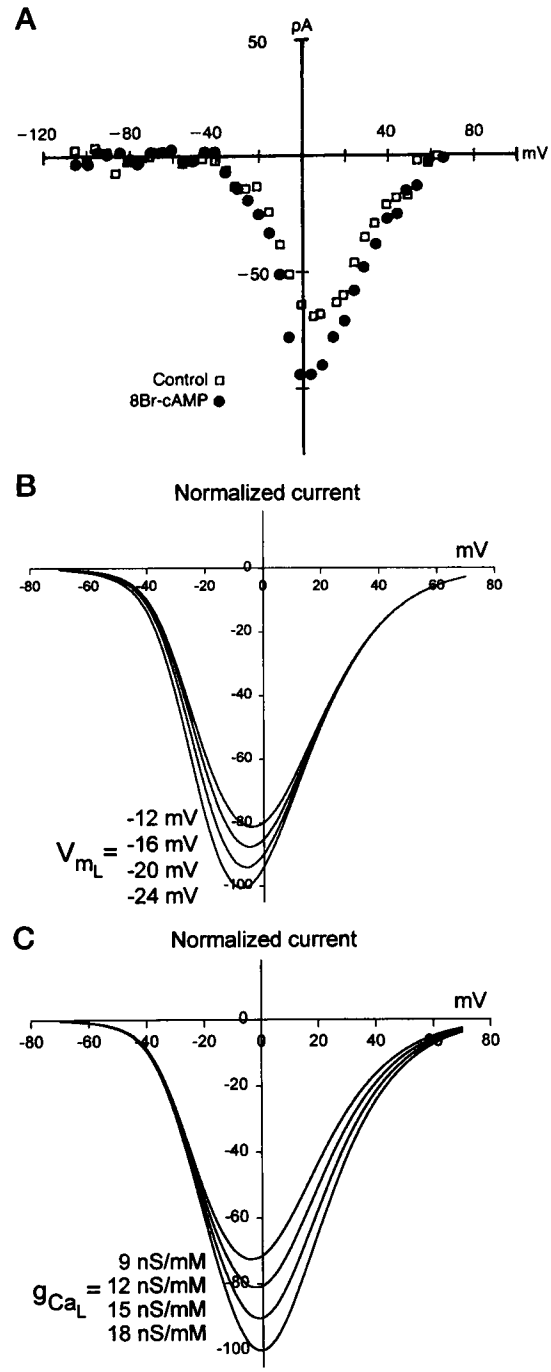

FIGURE 2 Modulation of corticotroph Ca^{2+} currents. (*A*) Experimental data, reprinted with permission from Luini et al. (1985), showing the effects of a cAMP analog 8Br-cAMP (10 μM) on the voltage-dependent Ca^{2+} current. The current-voltage curve was generated by stepping from −80 mV to test potentials over the range −120 to +70 mV, and recording the peak current. (*B*) Model voltage clamp simulations demonstrating the effect of making V_{m_L} more negative, from the control value of −12 mV. K^+ currents and the leak current were removed by setting the conductances of these currents to zero. Peak current was determined after depolarizing steps from −80 mV to test potentials over the range −70 to +70 mV. For convenience, the data were normalized to a maximum value of 100 pA to facilitate comparison with the experimental data. (*C*) As for *B*, except that here the effect of increasing the conductance of the L-type Ca^{2+} current (g_{Ca-L}, control value 9 nS/mM), rather than its voltage sensitivity, was determined.

lated whole-cell Ca^{2+} currents occurred, and 2) the shifts caused an increase in the peak amplitude of the whole-cell Ca^{2+} currents. Overall, the simulation data bear a good resemblance to the experimental data (Fig. 2 *A*).

Fig. 2 *C* shows the simulation experiments in which g_{Ca-L} was increased, from the control value of 9 nS · mM^{-1} to 12, 15, and 18 nS · mM^{-1}. Here, too, the amplitude of the peak current was enhanced, and the simulation data resemble the experimental data (Fig. 2 *A*).

The two simulated data sets are quite similar and both resemble the experimental data. Thus, although intuition might suggest that the data from Luini et al. (1985) support an increase in L-type Ca^{2+} conductance rather than a shift in the voltage dependence of activation, the model simulations suggest that either explanation is consistent with the experimental data.

Therefore we investigated the effects of either left-shifting the voltage sensitivity or increasing the conductance of the L-type Ca^{2+} current on the behavior of the model. To test the effects of a shift in the voltage sensitivity, V_{m_L} was made progressively more negative and changes in the membrane potential were monitored. This analysis is termed *bifurcation analysis*; the results are shown in Fig. 3. The resting, steady state condition of the model occurs at the right-hand side of the figure, at $V_{m_L} = -12$ mV. This corresponds to a membrane potential, V, of about −54 mV. As the value of V_{m_L} is made more negative, V gradually becomes less negative, and the system remains stable. However, when V_{m_L} reaches −16.0 mV, a "critical point" (point *q* in Fig. 3) is reached and the nature of the system changes: the steady-state solution becomes unstable (denoted by the *dashed line*), and a periodic solution emerges. The maximum and minimum values for V of the periodic solution, which is unstable, are denoted by the two branches extending from the critical point (*dotted lines*). Initially, these branches extend almost perpendicularly (bending slightly backward) with respect to the steady-state solution. When the amplitude of the periodic solution (i.e., the difference between the maximum and minimum values) is ~50 mV, the curve of the periodic solution turns around, the solution becomes stable (*solid lines*), and the amplitude increases more slowly as V_{m_L} is made more negative.

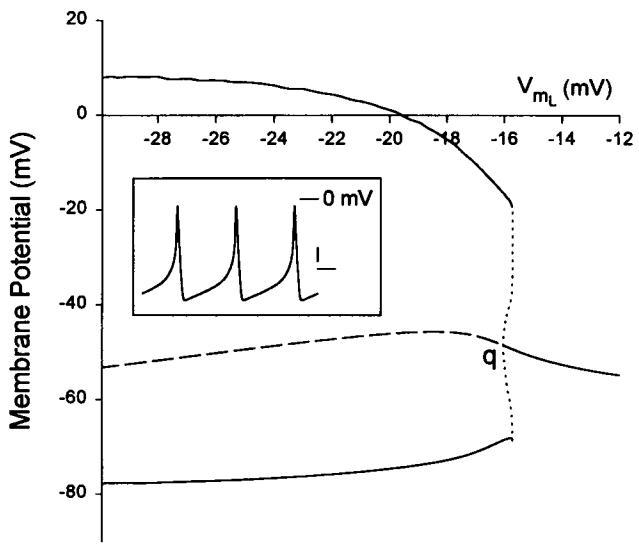

FIGURE 3 Bifurcation analysis of the model with respect to V_{m_L}. The initial state of the model, corresponding to the parameter values given in Table 1, occurs at $V_{m_L} = -12$ mV. Stable solutions of the model are represented by solid lines. Initially the model has a single, stable solution. As V_{m_L} is made progressively more negative, the model retains stability until $V_{m_L} = -16.0$ mV (point *q*), whereupon a periodic solution emerges, and the steady-state solution becomes unstable (*dashed line*). The periodic solution extends sharply away from the "critical point" and is unstable (*dotted line*). As V_{m_L} is made more negative, the periodic solution becomes stable (*solid lines*). (*Inset*) Model action potentials. Setting V_{m_L} to −18 mV results in the appearance of regenerating, all-or-none action potentials, that display features such as rapid upstrokes and downstrokes, afterhyperpolarizations and slow ramping of the membrane potential, leading to the firing of the next action potential.

In electrophysiological terms, the effect of shifting V_{m_L} to more negative potentials is to generate action potentials, once the critical point is crossed. The inset to Fig. 3 shows regenerative action potentials that occur at $V_{m_L} = -18$ mV. The action potentials display typical features such as a rapid upstroke, and similarly rapid downstroke, which overshoots the resting potential and thus generates an afterhyperpolarization. The membrane potential slowly ramps back up until the threshold is reached and another action potential is fired.

An important feature of the analysis shown in Fig. 3 is the way in which the periodic solution arises and extends from the critical point. Shifting V_{m_L} by up to 4 mV from its original value has little effect on the system, which would appear to still be in a "resting state." However, the effect of the almost perpendicular extension of the periodic solution from the critical point, with respect to the steady-state solution, is that a very small change in V_{m_L} results in the generation of action potentials with an amplitude of ~50 mV. The shift of V_{m_L} required to generate action potentials is as little as a fraction of a millivolt, as long as it takes the value across the critical point. This effect corresponds to the concept of "all-or-none" action potential firing. Thus, at the critical point, a change in a single parameter can radically alter the behavior of the system in a manner that corresponds to an observed biological phenomenon.

To test whether an increase in the conductance of the L-type Ca^{2+} current can also induce action potentials, we performed a similar bifurcation analysis in which the conductance of the L-type current (g_{Ca-L}) was increased (and V_{m_L} was kept at its resting value). This analysis showed that increasing g_{Ca-L} also resulted in the generation of action potentials. The critical value of g_{Ca-L} was 16.8 nS · mM^{-1} (the resting value was 9 nS · mM^{-1}), and, when this value was reached or exceeded, action potentials were generated in an all-or-none manner, as was found for the shift of V_{m_L}. The kinetics of the action potentials were also similar, although the amplitude of action potentials was increased (~80 mV for $g_{Ca-L} = 16.8$ nS · mM^{-1} compared with ~50 mV for $V_{m_L} = -16.0$ mV).

Thus both of the putative effects that were considered with regard to the data of Luini et al. (1985) also lead to the generation of action potentials in the model. However, this is not surprising, considering the functional form of the L-type Ca^{2+} current in the model (Eq. 2). Equation 2 shows that the L-type current is described by the product of g_{Ca-L} and m_L (and the driving force). The leftward shift of V_{m_L} results in an increase in the value of m_L. Therefore, both of the mechanisms described above will have essentially the same effect on I_{Ca-L}, that is, both will increase the numerical value of the current. Thus these investigations reveal that, in the model at least, an increase in the L-type Ca^{2+} current is sufficient (i.e., modulation of other ionic currents is not necessary) for the generation of action potentials. However, in its current form, the model cannot differentiate between the two possible underlying mechanisms. Previous experimental evidence, from other cell types, appears to favor a shift in the voltage sensitivity rather than an increase in the ionic conductance. However, further experimental investigation in corticotrophs is required to resolve this issue. For the additional investigations of the model behavior that we describe below, we use a shift of V_{m_L} as the mechanism underlying action potential generation. However, for all of these investigations, an increase in g_{Ca-L} could be substituted without any significant difference in the behaviors observed.

Fig. 4 shows model simulations in which action potential activity (*lower curve*) was induced by changing V_{m_L} from -12 mV to -18 mV. As in the experimental data (Fig. 1) from Guérineau et al. (1991), each action potential is associated with a single $[Ca^{2+}]_i$ transient (*upper curve*). The model $[Ca^{2+}]_i$ transients display kinetic features similar to those from the experimental data, such as a rapid rising phase, and a slower falling phase. Furthermore, during these trains of action potentials, $[Ca^{2+}]_i$ can be seen to fall most, but not all of the way, to its basal value before the next action potential.

In the experimental data shown in Fig. 1, the application of CRH (during the horizontal bar) induced action potentials, after a short delay, which continued for some time after the end of the CRH exposure. In the simulation shown in Fig. 4, to simulate the putative effects of CRH, V_{m_L} was instantaneously changed from its resting value of -12 mV to -18 mV, at which action potentials were induced. Cessation of action potentials occurred after a similar change back to -12 mV. Clearly, because the mechanisms underlying the generation of action potentials by CRH are not fully characterized, neither are the kinetics of the response well known. If, for example, a shift in the voltage sensitivity of the L-type Ca^{2+} current was the underlying mechanism, this would presumably occur as PKA phosphorylated the individual channels. The magnitude and rate of the shift in the macroscopic voltage sensitivity would be a function of the level of PKA activation. Conversely, loss of action potential activity, after the removal of CRH, would result from phosphatase-induced dephosphorylation of the channels (Wiechen et al., 1995) and a return of the voltage sensitivity to its basal condition. Because these effects remain uncharacterized, we have simulated them simply as instantaneous changes in the voltage sensitivity.

One feature of $[Ca^{2+}]_i$ transients that has been observed in corticotrophs (Guérineau et al., 1991) and in some other pituitary cells (Mollard et al., 1989, 1994) but not, apparently, in gonadotrophs (Li et al., 1995), is that the $[Ca^{2+}]_i$ continues to rise for between a few hundred milliseconds to 1–2 s, after the completion of the action potential (or return to the holding potential in voltage-clamp experiments). Such an effect does not (and cannot) occur in the model because Ca^{2+} influx ceases as soon as the membrane potential becomes repolarized. This suggests that more complex regulation of $[Ca^{2+}]_i$ occurs in the cells. We do not address this feature of a delayed $[Ca^{2+}]_i$ peak here, as this report is focused on the generation of action potentials. Instead, we investigate this mechanism in a later report in which we use more complex $[Ca^{2+}]_i$ handling mechanisms.

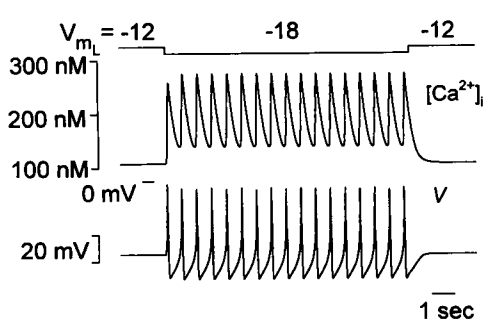

FIGURE 4 Model action potentials and associated $[Ca^{2+}]_i$ transients. Model simulations showing the effects on the membrane potential and $[Ca^{2+}]_i$ of changing V_{m_L} from -12 mV to -18 mV and then returning the value to -12 mV. As in the experimental data, each action potential evokes a single $[Ca^{2+}]_i$ transient.

Role of T-type current in the generation of action potentials

The fast activating and inactivating T-type Ca^{2+} channels are thought to act as a trigger for the generation of Ca^{2+} action potentials (Cohen and McCarthy, 1987). These channels appear to be well suited to this function, because of their low voltage activation threshold and rapid kinetics (voltage-sensitive Na^+ channels may play a similar role; Schlegel and Mollard, 1995). However, the lack of a specific blocker of the T-type current makes it difficult to investigate this scheme. The discovery of non-T-type currents that have activation thresholds between those of T- and L-type currents (a DHP-sensitive current with a low threshold, Corcuff et al., 1993; and a P-type Ca^{2+} current, Kuryshev et al., 1995a, 1996a) further adds to the question of the precise role of the T-type current. Because we have found that an increase in the L-type current was sufficient to generate action potentials, we wished to determine what influence the T-type current has on action potential generation in corticotrophs.

The relative contribution of the T-type current to the total ionic fluxes, during an action potential (Fig. 5 *A*) in the model, can be seen in Fig. 5 *B*. In terms of the magnitude of the individual fluxes, the contribution of the T-type current is small. However, this is not unexpected, considering the kinetics of the T-type current, and this current may still play a crucial role in action potential generation, depending on the relative ionic contributions at different times. Fig. 5 *C* shows the magnitudes of the two Ca^{2+} currents during the inter- and early-spike periods (see Fig. 5 *A*). The figure shows that the T-type Ca^{2+} current is larger than the L-type Ca^{2+} current during the interspike period, when V is slowly ramping up before the generation of the action potential. Thus, at this stage, the model behavior appears to be consistent with the "trigger" role of the T-type current.

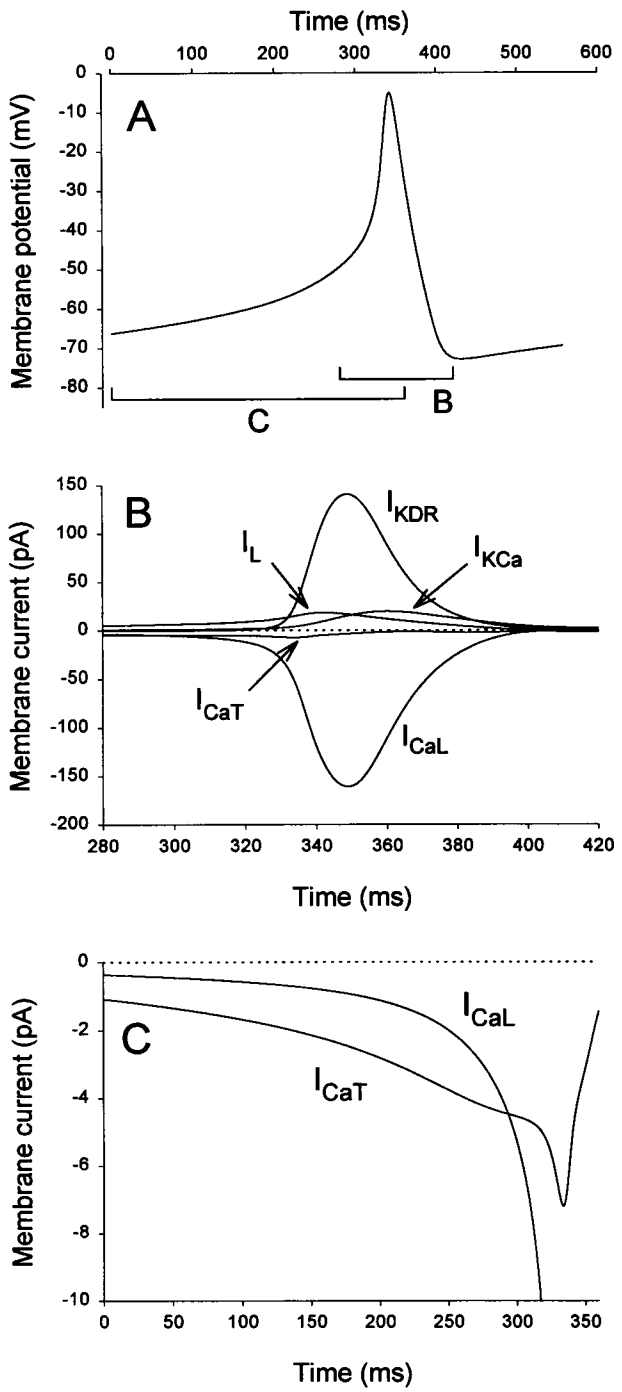

FIGURE 5 Characterization of the individual ionic currents of the model. (*A*) A single action potential at $V_{m_L} = -18$ mV. The time frames corresponding to *B* and *C* are indicated. (*B*) Magnitudes of the five individual ionic components during the action potential. The dotted line denotes the zero current line. (*C*) Individual Ca^{2+} currents during the interspike interval and early part of the action potential.

Although it is not currently possible to specifically block the T-type current in cells, our model allows us to simulate such an experiment, by setting the conductance of the T-type current, g_{Ca-T}, to zero. This resulted in a hyperpolarization of the membrane potential to approximately -67 mV and a decrease in the resting Ca^{2+} concentration to ~68 nM. These changes reflect the contribution of the T-type current to the resting membrane potential and basal $[Ca^{2+}]_i$, and may be considered as "passive" effects. For the T-type current to act as a trigger, we feel that this implies that the T-type current acts in a dynamic (i.e., in response to changes in V) manner in the generation of action potentials. Before investigating whether this is the case (by removing the dynamic actions of the T-type current), the passive effects must be separated out, so they can be retained in the model.

To do this, I_{Ca-T} was removed from the equations for V (Eq. 1) and Ca^{2+} influx (Eq. 14), and, to maintain Ca^{2+} homeostasis (i.e., $J_{in} \approx J_{eff}$ at rest), the numerical value of the steady-state influx of Ca^{2+} through T-type channels was calculated and added to the Ca^{2+} influx equation (Eq. 14) in place of I_{Ca-T}. As expected, influx via T-type channels accounts for virtually all Ca^{2+} influx at rest, and this level of influx now remains as a constant addition to the total influx.

To account for the electrical effects of the T-type current at rest, V_L, the reversal potential of the leakage current, was adjusted from -67 mV to -56 mV, to bring the resting membrane potential back to approximately -54 mV, as in the full model. This change essentially means that the T-type current is relegated to the leakage current. Although, in the model, the magnitude of the leakage current changes during the firing of an action potential (Fig. 5 *B*), this does not represent the opening and closing of channels, but rather represents a change in the current flow through each open channel (i.e., a change in the driving force).

The effects of these changes on the generation of action potentials, by a leftward shift of V_{m_L}, were investigated. We found that as V_{m_L} was shifted leftward, there was little change in the way oscillations arose, compared with the full model (not shown). The critical point occurred at $V_{m_L} = -15.7$ mV (-16.0 mV in the full model), and the branches of the periodic solution extended sharply away from the critical point. Thus removal of the dynamic aspects of the T-type current had virtually no effect on the generation of action potentials by a shift in the voltage dependence of the L-type Ca^{2+} current (or by an increase in g_{Ca-L}).

Action potentials, at $V_{m_L} = -18$ mV, and their associated $[Ca^{2+}]_i$ transients were also similar to those from the full model (not shown).

Role of closure of K^+ current in modulating membrane excitability

An experimentally observed effect of CRH on corticotroph electrical responses, distinct from the generation and modulation of action potentials, is a small depolarization of the membrane potential (Mollard et al., 1987; Kuryshev et al., 1995b, 1996a). The depolarization has been attributed to CRH-induced closure of a K^+ conductance that contributes to the resting membrane potential. Mollard et al. (1987) suggested that the current was similar to K^+ M-currents (Adams et al., 1982; Brown, 1988), whereas Kuryshev et al. (1996b) reported that the conductance displayed inwardly rectifying properties. Kuryshev et al. (1995b) demonstrated that the slow depolarization itself was not sufficient to induce action potential activity, and spontaneous action potential activity occurred in the absence of the CRH-induced depolarization. These results suggest that the inhibition of the K^+ conductance, although not critical for the generation of action potentials, may play an important modulatory role in the membrane electrical response to CRH.

To investigate the role of modulation of a K^+ conductance in the generation of action potentials in response to CRH, we tested the effects of manipulation of the resting membrane potential on the appearance of action potentials after a leftward shift of V_{m_L}. Manipulation of the membrane potential was achieved by changing V_L, the reversal potential of the leakage current. Because the putative K^+ current has not been characterized, it would be premature to include an explicit description of this conductance. Instead, manipulation of the membrane potential was used to simulate the effects of inhibition of the current. Kuryshev et al. (1995a, 1996a) found that in quiescent corticotrophs, exposure to CRH caused a slow depolarization of the membrane potential from about -60 mV to around -49 mV. Spontaneously active corticotrophs tended to have slightly less negative resting membrane potentials, but, after exposure to CRH, the slow depolarization was typically smaller in magnitude, such that the same membrane potential of approximately -49 mV was achieved.

To simulate these effects with the (full) model, the value of V_L was set to -68.5 mV, which gave a resting membrane potential of -60.4 mV. The effect of shifting V_{m_L} to the left (or increasing g_{Ca-L}) was then determined. To simulate the effects of inhibition of the K^+ conductance, V_L was then set to -64 mV, to give a membrane potential of -49.7 mV, and the effects on the generation of action potentials were again examined. Raising the membrane potential, to simulate closure of the K^+ conductance, caused a rightward shift of the critical value of V_{m_L}, from which action potentials arose, from -16.6 mV to -14.9 mV. When an increase in g_{Ca-L} was used to generate action potentials, the same change in V_L caused the critical point to shift from 18.6 to 13.9 nS · mM^{-1}. Therefore, depolarization of the plasma membrane means that a smaller shift of either V_{m_L} or g_{Ca-L} is required to generate action potentials. However, the depolarization itself was not sufficient to generate action potentials, which is consistent with experimental evidence (Kuryshev et al., 1995b).

DISCUSSION

To investigate possible mechanisms by which CRH, through PKA, induces membrane electrical activity, we describe here a model that includes explicit descriptions of the major ionic conductances that have been identified as being present in corticotrophs. Using this model, we have shown that an increase in the L-type Ca^{2+} current is sufficient to generate repetitive action potentials from a previously resting state of the model. This finding establishes a causal relationship between enhancement of the L-type current by the cAMP/PKA signaling system, observed experimentally (Luini et al., 1985; Mollard et al., 1987; Guérineau et al., 1991; Kuryshev et al., 1995b), and the generation of action potentials. Although modulation of other ionic conductances may participate in the integrated electrophysiological response, the model results suggest that this is not obligatory for action potential generation.

Two separate mechanisms, a leftward shift in the voltage sensitivity and an increase in the macroscopic conductance, led to an increase in the L-type current and thus the generation of action potentials. Experimental investigation is necessary to determine whether either, or possibly both, of these mechanisms are responsible for PKA-mediated enhancement of the L-type current in corticotrophs. Previous reports, but not from corticotrophs, have demonstrated that PKA caused a leftward shift in the voltage sensitivity of the L-type Ca^{2+} current (Nargeot et al., 1983; Mundiña-Weilenmann et al., 1991; Sculptoreanu et al., 1993a,b; Yuan and Bers, 1995). Shifts of 6–10 mV were typically reported. The shift required to generate oscillations in the model was ~4 mV, well within the experimentally observed range. Thus the leftward shift in the voltage sensitivity is a physiologically reasonable, experimentally well-supported mechanism.

The alternative mechanism for generating action potentials was an increase in the macroscopic conductance of the L-type current. The increase required was less than a doubling of the conductance. However, because we were unable to locate an experimental report describing an increase in the macroscopic conductance, we cannot say at present whether the increase required was physiologically reasonable. In the model, the macroscopic conductance represents the maximum conductance possible when all of the available channels are fully open. An increase in the conductance could be obtained if the total number of available channels were increased. This could occur either by the addition of new channels, or by the conversion of previously unavailable channels to an available state (McDonald et al., 1994).

The hypothesis that action potentials can be generated solely by an enhancement of the L-type Ca^{2+} current, and the mechanisms underlying this, must be tested experimentally. However, we have found that the generation of action potentials by the mechanisms described above is a robust feature of the model. In determining appropriate parameter values for the model, and testing the sensitivity of the model to changes in various parameters, the shift required to generate oscillations was always found to be within the range typically observed experimentally (for V_{m_L} at least). Furthermore, because of the way that the branches extend from the critical point (the point at which action potentials arise in the model) as either V_{m_L} is shifted leftward or g_{Ca-L} is increased, the model generates action potentials in an all-or-none manner, as typically observed (Guérineau et al., 1991; Kuryshev et al., 1995b, 1996a). Taken together, these findings give us confidence that the model behavior we describe here reflects genuine cellular effects.

In corticotrophs, experiments in which simultaneous measurements of membrane potential and $[Ca^{2+}]_i$ have been made show that each action potential is associated with a single Ca^{2+} transient (Guérineau et al., 1991). Furthermore, CRH-induced action potentials do not appear to fire in bursts (Guérineau et al., 1991; Kuryshev et al., 1995b, 1996a), as in pancreatic β cells (Sherman et al., 1988). Action potentials and Ca^{2+} transients in the model, induced by a shift of V_{m_L} or g_{Ca-L}, share these features with the experimental observations.

We report here that an enhancement of the L-type Ca^{2+} current is sufficient to generate action potentials. However, CRH will induce a number of cellular effects that may play modulatory roles to provide an integrated cellular response. Indeed, PKA has been found to cause changes in the level of phosphorylation of a number of corticotroph cell proteins (Liu, 1994), and CRH appears to utilize both PKA-dependent and PKA-independent mechanisms (Kuryshev et al., 1995b), to generate a coordinated cellular secretory response. Therefore, although a change in a single parameter can give rise to action potential activity, we would not expect that all features of the CRH-induced cellular response could be explained by this one change.

One CRH-induced effect in corticotrophs is a small depolarization of the membrane potential that precedes action potential firing (Mollard et al., 1987; Kuryshev et al., 1995b, 1996a). This depolarization results from the CRH-induced closure of a K^+ conductance that is active at rest. In the absence of PKA activity, inhibition of the K^+ current did not, in itself, cause the generation of action potentials (Kuryshev et al., 1995b), suggesting that this effect plays a modulatory role in the electrical response to CRH. A simulated depolarization in the model caused the critical point for action potential generation (by a leftward shift of V_{m_L}) to be shifted right by almost 2 mV, meaning that a smaller leftward shift of V_{m_L} was needed to generate action potentials. A similar effect occurred when g_{Ca-L} was used to induce action potentials. These results suggest that inhibition of the K^+ current raises the excitability of the plasma membrane, aiding PKA-induced generation of action potentials. Consistent with experimental observations (Kuryshev et al., 1995b), inhibition of the K^+ current by itself was not sufficient to generate action potential activity in the model.

T-type Ca^{2+} channels have been considered to play a trigger role in action potential generation. In light of our finding that an enhancement of the L-type current was sufficient to induce action potentials, we wished to investigate the role of the T-type current. Our analysis showed that if the steady-state effects of the T-type current were maintained, removing the voltage-dependent, or dynamic, effects of the current had virtually no effect on the generation of action potentials. This finding calls into question the trigger role and instead suggests that the T-type current plays more of a role in setting the excitability of the membrane.

In support of such a role, we have found that small changes in the voltage-sensitive kinetics of the activation and inactivation variables of the T-type current can cause considerable changes in the resting conditions of the model. This is not surprising, considering that the T-type current is active under resting conditions and the voltage sensitivities of both activation and inactivation variables are steep functions of V. Thus it appears that the T-type current may act in opposition to K^+ currents that are active at rest, in determining the resting excitability level of corticotrophs.

Spontaneous electrical activity is a commonly observed feature of corticotrophs (Guérineau et al., 1991; Kuryshev et al., 1995b, 1996a). However, the physiological significance of this activity is questionable, considering that in the single-cell preparations used for these studies, the cells are relieved of the normal constraints and regulation of their neighbor cells (Schlegel and Mollard, 1995). Furthermore, the complete absence of agonists, which equates to resting conditions in vitro, is a situation that may never actually occur in vivo (Alexander et al., 1994), where there is always some level of agonist present in the portal blood that perfuses the pituitary.

Despite these concerns, it is still important that the model be able to simulate commonly observed experimental results, such as spontaneous electrical activity. There has been no systematic experimental investigation into why some corticotrophs display spontaneous activity and others do not, and so there are few clues to indicate how spontaneous activity arises. Analysis of the model, however, provides a possible explanation: in a cell, different levels of expression of cellular constituents, such as an increased endogenous PKA activity, could result in the voltage sensitivity of the L-type current being close to the critical point. Fluctuations in ionic currents or other stochastic effects could then cause the system to cross the critical point for short periods, generating irregular, spontaneous action potentials.

The present study represents the first stage of a project whose overall goal is to construct a model describing the major aspects of the regulation of ACTH secretion in response to hypothalamic factors and other regulators of ACTH release. Through this approach, we hope to advance the current understanding of the regulation of ACTH secre-

tion, and, because the intracellular mechanisms involved in this response are common to many cell types, this investigation may also provide general insights into cellular signaling systems.

To develop a model for ACTH secretion, it is necessary to identify and investigate gaps in the current knowledge. The causal relationship between the enhancement of the L-type Ca^{2+} current and the generation of action potentials that we demonstrate here helps to establish and clarify the link between these two features of the response to CRH. Furthermore, our investigations have suggested two possible mechanisms for how the enhancement might occur, which are readily open to experimental investigation.

We thank Dr. Yue-Xian Li for advice and helpful discussion, and Prof. Julius Axelrod, Dr. Patrice Mollard, and the Endocrine Society for permission to reprint figures.

This work was supported by grants from the Canterbury Medical Research Foundation, the New Zealand Lottery Grants Board (AP047957), and the Marsden Fund of the Royal Society of New Zealand.

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