THE EFFECTS OF CALCIUM^{++} ON BURSTING NEURONS

A MODELING STUDY

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ABSTRACT Many observed effects of ionized calcium on bursting pacemaker neurons may be accounted for by assuming that calcium has multiple effects on the membrane conductance mechanisms. Two models are proposed that represent extreme cases of a set of possible models for these multiple effects. Both models are a priori designed to account for directly observed phenomena, and both are found to be able to simulate a posteriori certain observed phenomena, including persistent inactivation, increasing spike width, and decreasing after-polarization. Experimental tests are proposed for the decision of validity between the set of models discussed and the null hypothesis, and for the decision of validity between the two models themselves. Extensions of the models are discussed. One of these extensions leads to a simulation of the behavior of the cell when placed in a calcium-free bathing medium.

INTRODUCTION

Many spontaneously active pacemaker neurons display a form of activity known as bursting. This activity, endogenous within the cell (Alving, 1968), consists of the regular alternation of bursts of action potentials with silent periods during which the cell is hyperpolarized. A complete discussion of bursting cells, together with a review of the early literature, is given by Carpenter (1973). A mathematical model has been developed to simulate many of the features displayed by bursting pacemaker neurons (Plant and Kim, 1975, 1976). Other modeling and theoretical studies have also been conducted (Thompson; 1976, Both et al., 1976; Pinsker, 1977; Gulrajani et al. 1977).

The features simulated by the model of Plant and Kim include the existence of the slow wave in tetrodotoxin (TTX) (Strumwasser, 1968; Mathieu and Roberge, 1971), the phenomenon of resetting (Strumwasser, 1967), the N-shaped steady-state current voltage curve (Wilson and Wachtel, 1974; Gola, 1974), and the effects of ouabain and injected current (Junge and Stephens, 1973). While it is possible to develop very simple systems of equations whose solution has a bursting waveform, such systems do not incorporate known properties of the cell membrane, and it is unlikely that they will be able to predict or interpret behavioral features of the cell other than bursting. The crucial features of the model of Plant and Kim are two membrane conductance terms: a TTX-insensitive inward conductance, and an outward (potassium) conductance with very slow kinetics. At the time at which this model was developed, neither of these

terms had been specifically reported, although certain experimental results implied their existence (Junge and Stephens, 1973; Lux and Eckert, 1974). Subsequently, both of these conductance mechanisms have been identified experimentally (Smith et al., 1975, Eckert and Lux, 1975, 1976, Thompson, 1977). However, some of their properties are different from those incorporated in the original model.

With regard to the TTX-insensitive inward current conductance, the model assumed for simplicity that this conductance was voltage-independent, although the effect of incorporating voltage dependence was discussed (Plant and Kim, 1976). Experimental investigations have demonstrated that the conductance actually displays voltage-dependent activation and at least partial inactivation (Smith et al., 1975, Heyer and Lux, 1976*a*). Moreover, there is evidence that this activation is facilitated by earlier activation (Heyer and Lux, 1976*a*; Eckert et al., 1977).

The difference between the properties of the model's very slow potassium conductance and those of the actual membrane are more fundamental. While the model's conductance was assumed to be voltage-dependent, that of the actual membrane has been found to depend on the internal presence or concentration of calcium ions (Thompson, 1977, Thomas and Gorman, 1977). Moreover, internal calcium ions appear to affect the membrane potassium conductance in several ways. Heyer and Lux (1976*b*) have found that increased internal Ca^{++} concentration increases the "instantaneous" K^+ conductance, but reduces the conductance increase in response to a step depolarization during voltage clamping. Heyer and Lux interpret this to mean that increased internal Ca^{++} concentration inactivates the membrane K^+ conductance rather than activates it, as had been proposed earlier (Meech, 1972; Meech and Standen, 1975).

There is, however, an alternative interpretation consistent with this result. Namely, it is possible that Ca^{++} interacts with the membrane K^+ *both* as proposed by Meech and as proposed by Heyer and Lux. In other words, as proposed by Meech, increased internal Ca^{++} might directly increase the K^+ conductance of certain channels. During voltage clamp experiments, such a direct increase would be observed as an increase in the "instantaneous" conductance, since it would not depend directly on transmembrane potential. A second effect of Ca^{++} could be to "inactivate" a voltage-dependent conductance mechanism: i.e., to reduce its ability to respond to step changes in potential. This would give the reduced response observed by Heyer and Lux.

The present article attempts to incorporate the hypothesis presented in the previous paragraph into a mathematical model sufficiently precise to provide differential predictions permitting an experimental test of the hypothesis. Recently observed features of the membrane response of bursting neurons are incorporated into the model. Specifically, voltage-dependent activation is introduced into the TTX-insensitive inward current channel, and the K^+ channels are made calcium-dependent in the dual manner discussed above. Two separate models for calcium action are considered. As described in the next section, these models represent opposite ends of a spectrum in which the real system might be found. Then the predictive capabilities of the models are tested. The models are found to predict and allow a physical interpretation of

experimentally observed behavior that has not previously been explained. We then discuss experimental tests to decide the validity between the set of models proposed in this paper and the null hypothesis, as well as experiments to decide between the models themselves. Lastly, we discuss applications and possible modifications of the models.

As with previous studies, this one uses a formulation for activation and inactivation based on the product-of-variables approach of Hodgkin and Huxley (1952*b*). The question of to what extent this formulation represents the real system is still open (for reviews, see for example Keynes, 1975; Hille, 1976; and Armstrong, 1975). The present study takes the pragmatic, or "engineering" approach that this formulation is the best (i.e., simplest and most completely understood) one currently available. The question of direct correspondence of the variables to molecular mechanisms is not considered.

DEVELOPMENT OF THE MODELS

General Properties

The starting point for the development of the models of this section is the system of equations (1B, 3, 5, 6) of Plant and Kim (1976). This model may be represented as

$$dV/dt = (1/C)[I_I + I_K + I_L], \tag{1}$$

where V is the membrane potential, C is the membrane capacitance, and I_I, I_K, and I_L are inward, potassium, and leakage ionic currents, respectively, together with auxiliary equations for the conductance variables. Detailed arguments for this model are given in the earlier paper and will not be repeated here. Instead, the main assumptions will be listed, together with references from recent experimental literature supporting these assumptions.

The principle assumptions embodied in the model are:

(*a*) The slow wave underlying bursting is generated by a slowly varying potassium conductance (Junge and Stephens, 1973, Smith et al., 1975). This conductance variation is generated by the effect of calcium ions crossing the nerve membrane (Thompson, 1977, Thomas and Gorman, 1977).

(*b*) In addition to the spike-generating inward current, there is a slowly varying inward current resistent to TTX, which is carried by calcium and/or sodium ions (Smith et al., 1975; Eckert and Lux, 1976; Standen, 1975; Kostyuk et al., 1974; Eckert et al., 1977).

(*c*) Potassium accumulation does not play a major role in bursting (Lux, 1976; Heyer and Lux, 1976*a*). In addition, aside from providing a constant additive current, the electrogenic sodium pump does not play a role in bursting (Junge and Stephens, 1973).

(*d*) In addition to the slow conductance channels, the membrane contains inward and outward spike channels similar to those described by Hodgkin and Huxley (1952*b*) in the squid axon, and a fast potassium channel similar to that described by Connor

and Stevens (1971) in the molluscan soma (Neher, 1971, Neher and Lux, 1971, Kostyuk et al., 1975).

Based on these assumptions, we may write the differential Eq. 1 for voltage as

$$dV/dt = (1/C)[(g_I + g_T)(V_I - V) + (g_K + g_A + g_P)(V_K - V) + g_L(V_L - V)], \quad (2)$$

where g_I and g_T represent the TTX-sensitive and TTX-resistant sodium/calcium conductances, respectively: g_K, g_A, and g_P represent respectively the delayed, fast, and pacemaker potassium conductances; and g_L represents the leakage conductance. Two separate models, called Model I and Model II, are developed in this section. These models differ only in the form of the delayed potassium conductance, g_K. Hence, this channel will be treated separately.

INWARD CURRENTS Implicit in the formulation of Eq. 2 is the assumption that inward conductances may be multiplied by a single differential voltage. In fact, inward currents are carried by both sodium and calcium ions (Smith et al.; 1975, Kostyuk et al., 1974; Eckert and Lux, 1976; Standen, 1975). The equilibrium potential for Ca^{++} is considerably more positive (inside) than that for Na^+ (Standen, 1975, Heyer and Lux, 1976*a*). Nevertheless, the assumption of a mixed effective equilibrium potential has proven convenient in previous studies (e.g., Connor and Stevens, 1971, Plant and Kim, 1976) and should not materially affect results. The model expresses the total inward current as the product of voltage differential times the sum of two functionally independent conductances, g_I and g_T.

The conductance g_I represents the classical spike-generating conductance first described by Hodgkin and Huxley (1952*b*) for the squid axon. This conductance has been observed and described in several molluscan somata (Hagiwara and Saito, 1959, Neher, 1971, Connor and Stevens, 1971). We use the same expression for g_I as in the previous model (Plant and Kim, 1976), namely,

$$g_I = g_I x_I^3 y_I, \quad (3)$$

where

$$dx_I/dt = (\tau_{xI}(V))^{-1}(s_I(V) - x_I),$$
$$dy_I/dt = (\tau_{yI}(V))^{-1}(z_I(V) - y_I). \quad (4)$$

The functions s_I and z_I are monotonic, with

$$\lim_{V\to\infty} s_I(V) = \lim_{V\to-\infty} z_I(V) = 1,$$
$$\lim_{V\to-\infty} s_I(V) = \lim_{V\to\infty} z_I(V) = 0. \quad (5)$$

All other functions $s_j(V)$ and $z_j(V)$ also satisfy these properties. Minor changes have been made in certain parameters of the previous model but the basic form remains the same. Some of the functional values were incorrectly listed in the previous paper (I am grateful to Dr. D. Scriven for pointing out these errors). All values used in the present study are listed in the Appendix.

The TTX-resistant inward current conductance of the membrane is modeled by the term g_T. The current of this channel is carried by sodium and calcium ions, although there appears to be some variability among molluscan species as to the relative contribution of the two ions (Smith et al., 1975, Standen, 1975, Kostyuk et al., 1974, Eckert and Lux, 1976, Thompson, 1977). As has already been mentioned, this channel displays voltage-dependent activation and a partial inactivation, as well as an apparent facilitation (Heyer and Lux, 1976*a*, Eckert et al., 1977). In the present modeling study, we do not incorporate the last two phenomena. It must be emphasized that this does *not* imply a prediction or opinion that the phenomena are not present in the actual system. There are two reasons for not including these effects: the first and most obvious is that of simplicity and economy. The second is a desire to avoid wherever possible excessive "parameter fitting," i.e., the inclusion of many arbitrary parameters whose values may be adjusted to obtain any desired behavior. The consequences of this simplification are discussed under Properties of the Models and Discussion.

The T conductance is modeled by the expression

$$g_T = \bar{g}_T x_T,$$
$$dx_T/dt = \tau_{xT}^{-1}(s_T(V) - x_T). \tag{6}$$

Once again, the lack of dependence of τ_{xT} on V is a matter of simplicity. The conductance has been described as having a lower threshold than g_I, and does not appear to differ significantly from the other channels in its steady-state properties. These arguments led to the choice of parameters listed in the Appendix.

OUTWARD CURRENTS The outward current is modeled as consisting of a voltage difference times the sum of three potassium conductances; g_K, g_A, and g_P. The conductance channels g_A and g_P are identical in both versions of the model and will be described here. The two versions of g_K will be discussed later.

The so-called "fast potassium" channel, g_A, has been studied in detail by Connor and Stevens (1971) and by Neher (1971). The model described in this paper represents g_A as:

$$g_A = \bar{g}_A x_A^3 y_A, \tag{7}$$

where

$$dx_A/dt = \tau_{xA}^{-1}(s_A(V) - x_A)$$
$$dy_A/dt = \tau_{yA}^{-1}(z_A(V) - y_A). \tag{8}$$

Values of the parameters are based approximately on those of Neher (1971), and are listed in the Appendix.

The pacemaker channel is represented by g_P. Recent evidence (Thompson, 1977; Thomas and Gorman, 1977) indicates that this conductance is activated by the presence or concentration of internal calcium. This correlates well with observations of Meech (1972) and Meech and Standen (1975) on the effect of injected calcium on potassium conductance, as well as with experimental observations on the effect of illumination on potassium conductance (Brown and Brown, 1973; Nelson et al., 1976).

The role of processes involving ionized calcium has been extensively studied in the squid axon and other preparations (for reviews see Baker, 1972, and Reuter, 1973). Briefly summarizing: Ca^{++} that crosses the membrane during an action potential is removed from the cytoplasm by a combination of three processes: an active membrane transport process in which the extrusion of Ca^{++} is apparently coupled to the flow of Na^{+} down its electrochemical gradient; the sequestering of Ca^{++} in the mitochondria; and the effects of buffering agents contained in the cytoplasm. These three processes combine to maintain the concentration of Ca^{++} inside the cell at a very low level. Measurements indicate that the latter two processes are more important for the short-term, dynamic removal of Ca^{++}, while the membrane pump functions primarily to maintain a low steady-state concentration. For this reason the effects of the membrane pump are not included in this model. Furthermore, the combined effects of mitochondrial sequestering and cytoplasmic buffering are represented by a single model process.

Models have been developed to simulate the diffusion of calcium through the axoplasm of the squid giant axon (Blaustein and Hodgkin, 1969, Baker et al., 1971). Such a system could presumably be used here, based on a modification of the Nernst-Planck equations (Cohen and Cooley, 1965). Such a system would be extremely unwieldy, however, and would probably not contribute greatly to the accuracy of the model. In addition, the system would become dependent on two parameters, time and distance from the membrane, and hence would involve partial differential equations. The cost of solving such equations numerically would be prohibitive. Accordingly, a much simpler model of the distribution of Ca^{++} within the cytoplasm is used.

The model derives its form from the studies of Frankenhaeuser and Hodgkin (1956) of potassium accumulation outside the squid axon. It is assumed that there is a thin layer of uniform composition directly inside the membrane. Calcium accumulates in this layer at a rate proportional to the calcium current through the membrane, and is removed by a first-order rate process (there is, in fact, a certain amount of experimental justification for the first part of this assumption [Mullins, 1976]). Letting c denote the intracellular Ca^{++} concentration, measured in moles $\times 10^{-7}$, we have

$$dc/dt = k_{in} I_{Ca} - k_{out} c. \tag{9}$$

We will assume that the greater part of the calcium current is carried through the T channel and let I_{Ca} be proportional to $g_T x_T (V_{Ca} - V)$. The proportionality constant may be incorporated into k_{in}, yielding

$$dc/dt = k_{in} x_T (V_{Ca} - V) - k_{out} c. \tag{10}$$

We assume that the equilibrium potential V_{Ca} is constant. Actually, of course, V_{Ca} is a function of c, but the variations in internal ionized calcium concentration reported (e.g., Thomas and Gorman, 1977) would cause only about a 10% variation in V_{Ca}, and hence this simplifying assumption is justified.

Ionized calcium is assumed to interact with the P channel by a first-order process

$$p + c \underset{k_{-P}}{\overset{k_P}{\rightleftharpoons}} [pc] \tag{11}$$

in which p represents the unbound (closed) channel and pc the bound (open) channel. If K_p represents the dissociation constant of the reaction, and if it is assumed that the reaction is always in equilibrium (i.e., that the rate-limiting process is the diffusion of Ca^{++} into the membrane), then we may represent the conductance g_P by

$$g_P = \bar{g}_P c/(K_p + c). \tag{12}$$

This representation is used here. An alternative possibility is the assumption that reaction 11 is the rate-limiting step. This, however, leads to a model whose increased complexity cannot be justified by the present experimental knowledge. There is no information available to justify any particular choice of value for K_P. Hence, this value is chosen simply to make the model behave like the actual membrane. Details of this choice are given in the Appendix.

This completes the selection of processes common to both models. The electrogenic pump parameter I_{ep} (Plant and Kim, 1976) was not incorporated into the present model since no data are available to justify the selection of any particular value for it. For this reason, the present model will not simulate the effect of ouabain on the real cell (Junge and Stephens, 1973; Plant and Kim, 1976). This effect could easily be simulated by incorporating a nonzero term I_{ep} and slightly adjusting the nonpredetermined parameters.

Delayed Potassium Conductance

In order to account for the observation of Heyer and Lux (1976*b*) that increased internal ionized calcium concentration causes a reduction in the response of the membrane to step potential depolarizations, the model presented in this paper postulates that the delayed potassium conductance inactivates, and possibly activates, as a function of both voltage and internal Ca^{++}. Specifically, the equations governing this conductance are taken to be

$$\begin{aligned} g_K &= \bar{g}_K x_K^4 y_K, \\ dx_K/dt &= \tau_{xK}^{-1}(V)[s_K(V, c) - x_K], \\ dy_K/dt &= \tau_{yK}^{-1}[z_K(V, c) - y_K]. \end{aligned} \tag{13}$$

It should be noted that the restriction of the action of Ca^{++} to the K channel is a matter of convenience only. There is no reason why this effect could not be attributed to the A channel as well.

In the absence of any specific data, it is possible to imagine that the effect of potential and calcium on K channel inactivation may be represented by a function $z_K(V, c)$ lying somewhere between two extremes. Accordingly, these two extreme cases are the ones considered. In this way, a differential prediction between the extreme cases may

be obtained. This differential prediction may be used to determine which case is likely to correspond most nearly to reality.

The two extreme cases are as follows: In the first case (called Model I) Ca^{++} is assumed to act in a completely indirect manner by altering the apparent potential measured by the K channel. In the second case (called Model II), Ca^{++} is assumed to act in a completely direct manner; the function z_K is assumed to depend on c only, while s_K depends on V only.

MODEL I A simple way to incorporate a calcium effect into the voltage across the K channel is to assume that internal Ca^{++} binds to the K channel as proposed by Huxley (Frankenhaeuser and Hodgkin, 1957, Huxley, 1959) for the effect of external Ca^{++} on the sodium channel of the squid giant axon. Specifically, Ca^{++} is assumed to bind to negative sites located near the inside mouth of the conductance mechanism corresponding to the K channel. Note that the effect of binding to these internal sites is the opposite of that obtained by the binding of Ca^{++} to external sites as postulated for the squid axon. That is, by promoting binding to internal sites, increased internal Ca^{++} concentration will tend to depolarize the K channel (i.e., to make the argument of the functions $s_K(V)$ and $z_K(V)$ more positive or less negative). It would also be possible to include the effect of charge screening by Ca^{++} in the model (Gilbert and Ehrenstein, 1969). However, to do so would vastly increase the complexity, and the qualitative effect of charge screening is the same as that of binding (McLaughlin et al., 1971).

Huxley (1959) incorporates the effect of Ca^{++} into the squid axon model by adding a logarithmic term to the variable V. In the present study a slightly different approach is used. It is assumed that Ca^{++} binds to divalent sites σ according to the reaction $Ca^{++} + \sigma \rightleftharpoons Ca\sigma$, which is assumed to be always in equilibrium with a dissociation constant K_S. If $\bar{\sigma}$ represents the total number of divalent sites, then for an internal Ca^{++} concentration c we have

$$\sigma = K_S\bar{\sigma}/(K_S + c). \tag{14}$$

The voltage across the K channel is given by

$$V_{gK} = V + K_cK_S/(K_S + c). \tag{15}$$

for some K_c. Thus the equations of the delayed potassium channel of Model I are given by Eqs. 14 and 15 and

$$\begin{aligned} dx_K/dt &= (\tau_{xK}(V_{gK}))^{-1}[s_K(V_{gK}) - x_K], \\ dy_K/dt &= \tau_{yK}^{-1}[z_K(V_{gK}) - y_K]. \end{aligned} \tag{16}$$

MODEL II Model I represents the extreme case in which Ca^{++} is able to affect delayed potassium conductance only through variations in voltage across the channel. Model II represents the opposite extreme, in which Ca^{++} acts in a completely direct manner on the potassium conductance. This is accomplished by assuming that steady-state potassium depolarizing activation is a function purely of voltage, while

depolarizing inactivation is a function purely of internal Ca^{++} concentration. Thus, the equations of the K channel of Model II are

$$\begin{aligned} dx_K/dt &= (\tau_{xK}(V))^{-1}[s_K(V) - x_K], \\ dy_K/dt &= \tau_{yK}^{-1}[z_K(c) - y_K]. \end{aligned} \tag{17}$$

Values for the parameters of Eqs. 17 are given in the Appendix.

PROPERTIES OF THE MODELS

The following represents a summary of the equations of the two models. The equations of the components common to both models are

$$\begin{aligned} dV/dt = (1/C)\{&[\bar{g}_I x_I^3 y_I + \bar{g}_T x_T][V_I - V] + [\bar{g}_K x_K^4 y_K + \bar{g}_A x_A^3 y_A \\ &+ \bar{g}_P c/(K_P + c)][V_K - V] + g_L[V_L - V]\}; \end{aligned} \tag{18}$$

$$dx_i/dt = \tau_{xi}^{-1}[s_i(V) - x_i], \quad i = I, T, A; \tag{19a}$$

$$dy_j/dt = \tau_{yj}^{-1}[z_j(V) - y_j], \quad j = I, A; \tag{19b}$$

$$dc/dt = k_{in} x_T[V_{Ca} - V] - k_{out} c. \tag{19c}$$

The equations of the K channel of Model I are

$$\begin{aligned} dx_K/dt &= \tau_{xK}^{-1}[s_K(V_{gK}) - x_K], \\ dy_K/dt &= \tau_{yK}^{-1}[z_K(V_{gK}) - y_K], \end{aligned} \tag{20}$$

where

$$V_{gK} = V + K_c K_s \bar{\sigma}/(K_s + c).$$

The equations of Model II are

$$\begin{aligned} dx_K/dt &= \tau_{xK}^{-1}[s_K(V) - x_K], \\ dy_K/dt &= \tau_{yK}^{-1}[z_K(c) - y_K]. \end{aligned} \tag{21}$$

Values for the various parameters and functions, together with the rationale for selecting them, are given in the Appendix.

Fig. 1 shows the results of numerical solution of the voltage clamp potassium current, given by

$$I_K(t) = [\bar{g}_K x_K^4 y_K + \bar{g}_A x_A^3 y_A + \bar{g}_P c/(K_P + c)][V - V_K] + \bar{g}_L[V - V_L], \tag{22}$$

for the two models. Both models are seen to simulate the observed results of Heyer and Lux (1976*b*) in the following way. The initial value of I_K, that is, the value $I_K(0)$, is proportional to the "instantaneous" potassium conductance. This value increases with increasing initial value of c, representing increasing internal Ca^{++} concentration, as described by Heyer and Lux. This increase is due to the increase in the term $c/(K_P + c)$ in Eq. 22. In addition, the response to step voltage of the system is seen to ultimately

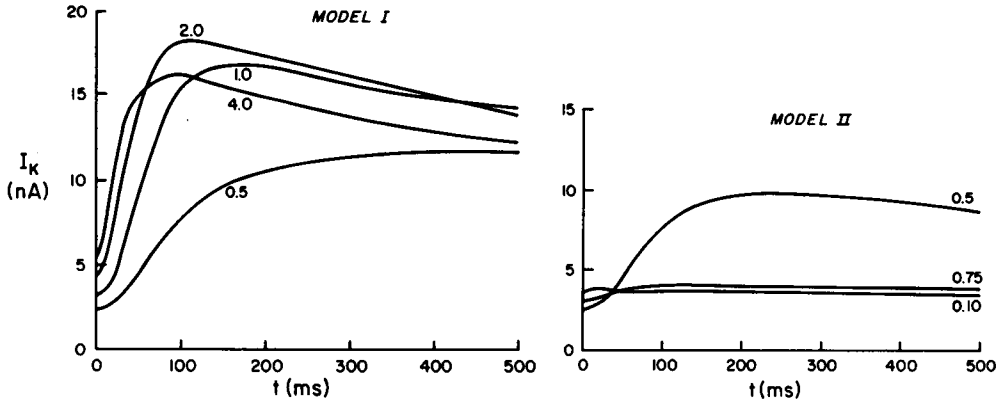

FIGURE 1 Simulation of the effects of increased internal Ca^{++} concentration on total potassium current in response to a 44 mV depolarization (Eq. 22) for (*left*) Model I and (*right*) Model II. Curves shown for various initial values of c as shown. The intersections of the curves with the V axis is proportional to the "instantaneous" conductance. To remove the effect of instantaneous conductance, curves should be shifted so as to intersect the V axis at the same point. This would result in a reduced relative steady-state potassium current for high values of c in both models.

decrease with increasing c, due to decreasing initial value of the variable y_K. This represents the calcium-dependent inactivation described by Heyer and Lux. Note in Fig. 1 *a* that initially the voltage response of Model I increases with increasing c. This is not a strong prediction of the model (the precise meaning of the term will be described later), since it may be eliminated by varying the parameters. Indeed, the voltage response is due to the fact that the initial value of x_K also depends on c, and may be removed by removing this dependence (i.e., by making the function s_K of Eqs. 20 depend on V and not V_{gK}). It seemed, however, somewhat more plausible to assume that both variables depend on V_{gK}.

Both models have the ability to simulate a well-known and interesting property of the potassium inactivation, first reported by Neher and Lux (1971). This property is that during voltage clamping, the recovery from inactivation does not display simple first-order kinetics, but rather persists and actually increases (i.e., the membrane becomes more inactivated) for a brief period after the return to a holding potential after step depolarization (c.f., Neher and Lux, 1971, Fig. 1; Heyer and Lux, 1976*a*, Fig. 9). The following procedure was used to simulate this experiment: Eqs. 19 and 20 or 21, respectively, were solved numerically, starting from a steady-state "holding" value of V given by $V = -54$. The value of V used in the solution was alternated in a "double pulse" between the holding value, and a "test" value, $V = -10$. The model's total potassium current, I_K, given by Eq. 22, was computed for this "voltage clamp" situation.

Fig. 2 *a* shows the response of Model I. The initial value of c was set at 1.0, a "large" value. It can be seen that inactivation is retained. This is a weak property of this model since for c initially equal to 0.5, recovery begins immediately. The reason for this is

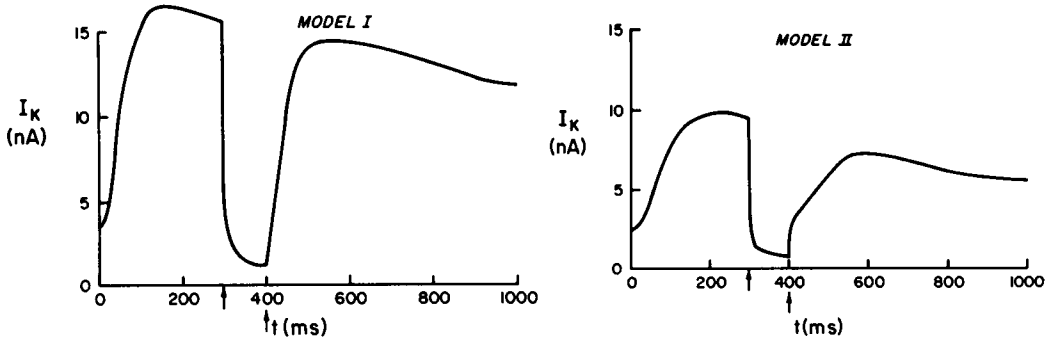

FIGURE 2 Simulated "double-pulse" experiment. Note increased inactivation (i.e., reduced peak current) in response to the second pulse. Pulses are 44 mV. (*left*) Model I, $c(0) = 1.0$; (*right*) Model II, $c(0) = 0.5$.

that the variable V_{gK} in Eq. 15 depends on both V and c, and the effect of making V more negative may be sufficient to overcome any effect of c.

Fig. 2 *b* shows the response of Model II. It is evident that inactivation continues after V is returned to the value $V = -54$, indeed, the rate of inactivation should be increased, (cf., Neher and Lux, Fig. 1). The reason for this is that in the model the conductance variable g_T is a continuous function of time (cf., Hodgkin and Huxley, 1952 *a*). Therefore, since calcium current is given by $I_{Ca} = g_T(V_{Ca} - V)$, the Ca^{++} current and the rate of Ca^{++} accumulation will initially increase after V is stepped from -10 to -54. Therefore Model II seems better able to simulate the observed retention of inactivation of Neher and Lux, although the results are inconclusive. The wave form of the bursting solution of the model is highly dependent on the specific values chosen for the various parameters, and therefore this aspect of the model was not studied extensively. Fig. 3 shows the wave form of a burst solution of Model II. The number of spikes and the duration of the burst could be increased by reducing the constants k_{in} and k_{out} in Eq. 10 (cf. Appendix). The spikes have the reduced undershoot commonly seen in bursting systems. This is due to the increasing inactivation of the K channel as c increases.

A well-known property of action potentials of bursting cells is increased spike width during the burst (Faber and Klee, 1972). It is of interest to inquire whether the model will simulate this phenomenon. The possible relation between retention of inactivation and increased spike width has been independently recognized by Getting and Thompson (1977). Fig. 4 shows a detailed plot of the spikes of Fig. 3. The spikes have been plotted so that their peaks coincide on the time axis. If the abnormally large first spike is excluded, the spike width increases as the burst progresses. This increase is not quite as large as that observed in the real cell, however. It is not clear whether other choices of the model parameters would improve this simulation, but it is evident that this phenomenon could also be due to a facilitating calcium channel. It should be noted that increased internal Ca^{++} concentration also affects the relaxation times of the potassium

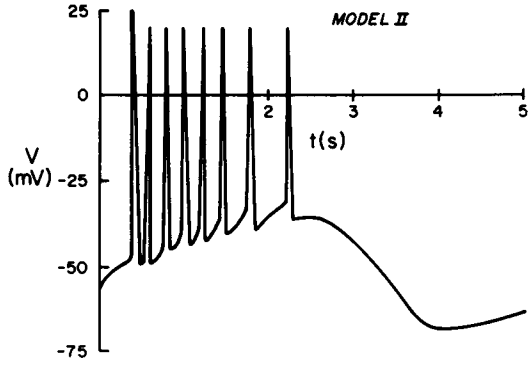

FIGURE 3

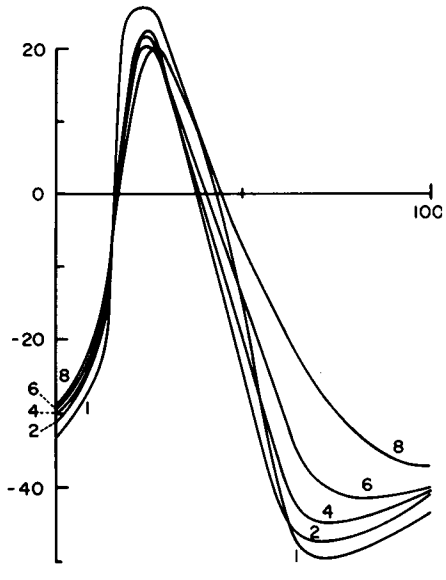

FIGURE 4

FIGURE 3 Bursting solution of Model II, under zero external current.

FIGURE 4 Superimposed action potentials from Fig. 3. Abscissa represents time in milliseconds. Ordinate represents membrane voltage in millivolts.

channels (Kostyuk et al., 1975), and this effect could also contribute to the increased spike width.

EXPERIMENTAL TESTS OF THE MODEL

With two alternative models, it is necessary to find differential predictions to provide some means of determining which is more likely to represent the real system, as well as provide tests for the validity of the concepts of the models themselves. In searching for such differential predictions, it is important to keep in mind that, in the absence of specific experimental values for the parameters of the equations, these tests must result in strong predictions of the model. In other words, the prediction must depend not on the particular chosen values of the parameters but rather on the qualitative features of the model. In order to develop experimental tests for the full set of models spanned by Models I and II, it is also necessary to formulate a null hypothesis.

There is an alternative description of the K channel which, in principle, should be able to reproduce the experimentally observed retention of inactivation. This alternative is to assume that the activation and inactivation processes are coupled. There is a rather extensive literature on the effects of coupling in models for the sodium channel in squid (e.g., Hoyt, 1968) and *Myxicola* giant axons, (e.g., Goldman, 1975); for a review, see Goldman (1976).

In the case of an inactivating potassium channel, one may assume that the conductance is represented as

$$\bar{g}_K = g_K x_K^4, \tag{23}$$

where x_K and the "hidden" variable y_K are governed by the equations

$$\begin{aligned} dx_K/dt &= a_1(V)x_K + a_2(V)y_K + a_3(V), \\ dy_K/dt &= b_1(V)x_K + b_2(V)y_K + b_3(V). \end{aligned} \tag{24}$$

Eqs. 24 might be viewed as a first-order Taylor series approximation of a more general system,

$$\begin{aligned} dx_K/dt &= f_1(V, x_K, y_K), \\ dy_K/dt &= f_2(V, x_K, y_K). \end{aligned} \tag{25}$$

The following heuristic argument suggests that a model incorporating a suitably chosen system of Eqs. 24 for the K channel would simulate the retention of inactivation. Suppose we differentiate the first equation in Eq. 24 and substitute the second equation for dy_K/dt, and the first equation for y_K. This operation results in the second-order equation

$$d^2x_K/dt - (a_1 + b_2)\,dx_K/dt + (a_1b_2 - b_1)x_K + a_3b_2 = 0, \tag{26}$$

where the arguments of the a_i and b_i have been suppressed. A well-known property of second-order equations, not possessed by first-order equations, is that of "memory" or "inertia." Hence we may expect that the variable x_K in Eq. 24 could have such a property.

A model incorporating Eqs. 24 rather than 20 or 21 could therefore in principle do an equally good job of predicting retention of inactivation. Of course such a model could not by itself simulate the Heyer-Lux observation of Ca^{++}-induced response decrement, but this effect could be obtained in the real cell in a completely different way. There is an obvious experimental test to distinguish between the two alternative models (Eqs. 20 or 21, and 24). This is to perform voltage clamp experiments on cells bathed in a medium containing a calcium antagonist such as cobalt, and compare the results with those obtained on cells in a normal medium.

Kostyuk et al. (1975) have reported on voltage clamp experiments on *Helix* cells bathed in a medium containing verapamil. These workers found that in this medium K (and *A*) channel inactivation was significantly reduced but not eliminated. This would be predicted by Model I. However, these results are inconclusive for two reasons. First, it is known (Eckert and Lux, 1976) that verapamil does not completely block the calcium current in these cells. Second, as has been emphasized throughout this paper, Models I and II are meant to represent the extremes of a continuum of models in which inactivation may be dependent on both calcium and transmembrane potential. Therefore, although the results of Kostyuk et al. provide compelling support for the models, and especially Model I, they are not conclusive.

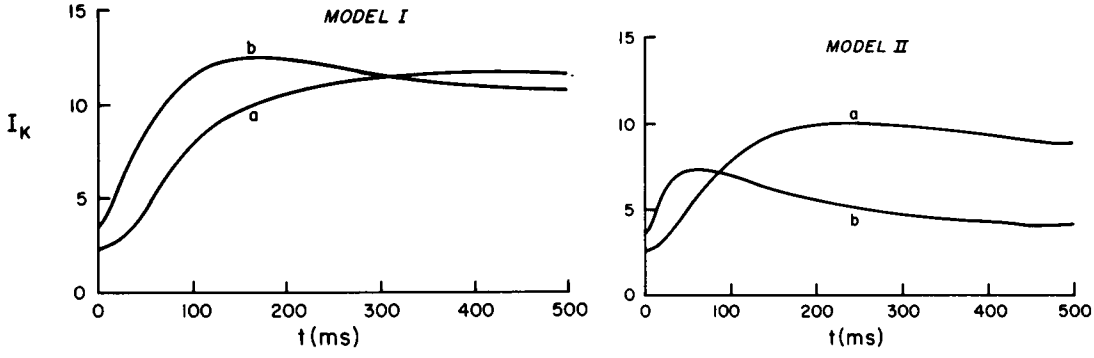

FIGURE 5 Differential prediction between Models I and II. (*left*) Model I; (*right*) Model II. Curves *a*: solution of Eq. 22 for V stepped from -54 to -10 with $c(0) = 0.5$. Curves *b*: solution (multiplied by 65/52.5) for V stepped from -66.5 to -22.5 with $c(0) = 1.0$.

One feature of the models that is strongly dependent on calcium is the retention of inactivation. Indeed, as pointed out earlier, this property is due to the continued influx of Ca^{++} after repolarization. Therefore, the models predict that the real cell should not display this retention in a medium containing Ca^{++}-blocking agents. If experiments demonstrate this retention in such a medium, then a calcium-dependent process as described under Properties of the Models cannot describe the system, and we must turn to some other model, such as that of Eqs. 24.

There is also a strong differential prediction between the extreme cases represented by Models I and II. This prediction is based on the fact that in Model I, internal Ca^{++} is able to affect the membrane only by displacing the potential measured by the K channel, and therefore the effect on this channel of an increased internal calcium concentration may be overcome by a suitable increase in membrane polarization. Such is not the case for Model II, in which inactivation is accomplished directly by increased internal calcium concentration.

Fig. 5 shows a comparison of the total potassium current in Models I and II for two voltage clamp cases. Curves *a* represent a step from $V = -54$ to $V = -10$, with an initial value of c equal to 0.5. Curves *b* represent a step from $V = -66.5$ to $V = -22.5$, with an initial value of c equal to 1.0 (the values of I_K in curves *b* are multiplied by 65/52.5 to normalize the differences in $V - V_K$). The initial surge in current in each model is due to the increased contribution of the A channel. However, while in Model I the currents at 500 ms are approximately equal, in Model II the current with "high calcium" is much lower.

DISCUSSION

Possible Extensions of the Model

In the present study, a very simple form is assumed for several of the conductances. It must be re-emphasized that the exclusion of various effects from the model in no way

represents a prediction or opinion that these effects are not present in the real cell. It is merely a simplification, and a recognition of the fact that the current knowledge of these phenomena is not sufficient to generate a verifiable model.

With regard to the T channel (Eq. 6), the simplifications have already been discussed. As mentioned earlier, the phenomenon of increased spike width could presumably be modeled better with the inclusion of a facilitating T-channel conductance. It should be noted that one way in which such facilitation might occur is if the channel conductance depends on one or more components of a coupled system of variables. This concept is similar to that discussed in developing the null hypothesis, except that in the present case both variables would govern depolarizing activation.

The mechanism assumed for the pacemaker channel, represented by Eq. 12, is perhaps the crudest approximation of the model. Some of the differences in wave form between the model and the real cell may be attributable to this crudeness. If we assume that the general idea of the mechanism—that of a very slowly varying conductance activated by internal calcium ions—is correct, then there are many questions about such a conductance which must be answered. These questions involve the exact means of entrance of the Ca^{++} ions which activate the P channel mechanism, the processes which are most important in inactivating or removing activation of this mechanism, and the problem of whether this activation and removal of activation is dependent on the rate of entry of ionized calcium or on the kinetics of the channel

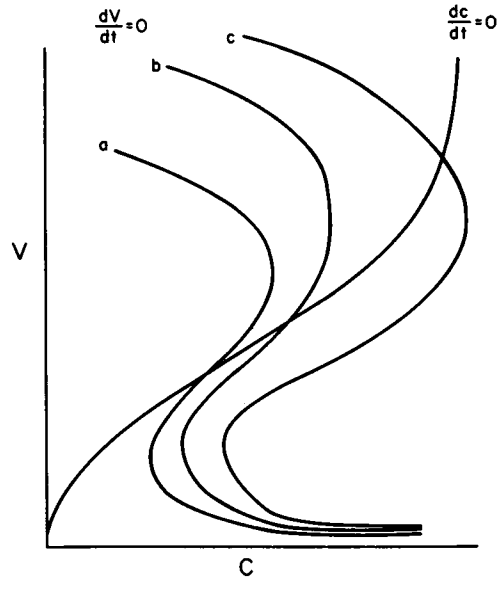

FIGURE 6 One way in which model could represent the effect of reduced external Ca^{++}. Curve *a* represents $dV/dt = 0$ curve for "normal" case, as in Fig. 8. Curve *b* represents increased amplitude burst; burst *c* represents bursting abolished by stabilizing of equilibrium point. In an actual model, the $dc/dt = 0$ curve would also vary; this variation is suppressed for simplicity.

itself. It is hoped that future modeling studies will aid in answering some of these questions.

There are additional calcium-related behaviors of the cell which cannot be accounted for by the double effect of Ca^{++} on the membrane proposed in the present model. One of the most prominent of these is the behavior of the *Aplysia* R15 cell in a nominally calcium-free bathing medium. This behavior has been studied by Carpenter and Gunn (1970), Barker and Gainer (1975), and Kim and Kim (unpublished). When the bathing solution of the cell is washed free of calcium, the bursting initially increases greatly in amplitude. Ultimately, bursting is abolished and the cell fires a continuous train of spikes which eventually dies away, leaving the membrane potential in a steady, depolarized state (Carpenter and Gunn, 1970; Barker and Gainer, 1975).

One obvious way of attaining an increased amplitude burst is shown in Fig. 6. In this scheme, the S-shaped curve defining the slow wave form is allowed to fold back on itself, resulting in an increased amplitude slow wave, and hence in increased amplitude bursts (Fig. 6, curve *b*). Ultimately, if the folding process is continued (Fig. 6, curve *c*), the intersection of the curves given by $dV/dt = 0$ and $dc/dt = 0$, which defines equilibrium point of the system, will become stable. The slow wave will then cease, and the voltage behavior will consist of an infinite train of spikes (cf. Plant and Kim, 1976, Fig. 5), which may die away due to a fatigue process. The folding back could be accomplished by, for example, a reduction in value of $\bar{g}_K$.

There is another, less obvious way in which the model can be modified to simulate low calcium behavior. This is illustrated in Fig. 7. This figure shows that if it is hypothesized that the effect of reduced external Ca^{++} is to shift the arguments V of the functions $s_I(V)$, $z_I(V)$, $\tau_{xI}(V)$, and $\tau_{yI}(V)$ a distance of -5 mV along the voltage axis (i.e., in the same direction as that proposed by Frankenhaeuser and Hodgkin [1957] for the squid axon), then in the model (Model II) bursting is abolished and a dying spike train emerges. This is interesting from a mathematical as well as a biologi-

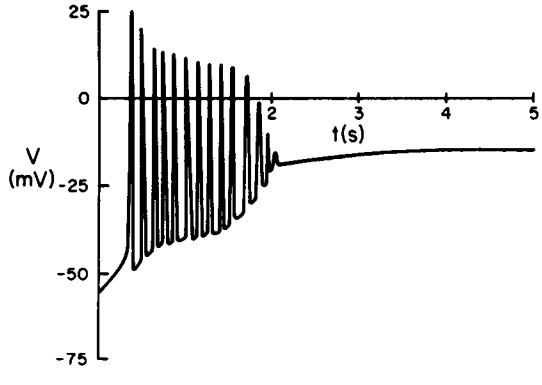

FIGURE 7 Alternative hypothetical effect of reduced external Ca^{++}. Solution of same system as Fig. 3, but with inward spike functions shifted by -5 mV along voltage axis, as postulated for squid axon. Abolishment of bursting results.

cal point of view, since it implies the emergence of an unexpected stable singular point in the nine-dimensional phase of the model.

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APPENDIX

In this section the values of the various constants and functions of the models are presented, together with the rationale for their selection. A summary of the values are given in Table I; the rationale is as follows. (*a*) The *I* channel. This channel is unchanged from the original model (Plant and Kim, 1976), except that the voltage axis of the functions is shifted slightly to give better spike properties, and the values of $\bar{g}_I$ is increased. (*b*) The *T* channel. For simplicity, the value of τ_{xT} is assumed constant, and is given the value 80 ms (Eckert and Lux, 1976). There are very few data as to the values of the other parameters. The values listed in Table I were chosen on the reasonable assumption that this channel has similar

TABLE I

PARAMETERS OF THE MODEL

$\alpha_i(V)$ and $\beta_i(V)$ are as given by FitzHugh (1969), based on the work of Hodgkin and Huxley (1952*a*).

$$\hat{V} = \frac{127}{105}V - \frac{8265}{105}, \qquad V^* = \hat{V} - 29, \qquad V^{**} = \hat{V} - 45,$$

$$s_I(V) = \frac{\alpha_m(V^*)}{\alpha_m(V^*) + \beta_m(V^*)}, \qquad \tau_{xI}(V) = \frac{1}{\alpha_m(V^*) + \beta_m(V^*)},$$

$$z_I(V) = \frac{\alpha_h(V^*)}{\alpha_h(V^*) + \beta_h(V^*)}, \qquad \tau_{yI}(V) = \frac{1}{\alpha_h(V^*) + \beta_h(V^*)},$$

$$s_K(V) = \frac{\alpha_n(V^{**})}{\alpha_n(V^{**}) + \beta_n(V^{**})}, \qquad \tau_{xK}(V) = \frac{1}{\alpha_n(V^{**}) + \beta_n(V^{**})} \text{ (see below).}$$

For other functions, $s_i(V) = (\exp(a_i[b_i - V] + 1)^{-1}$, $i = T, A$,

$$z_j(V) = (\exp(c_j[d_j - V] + 1)^{-1}, \; j = K, A$$

Parameter	Channel					
	I	T	K	A	P	L
$\bar{g}_i$	6	0.04	0.9	0.15	0.08	0.003
a_i	—	0.12	—	0.14	—	—
b_i	—	−45	—	−45	—	—
c_i	—	—	−0.07*	−0.27	—	—
d_i	—	—	−80*	−65	—	—
τ_{xi}	—	80	—	15	—	—
τ_{yi}	—	—	1,000	235	—	—

*Model I: $\sigma = -75$, $K_c = 1$, $K_S = 1$. In arguments of s_K and τ_{xK}, V^{**} replaced by $V_{gK} + 50$. (note at $V = 0$, $V_{gK} = .50$). Model II: $z_K = \exp(-15]58 - c]) + 1)^{-1}$; *P* channel; $K_P = 0.5$, $k_{in} = 8.0 \times 10^{-5}$, $k_{out} = 6.789 \times 10^{-3}$

properties to other subthreshold channels, such as the A channel. (c) The K channel. Values for the parameters of governing the behavior of x_K are unchanged from the model of Plant and Kim (1976). The value of $\bar{g}_K$ has been increased on account of the effect of the newly introduced variable y_K. The value of τ_{yK} (assumed constant), is set at 1 s (Kostyuk et al., 1975). The variables governing the voltage dependent behavior of s_K are also based on the results of Kostyuk et al. (1975). The variables governing the calcium-dependent portion of the behavior are obtained on a purely curve-fitting basis. (d) The A channel. Values for the parameters governing this channel were chosen to match approximately the observations of Neher (1971). (e) The P channel. Values governing this channel were chosen on a purely curve-fitting basis.

The curve-fitting procedure is identical to that used earlier (Plant and Kim, 1975, 1976). Specifically, the parameters are adjusted so that the curve $dV/dt = 0$ of the reduced second-order system in V and c,

$$0 = g_T s_T[V_I - V] + [g_{\underline{K}} s_{\underline{K}}^4 z_{\underline{K}} + g_A s_A^3 z_A + g_P(c/K_P + c)][V_{\underline{K}} - V] + g_L[V_L - V], \tag{A1}$$

is S-shaped, and the curve $dc/dt = 0$,

$$c = (k_{out}/k_{in}) s_T[V_{Ca} - V], \tag{A2}$$

intersects it in a region in which the equilibrium point is unstable (see Plant, 1977*a* and *b*) for complete description of this process). Eq. A1 cannot be solved explicitly due to the dependence of the K channel variables on c; the equation was solved numerically using a standard Newton iteration. Fig. 8 shows the graph of Eqs. A1 and A2, which results from the values

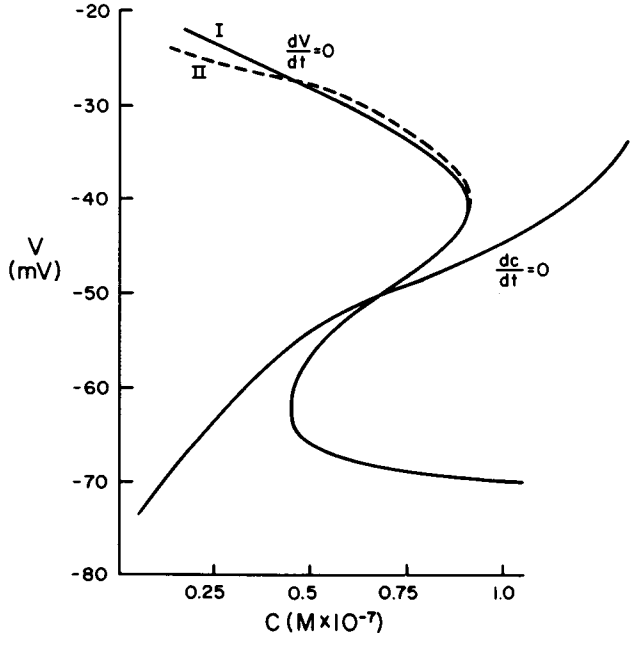

FIGURE 8 Null clines of reduced system. See text for discussion.

given in Table I. This graph was designed to given an oscillation in c roughly between values of 5×10^{-7} and 10×10^{-7} M, as described by Gorman et al. (1977).

Numerical solution of the differential equations followed the procedure given in an earlier paper (Plant and Kim, 1976), except that an Adams-Bashforth-Moulton method with externally varied step size was used to obtain the data for Figs. 3 and 4.

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