Slow inward Ca current in frog heart: theoretical evidence against a voltage-dependent inactivation

R Fischmeister, M Horackova

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RODOLPHE FISCHMEISTER[2] AND MAGDA HORACKOVA

Department of Physiology and Biophysics, Faculty of Medicine, Dalhousie University, Halifax, N.S., Canada B3H 4H7

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The validity of a Hodgkin–Huxley type voltage-dependent inactivation of slow inward Ca current (I_{si}) was tested in frog heart using a computer simulation. The time course of I_{si} was calculated during the development of a frog atrial action potential (AP). With a time constant of inactivation (τ_f) of 55 ms at a membrane potential (E_m) of −15 mV, the variation of I_{si} was biphasic; after a transient increase followed by a decrease to zero, I_{si} partially "reactivated" (at the beginning of the AP repolarization phase) and then fully deactivated. The "reactivation" phase of I_{si} developed whether τ_f was an increasing, decreasing, U-shaped, or bell-shaped function of E_m. The addition of an independent and slower process responsible for the recovery from inactivation only partly suppressed the "reactivation" phase. However, until now there was no experimental evidence supporting such a biphasic variation of I_{si} during AP repolarization. Thus our results indicate that the Hodgkin–Huxley type model of the voltage-dependence of I_{si}-inactivation process may not correctly represent the actual behavior of frog cardiac muscle.

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On a recouru à une simulation sur ordinateur pour vérifier, dans le coeur de grenouille, la validité d'une inactivation tension-dépendante du type Hodgkin–Huxley d'un faible courant entrant de Ca (I_{si}). On a calculé l'évolution temporelle de I_{si} pendant le développement d'un potentiel d'action (AP) auriculaire de grenouille. Avec une constante de temps d'inactivation (τ_f) de 55 ms à un potentiel de membrane (E_m) de −15 mV, la variation de I_{si} était biphasique; après une augmentation transitoire suivie d'une diminution à zéro, le I_{si} "se réactiva" partiellement (au début de la phase de repolarisation du AP) puis se désactiva totalement. La phase de "réactivation" de I_{si} se développa alors que τ_f était une fonction croissante, décroissante, en forme de U ou en forme de cloche de E_m. L'addition d'une fonction indépendante plus lente et responsable de la récupération de l'inactivation, ne supprima que partiellement la phase de "réactivation". Toutefois, jusqu'à présent, aucune expérience n'a encore révélé une telle variation biphasique de I_{si} pendant la repolarisation de AP. Ainsi, nos résultats indiquent que le modèle de type Hodgkin–Huxley du processus d'inactivation tension-dépendante d'un I_{si}, ne représente peut-être pas exactement le comportement réel du muscle cardiaque de grenouille.

[Traduit par le journal]

Introduction

Since its existence was first reported in heart by Rougier et al. (1969) and Beeler and Reuter (1970), the conductance of the slow inward channel in cardiac preparations has been argued to be voltage-dependent and to undergo activation and inactivation in a manner qualitatively similar to that originally described for the Na channel in nerve by Hodgkin and Huxley (1952). Voltage-clamp studies of the kinetics of the slow inward channel in cardiac tissues (see review by Coraboeuf 1980) show, however, considerable inter-species variation in the voltage dependence of the inactivation process, particularly when comparing mammalian (Beeler and Reuter 1970; Gettes and Reuter 1974; Kohlhardt et al. 1975) and amphibian heart (Besseau 1972; Horackova and Vassort 1976). Moreover, that voltage-dependent inactivation is a general feature of Ca channels is now called into question by findings that inactivation of Ca channels is Ca mediated in several noncardiac preparations (e.g., Tillotson 1979; Eckert and Tillotson 1981; Ashcroft and Stanfield 1981). Similarly, Schultze (1981) recently suggested, and recent investigations on frog (Fischmeister et al. 1981; D. Mentrard, G. Vassort, and R. Fischmeister, in preparation) and calf Purkinje fibres (Marban and Tsien 1981; Tsien and Marban 1982) partly confirm, that this also occurs in the heart on the slow inward channels.

In the present study, we have examined the validity of an interpretation of the slow inward channel kinetics, in frog heart, based on the Hodgkin–Huxley (H–H) model and on previously published voltage-clamp data. The slow inward current (I_{si}) was calculated, using a

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[2]Permanent address: Laboratoire de Physiologie Cellulaire Cardiaque, Bât 443, Université de Paris Sud, F. 91405, Orsay, France. Recipient of a Postdoctoral Scholarship from the Nova Scotia Heart Foundation.

simple iterative procedure, during the development of a frog atrial action potential (AP), and the physiological implications of the simulated variations in I_{si} were examined. Brief preliminary reports of these results have appeared (Fischmeister and Horackova 1982*a*, 1982*b*).

Theoretical considerations

The H–H type model of I_{si}

By analogy to the H–H interpretation of the fast sodium current, the slow inward current I_{si} (microamperes per square centimetre) could be described by the following equation:

$$[1] \quad I_{si} = \bar{g}_{si} \cdot d \cdot f (E_m - E_{Ca})$$

where $\bar{g}_{si}$ is the maximal slow inward channel conductance (microsiemens per square centimetre); d and f are the activation and inactivation gating variables, respectively; and E_m and E_{Ca} are the membrane and the I_{si}-reversal potentials (millivolts), respectively

The gating variables satisfy the first-order differential equation:

$$[2] \quad \frac{dy}{dt} = \alpha_y (1 - y) - \beta_y \cdot y$$

where $y = d$ or f and the rate constants α_y and β_y (per millisecond) are specifically voltage dependent. Equation 2 can be written in another form:

$$[3] \quad \frac{dy}{dt} = \frac{1}{\tau_y} (y_\infty - y)$$

where τ_y is the time constant (milliseconds) and y_∞ the steady-state value given by:

$$[4] \quad \tau_y = 1/(\alpha_y + \beta_y) \text{ and}$$

$$[5] \quad y_\infty = \alpha_y/(\alpha_y + \beta_y)$$

Description of the integration procedure

Mathematical models were written in FORTRAN IV on a Xerox Sigma 5 computer and were used by the investigators from an on-line Tektronix terminal. For the study of I_{si} during the course of an AP, a representative frog atrial AP was calculated with a model based on empirical equations describing I_{si} and the other ionic currents, using a Runge-Kutta-Fehlberg integration method (subroutine RKF45, Forsythe et al. 1977). The maximum accepted magnitude of the relative local truncation error was set at 10^{-3} for each of the integrated components. The truncation error estimate was used to control the step size of the integration procedure. The AP values were recorded over 600 ms, at 0.1-ms intervals for the first 40 ms of the AP and at 1 ms thereafter. These AP values were then used to calculate the corresponding I_{si} (and the related parameters) when different kinetics for I_{si} were considered. It may be noted that, instead of using the computed AP, the calculations with the iterative procedure described below could have been obtained from a drawn or recorded AP as well. However, the computation of the AP gives an easy way to check the accuracy of the procedure (see later).

The gating variables y ($y = d$ or f) of I_{si} were calculated from the AP by resolving Eq. 2 at each interval, using an iterative procedure similar to that described by Rush and Larsen (1978). Briefly, α_y and β_y were assumed to be constant during each time interval Δt (milliseconds) and the complete solution of Eq. 2 was

$$[6] \quad y = y_\infty - (y_\infty - y_0) \cdot \exp(-\Delta t/\tau_y)$$

In any iteration, y_0 was the value at the start and y_∞ the asymptotic value. The computed value of y on the left side of Eq. 6 became y_0 for the next iteration.

The intracellular calcium concentration ($[Ca]_i$) was assumed to be a constant during Δt, and I_{si} was calculated at each interval using Eq. 1. $[Ca]_i$ was then evaluated for the next interval with the rudimentary differential approximation:

$$[7] \quad [Ca]_{i,t+\Delta t} = [Ca]_{i,t} + \Delta t \cdot [d[Ca]_i/dt]_t$$

where the derivative $d[Ca]_i/dt$ is given later (see Results, Eq. 11).

The validity of the method and the choice of the sampling rate were assessed for the set of parameters used in the AP integration by comparing the I_{si}-related parameters computed during this integration of the AP with these parameters calculated later from the AP (by the iterative procedure). For each parameter the resemblance was perfect showing that the relative error made on each component, with this method, was comparable to that introduced by the integration procedure (i.e., 10^{-3}). All calculated parameters were then recorded at 1-ms intervals and stored for later graphical output.

Results

Figure 1 shows a computed frog atrial AP simulated to resemble those recorded experimentally in our laboratory in *Rana pipiens* with a double sucrose gap apparatus. Similar AP were reported by several investigators (e.g., Rougier et al. 1968; Horackova and Vassort 1973; Lenfant and Goupil 1977), using the same technique, on isolated bundles of frog atrial cells either from *Rana pipiens* or from *Rana esculenta*. When recorded with intracellular microelectrodes, slightly longer plateau and faster terminal repolarization are observed (M. Horackova and M. Desilets, unpublished results). However, all the kinetic studies of membrane currents in frog atrium have been performed with the double sucrose gap; thus, in the present study we have used the configuration of AP as recorded under these conditions. It may also be noted that longer AP (700–1000 ms) have been sometimes observed in bullfrog (*Rana catesbiana*) atrial preparations, either from isolated bundles (Hatae et al. 1980; Yatani et al. 1981) or from single enzymatically isolated cells (Hume and Giles 1981).

Description of I_{si} *in frog heart*

Time constants (τ_d and τ_f, Eq. 4) and steady-state values (d_∞ and f_∞, Eq. 5) of activation and inactivation, in various cardiac preparations, are assembled in a

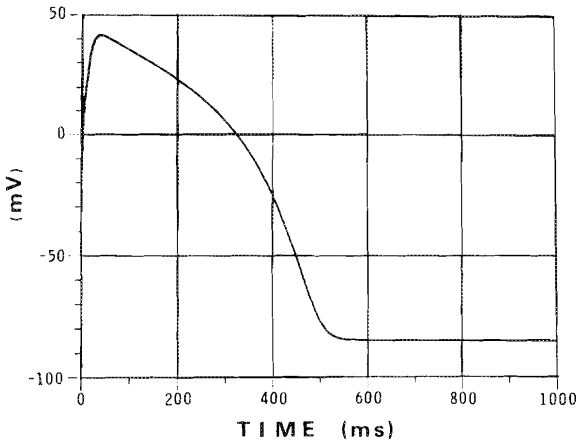

FIG. 1. A simulated representative frog atrial action potential.

recent review (Coraboeuf 1980). It appears that, except for bullfrog preparations, activation of I_{si} occurs faster in frog than in mammalian heart, with a time constant τ_d (under 15 ms) which decreases at high depolarizations. In the same preparations, the steady-state activation parameter d_∞ is similar to those reported in some mammalian preparations (e.g., cat ventricular muscle, Trautwein et al. 1975) taking into account that the original data of Besseau (1972) and Lenfant et al. (1972) were obtained assuming d, in Eq. 1, to be raised to a power of 3.

The following equations were used in our model to describe the activation of I_{si}:

$$[8] \quad \alpha_d = \frac{0.1 \exp [0.103(E_m + 16.7)]}{1 + \exp [8.75 \times 10^{-2}(E_m + 16.7)]}$$

$$[9] \quad \beta_d = \frac{7.75 \times 10^{-2} \exp [-8.89 \times 10^{-2}(E_m + 25.2)]}{1 + \exp [-6.5 \times 10^{-2}(E_m + 25.2)]}$$

Figure 2 shows the relationships of τ_d (Fig. 2A) and d_∞ (Fig. 2B) vs. E_m, as obtained from Eqs. 8, 9, 4, and 5. τ_d is a bell-shaped function of E_m, as is the time constant of activation used in the reconstructions of electrical activity in mammalian cardiac preparations (McAllister et al. 1975; Beeler and Reuter 1977; Yanagihara et al. 1980).

The relationship between τ_f and E_m presents a much larger variability among cardiac preparations (see Coraboeuf 1980). Whether τ_f is an increasing or decreasing function of E_m when $E_m > 0$ mV is still uncertain, although, in frog atrial fibres, the latter behaviour has been most frequently observed (Besseau 1972; Lenfant et al. 1972; Horackova and Vassort 1976). Except for bullfrog preparations, relatively smaller values of τ_f have been reported in frog atrial

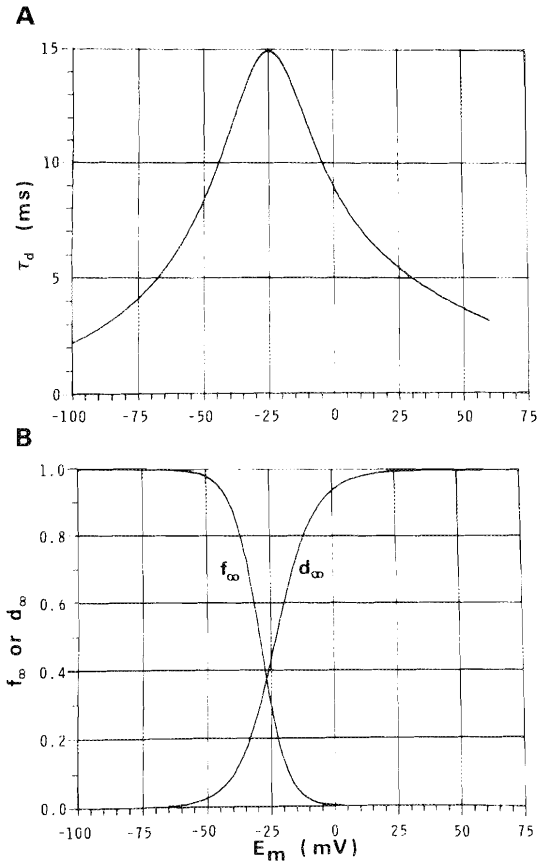

FIG. 2. (A) Voltage dependence of time constant of I_{si} activation, τ_d. (B) Voltage dependence of steady-state parameters of activation, d_∞, and inactivation, f_∞, of I_{si}.

fibres, compared with mammalian preparations: generally, $\tau_f \leq 55$ ms at $E_m \simeq -15$ mV (Coraboeuf 1980).

Assuming a given relationship between f_∞ and E_m, it was possible to determine the rate constant α_f and β_f for different $\tau_f - E_m$ relationships. The equation describing f_∞ used in our model was

$$[10] \quad f_\infty = \frac{1}{1 + \exp [(E_m + 29)/4.7]}$$

and was taken from Horackova and Vassort (1976) with a 10-mV shift towards more negative potentials to account for a lower resting potential (E_r) assumed in our conditions. The f_∞ curve vs. E_m used in our simulations is shown in Fig. 2B.

Owing to the large variability of the experimental data on $\tau_f - E_m$ relationships, six different functions were considered in our simulations of I_{si} (Fig. 3): two bell shaped (curves 1 and 2), one increasing (curve 3), one decreasing (curve 5), and two U-shaped (curves 4 and 6). The best-fit method was applied to determine α_f and β_f which fulfill Eq. 4 for a given τ_f when E_m is

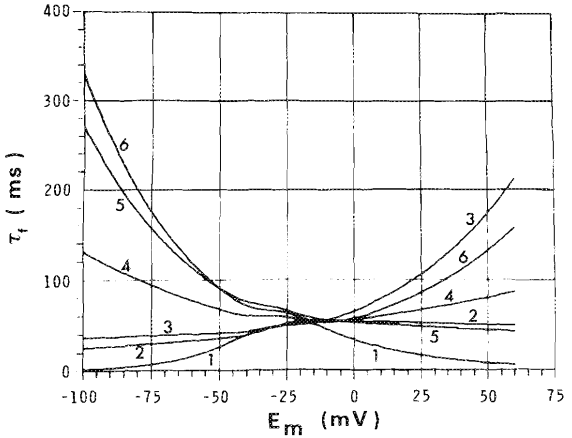

FIG. 3. Voltage dependence of various time constants of I_{si} inactivation, τ_f. τ_f is a bell-shaped (1 and 2), an increasing (3), a decreasing (5), and a U-shaped (4 and 6) function of E_m. For all $\tau_f - E_m$ relationships, $\tau_f \simeq 55$ ms at $E_m = -15$ mV.

outside the interval [−50 mV, 0 mV] and Eq. 5 for f_∞ given by Eq. 10 over the whole range of membrane potentials. τ_f was then calculated when E_m was inside the interval [−50 mV, 0 mV] and, when necessary, α_f and β_f were rescaled to satisfy the condition: $\tau_f \simeq 55$ ms at $E_m = -15$ mV. This procedure resulted in the presence of a small hump on some of the curves. The curve labelled "1" was calculated from the original experimental data of Horackova and Vassort (1976).

In the following simulations, I_{si} was calculated with a maximal conductance $\bar{g}_{si} = 0.11$ mS/cm^2. The I_{si} reversal potential, E_{Ca}, was calculated as the Nernst equilibrium potential for Ca ions at 20°C, with an extracellular concentration $[Ca]_0 = 1.8$ mM. $[Ca]_i$ was set at 10^{-7} M at the beginning of an AP and varied with time according to an equation similar to that used on mammalian heart by Beeler and Reuter (1977):

$$[11] \quad \frac{d[Ca]_i}{dt} = -6.84 \times 10^{-8} I_{si} + 5 \times 10^{-3}(10^{-7} - [Ca]_i)$$

The first term of Eq. 11 is the contribution of the influx of Ca ions through the slow inward channel to the total $[Ca]_i$, assuming a surface/volume ratio of 13 200 cm^{-1} (Hoerter et al. 1981) and the distribution of Ca ions inside the cell being instantaneous. An accumulation of Ca ions (not considered in the present model) might exist at the inner side of the membrane which would enhance the influence of I_{si} on $[Ca]_i$. However, it should be mentioned that, according to Eq. 11, the increase in $[Ca]_i$ due to I_{si} leads to an apparent $E_{Ca} \simeq 60$ mV, a value close to that observed experimentally (R. Fischmeister et al. 1982). The second term of Eq. 11 corresponds to a restitution of resting Ca level (by

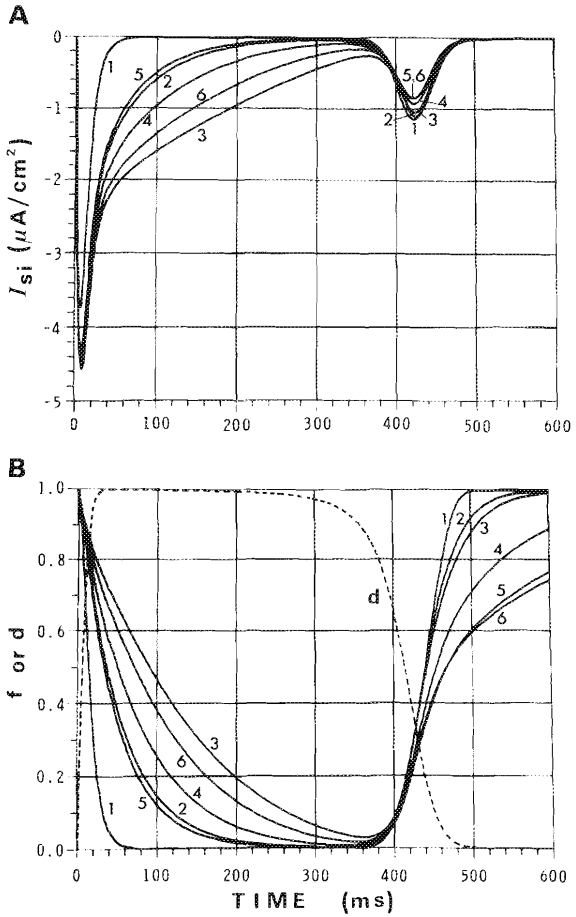

FIG. 4. Simulated variations of I_{si} (A) and inactivation parameter f (B), during the frog atrial AP of Fig. 1, for the different $\tau_f - E_m$ relationships of Fig. 3. All labels 1, 2, 3, 4, 5, and 6 refer to the corresponding curves of Fig. 3. Simultaneous variation of activation parameter d is also shown in (B).

intracellular stores and (or) by Ca efflux) with an assumed time constant of 200 ms, a value close to the time constant of tension relaxation in frog atrial muscle (Roulet et al. 1979). The influence of this term is, however, negligible during the development of I_{si}. Except during the initial rising phase, the time courses of I_{si} calculated with this model would be only slightly altered had a constant $E_{Ca} = 60$ mV been considered during the entire AP. The activation and inactivation variables d and f were given their resting steady-state values (at E_r) at the beginning of the AP.

Simulations of I_{si} during the course of the frog atrial AP

Figure 4A shows slow inward currents simulated with the H−H type model during the representative atrial AP of Fig. 1. Each curve is labelled to refer to the corresponding $\tau_f - E_m$ relationship of Fig. 3. Figure 4B gives the accompanying variations of the inactivation

and activation parameters, f and d, respectively. After the rapid increase in I_{si} during the rising phase of the AP, I_{si} declines more or less rapidly towards zero during the AP plateau. For the curve labelled "1" (Fig. 4A), which corresponds to the shortest τ_f at positive E_m, the amplitude of I_{si} does not reach its maximum, showing that some inactivation of I_{si} has occurred before full activation has been reached (see also Wong 1981). This is emphasized on the corresponding f curve whose intersection with the d curve shows that 30% of slow inward channels are already inactivated when only 70% are activated. The same phenomenon, although less pronounced, is apparent on all other curves.

The main feature of Fig. 4A is that a hump occurs on all I_{si} curves after 350 ms; during the subsequent 100−200 ms, I_{si} increases to 20−25% of its initial maximal value and then decreases again to zero. This "reactivation" phase of I_{si} corresponds to AP values of E_m between −10 mV and −50 mV (cf. Fig. 1) and is due to recovery of slow inward channels from inactivation occurring before the deactivation is completed (Fig. 4B). However, this may not have occurred if the removal of inactivation was due to an independent and slower process, as suggested by Kohlhardt et al. (1975) and Trautwein et al. (1975) in cat ventricular fibres.

To test this hypothesis, we incorporated into our model a second inactivation variable, l, using the representation of Kohlhardt et al. (1975). This parameter l was also of the H−H type and it differed from f only in its time constant, τ_l (milliseconds). We considered that τ_l was an increasing function of E_m (as in Kohlhardt et al. 1975; Trautwein et al. 1975). Owing to this assumption the voltage dependence of τ_l was derived from the $\tau_f - E_m$ relationship labelled "3" in Fig. 3 which exhibits this property; the rate constants α_l and β_l were correspondingly rescaled from α_f and β_f to satisfy the condition: $\tau_l \simeq 200$ ms at E_r. Finally, all rate constants (α_f, β_f, α_l, and β_l) were shifted 3.2 mV towards more positive potentials; this kept unchanged the position, on the voltage axis, of the new apparent steady-state inactivation parameter, $f_\infty l_\infty$ (or f_∞^2).

Figure 5 shows I_{si} curves simulated during the atrial AP of Fig. 1, with and without addition of parameter l. Curves labelled "1," "2," and "3" of Fig. 4A were compared with the corresponding curves "1,'" "2,'" and "3'" calculated when the parameter l was included in our model. In each case, the addition of parameter l decreased the amplitude of the reactivation phase (by about 40%) but did not suppress it completely.

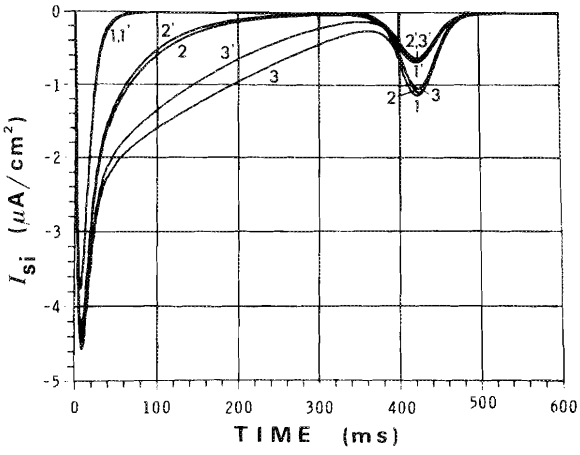

FIG. 5. Effect of the addition of an independent and slower process, responsible for the recovery from inactivation on the reactivation phase of I_{si}. Curves 1, 2, and 3 were taken from Fig. 4A and are compared with curves 1', 2', and 3' which were calculated with the additional inactivation parameter l (for further details, see text).

Discussion

The aim of the present study was to assess the validity of a Hodgkin−Huxley model of slow inward channel kinetics in frog atrium. The slow inward current (I_{si}) during the development of a standard AP was simulated using different time courses of the inactivation process. When the time constant of inactivation τ_l was set to 55 ms at $E_m = -15$ mV, the variation of I_{si} was biphasic: after a transient increase followed by a decrease to zero (during the upstroke and the plateau of the AP), I_{si} partially reactivated (at the beginning of the AP repolarization) and then fully deactivated. The reactivation phase of I_{si} developed whether τ_f was an increasing, decreasing, U-shaped, or bell-shaped function of E_m.

The development of a second surge of I_{si} was due to a relatively rapid recovery from inactivation and to the existence of an overlap between the $d_\infty - E_m$ and $f_\infty - E_m$ relationships (Fig. 2B). The size of this overlap was smaller in our conditions than in most mammalian cardiac preparations (e.g., Trautwein et al. 1975; Beeler and Reuter 1977) and was even smaller than from certain data from frog atria (e.g., Lenfant et al. 1972; Ducouret 1976). (A larger overlap would actually increase the amplitude of the reactivation phase of I_{si}.)

The recovery from inactivation was further examined. In the first simulations (Fig. 4) we considered that at each E_m removal of inactivation occurred with the same time constant as inactivation itself. This is consistent with the findings of Gettes and Reuter (1974), in mammalian myocardial fibres; however, it contradicts the data of Kohlhardt et al. (1975) and Trautwein et al. (1975) in cat ventricular fibres, indicating that recovery from inactivation is an independent and slower process. Such evidence was also obtained in frog atrial fibres by Mironneau et al. (1975) and Ducouret (1976), who

observed, at E_r, two different time constants of the process of recovery from I_{si} inactivation (τ_{f1} = 80–90 ms and τ_{f2} = 300–350 ms). More recently Shimoni (1981), in bullfrog atrial fibres, observed a time constant of recovery from inactivation at E_r of 200–312 ms.

The addition of an independent and slower process for removal of inactivation to our model, through a second inactivation parameter l (according to Kohlhardt et al. 1975), suppressed by only about 40% the reactivation phase of I_{si} (Fig. 5). To completely abolish the biphasic behaviour of I_{si}, l would have to reach a value close to zero at the end of the AP plateau and to recover more slowly its nonzero value during the AP repolarization. To achieve these conditions would require that τ_l be a steeply decreasing function of E_m, with a small value (<100 ms) when $E_m \simeq 0$ mV and a much larger value for E_m below −10 mV. However, no experimental evidence exists to date in cardiac muscle for such potential dependence of recovery from I_{si} inactivation. On the contrary, in frog atrium, a hyperpolarization is shown to facilitate, rather than to slow down, removal of inactivation and a high depolarization seems to avoid this process (D. Mentrard, G. Vassort, and R. Fischmeister, in preparation). Moreover, rapid kinetics for l at positive E_m would interfere with the inactivation mechanism itself, which would appear as a double, instead of single, exponentially decaying process. This effect is already significant on the I_{si} curves "2'" and "3'" (Fig. 5), which decrease from their initial peak value more rapidly than the corresponding curves "2" and "3."

Under these conditions the amount of Ca influx during this reactivation phase of I_{si} corresponds to 12% (Fig. 5, curve 3) and 65% (Fig. 5, curve 1) of that which entered the cell during the preceding phase. There are two observations which make uncertain the existence, under normal conditions, of a second surge of I_{si} during the AP repolarization. First, the additional influx of Ca ions occurs at a time when the twitch tension accompanying the AP is near its peak value and thus could possibly affect the relaxation phase of the contractile activity. However, there is no experimental evidence of any irregularity in the relaxation of contraction in frog atrium. Second, the presence of an important inward transient current is difficult to reconcile with the simultaneous increase in the total membrane outward current responsible for the repolarization (E_m = −10 to −50 mV). Two main currents participate in this phase of the frog atrial AP; the delayed outward current I_{xl} and the instantaneous outward current I_{K1}. Since I_{xl} is decreasing during this phase, the increase in I_{si} could be only compensated for by a substantial increase in I_{K1}. Thus, the rectification of the $I_{K1} - E_m$ relationship would be much larger than that observed experimentally (e.g., Brown et al. 1980).

It should be pointed out that, although the mammalian ventricular AP is shorter in duration than the amphibian atrial AP, a four to six times slower rate of I_{si} inactivation, during the AP plateau, was apparently necessary to properly reconstruct the mammalian ventricular AP (Beeler and Reuter 1977). However, such a large difference in the kinetics of the inactivation process of I_{si} is not a general observation (Coraboeuf 1980). In another AP model (McAllister et al. 1975) the authors had to consider that a certain fraction of slow inward channels in Purkinje fibres lack the property of inactivation. In addition, during their low amplitude AP plateau, E_m is inside the overlap between d_∞ and f_∞: this and the incomplete inactivation result in a significant net inward current in the plateau range, which compensates for the relatively rapid inactivation of I_{si} at these potentials. To simulate the proper configuration of the frog atrial AP, a different modification of the classical voltage-dependent model of I_{si} inactivation would be required.

Conclusion

Although the H–H representation of the slow inward channels has been already debated in mammalian cardiac preparations (Morad and Goldman 1976), it is to date most commonly accepted that the channel in heart is purely voltage dependent in a manner qualitatively similar to that described for the Na channel in nerve (Hodgkin and Huxley 1952). The present study shows, however, that concerning the inactivation of the slow inward channel in frog atrium, the classical H–H model applied in the range of the experimentally determined kinetical parameters leads to variations of I_{si} which are not supported by experimental data in terms of the corresponding mechanical and electrical events. Thus, these results question the validity of the present interpretation of I_{si} inactivation in frog heart as a purely voltage-dependent process.

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