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Action Potential-Like Responses due to the Inward Rectifying Potassium Channel

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Summary. This paper describes experiments carried out in the absence of sodium and calcium in the external solution. Frog atrial trabeculae were stimulated in current clamp with the double sucrose gap technique. The voltage responses looked like slow action potentials with a clear threshold. These responses were not suppressed in the presence of EGTA, in the presence of sodium or calcium channel blockers, or when sulfate ions replaced chloride. Guinea pig isolated ventricular myocytes were studied in whole cell clamp mode with a patch pipette. Under current clamp, they displayed also voltage responses with a threshold. These responses were resistant to cadmium (5 mM), and were suppressed by barium (0.5 mM). A negative slope conductance is required to take into account these results. The membrane current in current clamp can be estimated by plotting the response in the phase plane. This analysis shows that on both types of preparations, the current responsible for the negative slope is not time dependent. This current is suppressed by barium. It can be concluded that it is the outward current flowing through the inward rectifying potassium channels. To confirm this hypothesis, data obtained in voltage clamp on the same preparations were introduced into a computer model to predict the response in current clamp. The results were in agreement with the experiments. Similar responses could be recorded and analyzed on skeletal muscle in isotonic potassium solution. These results show that the inward rectifier can induce by itself properties looking like excitability on different preparations. The physiological significance of this effect in normal conditions is discussed. The voltage responses described in this paper look similar to the slow action potentials on heart, which are sensitive to modifications of the calcium channels, but also of the potassium channels. Some implications in cardiac pharmacology are discussed.

Key Words inward rectifier · I_{K1} · action potential · voltage clamp

Introduction

The inward rectifying potassium current decreases with voltage on depolarization, so that its slope conductance is negative for potentials positive to the potassium reversal potential (for review, *see* Stanfield et al., 1980). This current is therefore expected to produce by itself nonlinear membrane characteristics. It is, however, difficult in normal conditions to isolate the membrane properties due to this phenomenon.

We tried, in this study on different preparations, to estimate the properties of muscular cells underlain by this conductance. For this purpose, we used solutions free of sodium and of calcium to eliminate the presence of inward currents, and we compared the results obtained in current clamp and the results in voltage clamp. The phase plane representation was used to estimate the value of the membrane current in current-clamp conditions, and a computer model predicted the voltage response of a preparation whose properties are known from voltage-clamp experiments.

The experimental results, the phase plot analysis of the currents and the computer model consistently indicated that the inward-rectifying potassium current can elicit by itself depolarizing responses looking like action potentials.

Materials and Methods

Frog atrial trabeculae were studied in double sucrose gap (Rougier et al., 1968). To avoid the presence of Na or Ca in the clefts, the fibers were dissected and maintained in sodium- and calcium-free solution 20 min before the experiment. After a transient tension, they were relaxed before mounting into the chamber. The currents were stable for hours.

Solutions (concentrations in mM): KCl 15; $MgCl_2$ 2.8; $MnCl_2$ 0 to 3; pyruvic acid 2; sucrose 205; mannitol 0.4; Tris buffer 3; pH = 7.4. In some experiments, all the chloride anions were replaced by sulfate, and the difference in osmolarity was compensated by sucrose.

The resting potential was estimated by changing the central gap solution to isotonic potassium. It was ranging between −38 and −43 mV on different fibers in this solution.

Frog skeletal muscle bundles were studied in a double sucrose gap chamber (Rougier, 1964). The preparations were mounted in isotonic potassium solution where the resting poten-

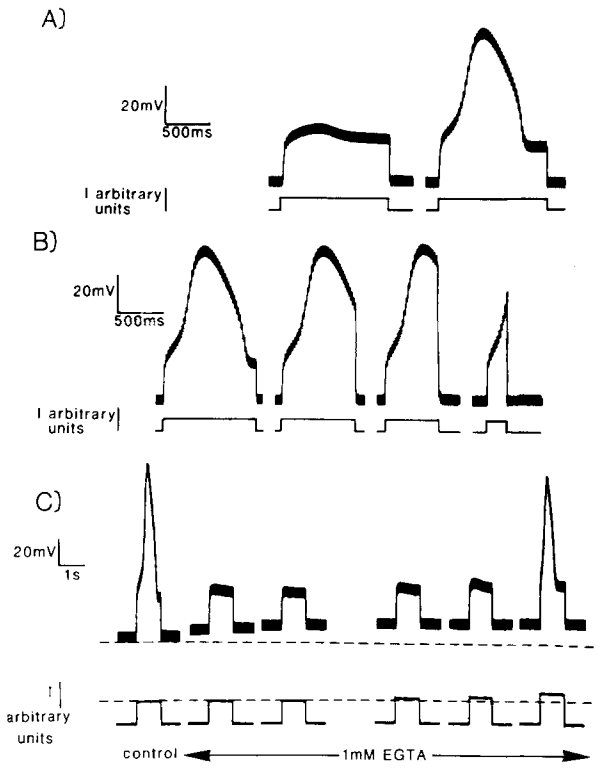

Fig. 1. Current clamp on frog atrial fibers in double sucrose gap. *See* text for details. (*A*) Two successive voltage responses to identical current stimulations. Notice that for the second stimulation, the voltage at the end of the response is less positive than the threshold voltage. (*B*) Effect of the stimulus duration on the response. (*C*) Effect of EGTA on the response. Fluctuations on voltage signals are due to power line 50 Hz

tial is zero, containing (mM): K_2SO_4 60; sucrose 60; $KHCO_3$ 2.4; pH = 8.0.

Guinea pig ventricular cells were dissociated enzymatically (Tourneur, Mitra and Morad, *in preparation*). The current or the potential was clamped by a giga seal pipette, with a DAGAN 8900 amplifier in whole cell clamp mode. The internal solution contained (mM): K-glutamate 115; NaH_2PO_4 10; beta-OH-butyrate 2; ATP 2; phosphocreatine 2; HEPES 5; pH = 7.2. The external solution contained (mM): KCl 5.4; $MgCl_2$ 1; $CdCl_2$ 5; mannitol 280; dextrose 10; pyruvic acid 5; HEPES buffer 5; pH = 7.3.

The action potential of the cell was controlled in normal tyrode before the experiment. It was at least 110 mV in amplitude and 200 msec in duration. When measured with a microelectrode, the membrane potential was close to −75 mV. The experimental chamber was continuously perfused and warmed at about 37°C.

The holding potential was the resting potential of the preparations. The difference between the membrane potential and the resting potential is referred to as *V*. All the preparations were stimulated at a constant rate between 0.2 and 0.05 Hz.

The oscilloscope traces of potential and current were recorded with a camera during the experiments and the traces were eventually digitized with a 7225 H.P. plotter-digitizer after enlargement. The computations were done on a SOLAR 16/40 minicomputer (BULL-SEMS, France). The equations are given in the Appendix.

Results

Frog Atrium

Figure 1*A* represents typical results in Na^+- and Ca^{2+}-free medium on frog atrium in double sucrose gap, in the presence of 3 mM Mn^{2+}. For two identical stimulations, the first voltage response resembles a local response, whereas the second one resembles a slow action potential, which indicates the presence of a threshold. The following experiments were carried out to determine the nature of this response.

Figure 1*B* shows the effect of the pulse duration. At any time, the membrane starts repolarizing instantly at the end of the stimulation, which indicates that the membrane current is net outward at any time during the voltage response (*see* Noble, 1975, Fig. 6-4*b*).

The 0 Ca solutions contained some traces of calcium in the micromolar range. We checked whether the remaining Ca^{2+} ions could be responsible for the depolarization observed. In Fig. 1*C*, the first trace represents the depolarizing response obtained in the same conditions as above. We then superfused the preparation with a similar solution in which 1 mM EGTA had been added. The following traces show that the resting membrane potential depolarizes as expected, and the response is rapidly suppressed. If the stimulus is increased, the depolarizing response reappears. A threshold response is therefore also observed in EGTA, and cannot be due to Ca^{2+} ions.

Similar responses were recorded in the presence of tetrodotoxin (5×10^{-7} M), when sulfate replaced Cl^- ions, and in the absence of manganese.

Guinea Pig Isolated Myocytes

Other experiments were carried out on isolated guinea pig ventricular cells in whole cell clamp mode using a patch pipette. Figure 2*A* shows the membrane response in current clamp. For negative pulses, the voltage traces are exponential. For low positive pulses, the traces are also exponential and reflect the presence of an inward rectification. By contrast, the rate of depolarization of the two most positive traces does not decrease to zero, and the repolarization looks delayed. A threshold discriminates two types of membrane behavior.

For longer current stimulations, the records are shown in Fig. 2*B* (other cell). The early phase of the

response looks like the initiation of an action potential, but the membrane keeps depolarizing during the whole stimulation, and repolarizes as soon as it ends.

The activation of an inward current seems unlikely in 5 mM cadmium, but in any case, it would be increased or at least unaffected in the presence of barium. Figure 2*C* represents the response of another cell. The control traces (left panel) were recorded in the same conditions as above. In the presence of 0.5 mM $BaCl_2$ (right panel), the voltage signal becomes exponential during the onset as well as during the offset of the response, as an electrotonus.

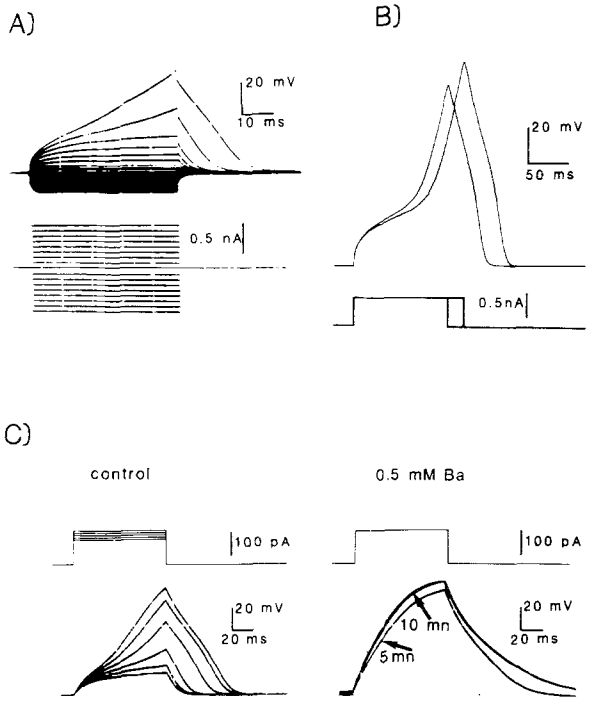

Fig. 2. Current clamp on guinea pig isolated myocyte with a patch-clamp pipette in whole cell clamp mode. *See* text for details. (*A*) Effect of the current amplitude on the membrane potential for short stimulations. (*B*) Effect of the current duration, for longer stimulations. (*C*) Other cell. Left panel: Effect of current amplitude on the response; Right panel: The largest current was applied continuously every 20 sec. The bath was then superfused with a Ba^{2+}-containing solution. The voltage traces obtained after 5 and 10 min superfusion in this solution are shown with arrows, and can be compared with the control (highest trace in left panel)

Use of the Phase Plane

The membrane current responsible for these responses was estimated in the phase plane representation. When the preparation is isopotential, the total current I_t is the sum of the membrane current I_m, and of the capacitive current CdV/dt, where C and t are the membrane capacitance and time, and V represents the depolarization from the resting potential. This can be written:

$$I_t = I_m + CdV/dt \tag{1}$$

or either

$$-dV/dt = (I_m - I_t)/C. \tag{2}$$

In current clamp, the total current I_t is the stimulation current, either zero or a constant positive value. When $I_t = 0$, for instance during the phase of repolarization, $-dV/dt = I_m/C$, then $-dV/dt$ represents I_m function of V, with a scaling factor $1/C$. During the depolarizing phase, $-dV/dt$ represents $(I_m - I_t)$, or I_m as a function of V, but shifted in the negative direction by a constant value.

Phase Plot of Guinea Pig Responses

Figure 3*B* shows the phase plot relative to the response shown in Fig. 3*A*. From rest, the arrows indicate the course of $-dV/dt$. The two curves corresponding to the depolarization and to the repolarization have the same shape, with a maximal value of $V = 25$ mV and a negative slope for more positive voltages. Within the experimental precision, these two curves can superimpose by a vertical shift. The membrane current I_m is then the same at a given voltage during the depolarization and during the repolarization. This result has been obtained for various current stimulations. It means that the current is depending on voltage, but is time independent at this time scale. The representation of the repolarization in this plane is quite similar to the representation of the normal action potential in mammalian ventricular preparation (Boyett et al., 1980).

More information can be obtained from this plot: the vertical distance between the traces relative to depolarization and repolarization is I_t/C, from Eq. (2). We know I_t and dV/dt, so we can estimate C. In this experiment, $I_t = 630$ pA and the distance between the two traces is 3.8 ± 0.1 V/sec, then $C = 630/(3.8 \pm 0.1) = 165 \pm 5$ pF. It is then possible to scale the phase plot in current amplitude. This has been done on the right vertical axis.

This "reconstructed" I/V curve can be compared to the "experimental" I/V curve obtained in voltage clamp on the same cell. The potential was clamped by the same suction pipette; the current after the outward capacitive transient appears time independent (Fig. 3*C*). The current measured at 25

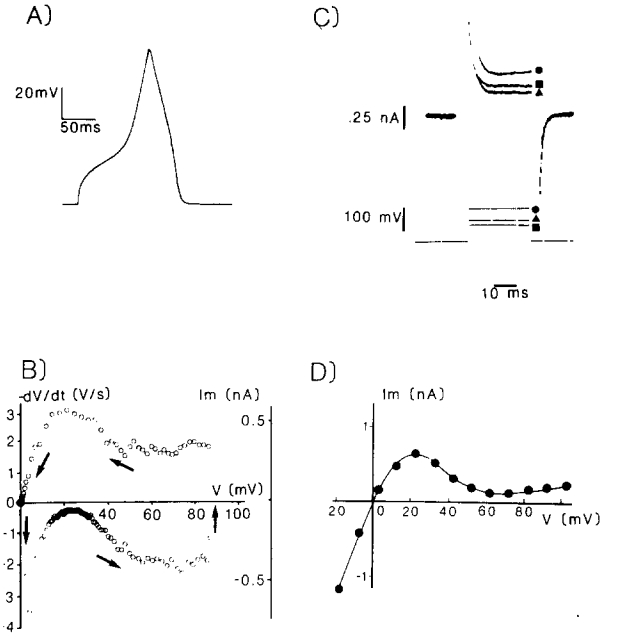

Fig. 3. Guinea pig myocyte: (*A*, *B*) Current clamp; time course (*A*) and phase plot (*B*) of the response (same as Fig. 2*B*). Note the initial instantaneous negative jump (positive jump in dV/dt), followed by the depolarizing phase (dV/dt positive, so $-dV/dt$ negative), the instantaneous jump at the end of the stimulation and the repolarization. The right vertical axis is scaled in current values, as described in the text. (*C*, *D*) Voltage clamp on the same cell: current signals for various voltage steps (*C*), showing a region of negative slope conductance (circles and triangles). The corresponding *I*/*V* curve is plotted in *D*. The current is measured at the end of the pulse

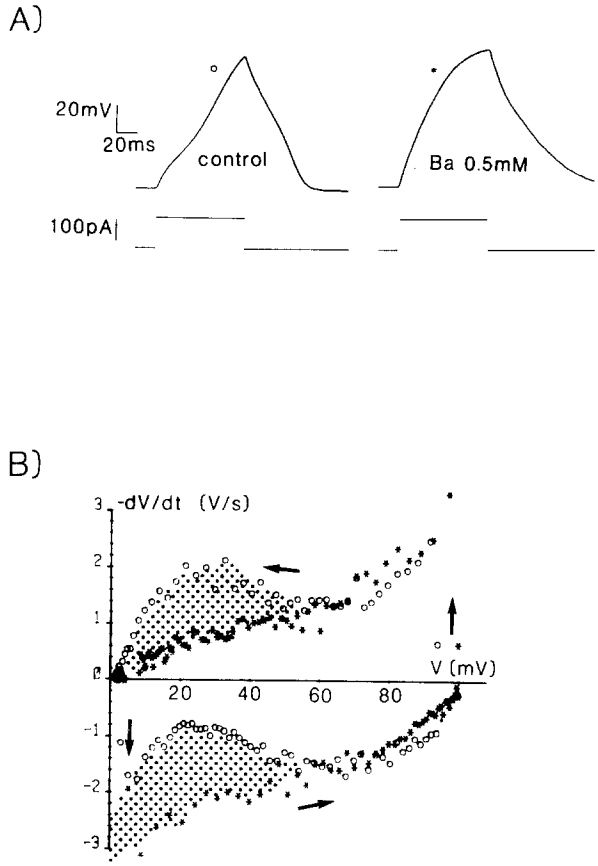

Fig. 4. Guinea pig myocyte: Same current-clamp experiment as in Fig. 2. *See* text for details. (*A*) Response in control (circles), and in the presence of barium (asterisks). (*B*) Phase representation of the preceding responses. Note that the traces relative to the depolarization (lower half of each curve), and to the repolarization (higher half) have the same shape in control (circles), and also in barium (asterisks). The dotted surface corresponds to the difference between control and barium

msec is plotted as a function of potential in Fig. 3*D*. The *I*/*V* curve obtained from voltage clamp and the "reconstructed" *I*/*V* curve are similar: they both reach a maximum value, $I_{max} = 0.6$ nA, at $V = 25$ mV, and have a negative slope.

The same analysis was applied to the previous barium experiment (Fig. 4*A*,*B*). The plot from the control response (open circles) has the features described earlier. In the presence of barium (asterisks), the two curves corresponding respectively to the depolarization and to the repolarization can superimpose. The current is therefore again in this case time independent. The vertical shift between these two curves is the same as in control, as expected from the theory. The dotted surface underlines the difference between control and barium. It represents either a time-independent barium inward current or an outward current which has been suppressed by Ba^{2+}. The hypothesis of a barium current can be discarded in these conditions:

(i) the experiments were carried out in 5 mM Cd^{2+} which blocks all the slow inward currents at this concentration (Noble, 1984).

(ii) The current is time independent in control as well as in barium, which has been confirmed in voltage clamp (*not shown here*).

This experiment represents then the suppression by barium of a time-independent outward current.

Phase Plot of Frog Atrium Responses

Similar analyses can be performed on the frog depolarizing responses, as shown in Fig. 5*A*,*B*. During the current stimulation, the value of $-dV/dt$ jumps instantly in the negative direction, then follows an initial course with a positive, then a negative slope as on guinea pig. The depolarization activates here an outward time-dependent current which in turn repolarizes the cell during the current pulse, and the

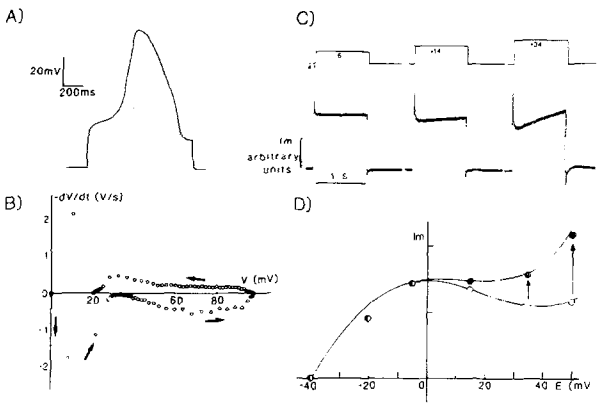

Fig. 5. Frog atrium: (*A*, *B*) Current clamp; time course (*A*) and phase plot (*B*) of the response. *See* text for details. (*C*, *D*) Voltage clamp on the same preparation: current signals for various voltage steps (*C*). Note the outward tail current after the last voltage step. The corresponding *I/V* curve is plotted in *D*. The current is measured after the end of the capacitive transient (open circles) and at the end of the voltage step (filled circles). The arrows represent the time dependence of the current

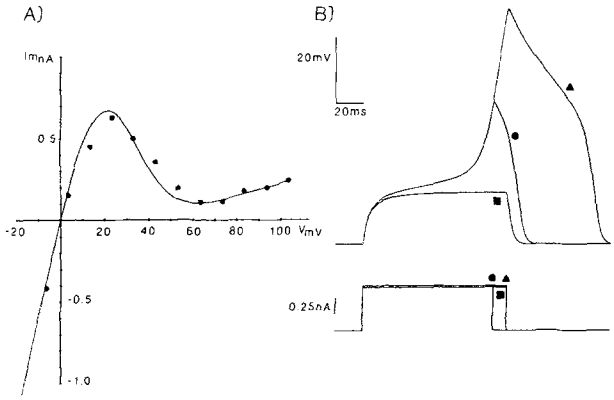

Fig. 6. Computer simulation of the guinea pig response. (*A*) Curve of the empirical *I/V* relation used for the calculations, together with the experimental values. The equations are described in the Appendix. (*B*) Simulated voltage response to three different current stimulations, differing by their amplitude (square, triangle) or their duration (circle, triangle)

phase plot runs into the positive half-plane, as shown by the arrows, up to 20 mV. In this case, the points relative to the initial and the final jumps are uncertain because of the sampling rate. This plot has a negative slope on depolarization, but also on repolarization, at the same potential. The corresponding current is then voltage dependent but time independent.

The plot can be compared to the results obtained on the same preparation in voltage clamp. Figure 5*C* shows the membrane current in response to three voltage steps. The initial current after a positive voltage jump is less outward when the potential is more positive, whereas the current at the end of the pulse increases with voltage. The time-dependent current is outward, as shown by its tail.

The *I/V* curve in Fig. 5*D* shows the initial (open circles) and the final (filled circles) values of the current for 1100 msec voltage steps. The curve corresponding to the initial value has a clear negative slope at the same potentials as the curve obtained on the phase plot. It corresponds to the time-independent part of the current.

The plots in the phase plane obtained on two different preparations show that the negative slope of the current voltage relation is similarly due to a time-independent current. On guinea pig this current is suppressed in the presence of barium; it is likely due to the negative slope conductance of the I_{K1} current, described on multicellular preparations (Noble, 1976; Trautwein & McDonald, 1978; Cleeman & Morad, 1979), and on isolated cells (Momose et al., 1984; Sakmann & Trube, 1984).

Computer Simulation

Simulation of Guinea Pig Depolarizing Responses

To check whether the hypothesis of I_{K1} underlying the depolarizing responses is physically reasonable, we introduced into a computer model the experimental properties of the preparation and simulated its response in current clamp by integration of Eq. (2) (*see* Appendix). Figure 6 shows a typical example on a guinea pig ventricular cell. The current-voltage relation is plotted in Fig. 6*A*. The membrane capacitance was estimated as described earlier. We determined empirically an analytic curve which fits the experimental *I/V* curve. We can observe on Fig. 6*B*, with two slightly different stimulations, that the simulated response has a clear threshold. The membrane repolarizes instantly at the end of the stimulus, as observed experimentally.

Simulation of Frog Depolarizing Responses

The same kind of model was applied to frog depolarizing responses. We assumed in the following model that the repolarizing current activating on depolarization has two components, I_{x1} and I_{x2}, as described by Ojeda and Rougier (1974). We assumed further that the activating parameters of these currents were unchanged by external potassium or sodium. The activation and time constant curves are plotted in Fig. 7*A*. Figure 7*B* shows the

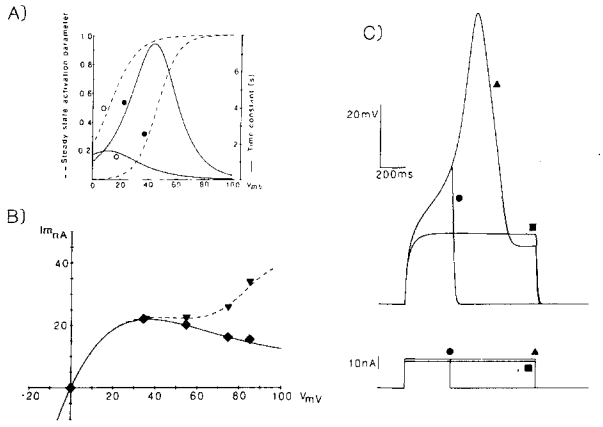

Fig. 7. Computer simulation of the frog response. (*A*) Plot of the time constants (full lines) and of the steady-state activation parameters (broken lines) of the two currents I_{x1} (open circles) and I_{x2} (filled circles). Equations are described in the Appendix. (*B*) Simulation of a voltage-clamp experiment; instantaneous current (full line) and current at 1100 msec (broken line). Diamonds and triangles represent the corresponding experimental currents. Similar fitting at $t = 600$ msec is not shown here for clarity. (*C*) Simulated voltage response to three current stimulations, using the preceding model, for three different stimulations. The value of the membrane capacitance was taken from Connor, Barr and Jakobsson (1975). The results show the existence of a threshold. Notice that the final value of potential after the repolarization is lower than the threshold, as experimentally (Fig. 1*A*)

I/V relation at $t = 0$ and at the end of a 1100 msec voltage step. The points represent experimental values. In order to fit the data, the ratio of the maximum activated conductance of I_{x1} and I_{x2} were found close to the ratio described by Ojeda and Rougier. The model represents therefore this preparation on a reasonable basis.

The simulation in Fig. 8*C* shows the existence of a threshold, and that the depolarization is similar to the experimental one and cannot maintain in the absence of stimulus.

Discussion

Action potential-like responses were obtained on frog atrial fibers and guinea pig isolated myocytes with two different techniques. These responses were obtained in the absence of sodium and calcium, in the presence of 1 mM EGTA, calcium blockers (Mn^{2+}, Cd^{2+}) and sodium blockers (TTX, Cd^{2+}). The anion could be chloride or sulfate. Barium, which crosses calcium channels, does not increase these responses, but suppresses them. It can therefore be excluded that these responses are due to a current flowing through the calcium channels, or to a chloride current.

A current with a negative slope conductance is required to produce a threshold. The phase plane analysis shows that this current is time independent, and is suppressed by barium. It can be concluded that it is the time-independent potassium current: the inward rectifier, called I_{K1} on heart. This assumption could be confirmed by computer simulation.

Similar responses are expected on other preparations: The inward rectifying K^+ current on frog skeletal muscle in isotonic potassium solution, has a negative slope conductance (Stanfield et al., 1980). Figure 8 shows that depolarizing responses with a threshold can be recorded under current clamp, the phase plot reflects an *I/V* curve similar to the experimental one, and the response can be simulated without the need of any inward current, as on heart.

Depolarizing responses with a threshold are elicited in conditions where no inward current activates. They differ from action potentials:

i) During the upstroke, the increase in dV/dt, when the slope conductance is negative, corresponds to a decrease in membrane conductance rather than an increase, as in a normal action potential.

ii) A maintained current is necessary, so the voltage depends on the stimulation, and is not "all-or-nothing."

iii) For the same reason the membrane response does not propagate from a local source of current stimulation on a large preparation (computations are in progress).

The threshold current, that is the maximum value of the outward current flowing through this channel, is reached at 20 to 30 millivolts positive to the resting potential (on skeletal muscle: Stanfield et al., 1980; on heart: Noble, 1975; Trautwein & McDonald, 1978; Sakmann & Trube, 1984). In normal conditions, this value is close to the threshold of the action potential. The inward rectifier must then contribute to this threshold, where its relative importance remains to be studied.

The voltage responses on heart resemble slow action potentials (for review, *see* Carmeliet, 1980), to which the inward rectifier must contribute. The rate of rise dV/dt is then not a direct reflection of the slow inward current. Some pharmacological agents may act on potassium channels, instead of on calcium channels: adenine which increases the K^+ permeability (Hartzell, 1979) suppresses these slow responses (Schrader et al., 1975), whereas formaldehyde which blocks the inward rectifier (Hutter & Williams, 1979), increases their duration (Kolhardt, 1983). Similarly, the current attributable to magnesium (Späh & Fleckenstein, 1979) and to manganese (Ochi, 1975) from current-clamp experiments, might at least partly flow through potassium channels.

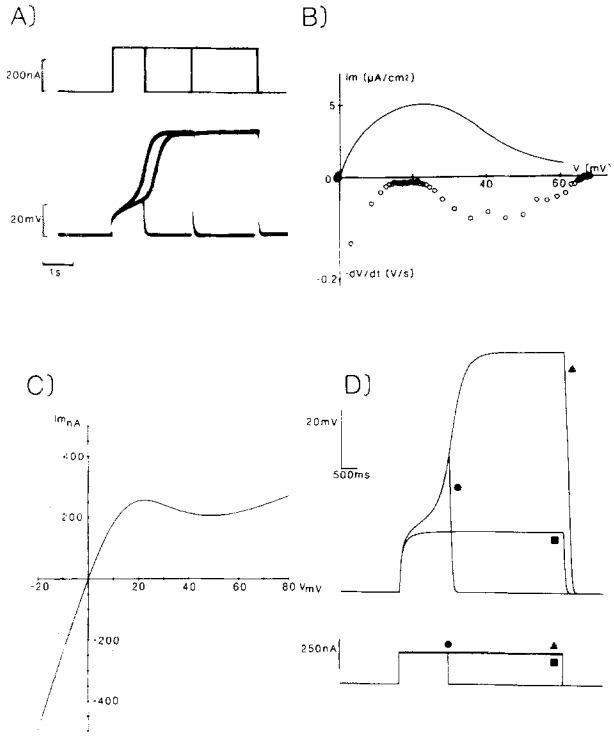

Fig. 8. Depolarizing responses obtained in current clamp on frog skeletal muscle in isotonic potassium sulfate. In this solution, the preparation is depolarized to zero mV, so the delayed K current is quite inactivated. (*A*) Responses obtained for various durations. Note that the current reaches a steady-state value. (*B*) Circles, lower vertical scale: phase plot of the response. It indicates that the current is outward and a negative slope conductance for potentials positive to 25 mV, as on heart. For potentials positive to 50 mV, the curve appears linear, possibly due to a leak component. Full line, upper vertical scale: current-voltage relation obtained by Standen and Stanfield (1978), in similar solution, after subtraction of the linear 'leak' component. This curve is redrawn by hand. (*C*, *D*) Computer simulation of the skeletal muscle response. (*C*) Current-voltage relation used for calculations. The current is the sum of a current as described by Standen and Stanfield (1978) and a linear leak. The equations are described in the Appendix. (*D*) Voltage responses for three different stimulations, showing the presence of a threshold, and which repolarizes as experimentally

Many pharmacological studies concerning the calcium channels on heart are based on slow action potential measurements. Our results show that their interpretation must be careful.

The experiments on frog atrial fibers were carried out with C. Ojeda and O. Rougier in Villeurbanne; the experiments on isolated guinea pig isolated cells with R. Mitra and M. Morad, in M. Morad's laboratory in Philadelphia; the skeletal muscle experiments were performed by O. Rougier. I thank R. Mitra, M. Morad, M. Ildefonse, C. Ojeda and O. Rougier for their useful comments, D. DiFrancesco for careful reading and J. Barbery for artwork. This study has been supported by grant INSERM C.R.E. 855021.

References

Boyett, M.R., Coray, A., McGuigan, J.A.S. 1980. Cow ventricular muscle: 1- The effect of extracellular potassium concentration on the current-voltage relationship. 2- Evidence for a time-dependent outward current. *Pfluegers Arch.* **389:**37–44

Carmeliet, E. 1980. The slow inward current: Non-voltage-clamp studies. *In:* The Slow Inward Current and Arrythmias. D.P. Zipes, J.C. Bailey, and V. Elharrar, editors. Nijhoff Publishers, The Hague/Boston/London

Cleeman, L., Morad, M. 1979. Extracellular potassium accummulation in voltage-clamped frog ventricular muscle. *J. Physiol. (London)* **286:**83–111

Connor, J., Barr, L., Jakobsson, E. 1975. Electrical characteristics of the frog atrial trabeculae in the double sucrose gap. *Biophys. J.* **15:**1047–1067

Hartzell, H.C. 1979. Adenosine receptors in frog sinus venosus: Slow inhibitory potentials produced by adenine compounds and acetylcholine. *J. Physiol. (London)* **293:**23–49

Hutter, O.F., Williams, T.L. 1979. A dual effect of formaldehyde on the inwardly rectifying potassium conductance in skeletal muscle. *J. Physiol. (London)* **286:**591–606

Kolhardt, M. 1983. Modifications of drug-induced cardiac slow inward current block by cooling, pH variations, and formaldehyde in mammalian ventricular myocardium. *J. Cardiovasc. Pharmacol.* **5:**968–977

Momose, Y., Giles, W., Szabo, G. 1984. Acetylcholine-induced K current in amphibian atrial cells. *Biophys. J.* **45:**20–22

Noble, D. 1975. The Initiation of the Heartbeat. Clarendon, Oxford

Noble, D. 1984. The surprising heart: A review of recent progress in cardiac electrophysiology. *J. Physiol. (London)* **353:**1–50

Noble, S. 1976. Potassium accumulation and depletion in frog atrial muscle. *J. Physiol. (London)* **258:**579–613

Ochi, R. 1975. Manganese action potentials in mammalian cardiac muscles. *Experientia* **31:**1048–1049

Ojeda, C., Rougier, O. 1974. Kinetic analysis of the delayed outward currents in frog atrium: Existence of two types of preparations. *J. Physiol. (London)* **239:**51–73

Palti, Y. 1971. Digital computer reconstruction of axon membrane action potential. *In:* Biophysics and Physiology of Excitable Membranes. W.J. Adelman, editor. V.N.R., New York

Rougier, O. 1964. La rectification de la membrane musculaire étudiée à l'aide du "sucrose gap". *Helv. Physiol. Acta* **22:**C36–C38

Rougier, O., Vassort, G., Stämpfli, R. 1968. Voltage clamp experiments on frog atrial heart muscle fibres with the sucrose gap technique. *Pfluegers Arch.* **301:**91–108

Sakmann, B., Trube, G. 1984. Conductance properties of single inwardly rectifying potassium channels in ventricular cells from guinea-pig heart. *J. Physiol. (London)* **347:**641–657

Schrader, J., Rubio, R., Berne, R.M. 1975. Inhibition of slow action potentials of guinea pig atrial muscle by adenosine: A possible effect on Ca^{++} influx. *J. Mol. Cell Cardiol.* **7:**427–433

Späh, F., Fleckenstein, A. 1979. Evidence for a new, preferentially Mg-carrying, transport system besides the fast Na and the slow Ca channels in the excited myocardial sarcolemmal membrane. *J. Mol. Cell Cardiol.* **11:**1109–1127

Standen, N.B., Stanfield, P.R. 1978. Inward rectification in skeletal muscle: A blocking particle model. *Pfluegers Arch.* **378:**173–176

Stanfield, P.R., Standen, N.B., Leech, C.A., Ashcroft, F.M.

1980. Inward rectification in skeletal muscle fibres. *In:* Molecular and cellular aspects of muscle function. *Adv. Physiol. Sci.* **5**:247–262

Trautwein, W., McDonald, T.F. 1978. Current voltage relations in ventricular muscle preparations from different species. *Pfluegers Arch.* **374**:79–89

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Appendix

The integration procedure is the same as used by Palti (1971). Potentials are in mV.
Analytical functions and numerical values for the simulation:

Guinea Pig Isolated Myocytes:

membrane capacitance: $C = 0.165$ nF

membrane current: $I_m = g_K(V - V_K) + g_L(V - V_L)$, with:

$g_K = 0.076/(1 + \exp(0.001 \cdot V^2 + 0.03625 \cdot V - 0.847))$ μS

$V_K = -3.1$ mV

$g_L = 0.039$ μS

$V_L = 42$ mV

integration step: $T = 0.5$ msec.

Frog Atrial Fibers:

membrane capacitance: 15 nF

membrane current: $I_m = I_{K1} + I_{x1} + I_{x2} + I_L = (g_{K1} + g_{x1} + g_{x2} + g_L) \cdot V$

$g_{K1} = 3.6/(1 + \exp((V + 12)/28))$ μS

$g_L = 0.0555$ μS

$g_{x1} = 0.013 \cdot n_1$

$g_{x2} = 0.26 \cdot n_2$

n_1, n_2 are the activation variables defined by:

$dn_i/dt = a_i \cdot (1 - n_i) - b_i \cdot n_i$ for $i = 1$ or 2, and

$a_1 = \exp(0.051 \cdot (V - 34))$ msec^{-1}

$a_2 = \exp(0.077 \cdot (V - 81))$ msec^{-1}

$b_1 = \exp(-0.05 \cdot (V + 12))$ msec^{-1}

$b_2 = \exp(-0.059 \cdot V)$ msec^{-1}

integration step: $T = 2$ msec.

Frog Skeletal Muscle:

membrane capacitance: $C = 800$ nF

membrane current: $I_m = I_K + I_L = (g_K + g_L) \cdot V$ with:

$g_K = 22/(1 + \exp((V - 17)/10))$ μS

$g_L = 3.33$ μS

integration step: $T = 5$ msec.