

Membrane Responses to Large Hyperpolarizations in Trabecles and Single Cells of Frog Atrium

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Abstract. Atrial trabeculae (studied in voltage-clamping conditions and in the presence of 0.5 mmol/l BaCl₂ to abolish gK_1) responded to 1 s hyperpolarizations to beyond approximately $E = -140$ mV (from HP of about $E = -80$ mV) with an inwardly directed current increasing with time. Quite similar results were obtained with enzymatically dissociated frog atrial cells studied in whole cell voltage clamp with a patch-clamp pipette. This behaviour could be accounted for by assuming the presence of an " i_f " current at this quite negative range of potentials or by the fact that the cell membrane may undergo reversible electropermeabilization when its potential is brought to values negative to about -140 mV (Stämpfli 1958). When a brief (1 ms) and large (150 mV) hyperpolarization was applied 1 s before the test pulse, an inwardly directed current increasing with time was elicited by test pulses to beyond approximately $E = -120$ mV. This current was neither abolished in the presence of 1 mmol/l CsCl nor greatly reduced in the absence of Na⁺ ions, unlike " i_f " (Di Francesco 1981). We conclude that this current having a time course similar to that of " i_f " is of different nature and we argue that it might be accounted for by electropermeabilization of the membrane (reversible within about 2.5 min) due to the electrical shock represented by a brief and large hyperpolarization.

Key words: Frog atrial cell membrane — Electric shock — Membrane injury — Single cells — Voltage clamp

Introduction

Since the work of Di Francesco (1981) showing that the current system iK_2 closely resembles that of " i_f " in the sino-atrial node (Brown and Di Francesco 1980; Di Francesco and Ojeda 1980; Yanagihara and Irisawa 1980), the existence of this current has been shown by Earm et al. (1983) in mammalian (sheep

and calf) atrium and more recently by Carmeliet (1984) in human atrium. Preliminary experiments made us hypothesize that " i_f " might also be present in frog atrium (Bonvallet and Ojeda: unpublished observations).

The present experiments were designed to reexamine this hypothesis. Indeed, we were able to record an inward current at very negative potentials that had some similarity with the " i_f " current (Bonvallet 1981). Nevertheless, the potential range for its apparent activation was far more negative than that reported for " i_f " in mammalian atria, and it did not display any of the known important properties of the " i_f " current.

We discuss whether the present results may be explained by a transient electropermeabilization phenomenon rather than by the existence of an " i_f " current.

Materials and Methods

Multicellular preparation:

Thin, free running trabeculae (0.06–0.1 mm in diameter, 2–3 mm in length) were isolated from the atrium of *Rana esculenta*.

The standard perfusing solution had the following composition (in mmol/l): NaCl 110; KCl 2.5; CaCl₂ 1.8; MgCl₂ 2.0; HEPES-NaOH buffer 10; glucose 10; pyruvic acid 5 (pH = 7.40). In all experiments, BaCl₂ (0.5 mmol/l) was used to reduce the background K⁺ permeability by blocking gK₁ (Cohen et al. 1983). When necessary, TTX (10⁻⁶ mol/l) was added to block the fast inward sodium current (Rougier et al. 1969; Connor et al. 1975) so that membrane potential "escapes" be prevented on return to holding potential (HP) after hyperpolarization.

Voltage-clamp experiments were performed at 17 ± 0.5°C using the double sucrose gap technique (Rougier et al. 1968). Both at the beginning and at the end of each experiment, the resting potential of the preparation was estimated under current-clamp conditions as described by Brown and Noble (1969). When values less negative than -70 mV were obtained, or when drops by more than 10% along an experiment occurred, the preparation was not considered in results. Holding potential (HP) was the resting potential of the preparation. Current and trans-gap potential were recorded both with a pen recorder (Gould Brush 2400) and with a four-channel FM tape recorder (Tandberg, Series 100) for subsequent analysis with a Solar 16/40 minicomputer (Bull-Sems, France). The superimposed traces in Fig. 1 were done with a HP-7225 plotter (Hewlett-Packard).

Single cell:

Frog atrial cells were enzymatically dissociated as described by Bonvallet (1987).

Cells retained for experiments were at rest with a resting potential equal to, or more negative than, -80 mV in standard superfusing solution.

The whole-cell-recording (WCR) configuration of the patch clamp technique (Hamill et al. 1981) was used for voltage clamping of single cells. Patch clamp electrodes were pulled on a modified vertical puller (Kopf model 700D, David Kopf Instruments, Tujunga, CA, USA) and their resistance ranged between 1 and 4 megaohms. They were filled with a solution containing (in mmol/l): KCl 120; HEPES-KOH 10; EGTA-KOH 1; NaCl 10; MgCl₂ 5; pH = 7.4. A patch-clamp RK300 amplifier (Bio-Logic, Grenoble, France) was used to monitor cell currents. After obtaining a giga

seal (10 to 100 gigaohms), the membrane patch was disrupted by applying a brief suction to the pipette interior to get whole cell voltage-clamp (Marty and Neher 1983). The series resistance was usually 5–15 megaohms, 50 to 80% of which could be compensated. This was assessed by continuous monitoring of both increases in input capacitance in the voltage clamp mode and changes in pipette potential in the current clamp mode. No series resistance compensation was used and appreciable distortions of fast currents were expected (Hume and Giles 1983). Traces were printed with a pen recorder (Gould Brush 220).

All the results reported herein were obtained in at least 10 separate preparations and the records shown are representative of the whole series. No exact numerical values are to be retained since this study did not aim to be more than qualitative.

Results

1 — *Effect of large and short hyperpolarizing prepulses on the membrane currents elicited by long hyperpolarizing pulses:*

Figure 1*A* shows membrane currents elicited by hyperpolarizing pulses from HP = -80 mV. The potential during test pulses (1 s) ranged from -90 to -170 mV in steps of 10 mV. The test pulses were applied in 3 min intervals. It can be noted that for potentials between -90 and -140 mV, the current reached a steady-state value about 0.1 s after the pulse start. For potentials from -140 to -170 mV, the current, after an initial decrease as above, progressively increased in the inward direction. Moreover, repolarization from these potentials evoked a slight inward tail current that caused the current trace to thicken after the pulse termination.

When a large and short hyperpolarizing command pulse (150 mV from HP, 1 ms) was applied to the same fibre 1 s before the same test pulses as shown in Fig. 1*A* (Fig. 1*B*, inset), the situation drastically changed (Fig. 1*B*). At the end of the short hyperpolarizing prepulse (further referred to as "the shock"), an inward tail current appeared, which did not completely return to the pre-shock level when a hyperpolarizing test pulse was applied. Both the amplitude and the time-course of this tail current in response to identical shocks was quite reproducible (Fig. 1*B*).

Notice that: i — the current response to hyperpolarization to -90 mV remained unchanged; ii — after shock, test pulses to -100 and -110 mV elicited larger current responses (Fig. 1*B*) than those without it (Fig. 1*A*) and after the former steady-state was reached within the pulse duration; iii — test hyperpolarizations to -120 mV and beyond evoked, in addition, an inward current that increased during hyperpolarization with time and with the amplitude of the test pulse; iii — on return to HP, an inward tail current was present for pulses to -120 mV and beyond and its amplitude was also potential-dependent.

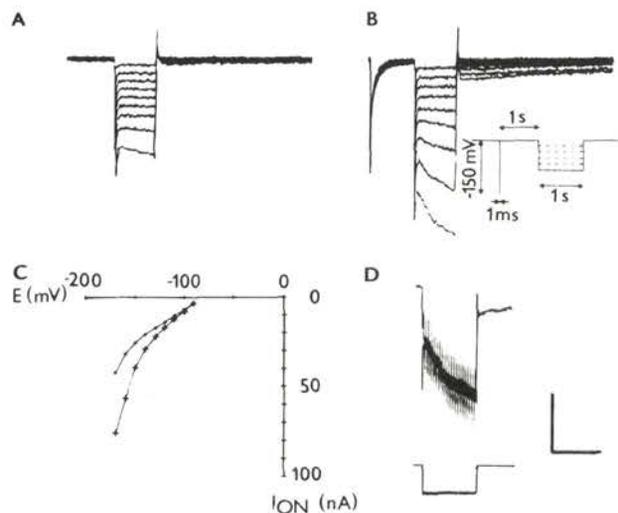


Fig. 1. *A*: Current responses to hyperpolarizing test pulses of 1 s duration bringing E from $HP = -80$ mV to values ranging from -90 to -170 mV in 10 mV steps. *B*: Current responses to the same hyperpolarizing test pulses as in *A* with a preceding short (1 ms) and large hyperpolarization (command potential -150 mV from HP) applied 1 s before (see inset). *C*: The amplitude of the current 900 ms after the start of the test pulse (I_{on}) plotted versus membrane potential (E), for control conditions (dots, corresponding to records in *A*) or after brief hyperpolarization (crosses, corresponding to records in *B*). *D*: In another preparation, a protocol similar to that shown in the inset to *B* was applied, except that HP was at -90 mV and that the test pulse was to -180 mV for 2.2 s. In order to measure the membrane conductance, short small hyperpolarizing pulses (40 ms, 10 mV) were superimposed with the test pulse (lower trace). The current responses (upper trace) to these small pulses (appearing as thickened vertical traces) increased with time, indicating an increase in membrane conductance to ions carrying the true inward current. Vertical bar: 50 nA. Horizontal bar: panels *A* and *B*: 1 s; panel *D*: 2 s.

Figure 1 *C* shows the current-voltage relations constructed from the records shown in Fig. 1 *A*, *B* for currents measured 900 ms after the start of the test pulse either without shock (filled circles) or 1 s after shock (crosses). Note that although both curves show an increase in slope conductance at most negative potentials, shock makes the curve larger and to start at less negative potentials than it is the case without shock. Short (40 ms) and small (-10 mV) hyperpolarizing pulses were applied to another preparation during a 2.2 s test pulse from -90 mV to -180 mV, in order to measure the membrane conductance. The pulse was preceded by the same shock protocol as above. Current responses to the short pulses (Fig. 1 *D*) increased with time as the current developed; this suggests that the membrane conductance was increasing with time. Bearing this in mind, the slight voltage displacement during the hyperpolarizing test pulse (Fig. 1 *D*) confirms that the time-dependent current was a true inward current.

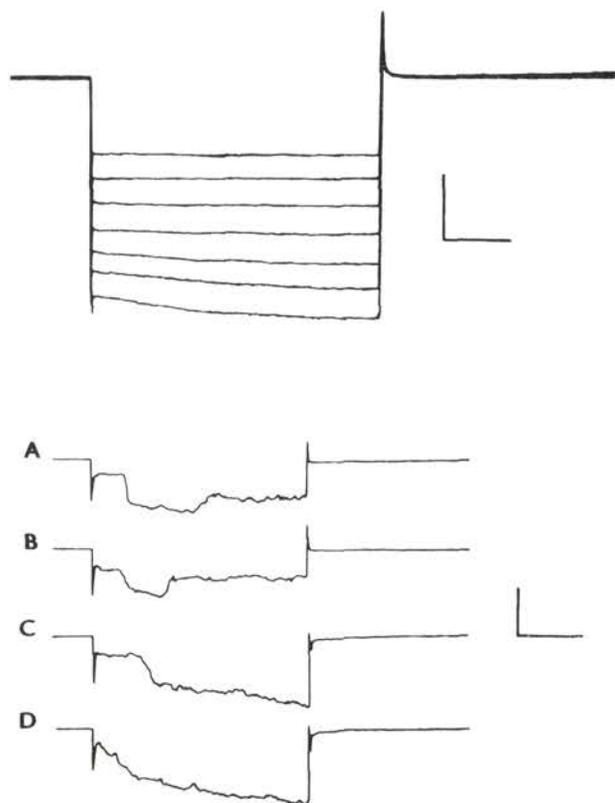


Fig. 2. Membrane currents recorded from a single dissociated frog atrial cell. The external medium did not contain barium ions. Holding potential was approximately $E = -80$ mV. *Upper panel:* photographically superimposed paper records of responses to 1.36 s pulses at $E = -120$ mV to -180 mV in 10 mV steps. Vertical bar: 20 pA, horizontal bar: 400 ms. *Lower panel:* responses of the same cell in the same conditions to pulses to $E = -260$ mV. Vertical bar: 200 pA, horizontal bar: 400 ms.

Single cells responses:

Isolated cells were voltage clamped at a HP about $E = -80$ mV in absence of Ba^{++} . They responded quite similarly as did multicellular preparations to long (1.36 s) hyperpolarizations applied in 30 s intervals (Fig. 2). An inwardly directed current creep was detectable when the membrane potential was brought to values more negative than -140 mV (Fig. 2, upper panel). The inward creep further increased with the magnitude of the hyperpolarizing pulse between $E = -180$ mV and $E = -200$ mV (not shown). At potentials more negative

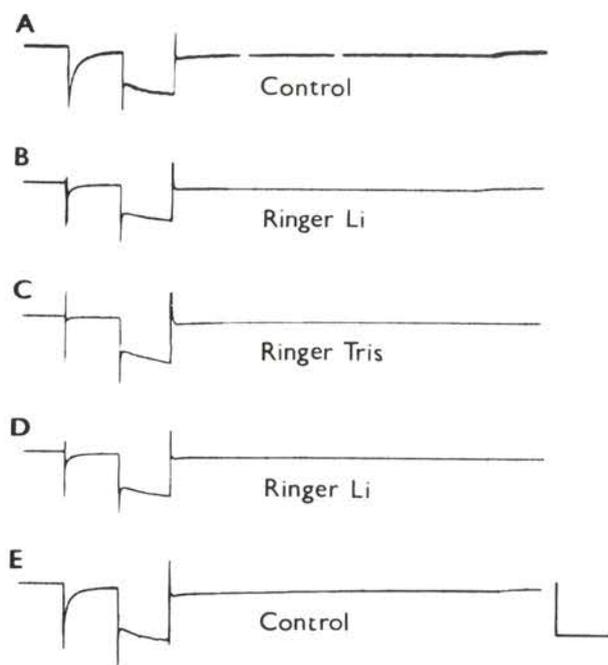


Fig. 3. Current records from the same fibre and with the same protocol as in Fig. 1 *B*. HP was at $E = -76$ mV and the test pulse brought E to -136 mV. *A*: control; *B* to *E*: each record was taken 10 min after switching the solution. *B*: all Na^+ ions replaced by Li^+ ions; *C*: all Na^+ ions replaced by Tris^+ ions; *D*: same as in *B*; *E*: return to control solution. Vertical bar: 50 nA. Horizontal bar: 1 s.

than -200 mV, the response for a given pulse became irregular, showing jagged deflections, as shown in response to pulses to -260 mV in Fig. 2, lower panel.

2 — Effect of sodium removal on the inward current:

It was shown that the " i_f " current is largely reduced in amplitude when Na^+ ions are partly or totally replaced (Di Francesco 1981; Earm et al. 1983).

Figure 3 illustrates an experiment performed on the same fibre as that in Fig. 1 *A* to *C*, with sodium ions replaced either by Li^+ ions (panel *B*) or Tris^+ ions (panel *C*). The inward current, recorded after 10 min in each medium, was elicited by a 950 ms hyperpolarizing pulse from -76 to -136 mV. The characteristics of the shock were 150 mV from HP, 1 ms in duration, delay 1 s. It can be seen that the inward current during the test pulse and the inward tail current

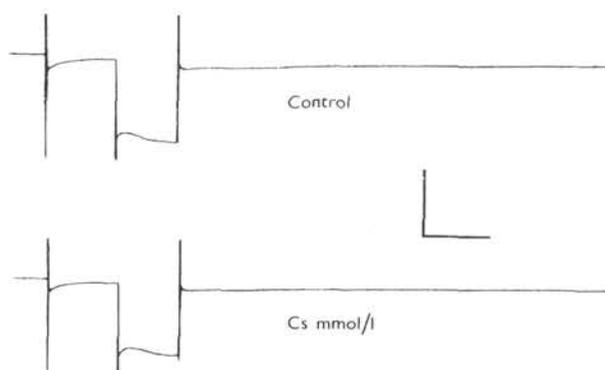


Fig. 4. Same protocol as in Fig. 1B except that HP was at $E = -84$ mV and that the test pulse brought E to -154 mV (900 ms). Upper panel: control solution; Lower panel: 20 min after the addition of 1 mmol/l CsCl. Vertical bar: 50 nA. Horizontal bar: 1 s.

on return to HP were slightly decreased at Na^+ ions removed (panels B—C). Nevertheless, the tail current induced at the end of the shock was very strongly decreased in LiCl (panel B) and was almost completely abolished in the presence of Tris^+ (panel C). Note that these effects were reversible (panels D—E).

3 — Effect of Cs^+ ions and D600:

According to Di Francesco (1981), the addition of 0.5 mmol/l CsCl to the bulk solution blocks the " i_f " current.

Figure 4 (lower trace) shows that 20 min after the addition of 1 mmol/l CsCl, there was no change in the inward current induced by a hyperpolarizing pulse from -84 to -154 mV and lasting 900 ms. The characteristics of the shock were 150 mV from HP, 1 ms in duration, delay 1 s.

In order to test possible release of neurotransmitters from nerve terminals that might be present in the preparations and could increase conductance other than $g\text{K}_1$ (see Carmeliet and Mubagwa 1985), we tested, on another fibre, the effect of 10^{-5} mol/l D600 (Fig. 5) on the inward current induced by hyperpolarizing pulses from HP = -80 mV. The potential during test pulses of 900 ms duration ranged from -140 to -160 mV in 10 mV steps. The shock had the same characteristics as before and TTX (10^{-6} mol/l) was present throughout the experiment. It can be seen that D600 had no effect on the inward current or the tail currents.

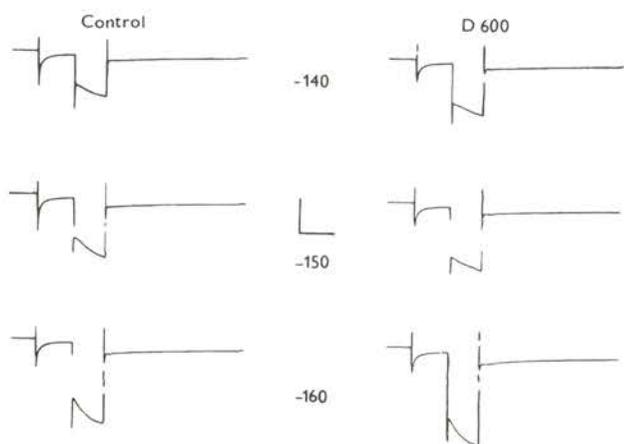


Fig. 5. Current records obtained in response to the same protocol as in Fig. 1 *B* with HP = -80 mV and in the presence of 10^{-6} mol/l TTX. *Left column*: control solution; *Right column*: 20 min after the addition of 10^{-5} mol/l D600. Each line was recorded with the same test pulse. The potential during the test pulse is shown by the figures between the records. Vertical bar: 100 nA. Horizontal bar: 1 s.

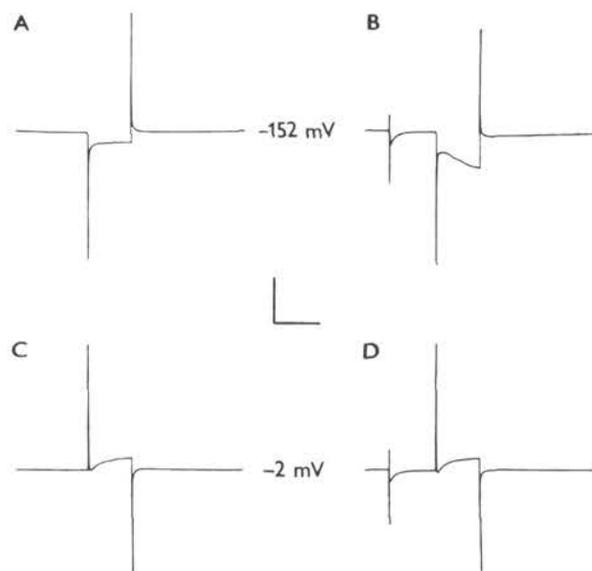


Fig. 6. Current records obtained on the same fibre as in Fig. 5. HP was at $E = -72$ mV. Panels *A* and *C* show current responses to a 1 s test pulse alone. Panels *B* and *D* show current responses with the same test pulse preceded (1 s) by a shock (-200 mV from HP, 1 ms). *A* and *B*: test pulse to $E = -152$ mV. *C* and *D*: test pulse to $E = -2$ mV. Note that the response to the depolarizing test pulse remained unaltered after the shock, whereas that to the hyperpolarizing test pulse was largely increased. Vertical bar: 200 nA. Horizontal bar: 1 s.

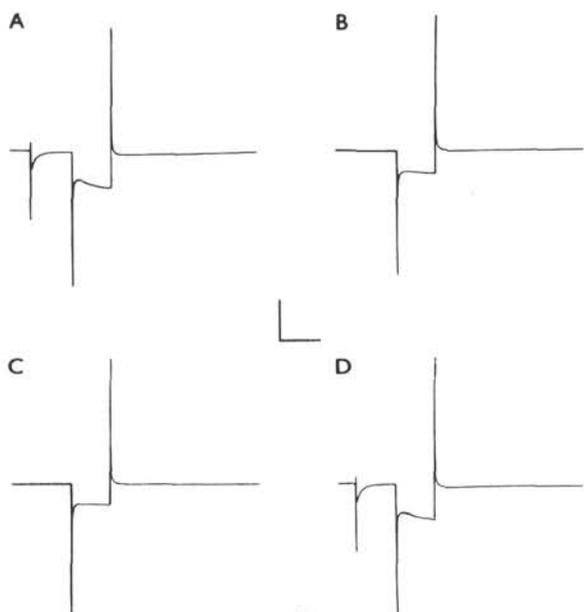


Fig. 7. Current traces recorded in the same fibre as shown in Fig. 6. HP was at $E = -72$ mV. The test pulse was to $E = -142$ mV. *A*: response to shock and test pulse separated by a 1 s time gap. *B* and *C*: responses to the test pulse alone respectively 30 s and 2 min 30 s after *A*. *D*: same as and 4 min after *A*. Vertical bar: 100 nA. Horizontal bar: 1 s.

4 — *Effect of shock on the membrane current elicited by a depolarizing pulse:*

Figure 6 shows results obtained with the same fibre in Ringer solution containing 10^{-6} mol/l TTX. The left panels illustrate the application of a hyperpolarizing pulse from -72 to -152 mV (upper panel) and a depolarizing pulse from -72 to -2 mV (lower panel) respectively. The pulse duration was 1 s. The upper right panel shows that a hyperpolarizing shock (200 mV from HP, 1 ms) applied 1 s before the same test pulse as shown in the upper left panel, evoked a larger inward current. When the same shock preceded the depolarizing test pulse (lower right), the current response remained unchanged as compared with that in control conditions (lower left).

5 — *Disappearance of the inward current after its induction:*

Figure 7 shows the evolution of the current after its induction (panel *A*) by the same pulse protocol and on the same fibre as in Fig. 6. Here, the hyperpolarizing test pulse brought the membrane potential from -72 to -142 mV. Panels *B* and *C* show current transients recorded 30 s and 2 min 30 s respectively after that

of panel A in response to the same test pulse but without repeating the shock. The magnitude of the inward current progressively increasing during the test pulse and that of the inward tail current on return to HP drastically decreased 30 s after the shock and returned to zero by 2 min 30 s. Meanwhile, the leak current (time independent) also decreased. Panel D shows that the effect of the shock was perfectly reproducible.

Discussion

The current traces in responses to hyperpolarizing pulses show an initial surge in the inward direction that reaches a steady-state by 0.1 s after the start of the pulse. This is too slow to be accounted for solely by the capacitive transient since in frog atrial trabeculae the time constant of the membrane is in the order of a few ms (Connor et al. 1975). On the other hand, it has been reported that for low Ba^{++} concentrations (below 1 mmol/l), the block of gK_1 is time and voltage dependent, and on hyperpolarization it is completed after about 100 ms in canine Purkinje fibres (Cohen et al. 1983), sheep Purkinje fibres (Di Francesco et al. 1984) and isolated ventricular cells of guinea pig (Tourneur, personal communication).

We noted that this initial transient was no longer visible when, for the same test pulse amplitude, any control current response (Fig. 1A) was digitally subtracted (not shown) from the corresponding one after shock application (Fig. 1B). This indicates that the shock did not influence the time dependent block of gK_1 by Ba^{++} and thus did not alter the properties of gK_1 .

The time-course of currents elicited by hyperpolarizations to beyond -140 mV in control conditions (Fig. 1A) or to beyond -120 mV after a shock (Fig. 1B) may resemble that of current responses to hyperpolarizations in sino-atrial node (Di Francesco and Ojeda 1980), Purkinje fibres (Di Francesco 1981; Cohen et al. 1983) or sheep atrial fibres (Earm et al. 1983), when gK_1 is blocked by Ba^{++} , namely the " i_f " current.

However, the potential range over which the time dependent current shown in Fig. 1A is recorded is far more negative than that within which " i_f " was found by the above authors.

In addition, the currents shown in Fig. 1A (lower traces) remained almost unchanged when Na^+ ions were replaced by either Li^+ or $Tris^+$ ions (Fig. 3) whereas " i_f " was largely reduced in amplitude when Na^+ ions were partly or totally replaced in Purkinje fibres (Di Francesco 1981) and in sheep atrial preparations (Earm et al. 1983).

Furthermore, the addition of 1 mmol/l CsCl to the bulk solution did not change the current shown in Fig. 4 whereas " i_f " was blocked by low Cs^+ concentrations (e.g. 0.5 mmol/l, Di Francesco 1981).

We therefore conclude that the time dependent increase in the inward current recorded either in control conditions or after a shock does not compare with " i_f " and requires a different interpretation.

Alternative explanations for this phenomenon include the possible release of neurotransmitters from nerve terminals present in small cardiac preparations. These terminals may respond to stimulation of subthreshold magnitude for these preparations (Blinks 1966; Glitsch and Pott 1978). Stimulation-induced release of acetylcholine may then increase K^+ conductance other than gK_1 (Carmeliet and Mubagwa 1985). However, the application of 10^{-6} mol/l TTX and 10^{-5} mol/l D600, which renders nerve membranes inexcitable, did not alter the current responses (Fig. 5). This indicates that neurotransmitter release did not play any significant role in the generation of the currents recorded in the present conditions. This is also confirmed by the results obtained on single cells (Fig. 2).

Another line of explanation may be proposed. It has been suggested by Stämpfli (1958) that excitable membranes of single Ranvier nodes of isolated frog nerve undergo reversible breakdowns of membrane resistance if the transmembrane potential is brought beyond a critical value of 120 to 140 mV (inside negative). On the same material, Ooyama and Wright (1961) have shown that an increasing inward membrane current developed during strong hyperpolarizations and that, when the membrane voltage was restored to and held at the resting level, an inward current persisted although it was somewhat smaller than during hyperpolarization. According to these authors, breakdown of the membrane of the Ranvier node leads to the formation of holes through which small ions like K^+ or Na^+ can pass freely. In the Ranvier node under voltage clamping conditions, a reversible membrane breakdown due to a large enough hyperpolarization causes an inward current developing within seconds to flow during the hyperpolarization, and on returning to HP a slowly decreasing inward tail current may be recorded (Ooyama and Wright 1961).

In our control conditions, both phenomena were observed (Fig. 1A) when the membrane was hyperpolarized to potentials negative to -140 mV, a value within the range of the threshold values reported for membrane electrical breakdown in response to durable hyperpolarizations in frog Ranvier node membrane (Stämpfli 1958; Ooyama and Wright 1961). These authors also noted a significant instability of the membrane resistance beyond threshold potentials for reversible breakdown appearing as sudden deflections on current traces in response to large depolarizations. It may be noticed that the two lower traces in Fig. 1B show jagged deflections as to the traces in the lower panel of Fig. 2.

The current response to short and large hyperpolarization (shock) in Fig. 1B may thus be interpreted on the basis of the membrane breakdown hypothesis.

It was largely assessed on cell membranes that pulses of large amplitude (more than 0.4 V) and of durations longer than about 1 μ s (electrical shocks) cause reversible breakdown of the membrane; this is interpreted as being due to the opening of "holes" or "pores" at lipid-protein interfaces, that heal up within several seconds to several minutes depending on the temperature and on the absolute membrane potential after the shock (see Zimmermann 1982 for review).

An analysis (not shown) of the time course of the current tail decay following shock (Fig. 1B) revealed one short time constant (of the order of 100 ms) and another longer one (of the order of 10 s). The value of the longer time constant would agree with the progressive reversal of membrane damage such as that due to reversible membrane breakdown. When a 1 s hyperpolarizing pulse is applied 1 s later (Fig. 1B), the membrane defects may have healed up incompletely and might be caused to reverse more slowly, or even to be reopened wider again, when the membrane potential is negative enough. An argument in favour of such an interpretation is brought about by the results shown in Fig. 7. When a test pulse was applied at various times after a supra-threshold shock, the amplitude of the extra-current induced during the test pulse decreased with time, returning to zero between 30 s and 2 min 30 s; this agrees with the slower time constant of the current tail decay following the shock (Fig. 1B) and with the time course of the reversal of electrical breakdown at the lipid-protein interface (Zimmermann 1982) as compared with the fast decay of transient permeabilization induced by electric shocks on pure lipid bilayer membranes (Teissie and Tsong 1981; Zimmermann 1982).

It should be noted that the real value of the membrane potential during the first few ms of a voltage clamp pulse cannot be known with precision in our voltage-clamping conditions. First, this is because some oscillation cannot be prevented, causing the real membrane potential during the first ms at the beginning of a pulse to be brought to values more negative than those of the command potential. Secondly, the time constant of the membrane of our preparations being in the order of several ms (Connor et al. 1975) the membrane capacitance cannot be charged completely within 1 ms; therefore, the real potential reached may be less negative than the command potential even in the case of an ideal clamp. Thus the real value of the membrane potential during the shock is uncertain as it results from the opposite influences of these two artifacts. However, by progressively increasing the value of the command potential during the shock (not shown) we noticed that a true threshold existed, within which the shock had no effect, and beyond which the effect of the shock, as evaluated by the magnitude of the tail current following it, were increasing with the shock command amplitude.

After a short hyperpolarizing shock, the current in response to a long-last-

ing hyperpolarization was altered at all potentials beyond -100 mV. If we consider the time dependent inward current creep during the pulse application, it can be seen to appear for pulses to -160 mV and beyond (Fig. 1A), and for pulses to -130 mV and beyond (Fig. 1B) after the application of a foregoing shock. This seems to correspond to the potential at which the current voltage relation (for the current measured at 900 ms after the start of the test pulse) departs from linearity (Fig. 1C) i.e. the behaviour of the membrane is no longer ohmic.

We did not observe a detectable effect of a preceding shock on the response to a depolarizing pulse (Fig. 6). This may be due to the fact that the membrane potential was brought to smaller absolute values at which reversible membrane breakdown does not occur, unlike in the case of large hyperpolarizing pulses.

It may be noted that the tail current following the shock and the one following the hyperpolarizing pulse applied one second later have different time courses, the latter being apparently devoid of the fast component of relaxation (Fig. 1B). However, both seem to be decreased in a similar way by substituting Li^+ or Tris^+ ions for Na^+ ions (Fig. 3). On the other hand, the inward current creep recorded during the hyperpolarizing pulse was quite insensitive to ionic substitutions. This suggests that the selectivity of the permeation mechanisms involved may be different. It should be examined in quantitative experiments with ionic substitutions in single cardiac cells.

We conclude that, in our control conditions, " i_f " seems to be absent in the frog atrial membrane. On the other hand, we propose that cardiac membranes may be as sensitive as nerve membranes to electrical breakdown. Such an interpretation seems able to account for most of the phenomena described here. However, the effects of sodium removal (Fig. 3), in particular upon lithium replacement remain to be interpreted within the scope of the membrane breakdown hypothesis. Our proposal obviously needs to be backed by further experiments attempting to estimate the real threshold for shock-induced breakdown and the possibility of artifacts due to the relatively imperfect technique used here (Johnson and Liebermann 1971). These points are being examined on isolated cardiac cells.

A possible application of our putative interpretation may concern artifacts that may arise when large voltage transients appear such as those due to oscillations of clamp potential; if they reach the threshold for electrical breakdown they may lead to distortions in the analysis of currents, especially in the hyperpolarization range (within which the threshold for membrane breakdown is more easily attained).

Another possible application of this interpretation might shed some light on the mechanism of resynchronization by defibrillating shocks applied on the heart as a whole. Resynchronization might be brought about by a reversible

membrane breakdown that might depolarize a large number of cells during seconds, thus delaying their reexcitability until the next pacemaker beat can propagate.

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