A new calcium current underlying the plateau of the cardiac action potential

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A small and very slow inward calcium current has been identified in isolated single ventricular cells using TTX and Cd²⁺ to block the sodium and fast calcium currents. Activation requires about 300 ms at the threshold potential of −60 mV, decreasing to 80 ms at the peak current voltage of −30 mV. Inactivation is five to ten times longer. Half steady-state activation and inactivation are at −50 and −45 mV respectively. The current is distinctively different in both its kinetics and pharmacology from the conventional calcium current described in single heart cells. It is proposed that it contributes significant current to help maintain a major portion of the long ventricular action potential.

Introduction

One of the most distinctive features of the cardiac action potential is the plateau, a region where the cell remains depolarized for tens of milliseconds (as in atrial or nodal cells) or for hundreds of milliseconds (as in the ventricle). The plateau is thought to be generated primarily by inward Ca²⁺ movements responsible also for initiating contraction of the working myocardium. The plateau is involved in controlling subsequent re-excitation of the cell, guarding it against entry of premature impulses. Because of these important roles in normal cardiac function, the ionic basis of the plateau has been the subject of intense investigation. However, as the number of ionic currents involved is fairly large and some of the smaller components are easily masked by larger ones, the traditional multicellular voltage clamp method is not fully adequate for clearly resolving the currents involved. Recent development of the single cell preparation (Powell & Twist 1976; Kao et al. 1980; Hume & Giles 1981; Bustamante et al. 1981; Isenberg & Klöckner 1982; Lee & Tsien 1984) and the successful study of ionic currents in single heart cells first performed by Lee et al. (1979b) and by Lee et al. (1979a) have demonstrated that, with appropriate protocols, much better resolution can be obtained. The studies so far have concentrated on well-established current components, but the improved resolution makes it likely that some of the more elusive currents previously undetected as separate currents in the plateau region can be resolved and identified as well.

We have used single guinea-pig ventricular cells and either suction pipettes or

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single microelectrodes for measuring membrane voltage and currents. In addition to the rapidly activated and inactivated main component of calcium current (which has been called $i_{\text{si}}$ in single cell work because it has been thought to correspond to $i_{\text{si}}$ recorded in multicellular preparations), we have found much smaller and slower components (Lee et al. 1983, 1984a). One of these is a component that we have temporarily called $i_{\text{si},3}$. It differs from $i_{\text{si}}$ in the fact that it cannot be eliminated by low levels of divalent blockers like cadmium. The purpose of the present paper is to describe the characteristics of this current and to assess its role in the plateau process.

**METHODS**

**Cell isolation**

Single ventricular cells were isolated from adult guinea-pig hearts according to the method described by Lee & Tsien (1984), a modified version of that originally described by Powell & Twist (1976) and, later, by Kao et al. (1980). Cells thus isolated do not need to be incubated in recovery solutions (Isenberg & Klockner 1982) or stored at $4^\circ\text{C}$ before use (Irisawa & Kokobun 1983). Since these cells exhibit stable, robust electrical and mechanical activities once isolated, they can be used immediately after separation or can be stored in 1 mM Ca-normal Tyrode solution at room temperature for subsequent use over the next 48 h. If kept longer under such conditions, the cells lose their distinct rod shape and muscle striations and their electrical and mechanical activities gradually diminish as well.

**Electrophysiological techniques**

An essential aim in these experiments was to keep the cells in as normal a physiological state as possible. We started our work using the perfusion electrode technique, but this has the disadvantage of reducing or eliminating the contraction of the cell, by removing or buffering internal calcium. For the work reported here, therefore, to avoid disruption of the intracellular physiological and ionic environments on which metabolically sensitive currents might depend, we have used the single microelectrode switch clamp method for monitoring potential and currents. The resistance of the microelectrodes was between 15 and 30 MΩ. As the maximum current we measure is only 1–2 nA and the time course is very slow, problems of non-uniformity and time-resolution should be minimal. We have tested this assumption in the following ways:

(i) Oscilloscope recordings of the capacity transient in response to small voltage steps show fast transients. Recordings of the faster calcium current, $i_{\text{si}}$, show a current similar in time course and magnitude to that obtained using the perfusion electrode technique (Lee & Tsien 1982). From these observations, we conclude that there cannot exist significant series resistance delays (cf. Noble & Powell 1983). For the slower currents, we have used pen recordings, as in this paper.

(ii) To determine whether significant voltage non-uniformity could exist we used nonlinear cable theory, with a polynomial representation of the current–voltage relation (Jack et al. 1975), to show that the maximum non-uniformity along a cell of 10 μm in diameter and 100 μm in length when 1–2 nA of inward current flows...
would be less than 0.2 mV. Even increasing the maximum current to 10 nA generates less than 2 mV of non-uniformity. Similar calculations also show negligible non-uniformity and delay would be expected in T tubules only 5 μm in length from the surface membrane.

(iii) In addition to studying the effect of low cadmium levels on the ionic currents, we have also recorded the action potentials. These should be consistent in speed and duration with the ionic currents recorded. Our results show this to be the case.

Solutions

The solutions used when recording were normal Tyrode containing 2.5 mM Ca²⁺. Except for the experiment shown in figure 1, all solutions contained 10–20 μM tetrodotoxin (TTX) with or without Cd²⁺. We also sometimes replaced K⁺ (5.4 mM) by Rb⁺ (2 mM) to reduce the background rectifying current, iK₁. Tetraethyl ammonium (TEA) at 2.5 and 5 mM were used in some experiments to reduce the delayed K⁺ current. All experiments reported here were done at room temperature (20–22 °C) rather than at normal body temperature (37 °C). This also greatly reduces the delayed K⁺ current, reduces noise (since a heat pump is not necessary) and improves temporal resolution.

Terminology

It may be helpful to explain the terminology since this is now somewhat confusing. The symbol isi has been used widely both for the total TTX-insensitive ‘second inward’ current in multicellular preparations and for the fast component identified recently in single cells. To avoid any confusion with either of these uses, we will refer to the slower components as isi₂ (when referring to the presumed [Ca]r-activated current (Lee et al. 1983) and isi₃ (when referring to the presumed very slow calcium current described in the present paper). The total ‘second inward’ current will then be the sum of these two components and the fast component. The symbol iCa₃ has also been used for isi₃ (see Noble 1984). This has some justification when referring to the current as the ‘slow calcium current’ to distinguish it from the fast current (for which the symbol iCa₁ might then be used). In this paper we have preferred to use the neutral terminology isi₃ since we are, in part, describing experiments to test the view that the current may be a new calcium channel.

Results

The large and long plateau

Freshly isolated cells display action potentials as shown in figure 1. They are characterized by a rapid initial upstroke at a speed of about 140 V s⁻¹ at room temperature. The upstroke overshoots to an average of 40–50 mV above zero membrane potential. It is followed by a very slow repolarization which, on average, takes about 600 ms to reach zero potential before fast repolarization takes place, driving the membrane potential back to its original resting value of about −85 mV. As the frequency of stimulation is increased, the shape of the action potential
Figure 1. A typical action potential recorded in a freshly isolated guinea-pig ventricular cell at room temperature. Bathing solution was normal Tyrode containing 2.5 mM Ca. Cell size was about 20 μm in diameter and 100 μm in length. The cell was impaled with a 30 MΩ microelectrode for potential measurement and current stimulation through a bridge circuit. The action potential was elicited by a 0.5 nA current pulse lasting for 20 ms. Arrow indicates the threshold potential, and, below it, a potential trace that failed to cross the threshold is superimposed. Current trace is shown at the bottom. (Note that, during the current pulse itself there is a voltage stimulus artefact due to the voltage drop across the single microelectrode. For estimates of threshold potential, the voltage after the stimulus artefact was used. This voltage is indicated here by the arrow.) In a good cell, stable impalement can be maintained for 2–3 h with electrical and mechanical activities undiminished at the end of the period. Percentage of good yield was between 20 and 50%. Cell number 6–24–C9.

remains unaffected up to 0.5 Hz. Higher frequency eventually reduces both the speed and amplitude of the upstroke. During each action potential the cell contracts forcefully. In addition to the accompanying mechanical activity, we think the size of the plateau is also an important indication of the robustness of the cell, and this is required for recording the very slow inward current, \( i_{si,3} \). Partly damaged cells exhibit action potentials with very brief plateaux and show short contractions, although the resting potential and overshoot potential may be similar to those of healthy cells.

**TTX-resistant inward currents**

What are the underlying currents responsible for generating and maintaining the large long plateau seen in ventricular cells? First, it can be shown that the maintained ‘window’ component of the sodium current (Attwell et al. 1979) is not strongly involved. We used a high concentration of TTX (20 μM) to eliminate the fast sodium current underlying the rapid upstroke (Lee et al. 1979a; Brown et al. 1981b). The resulting action potential (see figure 6a) has a characteristic slow upstroke but a normal, large plateau. This is probably because, in contrast to the situation in Purkinje fibres, the ventricular plateau is too positive for the sodium
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'window' current, which is usually significant only at potentials negative to about +10 mV. Under voltage clamp, inward currents with graded responses to depolarizing steps from a holding potential of -80 mV can be recorded. In the absence of any Ca current blockers, the current time course is quite complex (Lee et al. 1983). The largest component is a relatively fast current of up to 10 nA that reaches a peak in 2–5 ms (Lee & Tsien 1982, 1983; Mitchell et al. 1983). This current can be readily blocked by 0.2 mM Cd²⁺. Initially, this procedure leaves a second component which we have already briefly described and which is correlated with the contraction of the cell (Lee et al. 1983, 1984b). After a few minutes, the contraction becomes weaker and only a very slowly inactivated inward current remains. This is the current we report in this study.

Figure 2a illustrates the time course of block of the faster components of inward current. At 0 min, the control current record shows rapid activation to a peak which was almost constant from pulse to pulse. Then 0.2 mM Cd²⁺ was applied to the bath and the current started to decrease, initially slowly so that by the end of 4 min, the current was only decreased by about one half. Then it decreased more rapidly to reach a new constant level at 6 min. The onset of cadmium block in these experiments develops more slowly than it does in dialysed single cells (Lee & Tsien 1982), which we attribute to the presence of a component related to the contraction (Lee et al. 1983) and which may be activated by intracellular calcium. Dialysed cells do not contract and this component is then very weak or absent.

![Figure 2. Dissection of the slow inward current using cadmium. (a) Development of blockade of the faster inward components by Cd²⁺. Arrow indicates Cd²⁺ application. Number above each trace indicates minutes after addition of Cd²⁺ to the bathing solution. Experiment proceeded from left to right. Notice that the holding current level becomes slightly outward in the presence of Cd²⁺, an observation consistent with Cd²⁺ block of the Ca channel current in perfused cells. (b) Control currents recorded at different voltages. Eight current traces are shown, each one being elicited by a 1 s depolarizing pulse to the voltage levels in millivolts shown below. A 20 s rest period was allowed between successive pulses. (c) Current records 10 min after Cd²⁺ application to the bath. The eight records shown from left to right were elicited by the same voltage pulses as in (b). Dotted line indicates the zero current level. Calibration scale of (a) applies also to (b) and (c). Holding potential -80 mV. Cell number 5–27–C3.](http://rspb.royalsocietypublishing.org/Downloaded from http://rspb.royalsocietypublishing.org/)
Comparing the control current with that after 6 min in cadmium solution it can be seen that the eventual effect of cadmium is strikingly specific: total elimination of the faster components, but almost complete sparing of the very slow current, $i_{sl,3}$. Figure 2b, c show the effect of cadmium over a broad range of potentials. Traces in figure 2b show the currents at the potentials shown before application of cadmium. Traces in figure 2c show the currents at the same potentials after 10 min in cadmium. In all the traces the faster inward components are consistently removed by cadmium thus unmasking the very slow inward current, $i_{sl,3}$. In fact, between $-40$ and $-10$ mV, the maintained level of inward current becomes slightly larger once the faster transient components are removed, suggesting possible reduction of a Ca$^{2+}$-activated outward K$^+$ current or that cadmium may more directly reduce an outward K$^+$ current.

The combined use of TEA and low temperature may make the uncovering of $i_{sl,3}$ easier to achieve. Certainly, a current like $i_{sl,3}$ would be difficult to detect in cells showing a large amount of delayed K$^+$ current, which is very temperature dependent in these cells.

**Voltage gating of $i_{sl,3}$**

Both the amplitude and the time course $i_{sl,3}$ are sensitive to voltage change. Before leakage correction, the current starts to appear at $-60$ mV. It reaches a
peak at about $-40$ mV and becomes smaller again at more positive potentials (figure 2c). On strong depolarizations (to $+10$ mV and above) delayed outward K$^+$ current begins to appear in some cells. To reduce the K$^+$ current interference, 2.5–5 mM TEA and 1 mM 4-AP were added to the bathing solution. However, these agents cause deterioration of the cells and were therefore used only in experiments to test the extent of K$^+$ current contamination. By using these K$^+$ channel blockers, it was found that in most cells the K$^+$ current is not very significant between $+20$ mV and 0 mV, and is virtually absent at negative potentials. The absence of significant K$^+$ current at potentials negative to 0 mV is further supported by tail current measurements (figure 4a), for the envelope of the tails matches well with the time course of the inward current. The tail experiment also provides support for the view that $i_{\text{si,3}}$ is an inward current and that it activates and inactivates very slowly.

![Graph](image)

**Figure 4.** (a) Tail currents elicited by varying durations of a depolarizing pulse to $-20$ mV from a holding potential of $-80$ mV. Bottom trace shows the voltage step protocols. From left to right, the tail currents were produced by pulses lasting 25, 50, 200, 400, 600, 800 and 1000 ms, with a 5 s rest interval between each pulse. Standard Ca TTX and Cd solution. Cell number 5–26–C17. (b) Steady-state activation (open symbols) and inactivation (closed symbols) curves for $i_{\text{si,3}}$. Each set of symbols (open and closed) represents data collected from the same cell. Three cells were used to produce these results. Steady-state activation at each potential was obtained by fitting a straight line to the positive slope of the net Ca current–voltage relation. Then the ratio of peak current amplitude to the corresponding amplitude on the straight line at each potential was calculated. This approach gives only an approximation to the steady state activation curve and is probably not very reliable at potentials positive to $-10$ mV. Steady-state inactivation was obtained by depolarizing the cell with a 1 s test pulse to $-20$ mV from various holding potentials. Each pulse was separated by 20 s at each holding potential.
The slowness of $i_{si,3}$ is evident at all potentials (figure 2c). As shown in figure 3b (solid circles) the activation half time decreases from 170 ms at $-60$ mV to about 40 ms at $+10$ mV. Inactivation is very much slower still. Figure 3a shows semi-logarithmic plots of inactivation time courses obtained at different potential steps. At least two thirds of $i_{si,3}$ (up to about 700 ms) decays along a single exponential function with time constants ranging from 800 ms at $-60$ mV, to 400 ms at $-30$ mV. The relation between inactivation time constant and membrane potential is represented by open circles in figure 3b. Unlike activation, the voltage dependence of the time constant of inactivation, after reaching a minimum at about $-30$ mV, gradually increases again to about 700 ms at $+20$ mV. The slowing of inactivation at very positive potentials is not caused by K$^+$ current contamination since, in the presence of TEA and 4-AP, the inactivation time course hardly changes. In cells where significant K$^+$ current does exist, the K$^+$ channel blockers slightly flatten the current trace near the end of a 1 s pulse, making the slowing of inactivation more obvious at strong positive potentials. It is likely, therefore, that the slowing of inactivation towards positive potentials is a real channel property of $i_{si,3}$.

Steady-state activation and inactivation of $i_{si,3}$

The relations of steady-state activation (open symbols) and inactivation (solid symbols) to membrane potential are shown in figure 4b. According to the steady-state inactivation curve, the current is fully available up to $-60$ mV. It is roughly halved at $-45$ mV and becomes completely inactivated at potentials positive to $-30$ mV. Thus, on the same voltage axis, this inactivation curve lies about midway between that of the fast inward sodium current, $i_{Na}$ (Lee et al. 1981; Cachelin et al. 1983), and the fast calcium current (Isenberg & Klöckner 1980; Hume & Giles 1983). Steady-state activation starts at about $-70$ mV, becomes half-maximal at about $-50$ mV and reaches a peak at about $+20$ mV. Again, the activation curve of $i_{si,3}$ differs from that of the conventional Ca channel current by being more negative (Akaike et al. 1978; Hagiwara & Ohmori 1983), but resembles that of $i_{Na}$ in single rat ventricular cells (Brown et al. 1981b).

The main feature of this plot is the very extensive overlap of the activation and inactivation curves, spanning 40 mV between $-70$ and $-30$ mV. At $-50$ mV, where the degree of overlap is maximal, as much as half of the net inward current does not inactivate even at the end of a pulse as long as 20 s. This is reflected by the unusual amount of persistent inward current at the end of 1 s pulses recorded at most potentials.

Ionic basis of $i_{si,3}$

What ions carry $i_{si,3}$? We suspected that Ca$^{2+}$ might be involved since, when the current is flowing the cell contracts, although less strongly and much more slowly than in Tyrode solutions without added Cd$^{2+}$. To see whether calcium could be the charge carrier, we chose the direct approach of removing all external Ca ions, leaving Mg$^{2+}$ (1–5 mm) as the only divalent ion. Further, we removed Cd$^{2+}$. This was achieved by perfusing the cells with a solution containing Mg$^{2+}$ but no external Ca ions, the only divalent ion being Mg$^{2+}$.
as well at this stage since it is known that Cd\(^{2+}\) blocks the conventional calcium current in nerve (Hagiwara & Byerly 1981) and in heart (Lee & Tsien 1983) with increasing potency as external Ca\(^{2+}\) is reduced. The increased potency of Cd\(^{2+}\) might also reduce \(i_{\text{si,3}}\) under such conditions and so complicate the interpretation of the real effect of calcium removal.

As shown in figure 5a, the effect of calcium removal is very clear: it abolishes all time-dependent inward current, including the inward tail at the termination of the clamp step. Switching to current clamp conditions at this stage, it can be

![Graph](image-url)

**Figure 5.** Effect of Ca\(^{2+}\) removal on the slow inward current and on the corresponding action potentials generated by \(i_{\text{si,3}}\). The results shown here were obtained on the same cell.

(a) Currents at -50 mV. Inward current identified by the open circle is the slow current before Ca\(^{2+}\) removal. The time-independent outward current identified by the open triangle is the Ca\(^{2+}\)-insensitive leakage current obtained 2 min after removal of external Ca\(^{2+}\) and Cd\(^{2+}\). Shaded area indicates the total effect of Ca\(^{2+}\) removal, which gives an estimate of all Ca\(^{2+}\)-sensitive current obtainable at -50 mV. In zero Ca\(^{2+}\) solution, the holding current level becomes inward owing to increase of leakage current.

(b) Slow action potential generated by the current shown in (a). Again, the open circle indicates the record obtained in the presence of Ca\(^{2+}\), but the open triangles indicate the aborted action potential after only 30 s in Ca\(^{2+}\)-free solution. Longer exposure to zero Ca\(^{2+}\) solution will completely abolish the aborted action potential and the cell then depolarizes. Current stimuli are shown at the bottom.

(c) Peak current amplitudes plotted against the corresponding voltage steps. As in (a), the open circles represent the current obtained in Ca-containing solution and the open triangles show the currents obtained after Ca\(^{2+}\) removal. The closed circles show the difference between these two measurements. The arrow indicates the holding potential. All zero Ca\(^{2+}\) solutions contained 1 mM Mg\(^{2+}\), 20 \(\mu\)M TTX and no Cd\(^{2+}\). Cell number 6–28–C9.
shown that the corresponding action potential generated by $i_{s_i, 3}$ disappears as well, in spite of using an increased stimulus intensity (figure 5b). This result was consistently reproducible in each of six similar experiments. Increasing external Ca$^{2+}$ concentration from 1.8 to 2.5 mM slightly increased $i_{s_i, 3}$; this is the reason for using the higher calcium concentration in this study. The simplest interpretation of these experiments is that $i_{s_i, 3}$ is carried entirely by calcium ions.

By using the Ca$^{2+}$ removal approach, the size of the net Ca$^{2+}$ current and the amount of net Ca$^{2+}$ influx can be measured as the difference current before (open circles) and after (open triangles) Ca$^{2+}$ removal. This difference is represented by the shaded area for the $-50$ mV step illustrated in figure 5a. Similarly, the peak amplitudes of $i_{s_i, 3}$ at different membrane potentials can be obtained by taking the difference of the peak current amplitude before and after Ca$^{2+}$ removal at each potential. The result is expressed as the current–voltage plot (solid circles) in figure 5c. The net current–voltage curve thus obtained for $i_{s_i, 3}$ shows three interesting features: (i) unlike that for the fast calcium current, it starts to activate at a very negative potential, close to $-70$ mV; (ii) it peaks at about $-30$ mV at a maximum amplitude of about $-1.5$ nA, which then decreases only slightly with voltage, thus forming a very gentle positive slope; and (iii) an extrapolation suggests that it may reach a reversal potential at a very positive level of potential, which further reinforces the view that it may be carried by calcium ions.

Does $i_{s_i, 3}$ differ significantly from the fast calcium current?

The evidence presented so far suggests that $i_{s_i, 3}$ differs from the fast calcium current not only in time course and current–voltage relations, but also in pharmacology, which has allowed us successfully to separate $i_{s_i, 3}$ from the other inward currents by using cadmium ions (figure 2). There are also some other important differences in pharmacology. Thus $i_{s_i, 3}$ is not affected by 4 mM Mn$^{2+}$, nor is it enhanced by 2 μM isoprenaline, nor by equimolar substitution of Ca$^{2+}$ with Ba$^{2+}$. In these properties, the current differs very markedly from the conventional fast calcium channel.

Interestingly, caffeine (0.1 mM) increases $i_{s_i, 3}$ markedly. Since caffeine releases stored calcium, this suggests that the current magnitude may depend on intracellular calcium in some way.

At present, we have not yet found a specific blocker for $i_{s_i, 3}$, but high levels of cadmium (1 mM or more) or D600 (5 μM) will partly reduce it. Also, complete removal of extracellular K$^+$ ions will diminish the current, possibly through secondary effects caused by intracellular Na$^+$ loading in K$^+$-free solutions.

Is $i_{s_i, 3}$ an important plateau current?

So far, we have established in the guinea-pig ventricular cells a relatively Cd$^{2+}$-resistant slow Ca current, named provisionally $i_{s_i, 3}$. The fact that it is an inward current that inactivates very slowly means that it is very likely to be involved in maintaining depolarization during the plateau of the cardiac action potential. Direct evidence for this idea comes from the results shown in figure 6. The action potentials and ionic currents in this figure were obtained from the same cell. It can be seen that, in the presence of cadmium, an action potential plateau
Figure 6. Action potentials and membrane currents recorded from the same cell before and after Cd2+ application.

(a) Action potential recorded in normal Tyrode solution containing 2.5 mM Ca2+, 20 µM TTX only. A suprathreshold and just subthreshold response are shown.

(b) The corresponding current that generates action potential in (a).

(c) Seven minutes after 0.2 mM Cd2+ was applied to the bathing solution. A very slow, but long-lasting action potential is obtained. This type of Ca action potential was recorded in 20 cells showing isi3. The fact that this action potential is longer than that in (a) could be due to the smaller depolarization activating less delayed outward K+ current or to a smaller activation of a [Ca]i-dependent K+ current when the fast calcium current is blocked. As in (a), the traces show both a suprathreshold and a just subthreshold response. Note increased current stimulus compared to (a).

(d) The current record corresponding to (c). Voltage scale applies to (a) and (c), current scale to (b) and (d), time scale to all four sets of traces. Cell number 5–27–C9.

persists, though at a lower level than in the normal action potential. Its speed and amplitude correspond to the slow nature and amplitude of isi3. Thus, the plateau in cadmium solution is usually situated at about 0 mV, which is about the level of membrane potential at which the net ionic current becomes zero, while the very slow repolarization is consistent with the very slow inactivation of isi3.

Finally, it is worth mentioning that isi3 can best be recorded in cells with action potentials showing large and long plateaux, as in figure 1. Cells that are depolarized, or have relatively brief plateaux (lasting 200 ms or less at room temperature) always failed to show significant very slow inward current. In this respect, it is interesting to note that Hume & Giles (1983) have also reported a slow inward current resistant to cadmium in single frog atrial cells and they also maintain that a robust cell with a long action potential is essential for obtaining their slow inward current.
In this study, we have identified for the first time a TTX-insensitive and relatively Cd\textsuperscript{2+}-resistant slow inward current. The current appears to be carried by calcium ions and we have named it provisionally $i_{	ext{s}1,3}$ (the terminology $i_{\text{Ca},s}$ - meaning 'slow calcium current' - has also been suggested (see review by Noble (1984) for a more extensive discussion of possible terminology). The maximum amplitude of $i_{	ext{s}1,3}$ is very small, usually less than 1.5 nA in a single cell. Nevertheless, it is capable, \textit{in the absence of any other known inward current}, of generating a substantial and long-lasting Ca-dependent action potential (see figure 6). Moreover, during such action potentials, or during comparable voltage clamp depolarizations, the cell undergoes a small but maintained contraction.

Could $i_{	ext{s}1,3}$ alone be responsible for maintaining the normal ventricular action potential (figure 1)? We think this is unlikely since the cadmium-resistant action potential plateau is situated some 30–40 mV negative to the normal plateau level. It is probable therefore that the normal action potential plateau is generated by all the components of second inward current, including the very slow, and possibly \([\text{Ca}]_r\)-activated, current we have described elsewhere (Lee \textit{et al.} 1983).

On this view, the various components of $i_{	ext{s}1}$ would be important at different times during the cardiac action potential. The fast calcium current (usually called $i_{	ext{s}1}$ in single cell work, but clearly only a component of what has been called $i_{	ext{s}1}$ in multicellular work) is important in the early plateau, and with a time constant of inactivation in the range 10–20 ms, would be important for around 40–80 ms. The presumed \([\text{Ca}]_r\)-activated component, $i_{	ext{s}1,2}$ (Lee \textit{et al.} 1983; Brown \textit{et al.} 1983, 1984a, b; Mitchell \textit{et al.} 1984a, b; Noble & Powell 1984), may then carry a slow and small transient inward current, which together with the maintained current carried by $i_{	ext{s}1,3}$, may maintain the very long-lasting plateau. Preliminary calculations that we have done with a modification of DiFrancesco & Noble's (1984) equations that generate a ventricular action potential show that the amplitude and time course of $i_{	ext{s}1,3}$ reported in the present paper would enable it to contribute a significant fraction of the inward current required to maintain the plateau at potentials positive to 0 mV for several hundred milliseconds.

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