Early Outward Current in Rat Single Ventricular Cells

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SUMMARY. Voltage clamp experiments were conducted using single ventricular myocytes which had been dissociated enzymatically from adult rat hearts in order to examine further the membrane currents which contribute to the unusual plateau of the rat action potential. Membrane currents were recorded, using a single microelectrode (switching) voltage clamp circuit. From holding potentials near the resting potential (—80 to —90 mV), depolarizing clamp steps above —20 mV elicited an early outward current which overlapped in time with the slow inward current and displayed time-dependent inactivation. This is the first demonstration of a transient potassium current in an isolated ventricular myocyte. The early outward potential was voltage-inactivated at holding potentials of —50 to —40 mV and was blocked by 4-aminopyridine. The current was not dependent on Ca<sup>++</sup> or I<sub>K</sub> and was blocked by Ba<sup>2+</sup>. Double pulse experiments revealed that the time course for the recovery of the early outward current at —80 mV was rapid, and had a τ of 25 msec. The possible functional significance of this current is discussed. (Circ Res 54: 157–162, 1984)

THE ventricular action potential in the rat heart is characterized by a fast phase of repolarization, followed by a plateau of short duration (Hoffman and Cranefield, 1960). The unusual shape and the brevity of this action potential suggests that one or more of the underlying membrane currents may not be similar to those responsible for the ventricular action potentials of other species. The participation of the slow inward Ca<sup>++</sup> current (I<sub>Ca</sub>) in the genesis of the rat action potential plateau has already been discussed in a previous paper (Josephson et al., 1984). The presence of an early outward current has been suggested to explain the early phase of repolarization in rat heart (Coraboeuf and Vassort, 1968), but it has never been characterized in voltage clamp experiments using the ventricular muscle of rat or any other mammalian species. Such a transient outward current system, which shows voltage-dependent activation and inactivation, has been described in single cell preparations, such as snail neurone (Neher, 1971; Connor and Stevens, 1971), cat motoneurone (Barrett and Crill, 1972), egg cell membrane of a coelenterate (Hagiwara et al., 1981), and multicellular preparations such as cardiac Purkinje fibers from sheep and calf (Peper and Trautwein, 1968; Fozzard and Hiraoka, 1973; Kenyon and Gibbons, 1979; Siegelbaum and Tsien, 1980; Boyett, 1981).

The present paper will demonstrate the presence of an early outward current in single myocytes isolated from adult rat ventricle, describe some of its properties, and offer some ideas concerning its functional significance.

A preliminary account of these experiments has been presented (Josephson et al., 1982).

Methods

The methods used for preparing single ventricular cells, and the methods for voltage clamping these cells, have been described in the preceding paper (Josephson et al., 1984). Due to the limited time resolution of the single switching microelectrode voltage clamp method, the fast kinetics of activation of the early outward current were not studied in detail. The bathing solution used in all experiments was the normal Krebs solution containing 3.6 mm Ca<sup>++</sup> (see preceding paper for composition). The following agents were added to the solution reservoirs from concentrated stock solutions to give the following final concentrations: tetrodotoxin (TTX) (10<sup>−5</sup> M) to block the fast Na<sup>+</sup> current (I<sub>Na</sub>); CoCl<sub>2</sub> (3 x 10<sup>−3</sup> M) to block I<sub>K</sub>; and 4-aminopyridine (4-AP) (2 x 10<sup>−3</sup> M) to block the early outward current (I<sub>Na</sub>). All experiments were conducted at room temperature (18–22°C).

Results

The resting potentials of single rat ventricular cells, bathed in Tyrode's solution (3.6 mM Ca<sup>++</sup>) ranged between —80 and —90 mV (~84.3 ± 4.1, n = 15). Stimulation of these cells with brief intracellular current pulses produced action potentials (Fig. 1, lower trace) which were comparable in waveform to those recorded from intact ventricular muscle. The action potentials were characterized by a rapid, first phase of repolarization and a short plateau, yielding a sigmoidally shaped repolarization. However, upon depolarization of the membrane potential to —50 or —40 mV (with constant intracellular current), the resulting stimulated action potentials showed a markedly different appearance (Fig. 1, upper trace). The upstroke of the action potential was slowed due to the inactivation of the fast Na<sup>+</sup>...
current and the reliance on the slow inward current. Of even more interest was the fact that the duration of the slow action potential elicited at −45 mV was greatly enhanced, and showed a more gradual phase of repolarization, compared to the normal action potential (see Table 1).

The disappearance of the initial, fast phase of repolarization in the partially depolarized action potential suggested that a voltage-dependent inactivation of a previously unidentified outward current system might have occurred. Therefore, voltage clamp experiments were conducted to examine further the membrane currents flowing at plateau potentials in single rat ventricular cells. In the first series of experiments, membrane currents were recorded with use of a single microelectrode voltage clamp (Fig. 2, digitized traces) and were compared with recordings obtained using a standard, two-microelectrode voltage clamp method. Tetrodotoxin (TTX) 10−6 M and cobalt chloride (3 × 10−3 M) were added to the superfusing solution to block the fast Na+ current and the slow inward Ca2+ current. As can be seen in Figure 2, the application of depolarizing voltage clamp steps from a holding potential of −80 mV, to potentials more positive than −20 mV, resulted in the appearance of a transient, or early outward current (IETO). The time to peak amplitude of the early outward current (IETO) became more rapid at positive membrane potentials, ranging from 11.3 msec at −10 mV to less than 2 msec at +40 mV. This current persisted in the absence of the slow Ca2+ current (abolished by blockade with Co2+ (four experiments) or by Ca2+ removal from the bathing solutions, (five experiments)); therefore, it appeared that it was not

![Figure 1. Normal and slow action potentials recorded from a single rat ventricular cell. Lower trace: normal action potential elicited from a resting potential of −85 mV. Upper trace: slowly rising action potential elicited after the cell was depolarized to −45 mV by constant intracellular current. Time and voltage calibration as shown.](image)

![Figure 2. Early outward currents recorded from single rat ventricular cells: a comparison of the one-microelectrode (lower traces) and two-microelectrode (upper traces) voltage clamp methods. Tetrodotoxin (TTX) 10−6 M and cobalt chloride (3 × 10−3 M) were added to the superfusing solution to block the fast Na+ current and the slow inward Ca2+ current. As can be seen in Figure 2, the application of depolarizing voltage clamp steps from a holding potential of −80 mV, to potentials more positive than −20 mV, resulted in the appearance of a transient, or early outward current (IETO). The time to peak amplitude of the early outward current (IETO) became more rapid at positive membrane potentials, ranging from 11.3 msec at −10 mV to less than 2 msec at +40 mV. This current persist ed in the absence of the slow Ca2+ current (abolished by blockade with Co2+ (four experiments) or by Ca2+ removal from the bathing solutions, (five experiments)); therefore, it appeared that it was not shown. Original oscilloscope traces. Lower traces: digitized records of the early outward currents obtained using a single microelectrode (switching) voltage clamp technique. Holding potential −80 mV; holding current, 0.3 nA; numbers next to each trace indicate the clamp potential in millivolts.](image)

### Table 1

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<th>Characteristics of the Normal and Slow Action Potentials in Rat Ventricular Myocytes</th>
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<tr>
<td>Normal action potential</td>
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<td>n = 15</td>
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<td>RP (mV)</td>
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<td>AMP (mV)</td>
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<td>APD25 (msec)</td>
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<td>APD75 (msec)</td>
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<td>(current clamp)</td>
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<td>RP (mV)</td>
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<td>AMP (mV)</td>
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<td>APD25 (msec)</td>
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<td>APD75 (msec)</td>
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Data are expressed as mean ± so. RP = resting potential (under current clamp for slow AP); AMP = amplitude (peak-to-peak); OS = overshoot; APD25 = action potential duration measured at 25% of repolarization; APD75 = action potential duration measured at 75% of repolarization.
activated by a rise in [Ca], resulting from the flow of I_{Ca}.

In contrast to many voltage-dependent currents, the time course for the decay, or inactivation of I_{EO} was not clearly related to membrane potential. It was, however, generally found to be slightly faster at more positive potentials. For example, in one cell, it was fit with a single exponential with a time constant that decreased from 29 msec at 0 mV to 21 msec at +40 mV. In another cell, the time course for the inactivation of I_{EO} was slower at 0 mV (τ of 38 msec) than at +40 mV (τ of 35 msec).

The voltage dependence and kinetics of I_{EO} were also observed by means of standard two-microelectrode voltage clamp method in three experiments. However, this procedure resulted in a relatively large leakage current (see inset, Fig. 2). In addition to difficulties in measuring I_{EO}, the presence of the leakage current in the two-microelectrode experiments made necessary the application of a large holding current to maintain the membrane potential at −80 or −90 mV. Therefore, the subsequent experiments were carried out using the one-microelectrode voltage clamp, since the single impalement produced less injury to the membrane, resulting in relatively small leakage currents.

The current-voltage relation for the peak I_{EO} in a typical rat heart cell, elicited from a holding potential of −90 mV, is plotted in Figure 3. In this figure, and all subsequent figures, the magnitude of I_{EO} was defined as the peak transient outward current minus the holding current after blockade of I_{Na} and I_{K}. The threshold for I_{EO} was −30 to −20 mV, and the current increased at more positive membrane potentials displaying outward rectification, as can be seen. However, the magnitude of the current varied with the cell under study, ranging from 2 to 6 nA at a potential of +20 mV (n = 52 cells). In contrast to these results with rat ventricular cells, no I_{EO} was recorded when guinea pig ventricular cells were used under identical conditions (n = 12).

To identify the ionic nature of the early outward current, we conducted five experiments with 4-aminopyridine (4-AP). This agent was chosen since it is known to depress the transient K⁺ current in an egg cell membrane (Hagiwara et al., 1981), as well as the early outward current of sheep cardiac Purkinje fibers (Kenyon and Gibbons, 1979). Figure 4A shows the effects of 4-AP on the net membrane currents elicited by a voltage clamp step from −80 to +20 mV, thereby approximating the currents flowing near the peak overshoot of the action potential. The trace labeled "control" shows the net membrane currents obtained in normal Tyrode's solution, and the traces labeled 1 through 6 show the effects of 4-AP (2 mM) at 30-second intervals after its addition to the bathing solution. As the early outward current was diminished by 4-AP, the presence of the slow inward current became more visible (trace 6). The time-to-peak of the net outward current was also increased as the block of I_{EO} progressed, reflecting the increasing proportional contribution of I_{K}, to the net current. Correspondingly, it was found in three experiments that 4-AP (2 mM) produced a prolongation of the action potential duration by 20–50% (measured at 20% of repolarization). It should be emphasized that the time-to-peak I_{EO} shown in this experiment using the net currents appears to be long, compared with that shown in Figure 1 (done in solutions containing TTX and CoCl₂), because of the presence of the fast Na⁺ current (off-scale) and the slow inward current, which subtract from the initial values of I_{EO}.

In addition to 4-AP, exposure to Ba²⁺ (3.6 mM) or Cs⁺⁺ (10–20 mM) was also effective in blocking I_{EO}. An example of the effect of isosmotic substitution of Ba²⁺ for Ca²⁺ is shown in Figure 4B. The top trace is the current flowing upon a 140-msec depolarization from −80 mV to +0 mV. The lower trace shows the change in membrane current upon application of an identical voltage clamp step (voltage traces superimposed) 5 minutes after the switch to a Ba²⁺-Tyrode superfusion.

As was shown in Figure 1, the putative early outward current (as identified by its effect on the action potential), appeared to inactivate when the membrane potential was held depolarized beyond...
FIGURE 4. Panel A: time course of the 4-AP block of the early outward current. Control trace shows the net membrane currents elicited by a voltage step from −80 mV to +20 mV. Superimposed traces labeled 1 to 6 show the membrane currents elicited at 30-second intervals after the addition of 4-AP to the Tyrode's solution bathing the cell. Holding current, 0.2 nA. No TTX or Co** present. Panel B: the effects of Ba** ion substitution for Ca** ion in blocking /stro-

Superimposed current and voltage traces show currents recorded in normal Tyrode's solution (Ca_0 = 3.6 mM (top trace) and 5 minutes after switching to a Ba**-Tyrode (3.6 mM superfusion. Voltage clamp step from −80 to 0 mV, 140 msec duration. Calibrations for current, voltage and time are 2 nA, 100 mV, and 20 msec.

−50 mV. To test this observation, we examined the steady state inactivation of I_EO by applying 2-second conditioning clamp pulses at potentials ranging from −80 to −50 mV, and then stepping to +10 mV (Fig. 5). The magnitude of I_EO was large at a holding potential of −80 mV and was gradually reduced at less negative holding potentials, so that by −50 mV it was inactivated, unmasking the presence of I_a. A further reduction of the holding potential (i.e., positive to −40 mV) produced only a decrease in the magnitude of the peak I_a (due to partial inactivation; $f < 1$), but did not change the shape of the inactivation of I_a. This indicates that I_EO was inactivated fully by −50 mV. Additionally, after blockade of I_a with Cd** there was no hint of any residual I_EO at this holding potential.

At intermediate holding potentials (i.e., −60 mV in Fig. 5), a biphasic net current record was obtained which displayed an initial phase of I_a and a secondary phase of partially inactivated I_EO. Similar biphasic net currents are commonly seen in cardiac Purkinje fibers at even less negative holding potentials, (i.e., −40 or −30 mV). The incomplete inactivation of the transient outward current, I_{to}, at these potentials makes the examination of I_a difficult in Purkinje fibers.

A plot describing the steady state inactivation of I_EO from five experiments is shown in Figure 6. In these experiments, both TTX and CoCl_2 were present to block the inward currents. The membrane was voltage clamped for 2 seconds at potentials between −100 and −40 mV, and then stepped to +50 mV, a potential at which I_EO was fully activated. The resulting outward currents were normalized by divid-

FIGURE 5. Steady state inactivation of the rat early outward current. The membrane potential was clamped to the potentials indicated for 2 seconds before stepping to the test potential, +10 mV. The digitized records of membrane currents were obtained using the single micro-electrode voltage clamp. TTX (10^{-7} M) was present to block the fast Na+ current.

TABLE 6. Steady state inactivation of the rat early outward current. Data points represent the fraction of the maximal activated I_EO/I_EO_max available at a given holding potential. Two second conditioning pulse; +50 mV test voltage. TTX (10^{-7} M) and CoCl_2 (10^{-3} M) were present in the Tyrode's solution.

FIGURE 6. Steady state inactivation of the rat early outward current. Data points represent the fraction of the maximal activated I_EO/I_EO_max available at a given holding potential. Two second conditioning pulse; +50 mV test voltage. TTX (10^{-7} M) and CoCl_2 (10^{-3} M) were present in the Tyrode's solution.
ing them by the maximal $I_{EO}$ which was elicited at a holding potential of $-100$ mV. As can be seen, the inactivation of $I_{EO}$ was a sigmoidal function of membrane potential. Inactivation was almost completely removed near the normal resting potential ($-80$ to $-90$ mV), and $I_{EO}$ was fully inactivated at $-50$ to $-40$ mV.

During the rapid heart rates encountered in rats, the action potential duration might be affected by an incomplete recovery of $I_{EO}$ from inactivation. Therefore, it was of interest to examine the time course for the recovery of $I_{EO}$ at a potential near the normal resting potential ($-80$ mV). This was performed by a double-pulse procedure, where the interpulse interval was varied between 2 and 500 msec. The peak $I_{EO}$ elicited by the second pulse was expressed as a percentage of the $I_{EO}$ during the first or control pulse and was plotted in Figure 7. Although complete recovery of $I_{EO}$ required 500 msec, the current was 90% recovered in only 60 msec. With the exception of the first point recorded at 2 msec, the time course for the recovery of $I_{EO}$ could be described by a single exponential, with a time constant of 25.3 msec. The apparent delay in the recovery between 0 and 10 msec, in association with the small persisting value for $I_{EO}$ at 2 msec, suggests that a small fraction (less than 5%) of the current did not inactivate during the prepulse.

**Discussion**

An early outward current, which shows rapid kinetics of activation, inactivation, and recovery, can be recorded in single rat ventricular cells under voltage clamp conditions. This current may be conducted through $K^+$-permeable channels, since it was markedly reduced by the $K^+$-current blockers 4-AP, Ba$^{++}$, and Cs$^+$. Similarly, 4-AP depressed the transient outward $K^+$ current in an invertebrate egg membrane (Hagiwara et al., 1981) as well as in mammalian cardiac Purkinje fibers (Kenyon and Gibbons, 1979).

In the present study, the rat $I_{EO}$ did not require Ca$^{++}$ influx during $I_e$, for activation, since the current was present when Ca$^{++}$ influx was blocked by Co$^{++}$ substitution for Ca$^{++}$. Suppression of $I_{EO}$ by Ba$^{++}$ is therefore likely to be a direct effect in much the same way as Ba$^{++}$ blocks other $K^+$ currents; the Ba$^{++}$ effect cannot be interpreted as indicating a requirement for Ca$^{++}$. In other preparations, such as calf cardiac Purkinje fibers, the transient outward current has been reported to be activated by the internal [Ca] (Siegelbaum and Tsien, 1980), since pharmacological blockade of $I_a$ or removal of Ca$^{++}$ greatly depressed the transient outward current. However, in sheep Purkinje fibers, the major fraction of the transient outward current (which was 4-AP-sensitive) was found not to be Ca$^{++}$-activated (Boyett, 1981; see also Coraboeuf and Carmeliet, 1982). In addition, the transient (K$^+$) outward current in the egg cell membrane was unaffected by substitution of Mn$^{++}$ for Ca$^{++}$ in the external solutions (Hagiwara et al., 1981). There remains the possibility, however, that $I_{EO}$ may be modulated by the release of Ca$^{++}$ from an internal store, such as the sarcoplasmic reticulum.

Although the functional significance of the early outward current in the rat ventricular cell is not completely apparent, several speculative possibilities can be deduced from its characteristics. This current, which helps shorten the action potential duration (thereby keeping the refractory period brief), may be necessary at the high heart rates encountered in rats. In this regard, the rapid recovery of $I_{EO}$ from inactivation ensures that it remains functional in limiting the initial plateau duration, even at action potential rates which may depress the upstroke velocity (see Payet et al., 1981).

In addition, the early, fast phase of repolarization of the rat action potential, which appears to be caused by the activation of $I_{EO}$, rapidly returns the plateau membrane potential to levels below 0 mV. This may serve to limit the total amount of Ca$^{++}$ entering the cell during the plateau. Such a speculation may be strengthened by the observation that an elevated Ca$^{++}$ influx in the rat ventricular cell results in a negative inotropic state (Aronson and Capasso, 1980; Forester and Mainwood, 1974).

Finally, it is interesting to note that the early outward current may not be present during the early embryonic development of the rat heart, since, during this time, the cardiac action potential is conventionally shaped and has a plateau of long duration (Bernard and Gargouil, 1968). Future studies examining the electrophysiological changes occurring during development may help to clarify the role played by this unusual current.
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