Two Components of Transient Outward Current in Canine Ventricular Myocytes

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Repolarization during phase 1 of cardiac action potential is important in that it may influence both impulse conduction in partially depolarized tissue and action potential duration. Thus, it is important to know the properties and regulation of the underlying currents. In about 50% of canine ventricular myocytes, the action potential displays a phase 1 of fast repolarization and a prominent notch between phase 1 and the plateau. A transient outward current is responsible for both. This current is composed of two components: one (I_{to1}) blocked by 4-aminopyridine and the other (I_{to2}) blocked by manganese. In the present study, we characterized each of the components in isolation from the other. Both had an activation threshold between -30 and -20 mV. At the same voltage, I_{to1} was larger than I_{to2} and had a shorter time to peak. The peak current-voltage relationship for I_{to1} was almost linear, but that for I_{to2} was bell-shaped. I_{to1} decayed during sustained depolarization with a single exponential time course: \( \tau \approx 30 \text{ msec} \) at all voltages. It recovered from inactivation with a voltage-dependent time course: \( \tau \approx 70 \text{ msec} \) at -90 mV and 720 msec at -40 mV. I_{to2} was augmented by elevating \([Ca^{2+}]_0\) or by isoproterenol. It was inhibited by caffeine, ryanodine, or a preceding transient inward current, suggesting that it was activated by intracellular calcium released from sarcoplasmic reticulum. We conclude that I_{to1} and I_{to2} in canine ventricle are similar to those described for many other cardiac tissues, but the kinetics of I_{to1} are significantly faster than in other tissues. (Circulation Research 1989;64:633-647)

Transient outward current has been observed in nerve cells\(^1\) and cardiac tissues.\(^2\) This current is important in modulating the firing properties in "encoding" nerve cells.\(^1\) In cardiac cells, it has been suggested that transient outward current may modulate action potential duration,\(^3\) prevent arrhythmogenic slow-response action potentials,\(^3\) and help maintain a high resting membrane potential in latent pacemaker cells.\(^7\)

There have been controversies over the identity of the transient outward current. Earlier work attributed it to an influx of chloride ions.\(^8\)\(^9\) Later, it was identified as a potassium current that could be blocked by potassium channel blockers such as 4-aminopyridine (4-AP).\(^10\)\(^11\) In rabbit atrioventricular nodal cells the transient outward current can be blocked by 4-AP but not by calcium channel blockers,\(^7\) whereas in calf Purkinje fibers, it is activated by intracellular calcium but is not sensitive to 4-AP\(^6\) (however, see Reference 12). That there may be two different transient outward currents was first verified in studies on sheep Purkinje fibers by Coraboeuf and Carmeliet,\(^13\) who showed that one or the other could be isolated by appropriate experimental conditions.

In the past, it has been assumed that the transient outward current was absent from or negligible in ventricular muscle.\(^14\) In this study, we show that in about half of the canine ventricular myocytes there is a large transient outward current that causes the fast repolarization during phase 1 of the action potential and the notch between phase 1 and the plateau. The transient outward current is composed of two components: One is voltage dependent, and the other is dependent on calcium released from the sarcoplasmic reticulum (SR). A recent report, in which conventional microelectrode techniques were used, has shown that in canine ventricular epicardium, but not endocardium, the action potentials display a "spike and dome" morphology that is diminished or abolished by 4-AP and ryanodine or strontium treatment.\(^15\) A 4-AP-sensitive transient outward current has also been reported for dog ventricular myocardium voltage clamped with the double sucrose gap technique in the presence of calcium channel blockers.\(^16\) Stimulation of \(\beta\)-

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adrenoceptors can modulate the transient outward current in single canine Purkinje cells recorded under Ca"-free conditions.17 We report here that stimulation of β-adrenoceptors can greatly augment the intracellular Ca"-activated transient outward current in canine ventricular myocytes. The characteristics of the two transient outward current components we have identified may be useful in understanding the changes in impulse conduction and action potential duration in partially depolarized or Ca"-overloaded tissues under conditions such as myocardial ischemia and infarction.

Materials and Methods

Cell Preparation

Single myocytes were disaggregated from canine left ventricle by intracoronary perfusion of calcium-free Tyrode's solution containing collagenase (146 units/mg, 0.5 mg/ml) (type II, Worthington, Freehold, New Jersey) and subsequent trituration of chunks of deep myocardial tissue in the same medium.18 Cells were incubated in Minimal Essential Medium (GIBCO, Grand Island, New York), to which all salts were added to produce a standard Hank's balanced salt solution. The pH was adjusted to 7.3 with 5% CO2-95% O2. After disaggregation the cells were kept at 36° C for 1–2 hours before use.

Electrophysiological Experiments

The cells were allowed to adhere to a glass coverslip coated with poly-l-lysine that was placed on the bottom of a 0.7-ml lucite tissue chamber mounted on the stage of a Nikon inverted microscope. The cells were superfused continuously with normal Tyrode’s solution containing (mM) NaCl 137, NaHCO3 12, dextrose 5.5, NaH2PO4 1.8, MgCl2 0.5, KCl 4, and CaCl2 2. This solution was equilibrated with 5% CO2-95% O2 and maintained at a pH of 7.1 and a temperature of 35-37° C. To study the transient outward current by voltage clamp, we used either the switched-clamp method with a suction pipette. For the first method, we employed an AxoClamp-2 amplifier (Axon Instruments, Inc, Burlingame, California), and subsequent trituration of chunks of deep myocardial tissue in the same medium.18 Cells were incubated in Minimal Essential Medium (GIBCO, Grand Island, New York), to which all salts were added to produce a standard Hank’s balanced salt solution. The pH was adjusted to 7.3 with 5% CO2-95% O2. After disaggregation the cells were kept at 36° C for 1–2 hours before use.

One transient outward current component was blocked by 4-AP, and the other was abolished by either manganese, caffeine, or ryanodine. Similar components in sheep Purkinje fibers were called Ito and Iox, respectively, because the time course of the former was long (first time constant of decay, 80–100 msec; second time constant, 250–400 msec) and that of the latter brief (time constant of decay, 12 msec). A difference in the time course of this magnitude was not found for canine ventricular myocytes (see below). We thus chose to call the component blocked by 4-AP “Ito” and the other component “Iox.” To record Ito we used the continuous-clamp method with a suction pipette.

To quantify the magnitude of Ito, it was necessary to minimize other temporally superimposed currents (Ioa, ICa, and Iox). Ioa was largely blocked by 2 mM manganese in the external solution.21 Iox was suppressed both by the block of ICa using external manganese and by the 10 mM EGTA in the pipette solution since this current is activated by intracellular calcium6-13 (see below). Interference by Iox was largely avoided by adding tetrodotoxin (TTX) (15–30 μM) to the Tyrode’s solution. In some experiments, in addition to the use of TTX, Iox was decreased also by using a holding potential of -60 to -55 mV. In these myocytes, Iox is largely inactivated at -55 mV, as estimated from the relation between membrane potential and the maximum upstroke velocity of phase 0,20 whereas the potential for half-maximum inactivation of Ito is ~ -47 mV (see "Results"). Under such a condition, the transient outward current could be apparently abolished by 2 mM 4-AP (Figure 1A, left panel), confirming that only Ito remained. The amplitude of Ito was measured as the difference between the outward peak and a sloping baseline extrapolated from the current change between 100 and 120 msec after depolarization. We judged that contamination of
Two Transient Outward Currents in Heart Cells

Figure 1. Recording conditions and measurement of $I_{o1}$ and $I_{o2}$. Voltage clamp was done with continuous-clamp method. A (left panel): Upper trace (a) is current elicited in response to a step to +30 mV from -60 mV. There was 15.7 µM tetrodotoxin and 2 mM Mn²⁺ in external solution and EGTA (10 mM) in pipette solution. $I_{o1}$ amplitude was measured as difference between outward peak and sloping baseline extrapolated from current change between 100 and 120 msec after depolarization. Lower trace (b) is current induced by same voltage step after addition of 2 mM 4-aminopyridine (4-AP). $I_{o1}$ was apparently abolished. Right panel: 4-AP-sensitive current obtained by subtracting the current in the presence of 4-AP from that in its absence (a-b). Amplitude of $I_{o1}$ in this case was measured as difference between outward peak and holding current level. $I_{o1}$ amplitude measured by first method (from trace "a") was smaller than that measured by second method (from trace "a-b") by 5%. Currents during initial 2 msec after depolarization were capacitative transients and have been omitted. B (left panel): Current trace induced by a depolarization step from -80 mV to +50 mV. External solution contained 2 mM 4-AP and pipette solution contained 1 mM EGTA. Right panel: Current trace induced by same voltage step after addition of 1 µM isoproterenol (Isop). $I_{o2}$ was greatly enhanced. Amplitude of $I_{o2}$ was measured as difference between outward peak and sloping baseline extrapolated from current change between 50 and 100 msec after depolarization, as marked. Currents during initial 3 msec after depolarization are omitted.

Transient outward current by the delayed rectifier current was minimal because the latter activates with a time constant greater than 100 msec. In some experiments, the 4-AP-sensitive current was measured by subtracting the current in the presence of 2 mM 4-AP from that in its absence (Figure 1A, right panel). The amplitude of $I_{o1}$ was then measured as the difference between the outward peak and the holding current level. Since 4-AP at 2 mM did not significantly change the steady-state current-voltage relationship, the holding current level of the difference current was very close or equal to zero. The 4-AP-sensitive current subsided to almost zero at 100 msec after depolarization (Figure 1A, right panel). For the same cell, the amplitude of $I_{o1}$ measured by the first method was smaller than that measured by the second method by ≤6%.

Because $I_{o2}$ is activated by an increase in intracellular Ca²⁺ activity, to study this current we could not use methods that prevented intracellular Ca²⁺ transients. Therefore, we used either a single microelectrode and the switched-clamp method or a suction pipette containing only 1 mM EGTA. With the latter, we also used 1 µM isoproterenol to enhance both $I_{Ca}$ and calcium uptake by the SR, with the intent of increasing Ca²⁺ transients during depolarizations. Under these conditions, although the $I_{o2}$ was prominent, there was no visible mechanical activity. This may indicate that the calcium level that was required to elicit some $I_{o2}$ was lower than the mechanical threshold or that 1 mM EGTA did not suppress Ca²⁺ transients in the vicinity of the cell membrane as completely as in the bulk phase of the cytoplasm. $I_{o1}$ was eliminated by adding 2 mM 4-AP to the external solution. One example is shown in Figure 1B. With 1 mM EGTA in the pipette solution and in the presence of 2 mM 4-AP in the bath solution, there was only a very small transient outward current, the amplitude of which was difficult to measure. After the addition of 1 µM isoproterenol, the transient outward current was greatly enhanced. This outward current could be abolished by ryanodine (see “Results”). The amplitude of $I_{o2}$ was measured as the difference between the outward peak and a sloping baseline extrapolated from the current change between 50 and 100 msec after depolarization (Figure 1B, right panel). It is difficult to isolate $I_{o2}$ from $I_{Ca}$. Agents that block $I_{Ca}$ will also abolish $I_{o2}$ by decreasing intracellular Ca²⁺ transients. Ryanodine was used by...
FIGURE 2. Correlation between notch of action potential and transient outward current. A: Configuration of action potentials elicited from holding voltage ($V_h$) of −70 mV (left) or −40 mV (right). Notch was prominent at $V_h$ −70 mV but disappeared at $V_h$ −40 mV. Note also that plateau shifted positive and action potential duration was longer in absence of the notch. B: Superimposed current traces elicited by depolarization steps to +20 mV from $V_h$ of −75 or −35 mV. Transient outward current was totally inactivated at −35 mV. Action potentials were recorded using microelectrode; currents were recorded from different cell with continuous-clamp method with 2 mM Mn$^{2+}$ and 30 μM tetrodotoxin in external solution.

Kenyon and Sutko to isolate $I_{to2}$ from other currents. This agent abolishes $I_{to2}$ by inhibiting Ca$^{2+}$ release from the SR. However, in canine ventricular myocytes ryanodine can also prolong $I_{to}$ by decreasing inactivation mediated by intracellular Ca$^{2+}$ released from the SR. Thus, the interpretation of ryanodine-sensitive current is difficult. Since our method of measuring $I_{to}$ did not eliminate the overlapping $I_{ca}$, the amplitude of $I_{to2}$ probably was underestimated.

Results

As we have reported, in about 50% of canine ventricular myocytes a sizable transient outward current is recorded and the action potential displays fast repolarization during phase 1 and a notch between phase 1 and the plateau. The causal relation between the transient outward current and the action potential configuration during phase 1 and notch was shown by two observations in that study: 1) Both are suppressed by 4-AP and 2) both are interval dependent. Another example of the relation is shown in Figure 2. The notch in the action potential and the transient outward current are both eliminated by holding the membrane voltage at or positive to −40 mV before eliciting a response. The transient outward current is composed of $I_{to1}$ and $I_{to2}$, the former is blocked by 4-AP, and the latter abolished by manganese or caffeine, as is the case for other cardiac tissues. In three cells that did not have fast repolarization during phase 1 and a notch in the action potential, voltage clamp revealed the existence of a small transient outward current that was not blocked by 4-AP. It is thus likely that, in the cells that displayed such an action potential configuration, only $I_{to1}$, but not $I_{to2}$, was present.

Characteristics of $I_{to1}$

Activation of $I_{to1}$. To study the activation of $I_{to1}$, we clamped the membrane voltage for 300 msec to a test voltage ($V_t$) between −30 and +120 mV from a holding voltage ($V_h$) of −80 to −55 mV at an interval of 5–7 seconds. In all experiments the external solution contained TTX (15.7 μM) and manganese (2 mM). The amplitude of $I_{to1}$ was determined by either measuring the 4-AP-sensitive current or using the sloping baseline method (see "Materials and Methods"). Figure 3A shows 4-AP-sensitive current traces elicited by depolarization to different voltages, and Figure 3B depicts the peak...
Inactivation of $I_{\text{tol}}$. The voltage-dependent inactivation of $I_{\text{tol}}$ was studied by clamping to different conditioning voltages ($V_c$) for 500 msec and then applying a test pulse to $+40$ mV for 300 msec. The holding voltage was $-80$ mV, and the interval between test pulses was 7 seconds. The results of one representative experiment are shown in Figure 4. The inset shows the superimposed original current traces during the test pulses; the values of $V_c$ are indicated on the left. Peak current decreased as $V_c$ was made more positive, and the current-voltage relationship was almost linear. The peak current kept on increasing almost linearly for $V_c$ as positive as $+120$ mV (data not shown). The 4-AP-sensitive current showed an initial inward deflection in a voltage range from $-30$ to $+10$ mV (Figure 3A). This might be due to an enhancement of sodium channel block by TTX in the presence of 4-AP. This should cause an underestimation of $I_{\text{tol}}$ amplitude in this voltage range.

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Kinetic properties of $I_{\text{tol}}$. $I_{\text{tol}}$ rose to a peak and declined during maintained depolarization. The time course of activation and inactivation of $I_{\text{tol}}$ were studied by measuring the time to peak and the decay time course of the 4-AP-sensitive current. The decay time course of the 4-AP-sensitive current was studied between the moment when this current decayed to 95% of its peak amplitude and 100 msec after depolarization. Figure 5A illustrates three examples of fitting the decay time courses of the 4-AP-sensitive currents at different voltages with single exponential functions. It is clear that, at all three voltages, the fit was adequate. Figure 5B shows summarized results from this experiment. The time to peak of the 4-AP-sensitive current was 11.6 msec at the voltage of its activation threshold, $-30$ mV, and decreased monotonically at more positive voltages. The decay time constant was 28.5 msec at $-20$ mV, decreased to 16 msec at $+20$ to $+30$ mV, and increased again at still more positive voltages, thus showing a U-shaped voltage dependence. Similar results were obtained in two other experiments in which the 4-AP-sensitive currents were measured. The time course of decay of $I_{\text{tol}}$ in canine ventricular cells thus differs from that reported for other cardiac tissues, such as sheep Purkinje fibers and rabbit atroventricular nodal cells, in that it can be described by a single rather than a double exponential function. Moreover, the time constant is shorter: In a voltage range from $-20$ to $+70$ mV, the time constant was less than 30 msec in canine ventricular cells whereas the first time constant was 80–100 msec in sheep Purkinje fibers in a similar voltage range.

The time course of recovery of $I_{\text{tol}}$ from inactivation (reactivation) was studied by a double-pulse protocol: Two 100-msec pulses, each to $+60$ mV with a varying interpulse interval, were applied every 10 seconds from each of several holding voltages. The interpulse interval ranged from 10 to 5,000 msec. Figure 6A shows two examples of original current traces elicited from the same cell by this protocol at holding voltages of $-60$ and $-80$ mV. $I_{\text{tol}}$ was absent or very small with an interpulse interval of 10 msec, and increased gradually as the interval was prolonged. The rate of increase was
The current-voltage relationship was determined by a two-step voltage-clamp protocol illustrated in Figure 5. A: Examples of fit at -10, +30, and +60 mV. For each trace, dots represent recorded current levels and superimposed smooth curve is calculated from the single exponential function that best fits the current change. It is clear that the fit with single exponential functions was adequate. B: Summarized results from this experiment. Time to peak current was determined as difference between start of depolarization and moment of peak outward current. Time to peak current decreased as the test voltage became more positive. The decay time constant showed a U-shaped voltage dependence.

Figure 6. Kinetic properties of $I_{\text{tot}}$. A: Aminopyridine-sensitive currents were measured at different depolarization voltages and time courses of current change between time when current decayed to 95% of its peak amplitude and 100 msec after depolarization were fit with single exponential functions. A: Three examples of fit at -10, +30, and +60 mV. For each trace, dots represent recorded current levels and superimposed smooth curve is calculated from the single exponential function that best fits the current change. It is clear that the fit with single exponential functions was adequate. B: Summarized results from this experiment. Time to peak current was determined as difference between start of depolarization and moment of peak outward current. Time to peak current decreased as the test voltage became more positive. The decay time constant showed a U-shaped voltage dependence.

Current-voltage relationship and reversal potential of $I_{\text{tot}}$. For the above experiments $I_{\text{tot}}$ was measured at voltages positive to -30 mV, and it was isolated from other currents by TTX and manganese. To determine the current-voltage relationship of $I_{\text{tot}}$, we had to use voltages negative to -30 mV and this caused interference from the inward rectifier current, $I_{K1}$, especially at voltages negative to -70 to -100 mV when $I_{K1}$ displayed time-dependent changes. Aminopyridine-sensitive currents were measured at different depolarization voltages and time courses of current change between time when current decayed to 95% of its peak amplitude and 100 msec after depolarization were fit with single exponential functions. A: Three examples of fit at -10, +30, and +60 mV. For each trace, dots represent recorded current levels and superimposed smooth curve is calculated from the single exponential function that best fits the current change. It is clear that the fit with single exponential functions was adequate. B: Summarized results from this experiment. Time to peak current was determined as difference between start of depolarization and moment of peak outward current. Time to peak current decreased as the test voltage became more positive. The decay time constant showed a U-shaped voltage dependence.

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faster at -80 mV than -60 mV. The reactivation time course was quantified by calculating the percent of decrease in $I_{\text{tot}}$ amplitude during the second pulse (percent of inactivation) and plotting this value on a semilogarithmic scale against the interpulse interval, as shown in Figure 6B for the current traces in Figure 6A. The reactivation time course at $V_h$ of -60 mV could be well described by a single exponential function with a time constant of 380 msec. At $V_h$ of -80 mV the reactivation followed a double exponential time course with a fast component (86%, 95 msec) and a slow component (14%, 610 msec). The reactivation time course of $I_{\text{tot}}$ was thus a function of holding voltage. Similar results were obtained from five other cells. The relation between the holding voltage and the average time constant of reactivation of $I_{\text{tot}}$ (the fast time constant considered if a two exponential function fit better) for these six cells is summarized in Figure 6C. Although there was some variability among cells in the reactivation time constant at the same holding voltage, as shown by the standard error bars, the general trend was clear: The reactivation time constant was long at less negative holding voltages (>700 msec at -40 mV) and decreased as the holding voltage was made more negative (<100 msec at -90 mV). The reactivation time constant of $I_{\text{tot}}$ in canine ventricular myocytes is thus shorter than in other cardiac tissues, for example, 100 msec in canine ventricular cells at -80 mV versus 500 msec in sheep Purkinje fibers at -84 mV.9
FIGURE 6. Time course of reactivation of $I_{\text{to}}$. Voltage-clamp protocol is explained in text. A: Original current traces at holding voltage ($V_h$) of $-60$ and $-80$ mV. Traces on left show $I_{\text{to}}$ during first pulses at these two holding voltages; superimposed traces on right show $I_{\text{to}}$ during second pulses. Interpulse intervals are marked above traces. B: Time course of reactivation of $I_{\text{to}}$ for $V_h=-60$ and $-80$ mV shown on a semilogarithmic scale. Ordinate is percent of inactivation (explained in text). At $V_h$ of $-60$ mV, time course can be described by single exponential function with time constant of 380 msec. At $V_h$ of $-80$ mV, time course is better fit by double exponential function with time constants of 95 and 610 msec. C: Average time constants of reactivation at various holding voltages obtained for six cells. Bars represent standard errors of the mean. Time constants at $-40$ and $-90$ mV represent one observation at each voltage. Reactivation was accelerated by making holding voltage more negative.

The inset of Figure 8. From a holding voltage of $-70$ mV, the voltage was clamped to $+60$ mV for 5 msec and then to test voltages ($V_t$) ranging from $+50$ mV to $-110$ mV. Tests were made at an interval of 7 seconds. Figure 8 shows the original tail current traces during the test pulses at 4 and 40 mM [K+]o. At 4 mM [K+]o, and voltages positive to $-40$ mV there were obvious outward tail currents that decayed with an apparent single exponential time course (time constant: 8.5, 8.6, and 6 msec at $+30$, 0, and $-20$ mV, respectively). In the voltage range between $-40$ and $-60$ mV, the tail current was very small after the decay of the capacitative transient. At still more negative voltages obvious inward tail currents occurred; at $-110$ mV the inward tail current decayed with a time constant of 4.3 msec. When [K+]o was elevated to 40 mM, at voltages positive to $-20$ mV outward tail current was smaller at each test voltage than with 4 mM [K+]o. The tail current was very small after the decay of the capacitative transient in the voltage range from $-20$ to $-40$ mV, and a distinct inward tail current occurred at $-50$ mV or more negative voltages.

The amplitude of outward tail current was determined by fitting the current change after the outward peak with a single exponential function and extrapolating to the time of repolarization. For inward tail current the amplitude was determined by fitting the tail with a double exponential function, the fast component being the capacitative transient (time constant, 0.4–0.5 msec) and the slower component being the inward $I_{\text{to}}$ tail. Figure 9A illustrates some examples of the fit to the outward and inward tail currents at 4 and 40 mM [K+]o. It is clear that the fit was adequate. When the reversal potential was approached and fitting was not possible, the amplitude of tail current was estimated by the current level 4 msec after repolarization. The current-voltage relationships at the two concentrations of external potassium are depicted in Figure 9B. The shape of the current-voltage relationship was similar, but the reversal potential was shifted in the positive direction with the [K+]o higher. The reversal potential of $I_{\text{to}}$ in 4 mM [K+]o was about $-50$ mV and significantly more positive than the reversal potential of $I_{\text{k1}}$ determined in the same cell ($-95$ mV, Figure 7A). Similar current-voltage relationships in 4 mM [K+]o were obtained in a total of six experiments; the average reversal potential was $-47±16$ mV (mean±SD). If we assume that sodium as well as potassium is a charge carrier,7,9 that the intracellular sodium concentra-
FIGURE 1. Different effects of barium on inward rectifier current (I_K) and I_lo. A: Steady-state current-voltage relationship obtained under control conditions and after addition of 1 mM Ba^2+. [K+]o was 4 mM and there was 15.7 μM tetrodotoxin and 2 mM Mn^2+ in external solution. Current recorded at voltages negative to −30 mV, I_K, was largely eliminated. B: Results of experiment done in same cell as that in A. Left panel: I_lo induced by step from −60 mV to +40 mV. Right panel: I_lo induced by same step after addition of 1 mM Ba^2+. Barium decreased amplitude of I_lo by only 13% (from 1,260 pA to 1,100 pA).

Properties of I_lo

Effects on I_lo of [Ca^{2+}]_o and isoproterenol. Figure 10 shows the effects of elevating [Ca^{2+}]_o and of isoproterenol on I_lo. For the experiment shown in Figure 10A we used the switched-clamp method in the presence of 2 mM 4-AP. At 2 mM [Ca^{2+}]_o, a depolarizing pulse from −80 to +50 mV activated only a small and slow I_lo. Time to peak was 20 msec, and peak amplitude was hard to measure. After [Ca^{2+}]_o had been increased to 5 mM, the amplitude of I_lo increased (250 pA, measured by the sloping baseline method) and time to peak decreased to 17 msec. Figure 10B shows that isoproterenol augmented both I_c and I_lo. For this experiment we used the continuous-clamp method with EGTA concentration of 1 mM in the pipette solution. There was no 4-AP present because this cell displayed only very small I_c (Figure 10B, left panel). The onset of the effect of isoproterenol on I_c lagged behind the increase in I_c caused by isoproterenol (data now shown), suggesting that the augmentation of I_lo was due to an increase in intracellular Ca^{2+} level subsequent to the enhancement of I_c by isoproterenol. Since elevating [Ca^{2+}]_o or isoproterenol augmented I_c, the real increase in I_lo probably was larger than these records indicated. It is clear from the right panel of Figure 10B that the time to peak of I_lo was longer at more positive voltages and its amplitude first increased and then decreased as the voltage became more positive (see below).

Activation of I_lo. To study the activation of I_lo, we applied depolarizing pulses to different test voltages from a holding voltage of −80 mV at an interval of 7 seconds. The left panel in Figure 11A shows superimposed current traces in response to this voltage-clamp protocol. The voltage clamp was done with the continuous-clamp method. The pipette solution contained only 1 mM EGTA. The Tyrode's
solution contained 2 mM 4-AP and 1 μM isoproterenol. As the test voltage was made more positive, the amplitude of \( I_{\text{to2}} \) increased until the voltage reached +70 mV. At still more positive voltages, the amplitude of \( I_{\text{to2}} \) declined. To exclude the possibility that the decline of \( I_{\text{to2}} \) at strongly positive voltages was due to activation of the delayed rectifier current, the voltage-clamp protocol was repeated in the same cell after the addition of 3 μM ryanodine (Figure 11A, right panel). \( I_{\text{to2}} \) was abolished, but there was no apparent increasing outward current during the first 100 msec after depolarization. \( I_{\text{to2}} \) may display a “staircase” phenomenon, either positive or negative, depending on the voltage-clamp protocol.26 The bell-shaped peak current-voltage relationship was not due to a staircase because the relationship stayed the same with depolarizing steps at randomly selected voltages. Figure 11B depicts the peak current-voltage relationships of \( I_{\text{to2}} \) recorded from different cells, including the one shown in Figure 11A (represented by •). The activation threshold of \( I_{\text{to2}} \) was −20 mV, and the current-voltage relationship in each of the five cells was bell-shaped, with the maximal \( I_{\text{to2}} \) occurring between +30 mV and +70 mV. Thus, the peak current-voltage relationship of \( I_{\text{to2}} \) is dramatically different from that of \( I_{\text{to1}} \), which does not decline at a voltage as positive as +120 mV. The mechanisms for the bell-shaped peak current-voltage relationship of \( I_{\text{to2}} \) and the variability in the voltage that gave the maximal \( I_{\text{to2}} \) will be discussed in “Discussion.”

Inactivation of \( I_{\text{to2}} \). To study the voltage-dependent inactivation of \( I_{\text{to2}} \), we applied a test clamp to +50 mV after a 500-msec conditioning step to different voltages. The holding voltage was −80 mV, and the interval between test clamps was 7 seconds. Figure 12 illustrates the results of one representative experiment. The continuous-clamp method was used. Figure 12A shows the original current traces during the test pulses after different conditioning voltages (Vc). The peak amplitudes of \( I_{\text{to2}} \) during test pulses were plotted against conditioning voltages in Figure 12B. \( I_{\text{to2}} \) disappeared after Vc positive to −18 mV. As the Vc was made more negative, the amplitude of \( I_{\text{to2}} \) increased, attained a plateau between −40 and −60 mV, and increased again at still more negative Vc. Similar voltage-dependent inactivation of \( I_{\text{to2}} \) was observed in two other experiments using the switched-clamp method. Thus, the voltage dependence of inactivation of \( I_{\text{to2}} \) is different from that of \( I_{\text{to1}} \). The former is nonsigmoidal and extends over a wide voltage range from −70 to −10 mV, whereas the latter is sigmoidal and occurs between −70 and −30 mV. The nonsigmoidal nature of the voltage dependence of inactivation of \( I_{\text{to2}} \) was not due to a staircase phenomenon.
FIGURE 10. Effects of elevating \([Ca^{2+}]_o\) or isoproterenol on \(I_{\text{to2}}\). A: Voltage clamp was done with switched-clamp method using single micro-electrode. Two current traces were elicited by same voltage-clamp protocol: depolarization to +50 mV from holding voltage of −80 mV at an interval of 7 seconds. Left trace was obtained in presence of 2 mM Ca\(^{2+}\) and 2 mM 4-amino-pyridine (4-AP); the right, 5 mM Ca\(^{2+}\) in the continuous presence of 4-AP. B: Experiment was done with continuous-clamp method with only 1 mM EGTA in pipette solution. \([Ca^{2+}]_o\) was 5 mM. Holding voltage was −80 mV, and currents were induced by steps of depolarization from 0 to +70 mV, in increments of 10 mV. Although there was no 4-AP present, this cell displayed only very small \(I_{\text{to1}}\) (a). Addition of 1 μM isoproterenol (Isop) greatly increased both \(I_{\text{to1}}\) and \(I_{\text{to2}}\) (b). Difference currents (b-a) more clearly show effects of isoproterenol on membrane currents. Horizontal line denotes zero current level.

because the relationship stayed the same with conditioning pulses of random amplitudes.

Dependence of \(I_{\text{to2}}\) on the function of the sarcoplasmic reticulum. Figure 13A shows that 10 mM caffeine first decreased the amplitude and time to peak of \(I_{\text{to2}}\) and then abolished this current. The effect of 3 μM ryanodine on \(I_{\text{to2}}\) is shown again in Figure 13B, with an expanded time scale. Ryanodine decreased the amplitude of \(I_{\text{to2}}\) and then abolished it, without changing its time to peak. The similar effects of caffeine and ryanodine on \(I_{\text{to2}}\) amplitude and their different effects on time to peak of \(I_{\text{to2}}\) resemble the effects of these agents on transient inward current,\(^{27}\) which is known to be dependent on Ca\(^{2+}\) released from the SR.\(^{28}\) More direct evidence for the dependence of \(I_{\text{to2}}\) on SR function is provided by Figure 13C. When a transient inward current preceded the depolarizing pulse activating \(I_{\text{to2}}\), the amplitude of \(I_{\text{to2}}\) was decreased. This has also been shown by Lipsius and Gibbons.\(^{26}\) Note that in Figure 13C \(I_{\text{to2}}\) decayed in two phases and both were suppressed by the preceding transient inward current.

Discussion

There is a transient outward current in various cardiac tissues: Purkinje fibers (sheep\(^{13}\) and calf\(^{6,12}\), ventricular muscle (rabbit\(^5\) and monkey\(^{29}\), ventricular myocytes (rat\(^4\), and atrial myocytes (rabbit crista terminalis,\(^3\) rabbit atrioventricular node,\(^7\) and human\(^{23}\). Single-channel currents have been recorded from some of these tissues (calf Purkinje cells,\(^{30}\) sheep and rabbit Purkinje cells,\(^{31}\) rabbit atrioventricular nodal cells,\(^7\) and mouse ventricular myocytes\(^{32}\)). From these studies it is known that there are two distinct transient outward currents that differ in terms of pharmacological and kinetic properties and single-channel conductance. In the present study, both components of the transient outward current were found in and characterized for canine ventricular myocytes. We used recording conditions that minimized interference between \(I_{\text{to1}}\) and \(I_{\text{to2}}\) and by other membrane currents. Among the interventions, 2 mM manganese was added to eliminate \(I_{\text{to2}}\) when studying \(I_{\text{to1}}\) and 2 mM 4-AP was added to eliminate \(I_{\text{to1}}\) when studying \(I_{\text{to2}}\). However, manganese might cause a positive voltage shift in the activation and inactivation of \(I_{\text{to1}}\),\(^{33}\) and 4-AP also might affect \(I_{\text{to2}}\).\(^{13}\)

Properties of \(I_{\text{to1}}\)

In canine ventricular myocytes, \(I_{\text{to1}}\) is activated by a step depolarization to −30 mV or more positive voltages. This current has an almost linear peak current-voltage relationship and a sigmoidal voltage dependence of inactivation, similar to the transient outward current seen in other cardiac tissues. There is very little overlap, if any, of the voltage ranges for activation and inactivation of \(I_{\text{to1}}\), as is the case for transient outward current in nerve cells.\(^1\)

\(I_{\text{to1}}\) decays during sustained depolarization. The decay of the 4-AP-sensitive current (\(I_{\text{to1}}\)) between its peak and 100 msec after depolarization, when the current has subsided to near zero, is adequately fit by a single exponential function with a time constant of 10–30 msec. The counterpart of \(I_{\text{to1}}\) in other cardiac tissues very often decays with a double exponential time course, although the time constant of the second phase tends to vary widely, and sometimes this time-dependent change in current can be attributed to activation of the delayed rectifier current.\(^7\) Moreover, \(I_{\text{to1}}\) in canine ventricular cells decays faster than in other cardiac tissues: In a voltage range from −20 to +70 mV, the time constant of decay is less than 30 msec in the canine ventricular cells but 300 msec in atrial cells from
A 4-AP + Isop 4-AP + Isop + Ryanodine

B

Peak I_{o2} (pA)

DEPOLARIZATION (mV)

FIGURE 11. Voltage-dependent activation of I_{o1}. A (left panel): Superimposed current traces induced by depolarizing steps from -80 mV to +30 to +100 mV (from lowest trace to highest trace, in increments of 10 mV). Interval between test pulses was 7 seconds. Voltage clamp was done with continuous-clamp method. Pipette solution contained only 1 mM EGTA. External solution contained 2 mM Ca^{2+}, 2 mM 4-aminopyridine (4-AP), and 1 μM isoproterenol (Isop). Right panel: Superimposed current traces induced by same voltage-clamp protocol after addition of 3 μM ryanodine. Currents during initial 3 msec were omitted. B: Peak current-voltage relationships of I_{o1} obtained in five experiments. ▲ and ●, Experiments done with switched-clamp method; ○, ○, ○, experiments done with continuous-clamp method. ●, experiment shown in A.

Figure 12. Voltage-dependent inactivation of I_{o2}. Conditioning step to different voltages (V_c) for 500 msec followed by test clamp to +50 mV was applied from holding voltage of -80 mV every 7 seconds. Voltage clamp was done with continuous-clamp method, with 2 mM Ca^{2+}, 2 mM 4-aminopyridine, and 1 μM isoproterenol in external solution. A: Original current traces during test pulses, with conditioning voltages marked above. Capacitive transients are omitted. B: Peak amplitude of I_{o2} plotted against conditioning voltages.

rabbit crista terminalis; in sheep Purkinje fibers at comparable potentials, t_1 is 80-90 msec and t_2 is 250-300 msec. Similar kinetic properties of a 4-AP-sensitive transient outward current, that is, single exponential decay with a time constant ≤30 msec, have recently been described for dog ventricular myocardium voltage clamped with the double-sucrose gap technique.

The reversal potential of I_{o1} in canine ventricular myocytes at 4 mM [K+]_o is -47±16 mV (n=6). The current-voltage relationship of this current in canine ventricular myocytes displays an outward as well as an inward rectification at voltages 10-20 mV positive and 30-40 mV negative, respectively, to the reversal potential. Several points need to be considered concerning the experimental conditions we used to determine the current-voltage relationship and reversal potential of I_{o1}. First, the inward rectifier current (I_K) interferes with the measurement of I_{o1} tail currents at voltages negative to -30 mV. We used 1 mM barium to eliminate this current. Since the blockade of I_K by barium is voltage- and time-dependent, it was possible that the I_{o1} tail current might have been contaminated by a time-dependent change in I_K, caused by a gradual development of barium blockade of I_K at negative voltages. However, the development of barium blockade of I_K is slow: In guinea pig ventricular myocytes at 32-35°C and with [Ba^{2+}]_o=0.4 mM, the time constant of this process is on the order of hundreds of milliseconds. Therefore, the contamination from such a slow development of barium blockade of I_K in the measurement of I_{o1} tail currents, which decayed with a time constant ≤10 msec, was only negligible. Second, although the I_{o1} tail currents decayed with apparent single exponential time courses at all voltages, the underlying conductance change might be more complicated.
According to the Hodgkin-Huxley formalism, at voltages positive to $-80$ mV the decay of tail current was caused by both inactivation and deactivation of the channels. Only at voltages negative to $-80$ mV when the inactivation of the $I_{to}$ channels was totally removed could the decay of tail current be attributed to a pure deactivation process. At present we do not have quantitative data on the time course of deactivation of $I_{to}$ in a wide voltage range. If the deactivation process of this current was extremely fast at some voltages so that the deactivation was largely complete during the capacitative transient, the tail current amplitude at these voltages would be underestimated, resulting in a distortion of the current-voltage relationship and possibly an error in the determined reversal potential. However, as shown in Figure 8, the time constant of decay of inward $I_{to}$ tail current at $-110$ mV was 4.3 msec. Since this time constant should reflect the time constant of a pure deactivation process and it was much longer than the time constant of the capacitative transient (0.4 msec), we do not think that the deactivation of $I_{to}$ could cause a serious problem in the determination of the current-voltage relationship and the reversal potential of $I_{to}$. Third, the tail currents at voltages close to the reversal potential were very small. This made an accurate determination of the reversal potential very difficult. The final solution to these problems would be to apply single-channel recording of $I_{to}$ current to our preparation at physiological $[K^+]_o$.

When compared in the same cell under identical conditions, the reversal potential of $I_{to}$ was significantly less negative than that of $I_{K}$ (Figures 7 and 9). The reversal potential of $I_{to}$ shifted in the positive direction by only about 25 mV when $[K^+]_o$ was elevated tenfold (Figure 9). These observations indicate that potassium is an important, but not the only, charge carrier of $I_{to}$. Sodium may be another charge carrier. Calculating the permeability ratio of sodium to potassium, calculated using the constant field equation assuming $[K^+]_o$ equals 145 mM and $[Na^+]_o$ is negligible, was 0.11 in both 4 and 40 mM $[K^+]_o$. Calculating the permeability ratio using the change in reversal potential when changing $[K^+]_o$, without the assumption about $[K^+]_o$ and $[Na^+]_o$, generates a similar value of 0.12. The reported reversal potentials of this current in different cardiac tissues differ: With 5.4 mM $[K^+]_o$ the reversal potential is $-18$ mV in sheep Purkinje fibers and $-30$ to $-40$ mV in rabbit atrioventricular nodal cells. This variability may be partially explained by contamination to various degrees by other current components and a slow speed of voltage clamp, especially in multicellular preparations. The reversal potential of $I_{to}$ in rabbit atrial cells from crista terminalis reported by Giles and van Ginneken was $-70$ to $-80$ mV in 5 mM $[K^+]_o$. This value probably represents a close estimation of the reversal potential of $I_{to}$ in this
preparation because in this preparation there is no interference from \( I_{K1} \) and the voltage clamp was made fast by the "supercharging" technique.

The time course of recovery of \( I_{lo2} \) from inactivation (reactivation) is voltage-dependent, as in other cardiac tissues.\(^9\) At \(-60\) mV or less negative voltages, the reactivation of \( I_{lo2} \) follows an apparent single exponential time course. At more negative voltages the reactivation time course is better fit with a double exponential function, a fast component of 85–95%, and a slower component of 5–15%. The time course of reactivation of \( I_{lo2} \) is faster in canine ventricular cells than in some other cardiac tissues. For example, at a holding potential of \(-60\) mV, the time constant of reactivation of \( I_{lo2} \) is 1,000 msec\(^9\) in sheep Purkinje fibers but 300 msec in canine cells. The time constant of reactivation of \( I_{lo2} \) seems to be even faster in rat ventricular cells. At \(-80\) mV it is 25 msec in rat cells,\(^4\) but the fast time constant at \(-80\) mV is 110 msec in canine cells. The time course seems to be similar in canine ventricular myocytes and rabbit atrial cells from crista terminalis.\(^3\) The fact that reactivation of \( I_{lo2} \) is faster in canine ventricular and other cell types than in sheep Purkinje fibers and rabbit atrioventricular nodal cells indicates that this current is more important at physiological heart rates in the former.

\( I_{lo1} \) in canine ventricular myocytes seems less sensitive to 4-AP than in sheep Purkinje fibers. In the latter, this current can be totally suppressed by 1 mM 4-AP,\(^13,34,35\) whereas in the former, 2 mM 4-AP is needed. In the canine cells, total transient outward current was inhibited by 70–80% at 1 mM 4-AP and by 80–90% at 2 mM 4-AP. No further inhibition was obtained by increasing 4-AP to 5 mM. The remaining transient outward current was totally inhibited by manganese, caffeine, or ryanodine (data not shown).

Properties of \( I_{lo2} \)

From the inhibitory effects of caffeine, ryanodine, and preceding transient inward current on \( I_{lo2} \) (Figure 13), it is clear that \( Ca^{2+} \) released from the SR is important for the activation of \( I_{lo2} \). Similar inhibition of this current by caffeine and ryanodine have been reported for cardiac Purkinje fibers,\(^13,33\) and similar conclusions about the dependence of \( I_{lo2} \) on the function of the SR have been reached by these investigators. Single-channel studies on this current in calf Purkinje cells have shown that these channels have a large single-channel conductance (120 pS at 10.8 mM [K\(^+\)]) and are modulated by both voltage and intracellular calcium,\(^30\) like the \( Ca^{2+} \)-activated potassium channels in various neurons and muscles.\(^1\)

In canine ventricular myocytes, \( I_{lo2} \) has a bell-shaped peak current-voltage relationship, which is not an artifact due to the overlapping activation of the delayed rectifier current (Figure 11A) or to a staircase phenomenon.\(^26\) The voltage dependence of activation of \( I_{lo2} \) is not sigmoidal. These properties of activation and inactivation of \( I_{lo2} \) are hard to reconcile with a purely voltage-dependent gating mechanism. Two other mechanisms likely contribute to this behavior of \( I_{lo2} \): voltage-dependent \( Ca^{2+} \) influx and voltage- and/or calcium-dependent \( Ca^{2+} \) release from the SR. The first mechanism is responsible, at least partially, for the bell-shaped peak current-voltage relationship of \( I_{lo2} \). As the voltage is made more positive and approaches the calcium equilibrium potential, there will be less calcium influx and thus a smaller intracellular calcium transient, causing less activation of \( I_{lo2} \). Similar observations and conclusions were obtained by other investigators for calf Purkinje fibers\(^6,12\) (however, see Reference 26). However, the single-channel current-voltage relationship for calf Purkinje cells, measured in cell-attached or cell-free patches, also is bell-shaped.\(^30\) It may be possible that at positive voltages the \( I_{lo2} \) channel shows inward rectification. The variability in the voltage that gave the maximal \( I_{lo2} \) (Figure 11B) probably is due to differences in cellular conditions. Since for recording \( I_{lo2} \) we did not totally suppress intracellular \( Ca^{2+} \) transients, there might be variations in the subsarcolemmal \( Ca^{2+} \) level among different cells. A change in the intracellular \( Ca^{2+} \) level will alter the \( Ca^{2+} \) equilibrium potential. Moreover, the rectification voltage of \( I_{lo2} \) may be altered.

The inactivation of \( I_{lo2} \) extends over a wide voltage range from \(-70\) to \(-10\) mV and displays a plateau between \(-60\) and \(-40\) mV. The gradual decrease in \( I_{lo2} \) in the voltage range from \(-40\) to \(-10\) mV probably is due to inactivation of the L type \( Ca^{2+} \) current over a similar voltage range.\(^20\) In canine ventricular myocytes, there is a second type of \( Ca^{2+} \) current, the low-threshold or T-type \( Ca^{2+} \) current\(^26\) that inactivates between \(-70\) and \(-50\) mV. This current may serve as a trigger for \( Ca^{2+} \) release from the SR.\(^37\) It is possible that part of the \( Ca^{2+} \) activating \( I_{lo2} \) derives from \( Ca^{2+} \) released from the SR by the T-type current; thus, inactivation of the T-type current leads to a decrease in \( I_{lo2} \). However, we cannot exclude the possibility that depolarization to between \(-70\) and \(-50\) mV affected the function of or \( Ca^{2+} \) store in the SR.\(^38\) So that less \( Ca^{2+} \) was released during the following test pulse.

Since we did not find an ideal way to separate \( I_{lo2} \) from \( I_{lo1} \), a precise measurement of the kinetic properties of \( I_{lo2} \) was not possible. The apparent time to peak of \( I_{lo2} \) was between 15 and 20 msec, and the current seemed to subside totally by 50 msec after the start of depolarization. Thus, the apparent kinetics of activation and inactivation of \( I_{lo2} \) are not very different from those of \( I_{lo1} \), contrary to what has been observed for sheep Purkinje fibers.\(^13\) The voltage dependence of the kinetics of \( I_{lo2} \) is different from that of \( I_{lo1} \). With more positive voltages, the time to peak \( I_{lo2} \) became longer and the decay was slower (most apparent in Figures 10B and 11A); this is unlike the acceleration of the time to peak and decay of \( I_{lo1} \) by more positive voltages (Figure 5).
Since $I_{o2}$ is superimposed on $I_{Ca}$, the actual time to peak $I_{o2}$ probably is shorter and the decay faster than we have reported. Moreover, the decay of $I_{o2}$ probably is more complicated than a monophasic decline. For example, Figure 13C shows that $I_{o2}$ decayed in two phases and both were sensitive to the preceding transient inward current. This wave-form of $I_{o2}$ is reminiscent of that of delayed after-depolarizations, which sometimes show a series of dampened oscillations.

As stated, we did not separate $I_{o2}$ from $I_{Ca}$. This hinders attempts to study the kinetics of activation and inactivation of $I_{o2}$, its instantaneous current-voltage relationship, and its reversal potential. The techniques used here, that is, whole-cell current recording using a single microelectrode or a suction pipette, do not enable us to distinguish between regulation of $I_{o2}$ by intracellular $Ca^{2+}$ and voltage. Single-channel recording of $I_{o2}$ and a precise control of the calcium level at the intracellular surface of the cell membrane are needed.

Possible Physiological Functions of the Two Transient Outward Current Components

Up to now, both transient outward current components have been found in sheep Purkinje fibers, calf Purkinje fibers, canine ventricular myocytes, adult human atrial cells, and rat ventricular myocytes. In other preparations, only one or the other has been identified. It is possible that both components exist in all cardiac tissues but that there are species/tissue variations in the relative amplitudes of the two components. For example, in sheep Purkinje fibers $I_{o1}$ is larger than $I_{o2}$, while the reverse might be true for calf Purkinje fibers when only one component is identified, the experimental conditions may not be optimal for the observation of the other. For example, in rabbit atrioventricular nodal cells only $I_{o1}$ was reported. However, these investigators used EGTA in their pipette solution (1 mM) without using isoproterenol or other interventions to enhance calcium current and calcium sequestration by the SR. Thus, intracellular calcium transients may not have been large enough to activate $I_{o2}$.

Several suggestions about the physiological functions of transient outward current in cardiac tissues have been offered: modulation of action potential duration, offsetting calcium current and thus preventing arrhythmogenic slow-response action potentials, and maintaining a high resting membrane potential in latent pacemaker cells. According to our data, at plateau voltages the amplitude of $I_{o1}$ can be 1 nA or larger and it decays with a time constant of 10-30 msec. Therefore, $I_{o1}$ is an important factor influencing phase 1 repolarization and early plateau voltage. By affecting the voltage of the initial plateau phase and thus modifying both activation and inactivation of other plateau currents, for example, $Ca^{2+}$ current and delayed rectifier current, $I_{o1}$ can modulate action potential duration.

Reuter and Scholz have shown that a positive shift in the voltage of the initial part of the plateau will prolong the action potential in ventricular muscle. This may partially explain the prolongation of the action potential in canine ventricular cells when $I_{o1}$ is suppressed either by a holding potential positive to -40 mV (Figure 2 of this study) or by 4-AP. In canine ventricular myocytes, only about half the cells displayed a large $I_{o1}$ associated with fast repolarization during phase 1 of the action potential and a prominent notch. Whole tissue studies show that these action potential characteristics are seen in subepicardial, but not in subendocardial, ventricular myocardium. It is possible that $I_{o2}$ is responsible, at least partially, for the fact that action potentials in subepicardial tissues are shorter than in subendocardial tissues. Without any interventions, the amplitude of $I_{o2}$ is only 10-20% of that of $I_{o1}$, and thus, $I_{o2}$ does not seem to be important for the formation of notch in the action potential (see Figure 5A of Reference 20). However, $I_{o2}$ can be enhanced by elevating [Ca$^{2+}$]$_i$, or isoproterenol (Figure 10). When enhanced, this current can decrease Ca$^{2+}$ current activation by making the initial plateau voltage less positive; $I_{o2}$ thus serves as a feedback mechanism to control Ca$^{2+}$ influx. A modulation by β-adrenoceptors stimulation of transient outward current recorded under Ca$^{2+}$-free conditions in canine Purkinje cells has been reported recently. We here offer another mechanism for a modulation of transient outward current and, thus, action potential configuration by β-adrenoceptor stimulation in cardiac tissues.

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References


32. Beindorf K, Markwardt F, Nilius B: Two types of transient outward currents in cardiac ventricular cells of mice. Pflugers Arch 1987;409:641-643


34. Bogaert PP, Snyders DJ: Effects of 4-aminopyridine on inward rectifying and pacemaker currents of cardiac Purkinje fibres. Pflugers Arch 1982;339:230-238


37. Morad M, Cleemann L: Role of Ca2+ tolerance, inward-rectifying and pacemaker currents of cardiac Purkinje fibres. J Gen Physiol 1984;73:139-157


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