Existence of a transient outward \( K^+ \) current in guinea pig cardiac myocytes

GUI-RONG LI,1,2 BAOFENG YANG,1 HAIYING SUN,1 AND CLIVE M. BAUMGARTEN2

1Department of Medicine, Montreal Heart Institute and University of Montreal, Montreal, Quebec, Canada H3C 3J7; and 2Department of Physiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298

Received 18 August 1999; accepted in final form 7 January 2000

Li, Gui-Rong, Baofeng Yang, Haiying Sun, and Clive M. Baumgarten. Existence of a transient outward \( K^+ \) current in guinea pig cardiac myocytes. *Am J Physiol Heart Circ Physiol* 279: H130–H138, 2000.—A novel transient outward \( K^+ \) current that exhibits inward-going rectification \( (I_{to,ir}) \) was identified in guinea pig atrial and ventricular myocytes. \( I_{to,ir} \) was insensitive to 4-aminopyridine (4-AP) but was blocked by 200 \( \mu \text{mol/l} \) \( \text{Ba}^{2+} \) or removal of external \( K^+ \). The zero current potential shifted 51–53 mV/decade change in external \( K^+ \). \( I_{to,ir} \) density was twofold greater in ventricular than in atrial myocytes, and biexponential inactivation occurs in both types of myocytes. At \(-20\) mV, the fast inactivation time constants were 7.7 ± 1.8 and 6.1 ± 1.2 ms and the slow inactivation time constants were 85.1 ± 14.8 and 77.3 ± 10.4 ms in ventricular and atrial cells, respectively. The midpoints for steady-state inactivation were \(-56.4 ± 0.3\) and \(-51.6 ± 0.4\) mV, and recovery from inactivation was rapid near the resting potential (time constants = 7.9 ± 1.9 and 8.8 ± 2.1 ms, respectively). \( I_{to,ir} \) was detected in \( \text{Na}^+ \)-containing and \( \text{Na}^+ \)-free solutions and was not blocked by 20 \( \text{mmol/l} \) saxitoxin. Action potential clamp revealed that \( I_{to,ir} \) contributed an outward current that activated rapidly on depolarization and inactivated by early phase 2 in both tissues. Although it is well known that 4-AP-sensitive transient outward current is absent in guinea pig, this \( \text{Ba}^{2+} \)-sensitive and 4-AP-insensitive \( K^+ \) current has been overlooked.

transient outward potassium current; whole cell patch clamp; repolarization; excitability; action potential configuration

**DEPOLARIZATION-ACTIVATED OUTWARD currents have been distinguished in the myocardium on the basis of their time and voltage dependence and their pharmacological sensitivity (1).** Delayed rectifier (30) and transient outward currents \( (I_{to}) \) (9–11) are observed. Rapid and slow delayed rectifier \( K^+ \) currents \( (I_{Kr} \) and \( I_{Ks} \), respectively) are found in guinea pig (31, 32), dog (37), and human (22) cardiac myocytes. Ultrarapid delayed rectifier \( K^+ \) currents \( (I_{Kr,ir} \) or \( I_{Ks,ir} \) also are present in rat (4), dog (38), and human (36) atrial cells. \( I_{to} \) was identified with whole cell voltage-clamp techniques in cardiac cells from a wide range of species, including the rat (8, 17), rabbit (10), dog (24, 35, 37), elephant seal (27), ferret (5), and human (2, 21). Kenyon and Gibbons (18) reported that 4-aminopyri-

dine \( (4-\text{AP}) \) decreased \( I_{to} \) in sheep cardiac Purkinje fibers. Subsequently, 4-AP has been used as a selective inhibitor of transient outward \( K^+ \) current, and 4-AP-sensitive and 4-AP-resistant components of \( I_{to} \) have been reported in sheep Purkinje fibers (7) and rabbit (40, 41) and dog (35, 37) cardiac myocytes. The 4-AP-sensitive and 4-AP-resistant components often are termed \( I_{to,1} \) and \( I_{to,2} \), respectively, after Tseng and Hoffman (35), and \( I_{to,1} \) is a \( \text{Ca}^{2+} \)-activated transient outward \( \text{Cl}^- \) current \( (I_{Cl,ca}^+) \) (37, 40, 41). \( I_{to,1} \) and \( I_{to,2} \) play important roles in the repolarization of the cardiac action potential (1, 2, 9, 10, 17, 21, 37).

We recently described inward-going rectifying \( I_{to} \) \( (I_{to,ir}) \) in dog ventricular cells (23). \( I_{to,ir} \) is another 4-AP-insensitive transient outward current that is carried by \( K^+ \), exhibits inward-going rectification, and is blocked by low concentrations of \( \text{Ba}^{2+} \). The biophysical and pharmacological properties of \( I_{to,ir} \) are distinct from those of the classic \( I_{to,1} \) and \( I_{to,2} \) (37, 35, 40, 41). To document whether \( I_{to,ir} \) is present in other species and in atria, we studied guinea pig cardiac myocytes. Classic \( I_{to} \) is not present in the guinea pig under physiological conditions (8, 12, 13, 15), and \( I_{Kr} \) and \( I_{Ks} \) are thought to be responsible for repolarization of the action potential in the absence of \( I_{to} \) (39). We found that \( I_{to,ir} \) also is present in guinea pig atrial and ventricular cells. \( I_{to,ir} \) is likely to contribute to repolarization and may be more broadly distributed than previously recognized.

**MATERIALS AND METHODS**

**Cell preparation.** Single atrial and ventricular myocytes were isolated using a modification of a procedure described previously (20). Briefly, guinea pigs were killed by cervical dislocation, and the heart was quickly removed and placed in oxygenated Tyrode solution. The heart was mounted on a Langendorff column and initially perfused with oxygenated Tyrode solution (37°C, ~5 min) to wash out the blood. After perfusion with \( \text{Ca}^{2+} \)-free Tyrode solution for 5–8 min, the heart was enzymatically digested with a solution containing 0.06% collagenase (type II, Worthington Biochemical) and 0.1% BSA (Sigma Chemical) until it softened and shed cells.

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The left atrium and/or ventricle were excised and placed in a high-K⁺ storage medium (see Solutions). Small aliquots of the solution containing isolated cells were placed in an open perfusion chamber (1 ml) mounted on the stage of an inverted microscope. Experiments were conducted at room temperature (22°C), except as noted for action potential recordings. Only quiescent rod-shaped cells showing clear cross striations were used.

Solutions. Action potentials were recorded from myocytes bathed in Tyrode solution, which contained (in mmol/l) 136 NaCl, 5.4 KCl, 1 MgCl₂, 1.8 CaCl₂, 0.33 NaH₂PO₄, 10 glucose, and 10 HEPES; pH was adjusted to 7.4 with NaOH. Voltage-clamp studies were conducted in K⁺-containing and K⁺-free bathing solutions designed to isolate currents of interest. NaCl in Tyrode solution was replaced by equimolar choline chloride to block the Na⁺ current, except where indicated (see Figs. 8 and 9). Atropine (1 μmol/l) was added to block possible muscarinic receptor-mediated current activated by choline. In addition, 200 μmol/l Cd²⁺ was used to block Ca²⁺ currents, 5 μmol/l E-4031 to block Iₖᵣ, and 10 μmol/l 293B to block Iₖₛ (3). The K⁺-free version was made by also omitting KCl. The pipette solution contained (in mmol/l) 20 KCl, 110 potassium aspartate, 1 MgCl₂, 10 HEPES, 5 EGTA, 0.1 GTP, and 5 Mg₂ATP; pH was adjusted to 7.2 with KOH. The high-K⁺ storage medium contained (in mmol/l) 20 KCl, 10 KH₂PO₄, 70 potassium glutamate, 20 taurine, 10 β-hydroxybutyric acid, 25 glucose, 20 mannitol, and 5 EGTA and 0.1% albumin; pH was adjusted to 7.2 with KOH.

Electrophysiology and data analysis. Membrane currents and action potentials were recorded with the tight-seal whole cell patch-clamp technique with use of an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Data were acquired and command pulses were generated by analog-to-digital and digital-to-analog converters (12-bit) controlled by pClamp 6 software (Axon). Recordings were low-pass filtered at 2 kHz and stored on the hard disk of an IBM-compatible computer.

Borosilicate glass (1.0 mm OD) pipettes were prepared (model P87, Sutter Instruments) to give a resistance of 2–3 MΩ when the pipettes were filled with pipette solution. After the pipette was zeroed in bath solution, a gigaseal was obtained, and the cell membrane was ruptured by gentle suction to establish the whole cell configuration. The liquid junctional potentials were determined by immersing the pipette into the bath filled with pipette solution, zeroing the voltage reading, and switching to the bath solution used for voltage clamp. The average liquid junction potential was 10.6 ± 0.3 mV (n = 14 pipettes) and was not corrected in the experiments, except as noted.

The membrane capacitance was determined by integrating the capacitive response to a 5-mV hyperpolarizing pulse from a holding potential of −60 mV and dividing by the voltage drop. Membrane capacitance was 105 ± 8 and 44 ± 3 pF in ventricular (n = 25) and atrial (n = 21) cells, respectively. Series resistance was electronically compensated.

Nonlinear curve-fitting techniques (Clampfit in pClamp and Sigmaplot, SPSS, Chicago, IL) based on the Marquardt procedure were used to fit equations to experimental data. Paired and unpaired Student’s t-tests were used as appropriate to evaluate the statistical significance of differences between two group means. ANOVA was used for multiple groups. P < 0.05 was considered to indicate significance. Group data are expressed as means ± SE.

RESULTS

Transient outward current in guinea pig ventricular and atrial myocytes. To study whether Iₒ is present in guinea pig ventricular cells, membrane currents were elicited by 200-ms depolarizing voltage steps from a

![Graph showing transient outward currents in a ventricular myocyte in Na⁺-free bathing media containing 5 μmol/l E-4031 to block rapid delayed rectifier K⁺ current (Iₖᵣ), 10 μmol/l 293B to block slow delayed rectifier K⁺ current (Iₖₛ), 200 μmol/l Cd²⁺ to block Ca²⁺ current (Iₐᵥ), and 1 μmol/l atropine to block any muscarinic receptor-mediated current activated by choline. A: currents elicited by 200-ms depolarizations from −80 mV to test potentials between −70 and 0 mV. Some of the negative-going capacity spike was omitted to optimize presentation of the currents (voltage-clamp protocol shown in inset of B; calibration in B applies to A − C). B: omission of extracellular K⁺ (Kₒ) abolished the transient outward current and decreased the holding current. Only capacitive transients and a small leak current remained. C: K⁺-sensitive currents obtained by subtracting currents before and after removal of Kₒ. D: crossover of inactivating current traces and inward rectification of transient current (same currents as in C) at higher gain and time resolution. Arrows, zero current level.](http://node210361.iaf.unicamp.br/images/3e2f93232a41bfb12631b2e75f4f6b33/figure1.png)
holding potential of −80 mV to test potentials between −70 and 0 mV in 10-mV increments; steps were applied every 10 s. Figure 1A shows that depolarization elicited a transient outward current at −60 mV and more positive potentials. This current was sensitive to external K⁺ (Kₒ) and was fully abolished by the removal of Kₒ (Fig. 1B). Only membrane capacitive transients and small leak currents were detected in K⁺-free bathing media. The family of Kₒ-sensitive currents, which were obtained by subtracting currents before and after removal of Kₒ, are shown in Fig. 1C and at higher gain in Fig. 1D. The original (Fig. 1A) and the Kₒ-sensitive currents undergo voltage-dependent inactivation. Moreover, the current inactivation appears to be more rapid at more positive potentials, producing a characteristic crossover of the current traces. Finally, the magnitude of the transient current appears to saturate at positive voltages, indicating inward-going rectification (see Fig. 4B). Similar results were obtained in 21 of 27 ventricular myocytes. The characteristics of the transient outward current in guinea pig ventricular myocytes are similar to those of Iₒ(tot) described previously in dog ventricular cells (23). All the K⁺-sensitive current is not Iₒ(tot); however, the inward rectifier, Kcl, should not be affected by the cocktail of ion channel blockers employed, and therefore the steady-state K⁺-sensitive current is expected, at least in part, to represent I-Kcl.

Iₒ(tot) in dog ventricle is inhibited by low concentrations of Ba²⁺ (23). Consistent with this result, the transient outward current in guinea pig ventricular myocytes also was sensitive to Ba²⁺ and was fully blocked by 200 μmol/l Ba²⁺ (n = 10 cells; data not shown).

To examine whether Iₒ(tot) also is expressed in guinea pig atrial cells, we used the same protocol used in ventricular cells. Figure 2A displays a family of transient outward currents elicited in an atrial myocyte. As in the ventricle, the current was sensitive to K⁺ and abolished by omission of Kₒ (Fig. 2B). The Kₒ-sensitive currents are displayed in Fig. 2C and at higher gain in Fig. 2D. As was the case for Kₒ-sensitive transient outward current in ventricular cells, the transient outward current in atrial cells also undergoes voltage-dependent inactivation and exhibits inward rectification. Similar results were obtained in 17 of 25 atrial cells, indicating that Iₒ(tot) is present throughout the working myocardium of the guinea pig.

Effect of 4-AP on transient outward current. It is well known that Iₒ(tot) is sensitive to 4-AP. To test whether the K⁺-sensitive transient outward current in the guinea pig is sensitive to 4-AP, membrane currents were measured in the absence and presence of 5 mmol/l 4-AP. Figure 3 displays the Kₒ-sensitive difference current before (A) and after (B) the application of 4-AP in a ventricular myocyte. A comparison of the traces indicates that the transient outward current was essentially unaffected by 4-AP. This is more clearly shown in Fig. 3C, in which the 4-AP-sensitive difference currents are plotted. For a 200-ms voltage step from −80 to −20 mV, for example, Iₒ(tot) measured as the peak minus the quasi-steady-state K⁺-sensitive current was 4.1 ± 0.5 pA/pF in control and 4.2 ± 0.6 pA/pF after the application of 5 mmol/l 4-AP (n = 5, P = NS). Similarly, 4-AP did not affect the transient outward current in the four atrial cells tested. 4-AP resistance is a characteristic of the K⁺ current Iₒ(tot) in canine myocytes (23) but is distinct from the pharmacology of the classic I-Kcl.

Current-voltage relationship of Iₒ(tot). The current-voltage (I-V) relationship of Iₒ(tot) was assessed in atrial and ventricular myocytes, as illustrated in Fig. 4A with a recording from a ventricular cell. Iₒ(tot) was taken as the transient component of K⁺-sensitive membrane current (peak minus quasi-steady-state current at 200 ms), and I-SSt, the steady-state current, was measured from the zero current level, as was the holding current (I-hold). Currents were expressed as current densities normalized by membrane capacitance to control for variation in cell size and allow more direct comparison results from atrial and ventricular cells.
atrial cells, close to the calculated $E_K$ in ventricular cells and a 51 mV in atrial cells. On correction for the 10.6-mV liquid junction potential, the zero current potentials were 74.6 mV in ventricular cells and 79.6 mV in atrial cells. The transient component was best fit by a biexponential function with time constants of 7.8 and 85.2 ms. The voltage dependence of the time constants in atrial ($n = 12$) and ventricular ($n = 16$) cells is shown in Fig. 5B. The fast inactivation time constant ($\tau_f$) was voltage dependent ($P < 0.01$) in both cell types, and rate of rapid inactivation increased on depolarization. The voltage dependence of $\tau_r$, combined with the depolarization-induced increase in the amplitude of $I_{to.ir}$ (Fig. 4B), explains the crossover of the current traces when a family of currents is recorded (Figs. 1 and 2). In contrast to $\tau_f$, the slow inactivation time constant ($\tau_s$) was not significantly affected by voltage. Both $\tau_f$ and $\tau_s$ were slightly faster in atrial than in ventricular cells, but the difference was not statisti-

Figure 4B shows the $I-V$ relationships for the $K_{to.ir}$-sensitive currents in ventricular ($n = 19$) and atrial ($n = 17$) cells. The density of $I_{to.ir}$ increased on depolarization, reaching a plateau at potentials positive to $-20$ mV. The $I-V$ curve for $I_{SS}$ displayed even stronger inward rectification; $I_{SS}$ decreased at potentials positive to $-30$ mV. This behavior is similar to that of $I_{Kt}$ and suggests that $I_{SS}$ is largely $I_{Kt}$. The possibility that the channels responsible for $I_{to.ir}$ do not fully inactivate and contribute to $I_{SS}$ is considered below. Although the shapes of the $I_{to.ir}$ and $I_{SS}$ $I-V$ curves were similar in atrial and ventricular cells, the current densities were significantly greater in ventricular cells.

$I_{to.ir}$ is most likely a $K^+$ current on the basis of the fact that the current was outward at all potentials positive to $K^+$ equilibrium potential ($E_K$) and that it disappeared on removal of $K^+$. When the $I-V$ curves were extrapolated to the voltage axis, the zero current potential for $I_{to.ir}$ was $-69$ mV in ventricular cells and $-64$ mV in atrial cells. On correction for the 10.6-mV liquid junction potential, the zero current potentials were $-79.6$ mV in ventricular cells and $-74.6$ mV in atrial cells, close to the calculated $E_K$ of $-81.2$ mV. Moreover, a 10-fold change in $K^+$ led to a $53 \pm 3$ mV/decade ($n = 6$) shift in the zero current potential in ventricular cells and a $51 \pm 2$ mV/decade ($n = 5$) shift in atrial cells, in agreement with the prediction of the Nernst equation. Taken together, these data suggest that $I_{to.ir}$ is carried by $K^+$ in guinea pig myocytes as in dog ventricular cells (23).

**Kinetics of $I_{to.ir}$**. The kinetics of $I_{to.ir}$ inactivation were determined by analyzing the $K_{to.ir}$-sensitive current. Figure 5A shows the difference current obtained for a 200-ms voltage step from $-80$ to $-20$ mV in a ventricular cell. The transient component was best fit by a biexponential function with time constants of 7.8 and 85.2 ms. The voltage dependence of the time constants in atrial ($n = 12$) and ventricular ($n = 16$) cells is shown in Fig. 5B. The fast inactivation time constant ($\tau_f$) was voltage dependent ($P < 0.01$) in both cell types, and rate of rapid inactivation increased on depolarization. The voltage dependence of $\tau_r$, combined with the depolarization-induced increase in the amplitude of $I_{to.ir}$ (Fig. 4B), explains the crossover of the current traces when a family of currents is recorded (Figs. 1 and 2). In contrast to $\tau_f$, the slow inactivation time constant ($\tau_s$) was not significantly affected by voltage. Both $\tau_f$ and $\tau_s$ were slightly faster in atrial than in ventricular cells, but the difference was not statisti-
to then currents were recorded during 200-ms test pulses.

The voltage dependence of inactivation of conductance and fits to Boltzmann distributions. The half-inactivation voltage \( V_{0.5} \) was more negative in atrial cells, \( -51.6 \pm 0.4 \) mV \((n = 10, P \leq 0.05)\), than in ventricular cells, \( -36.4 \pm 0.3 \) mV \((n = 12, P < 0.05)\), whereas the slope factors, 13.4 \pm 0.3 and 12.1 \pm 0.3 mV, respectively, were not distinguishable. The different \( V_{0.5} \) values may reflect intrinsic differences between the underlying channels in atrial and ventricular myocytes.

The time dependence of recovery from inactivation of \( K^+ \)-sensitive \( I_{\text{to.ir}} \), studied with the paired-pulse protocol illustrated in Fig. 7A. Identical 200-ms pulses \((P1 \text{ and } P2)\) from \(-80 \) to \(-20 \) mV were delivered every 10 s with varying intervals between \( P1 \) and \( P2 \). The current during \( P2 (I_2) \) relative to the current during \( P1 (I_1) \) is plotted as a function of the \( P1-P2 \) recovery interval in Fig. 7B. The recovery of \( I_{\text{to.ir}} \) was well fit by monoexponential functions with time constants of 8.8 \pm 2.1 and 7.9 \pm 1.9 ms in atrial \((n = 11)\) and ventricular \((n = 10)\) cells, respectively \((P = NS)\). Thus recovery from inactivation was rapid at diastolic potentials.

![Graph A](Image 343x169 to 545x479)  
**Fig. 5.** Inactivation of \( I_{\text{to.ir}} \) is voltage dependent. A: inactivation of \( K^+ \)-sensitive \( I_{\text{to.ir}} \) in ventricular cell during a 200-ms depolarization from \(-80 \) mV to a test potential of \(-20 \) mV was fitted by a biexponential function with time constants of 7.8 and 85.2 ms. Inset: voltage-clamp protocol. B: rapid and slow time constants \((\tau_1 \text{ and } \tau_2)\) of \( I_{\text{to.ir}} \) inactivation obtained as illustrated in A for atrial \((n = 12)\) and ventricular \((n = 16)\) cells. Both \( \tau_1 \) and \( \tau_2 \) were slightly smaller in atrial than in ventricular cells. Voltage dependence of \( \tau_1 \) was significant \((P < 0.01)\), and this component of inactivation became faster at more positive potentials in atrial and ventricular cells. In contrast, \( \tau_2 \) was not significantly affected by voltage. C: fractional amplitude of current decaying in the fast component \((A_1/A_{\text{total}})\) plotted as a function of voltage. The fraction of current decaying rapidly significantly increased at more positive potentials \((P < 0.01)\) in both types of myocytes. \( K^+ \)-sensitive current was obtained as described in legends of Figs. 1C and 2C. Values are means \( \pm \) SE.

physically significant. At a test potential of \(-20 \) mV, the fast component accounted for 75% of inactivation in atrial and ventricular myocytes. The voltage dependence of the fraction of current, \( A_1 \), that inactivates with \( \tau_1 \) is presented in Fig. 5C. \( A_1 \) significantly increased on depolarization as \( \tau_1 \) became faster.

The voltage dependence of steady-state inactivation of the conductance underlying \( K^+ \)-sensitive \( I_{\text{to.ir}} \), was determined as illustrated in Fig. 6A. Prepulses, 300 ms or, in some cases, 1,000 ms in duration, were applied at conditioning potentials between \(-110 \) and 0 mV, and then currents were recorded during 200-ms test pulses to \(+10 \) mV before and after removal of \( K^+ \). The conductance inactivation variable was calculated by dividing \( I_{\text{to.ir}} \) at a given prepulse potential by the maximum \( I_{\text{to.ir}} \) and the results with 300- and 1,000-ms prepulses were indistinguishable. Figure 6B shows the voltage dependence of inactivation of conductance and fits to Boltzmann distributions. The half-inactivation voltage \( V_{0.5} \) was more negative in atrial cells, \(-51.6 \pm 0.4 \) mV \((n = 10)\), than in ventricular cells, \(-36.4 \pm 0.3 \) mV \((n = 12, P < 0.05)\), whereas the slope factors, 13.4 \pm 0.3 and 12.1 \pm 0.3 mV, respectively, were not distinguishable. The different \( V_{0.5} \) values may reflect intrinsic differences between the underlying channels in atrial and ventricular myocytes.

![Graph B](Image 78x423 to 281x725)  
**Fig. 6.** Voltage dependence of steady-state inactivation of \( I_{\text{to.ir}} \). A: protocol and representative \( K^+ \)-sensitive currents used to assess the voltage dependence of steady-state inactivation of \( I_{\text{to.ir}} \) in a ventricular cell. Inset: voltage-clamp protocol. B: currents \((i)\) recorded at \(+10 \) mV after 300-ms steps to the condition potential \((CP)\) were normalized by the maximum current obtained \((I_{\text{max}})\). Data were fit to the Boltzmann relationship: 

\[
I/I_{\text{max}} = \left[ 1 + \exp \left( \frac{V-V_{0.5}}{\Sigma} \right) \right]^{-1},
\]

where \( V \) is the conditioning potential, \( V_{0.5} \) is the potential for half-maximal inactivation, and \( \Sigma \) is the slope factor. The midpoint of the inactivation curve was more negative in atrial \((n = 10)\) than in ventricular cells \((n = 12, P < 0.05)\). \( K^+ \)-sensitive current was obtained as described in legends of Figs. 1C and 2C. Values are means \( \pm \) SE.
Effect of intracellular Mg$^{2+}$ and spermine on $I_{to,ir}$. Intracellular Mg$^{2+}$ (Mg$^{2+}_{i}$) and polyamines, such as spermine, are responsible for inward rectification of native $I_{K1}$ and cloned channels that exhibit inward rectification (16, 26, 29). A decrease in Mg$^{2+}_{i}$ reduces the extent of inward rectification and thereby increases the amplitude of outward current at positive potentials, whereas addition of polyamines has the opposite effect (29). To study whether Mg$^{2+}$ or spermine modulates $I_{to,ir}$, we omitted Mg$^{2+}$ or included 10 μM spermine in the pipette solution and observed whether $I_{to,ir}$ changed during cell dialysis. For a voltage step from −80 to −20 mV, $I_{to,ir}$ recorded immediately after membrane rupture was 4.5 ± 0.6 and 2.1 ± 0.3 pA/pF in ventricular ($n = 4$) and atrial ($n = 4$) myocytes, respectively, and 4.6 ± 0.5 and 2.3 ± 0.4 pA/pF after 10 min of dialysis of Mg$^{2+}$-free pipette solution. In five ventricular and four atrial cells, the inclusion of 10 μM spermine in pipette solution also did not affect $I_{to,ir}$. These results were similar to those previously obtained in dog ventricular myocytes (23).

$I_{to,ir}$ in the presence of external Na$^{+}$. As described above, $I_{to,ir}$ was determined in the absence of external Na$^{+}$, a nonphysiological condition. Because Inoue and Imanaga (15) also described a transient outward K$^{+}$ current in guinea pig ventricular myocytes under a nonphysiological condition, removing external Ca$^{2+}$, it is important to clarify whether $I_{to,ir}$ can be observed in the presence of external Na$^{+}$.

Recordings of $I_{to,ir}$ in a ventricular myocyte in the presence of Na$^{+}$ are shown in Fig. 8. Figure 8A shows a transient $I_{to,ir}$ at −60 and −50 mV, but $I_{to,ir}$ was obscured by the Na$^{+}$ spike at −40 and −30 mV. Figure 8B displays $I_{to,ir}$ after Na$^{+}$ channels were blocked with 20 nmol/l saxitoxin (STX); $I_{to,ir}$ was observed over the entire voltage range examined. Similar results were obtained in a total of four cells. $I_{to,ir}$ at −50 mV was 2.3 ± 0.2 pA/pF in control and 2.4 ± 0.3 pA/pF after STX ($P = NS$). The results indicate that activation of $I_{to,ir}$ does not require removal of external Na$^{+}$ and that the current is not K$^{+}$ efflux through Na$^{+}$ channels.

Contribution of $I_{to,ir}$ to the action potential. To gain insight into $I_{to,ir}$ during the action potential and its contribution to repolarization, we used the action potential-clamp technique to measure $I_{to,ir}$. Representative action potentials were recorded from atrial and ventricular cells in normal Tyrode solution at 36°C under current-clamp conditions (stimulation rate 0.5 Hz). These waveforms were latter used as the voltage-clamp command voltage to elicit membrane currents under the conditions previously used to record $I_{to,ir}$ with square voltage pulses.

Figure 9 illustrates an experiment on a ventricular cell. Figure 9A shows the action potential waveform, and Fig. 9B displays the currents recorded in response to the action potential clamp before and after the removal of K$^{+}$. The K$^{+}$-sensitive current, obtained by subtraction, is shown in Fig. 9C. Two transient compo-
DISCUSSION

The present study demonstrated that $I_{\text{to,ir}}$, a transient outward $K^+$ current that undergoes inward rectification, is present in guinea pig atrial and ventricular myocytes. $I_{\text{to,ir}}$ inactivated and recovered from inactivation very quickly, was fully suppressed by the removal of $K^+_o$ or by $Ba^{2+}$ (200 µmol/l), and was insensitive to 4-AP (5 mmol/l). The current is most likely a $K^+$ current, because it was outward over a wide range of voltage positive to $E_K$, and the zero current potential shifted 51–53 mV/decade in response to altered $K^+_o$.

$I_{\text{to,ir}}$ is not $K^+$ efflux via $Na^+$ channels, because it was not blocked by CTX. Action potential-clamp experiments showed that $I_{\text{to,ir}}$ generated a significant outward current at the time of phase 1 repolarization. Although the current density was less in atrial than in ventricular myocytes, $I_{\text{to,ir}}$ is expected to affect the action potential configuration of both types of cells. $I_{\text{to,ir}}$ also is present in canine (23) as well as in human and rabbit (unpublished observations) ventricular myocytes. This indicates that $I_{\text{to,ir}}$ is widespread in mammalian hearts as well as in atria and ventricles.

$I_{\text{to,ir}}$ is a novel $K^+$ current. The biophysical and pharmacological properties of $I_{\text{to,ir}}$ were distinct from those of the two types of $I_K$ that have been well studied in mammalian cardiac myocytes (35, 37, 40, 41). $I_{\text{to1}}$ is a 4-AP-sensitive $K^+$ current, and $I_{\text{to2}}$ is $Ca^{2+}$-activated $Cl^-$ current. The threshold potential for $I_{\text{to1}}$ activation is –20 or –30 mV, and its $I-V$ relationship is bell shaped (2, 21, 23, 37). $I_{\text{to2}}$ activates near –30 mV, corresponding to the activation of $I_{Ca}$ and the intracellular $Ca^{2+}$ transient, and its $I-V$ relationship is bell shaped (37, 40, 41). In contrast, $I_{\text{to,ir}}$ was apparent at –60 mV in guinea pig cardiac myocytes, and its $I-V$ relationship was neither linear nor bell shaped. Moreover, $I_{\text{to,ir}}$ was not sensitive to the $I_{\text{to1}}$ blocker 4-AP and was present under conditions (5 mmol/l EGTA in pipette solution and 200 µmol/l Cd$^{2+}$ in superfusate) that fully suppress $I_{\text{to2}}$. In view of these biophysical and pharmacological differences, it is reasonable to suggest that an ion channel distinct from those underlying $I_{\text{to1}}$ and $I_{\text{to2}}$ is responsible for $I_{\text{to,ir}}$.

Martin et al. (25) reported a 3,4-diaminopyridine-insensitive, $Ca^{2+}$-independent transient outward $K^+$ current in feline cardiac ventricular myocytes. This current was not initially observed on establishment of whole cell conditions; its activation depended on the duration of dialysis, and therefore, it was named the patch-duration-dependent $K^+$ current, $I_{Kr,(PDD)}$. $I_{Kr,(PDD)}$ shares some properties with $I_{\text{to,ir}}$, including 4-AP insensitivity, $Ba^{2+}$ sensitivity, and voltage-dependent inactivation. In contrast to $I_{Kr,(PDD)}$, $I_{\text{to,ir}}$ was present immediately on membrane rupture, and its inwardly rectifying $I-V$ relationship was clearly different from the $I-V$ relationship for $I_{Kr,(PDD)}$, which is linear between –40 and +60 mV.

A transient outward $K^+$ current previously was described in the guinea pig only after removal of external $Ca^{2+}$, a nonphysiological condition (15). This and our experiments under $Na^+$-free conditions raised the possibility that $I_{\text{to,ir}}$ was only present under conditions not relevant to normal function. However, Fig. 8 shows that $I_{\text{to,ir}}$ can be observed under physiological conditions in the presence of $Ca^{2+}$ and $Na^+$, and it does not reflect $K^+$ efflux through $Na^+$ channels.

Although $I_{Kr}$ exhibits inward rectification at positive potentials (31, 32), it is unlikely that $I_{Kr}$ contributed to the present observations. In contrast to $I_{\text{to,ir}}, I_{Kr}$ has a “bell-shaped” $I-V$ relationship (31, 32). Furthermore, $I_{\text{to,ir}}$ was studied in the presence of the $I_{Kr}$ channel blocker E-4031 (5 µmol/l). E-4031 is an highly effective $I_{Kr}$ blocker (31, 32), and the block of $I_{Kr}$ is voltage and
time independent (6). Therefore, it is unlikely that \( I_{\text{to,ir}} \) reflects time- and voltage-dependent “unblock” of \( I_{Kr} \).

Is \( I_{\text{to,ir}} \) a component of \( I_{K1} \)? Several characteristics of \( I_{\text{to,ir}} \) in the guinea pig and dog are similar to characteristics of \( I_{K1} \): both currents are blocked by low concentrations of \( \text{Ba}^{2+} \) and by removal of \( K^+ \), and both display inward-going rectification. Therefore, it is important to consider whether \( I_{\text{to,ir}} \) could be a component of \( I_{K1} \). One might imagine, for example, that the decay of \( I_{\text{to,ir}} \) could be due to the voltage-dependent block of \( I_{K1} \) by \( \text{Mg}^{2+} \) that gives rise to rectification (16, 26). This idea seems unlikely. At only 500 \( \mu \text{mol/l} \) \( \text{Mg}^{2+} \), a concentration lower than that present here, \( I_{K1} \) rectification on depolarization appears to be instantaneous in guinea pig myocytes (16). Furthermore, omitting \( \text{Mg}^{2+} \) or adding spermine to the pipette solution did not modulate \( I_{\text{to,ir}} \), whereas outward \( I_{K1} \) should have been increased with \( \text{Mg}^{2+} \)-free pipette solution (16, 26) and depressed by spermine (29). A selective blocker that distinguishes between \( I_{K1} \) and \( I_{\text{to,ir}} \) would further clarify this question, but such an agent has not been identified.

In the absence of a blocker that distinguishes between \( I_{K1} \) and \( I_{\text{to,ir}} \), we empirically defined \( I_{\text{to,ir}} \) as the transient component of \( K^+ \)-sensitive current remaining after other outward \( K^+ \) currents were blocked. However, we cannot rigorously exclude the possibility that a rapidly inactivating component of \( I_{K1} \) contaminated the currents measured as \( I_{\text{to,ir}} \). In theory, this might have affected the shape of the \( I_{\text{to,ir}} \) \( I-V \) curve (Fig. 4B) and the analyses of its kinetics. The presence of \( I_{K1} \) also made it difficult to measure the reversal potential of \( I_{\text{to,ir}} \) with use of deactivating tail currents. We therefore estimated the reversal potential as the zero current potential for \( I_{\text{to,ir}} \) by extrapolating the \( I-V \) curves to the voltage axis. This approach can be criticized because the voltage dependence of activation might influence the zero current potential. Nevertheless, other factors strongly support the conclusion that \( I_{\text{to,ir}} \) is largely a \( K^+ \) current. Not only was \( I_{\text{to,ir}} \) absent in \( K^+ \)-free bathing media, but also the zero current potential shifts 51–53 mV/decade change in \( K^+ \). Furthermore, \( I_{\text{to,ir}} \) is outward over a wide range of potentials from near \( E_K \) to +40 mV. No other ion can readily explain the direction of current flow.

**Physiological implications.** In view of its rapid activation and inactivation, \( I_{\text{to,ir}} \) should have an important contribution to \( K^+ \) efflux during phase 1, especially in guinea pig myocytes in which 4-AP-sensitive \( I_{Na} \) is absent (8, 12). Previously, repolarization in the guinea pig largely has been attributed to the delayed rectifier \( K^+ \) currents (13, 39), a conclusion that now should be revisited. The rapid recovery of \( I_{\text{to,ir}} \) from inactivation suggests that its contribution to the action potential should be independent of heart rate. The activation of \( I_{\text{to,ir}} \) (Fig. 4B) and steady-state inactivation curve (Fig. 6B) overlapped between −60 and 0 mV. This implies that a fraction of \( I_{\text{to,ir}} \), up to 10 to 20% of peak current, does not inactivate over this voltage range. Because the plateau of the guinea pig action potential is positive to 0 mV, the potential at which rapid and complete inactivation occurs, noninactivating \( I_{\text{to,ir}} \) should have little direct effect during the plateau or on action potential duration. On the other hand, a noninactivating, \( \text{Ba}^{2+} \)-sensitive \( K^+ \) current arising from \( I_{\text{to,ir}} \) might easily be mistaken for \( I_{K1} \) in voltage-clamp experiments, and we suspect that the noninactivating component of \( I_{\text{to,ir}} \) contributed to the steady-state \( K^+ \)-sensitive current (Fig. 4B, \( I_{SS} \)).

Cell excitability has generally been associated with the ability of inward currents to generate an action potential upstroke. More recent studies suggest that \( I_{K1} \) may also play a role in the excitability of cardiac cells (14, 33) by stabilizing the resting potential (29). Because significant outward currents carried by \( I_{\text{to,ir}} \) can be elicited by depolarization at very negative potentials over a time course comparable to \( I_{Na} \), \( I_{\text{to,ir}} \) also may play a role in excitability. The density of \( I_{\text{to,ir}} \) was lower and the voltage-dependent inactivation (\( V_{0.5} \)) of \( I_{\text{to,ir}} \) was more negative in atrial than in ventricular cells. Whether these differences contribute to electrophysiological variation between atrial and ventricular cells remains to be studied.

**Molecular identity.** The molecular identity of \( I_{\text{to,ir}} \) is unknown. In contrast, much has been learned about the proteins responsible for \( I_{to1} \). The preponderance of the evidence links \( I_{to1} \) with \( K^+ \) in humans and perhaps the rat, with \( K^+ \) in the rabbit and perhaps the ferret. In addition, \( K^+ \) and \( K^+ \) may not be uniformly distributed across the ventricular wall, giving rise to fast and slow components of \( I_{to1} \) (for review see Refs. 28 and 34). We are unaware of studies demonstrating the expression of these genes in guinea pig myocytes. Several other cloned \( K^+ \) channels also generate transient outward currents on expression. Some properties of \( I_{\text{to,ir}} \) are similar to those of the outward current carried by the recently cloned human TWIK-1 \( K^+ \) channel, which is expressed predominantly in the heart and brain (19). Currents corresponding to the behavior of TWIK-1 have not been identified in native cells. Expression of TWIK-1 in oocytes gives rise to a fast activating and rapidly decaying transient outward current that displays inward rectification over a voltage range similar to \( I_{\text{to,ir}} \). TWIK-1 current is blocked by \( \text{Ba}^{2+} \) (\( IC_{50} = 100 \mu \text{mol/l} \)) and insensitive to 4-AP (19), properties also shared by \( I_{\text{to,ir}} \). On the other hand, TWIK-1 produces a noninactivating current over a wide range of negative potentials, whereas \( I_{\text{to,ir}} \) activated positive to −70 mV. This apparent difference may not be real. Because \( I_{\text{to,ir}} \) was defined as the transient component of current, any noninactivating component was included in \( I_{SS} \).

In conclusion, a novel transient outward current, \( I_{\text{to,ir}} \), is present in guinea pig atrial and ventricular myocytes as well as in dog myocytes. \( I_{\text{to,ir}} \) is likely to contribute to phase 1 repolarization. Because it is insensitive to 4-AP and sensitive to low concentrations of \( \text{Ba}^{2+} \), \( I_{\text{to,ir}} \) may easily be confused with other membrane currents. Further work is required to fully understand the physiological role of \( I_{\text{to,ir}} \) in various species and to identify the molecular entity responsible for this current.
REFERENCES


