Extracellular Ba\textsuperscript{2+} blocks the cardiac transient outward K\textsuperscript{+} current

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Shi, Hong, Hui-Zhen Wang, and Zhiguo Wang. Extracellular Ba\textsuperscript{2+} blocks the cardiac transient outward K\textsuperscript{+} current. Am. J. Physiol. Heart Circ. Physiol. 278: H295–H299, 2000.—Ba\textsuperscript{2+} is widely used as a tool in patch-clamp studies because of its ability to block a variety of K\textsuperscript{+} channels and to pass Ca\textsuperscript{2+} channels. Its potential ability to block the cardiac transient outward K\textsuperscript{+} current (I\textsubscript{to}) has not been clearly documented. We performed whole cell patch-clamp studies in canine ventricular and atrial myocytes. Extracellular application of Ba\textsuperscript{2+} produced potent inhibition of I\textsubscript{to}, with an IC\textsubscript{50} of \textasciitilde40 µM. The effects were voltage independent, and the inactivation kinetics were not altered by Ba\textsuperscript{2+}. The potency of Ba\textsuperscript{2+} was \textasciitilde10 times higher than that of 4-aminopyridine (a selective I\textsubscript{to} blocker with an IC\textsubscript{50} of 430 µM) under identical conditions. By comparison, Ba\textsuperscript{2+} blockade of the inward rectifier K\textsuperscript{+} current was voltage dependent; the IC\textsubscript{50} was \textasciitilde20 times lower (2.5 µM) than that for I\textsubscript{to}, when determined at –100 mV and was comparable to I\textsubscript{to}, as determined at –60 mV (IC\textsubscript{50} = 26 µM). Ba\textsuperscript{2+} concentrations of \textasciitilde1 mM or higher failed to block ultrarapid delayed rectifier K\textsuperscript{+} current. Our data suggest that Ba\textsuperscript{2+} can be considered a potent blocker of I\textsubscript{to}.

METHODS

Cell isolation. Single ventricular and atrial myocytes were isolated from the left ventricular epicardium and the right atrium, respectively, from adult mongrel dogs (20–26 kg) of either sex with previously described Langendorff perfusion techniques (12, 13, 17, 18). The preparation was perfused with Ca\textsuperscript{2+}-containing Tyrode solution at 37°C until the effluent was clear of blood, and the perfusate was then switched to Ca\textsuperscript{2+}-free Tyrode solution for 20 min at a constant rate of 12 ml/min, followed by perfusion with the same solution containing collagenase (110 U/ml CLS II collagenase; Worthington Biochemical, Freehold, NJ) and 0.1% BSA (Sigma Chemical, St. Louis, MO). The dispersed cells were stored in Kraftbrühe (KB) medium at 4°C for later electrophysiological experiments.

Patch-clamp recording. Patch-clamp recording techniques used in this study have been described in detail elsewhere (12, 13, 17, 18). Ionic currents were recorded with the whole cell voltage-clamp method, using an Axopatch 200B amplifier (Axon Instruments, Burlingame, CA). Borosilicate glass electrodes (1 mm outer diameter) had tip resistances of 1–3 M\Omega when filled with pipette solution. Junction potentials were zeroed before formation of the membrane-pipette seal in Tyrode solution. Mean seal resistance averaged 15 ± 2 G\Omega. Several minutes after seal formation, the membrane was ruptured by gentle suction to establish the whole cell configuration. The capacitance and series resistance were electrically compensated to minimize the duration of the capacitive surge on the current recording and the voltage drop across the clamped cell membrane. Leak currents were linearly subtracted. Cells with changing leak current (indicated by \textasciitilde10-pA changes in holding current at –50 mV) were rejected. Experiments were conducted at 36 ± 1°C unless otherwise specified.

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The Tyrode solution for cell isolation and whole cell patch-clamp recordings had the following composition (in mM): 136 NaCl, 5.4 KCl, 1 MgCl₂, 10 HEPES, 10 glucose, and 1 CaCl₂ (pH 7.4). The KB medium for cell storage contained (in mM) 20 KCl, 10 K₂PO₄, 25 glucose, 70 potassium glutamate, 10 β-hydroxybutyric acid, 20 taurine, 10 EGTA, and 40 mannitol, as well as 0.1% albumin (pH 7.4). The pipette solution contained (in mM) 0.1 GTP, 110 potassium aspartate, 20 KCl, 1 MgCl₂, 5 Mg-ATP, 10 HEPES, 10 EGTA, and 5 phosphocreatine (pH 7.3). Contamination by Na⁺ was diminished to the same extent as ventricular experiments is shown in Fig. 1B. Inactivation of the cells to Ba²⁺ current, and complete reversion of the effects was achieved by 100 µM Ba²⁺ (10 µM to prevent ATP-sensitive K⁺ current) and Cd²⁺ (200 µM; to suppress Ca²⁺ current). All chemicals were purchased from Sigma Chemical. BaCl₂ and 4-aminopyridine (4-AP) were prepared from a stock solution before experiments, and the pH of the recording solution with 4-AP was properly adjusted.

RESULTS

Depolarizing pulses activated a rapidly activating and inactivating outward current, known to be a 4-AP-sensitive Iₒ in both ventricular and atrial myocytes. Exposure of the cells to Ba²⁺ markedly diminished the current, and complete reversion of the effects was achieved on washout of Ba²⁺. Figure 1A displays a typical example from a representative ventricular myocyte. Ba²⁺ at a concentration of 100 µM produced ~60% reduction in the current amplitude. Notice that in the absence of Ba²⁺, there is a time-independent outward component, presumably caused by overlapping I₁K₁. This component was indeed depressed by Ba²⁺.

The inhibition of Iₒ seen with Ba²⁺ could have been confounded by potential contamination caused by concomitant blockade of I₁K₁. To exclude this possibility, effects of Ba²⁺ on Iₒ in atrial myocytes were also evaluated because atrial I₁K₁ is known to have a negligible outward component (20, 23). An example of such experiments is shown in Fig. 1B. Atrial Iₒ was diminished to the same extent as ventricular Iₒ by 100 µM Ba²⁺ in the bathing solution. Similar results were obtained from another three atrial myocytes.

Ba²⁺ is known to be a blocker of I₁K₁. To compare the relative potency of Ba²⁺ on Iₒ and I₁K₁, effects of Ba²⁺ on ventricular I₁K₁ were assessed. The results from a representative experiment are shown in Fig. 1C. Because Ba²⁺ blockade of I₁K₁ is voltage dependent, currents recorded at test potentials of −100 and −60 mV are displayed for comparison. These two voltages were chosen because an inward current was elicited at −100 mV and the maximum outward I₁K₁ was seen at −60 mV. As expected, Ba²⁺ at a concentration of 100 µM nearly abolished the inward I₁K₁ at −100 mV, whereas the effect was considerably attenuated with less negative potentials, in this case at −60 mV (Fig. 1C, right).

To investigate whether the inhibition of Iₒ by Ba²⁺ was also voltage dependent, mean data of current-voltage relationships of ventricular Iₒ before and after Ba²⁺ are presented in Fig. 2A. Ba²⁺ blocked Iₒ equally at various voltages tested without showing voltage dependence (P > 0.05, F-test). For example, the percent inhibition was 58% at −10 mV and 60% at +40 mV (P > 0.05, t-test). In addition, Ba²⁺ did not alter the inactivation kinetics either. For example, under control conditions, the inactivation time constants calculated by the double-exponential fits to the decaying phase of Iₒ were 3.8 ± 0.1 and 19.0 ± 2.2 ms for the rapid and slow components, respectively. The same analysis yielded time constants of 3.6 ± 0.3 and 20.7 ± 2.7 ms, respectively, in the presence of 100 µM Ba²⁺.

Concentration-dependent blockade of Iₒ was as well as I₁K₁ by Ba²⁺ was analyzed using the Hill equation, and the data are shown in Fig. 2B. The IC₅₀ (concentration needed for half-maximal inhibition) for Iₒ inhibition in ventricular cells was 41.7 ± 1.6 µM (Hill coefficient = 0.6). Whereas Ba²⁺ blocked the inward I₁K₁ recorded at −100 mV with greater potency, as indicated by the smaller IC₅₀ (2.6 ± 0.6 µM; Hill coefficient = 0.7), a much higher concentration of Ba²⁺ was required to achieve the same degree of block of the outward I₁K₁ elicited at −60 mV (IC₅₀ = 26.5 ± 2.2 µM; Hill coefficient = 0.8).

4-AP is considered to be a selective blocker of Iₒ. To compare the relative potency of 4-AP with that of Ba²⁺, additional experiments were performed with serial concentrations of 4-AP. Our results demonstrated that 4-AP was ~10 times less potent (IC₅₀ = 425.4 ± 38.6 µM; Hill coefficient = 0.6) than Ba²⁺ in blocking Iₒ under the same conditions (Fig. 2B). 4-AP at 2 mM completely suppressed the portion of Iₒ that was not inhibited by 100 µM Ba²⁺, as well as I₁K₁ (data not shown).

It was noticed that, as shown in Fig. 1B, although Ba²⁺ produced significant inhibition of Iₒ, it left I₁K₁ unaltered in atrial myocytes, as suggested by the lack of change in the sustained component remaining after complete inactivation of Iₒ. However, the analysis of this current was complicated by the overlapping Iₒ in these experiments. To clarify this issue, we conducted additional experiments at 22°C. Voltage protocols shown in Fig. 3, inset, activated a rapidly activating and slowly inactivating delayed rectifier current with typical characteristics of I₁K₁ (23, 24). Bath application of
Fig. 1. Representative experiments showing Ba²⁺ block of cardiac transient outward K⁺ current (I_{to}) and inward rectifier K⁺ currents (I_{K1}). I_{to} in canine ventricular (A) or atrial (B) myocytes were activated with voltage protocols shown in inset. Pulse duration was 200 ms, and interpulse interval was 10 s. For the sake of clarity, only current traces of the first 100 ms are shown. C: inward rectifier K⁺ currents (I_{K1}) elicited by voltage steps ranging from −100 mV to 0 mV from a holding potential of −50 mV. Dashed lines indicate zero current levels. Only current traces obtained at −100 and −60 mV are shown for the sake of clarity and better comparison of voltage dependence of Ba²⁺ action.

Fig. 2. Mean data on Ba²⁺ blockade of I_{to} and I_{K1}. A: current-voltage relationships (I-V curves) of I_{to}. B: dose-response curves of I_{to} and I_{K1} blockade by Ba²⁺ determined with 3 different voltage protocols. V_{Ito}, ventricular I_{to}; V_{Ik1}, ventricular I_{K1}. Symbols represent averaged experimental data (n = 6 for V_{Ito} (Ba²⁺), n = 8 for V_{Ito} (4-AP), n = 4 for V_{Ik1}) at varying concentrations; lines represent best fits to Hill equation: B = 100(1 + (IC_{50}/D)^nH), where B is percent change of I_{to} or I_{K1} at a drug concentration D, IC_{50} is Ba²⁺ concentration that produces 50% inhibition of current, and nH is Hill coefficient. IC_{50} was determined with a test potential of +50 mV for I_{to}. A test potential of −100 mV was used to evaluate Ba²⁺ effects on inward I_{K1}, and a test potential of −60 mV was used to assess inhibition of outward I_{K1}. [Drug], drug concentration.
Fig. 3. Lack of effects of Ba^{2+} on ultra-rapid delayed rectifier K^{+} current ($I_{K_{\text{ard}}}$) in canine atrial myocytes. Voltage protocols are shown in inset. To inactivate $I_{t_0}$, a 200-ms prepulse $+50$ mV was added 10 ms before each test pulse. Experiments were conducted at $22^\circ$C to provide better resolution of the initial activation phase of the current. Similar results were obtained from a total of 5 cells.

Ba^{2+} failed to alter $I_{K_{\text{ard}}}$ even at elevated concentrations of $\leq 1$ mM or higher.

**DISCUSSION**

We have demonstrated in this study that Ba^{2+} produced significant inhibition of $I_{t_0}$ in addition to its well-known effects on $I_{K_1}$, in cardiac myocytes. Noticeably, the potency of $I_{t_0}$ blockade was comparable to inhibition of the outward $I_{K_1}$ at $-60$ mV, and $I_{t_0}$ was actually more sensitive to Ba^{2+} than to 4-AP. On the other hand, Ba^{2+} did not affect $I_{K_{\text{ard}}}$. Although Ba^{2+} has been shown to block several types of K^{+} currents (1, 5, 7, 8, 11, 14–16, 20, 21), only one report (9) has presented one example showing the inhibition of rat ventricular $I_{t_0}$ by a high concentration of Ba^{2+} (3.6 mM). To date, no aimed and detailed characterization of Ba^{2+} blockade of $I_{t_0}$ has yet been documented despite the fact that Ba^{2+} is used so extensively for patch-clamp studies. This report represents the first aimed study of Ba^{2+} blockade of $I_{t_0}$. It is unlikely that the Ba^{2+} block of $I_{t_0}$ seen in our experiments was caused simply by surface charge effects because Ba^{2+} did not alter the voltage-dependent steadystate inactivation of $I_{t_0}$ (data not shown). In addition, the effects of Ba^{2+} on $I_{K_1}$ were also consistent with previous reports (5, 11, 14, 20) in terms of the voltage and time dependence. There should have been minimal contamination of $I_{t_0}$ measurement by other currents because Na^{+} current, Ca^{2+} current, and ATP-sensitive K^{+} current were suppressed throughout the experiments. In addition, with the concentrations of Ba^{2+} used in our study, $I_{K_{\text{ard}}}$ was not affected. Only $I_{t_0}$ currents elicited at $+50$ mV were used for evaluating the concentration-dependent Ba^{2+} effects because at less positive voltages (between $-40$ and $+10$ mV) there are outward $I_{K_1}$ currents, and effects of Ba^{2+} on these currents could complicate the analysis of $I_{t_0}$.

Our finding bears several important implications. An interesting finding is that Ba^{2+} blocks $I_{t_0}$ with $-10$ times higher potency than 4-AP ($IC_{50} = 40 \mu$M vs. 430 μM for Ba^{2+} vs. 4-AP, respectively), a compound commonly used as a selective $I_{t_0}$ blocker (9, 10, 18, 19, 23). This indicates that Ba^{2+} could be used as an $I_{t_0}$ blocker in some applications when $I_{t_0}$ needs to be removed to minimize overlapping currents. This is particularly true when studying $I_{K_{\text{ard}}}$. To date, there are no pharmacological tools for separating $I_{K_{\text{ard}}}$ and $I_{t_0}$ from each other because 4-AP at concentrations that block $I_{K_{\text{ard}}}$ also significantly inhibit $I_{t_0}$ (19, 23). Our finding that Ba^{2+} at 1 mM completely suppresses $I_{t_0}$ and $I_{K_1}$ but does not alter $I_{K_{\text{ard}}}$ at all implies that Ba^{2+} provides an ideal tool for studying $I_{K_{\text{ard}}}$ in a condition free of contaminating currents.

Moreover, when the potency was compared between $I_{t_0}$ and $I_{K_1}$, it was found that although the inward $I_{K_1}$ at $-100$ mV was more sensitive to Ba^{2+} than was $I_{t_0}$, the sensitivity of the outward $I_{K_1}$ at $-60$ mV was in the same range as that of $I_{t_0}$. It has been demonstrated that Ba^{2+} could alter cardiac electrical activities such as membrane depolarization and induction of pacemaker activity, changes presumably caused by reduction in outward currents. These Ba^{2+}-induced alterations have been interpreted as a result of $I_{K_1}$ inhibition. Our finding here suggests that Ba^{2+} blockade of $I_{t_0}$ may also contribute to the electrophysiological effects of Ba^{2+} in the heart. Future studies are certainly required to test this possibility.

Our finding also suggests that Ba^{2+} can be used to explore the structure-function relationships of A-type K^{+} channels in the light of its ability to block $I_{t_0}$ potently. In fact, Ba^{2+} has been used as a probe for investigating the pore properties of Shaker K^{+} channels (6, 7), and two external Ba^{2+} binding sites were identified. It has been strongly suggested that Kv4.2 and Kv4.3 channels are the major components of the native $I_{t_0}$ (2, 3), and detailed studies on the interactions of Ba^{2+} with these channels may help us understand mechanisms of Ba^{2+} blockade of $I_{t_0}$. However, whether Ba^{2+} also blocks these channels is still unknown, and future studies are absolutely needed to address this interesting and important issue in detail.

Finally, our study also suggests that caution must be exercised when Ba^{2+} is used to block $I_{K_1}$ to allow the study of $I_{t_0}$, because, as has actually happened in many
studies (10, 22), the interpretation and conclusion can be profoundly obscured by the concomitant inhibition of $I_{\text{to}}$ as the result of a significant underestimation of $I_{\text{to}}$ magnitude. Thus the use of Ba$^{2+}$ cannot be easily validated and justified for experiments involving $I_{\text{to}}$.

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