Effects of Cadmium and Nisoldipine on the Delayed Rectifier Potassium Current in Guinea Pig Ventricular Myocytes

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Received for publication September 18, 1996.

ABSTRACT

Block of the slow inward calcium current (I_{Ca-L}) during assessment of the delayed rectifier potassium current (I_K) of cardiac ventricular myocytes is commonly achieved by use of either inorganic compounds such as cadmium or dihydropyridine derivatives such as nisoldipine. Effects of these two I_{Ca-L} blockers on I_K characteristics of guinea pig ventricular myocytes were compared in this study. Currents were measured in the whole cell configuration of the patch-clamp technique and I_K tail amplitudes were measured at −40 mV after depolarizations to various test potentials (voltage steps, −20 to +50 mV) for either 250 (I_K250), 450 (I_K450) or 5000 (I_K5000) msec. Activating and tail currents measured in the presence of cadmium were of greater amplitudes when voltage steps were more positive than 0 mV but were of smaller amplitudes at V_{test} ≤ 0 mV compared to currents measured in the presence of nisoldipine or Tyrode solution. In the presence of the rapid component of the delayed rectifier E-4031, a blocker of cadmium increased I_{K_r} amplitude during high voltage tests and caused a positive shift in the voltage dependence of I_{K_r} activation at low depolarizing potentials. In contrast, no effect on I_{K_r} was observed when nisoldipine was added to Tyrode solution. In conclusion, results obtained in this study suggest that cadmium depresses and/or shifts the activation curve of the rapid component and increases and positively shifts the slow component of I_K in guinea pig ventricular myocytes. These observations lead us to propose that nisoldipine may be a better tool to inhibit long lasting inward calcium current during assessment of I_{Ca-L}.

Elaboration of L-type calcium current (I_{Ca-L}) is commonly required during pharmacological or physiological studies assessing modulation of I_K in ventricular myocytes. Successful block of I_{Ca-L} has been achieved by use of either organic or inorganic calcium channel antagonists (Reuter et al., 1985; Kamp and Miller, 1987; Kamp et al., 1988). However, specificity of block of calcium channel antagonists relative to cardiac potassium currents is of great concern in order not to bias interpretation of records obtained.

Previous studies have suggested that calcium channel antagonists may not be as specific as generally thought (Hume, 1988). However, specificity of block of calcium channel antagonists relative to cardiac potassium currents is of great concern in order not to bias interpretation of records obtained.

Abbreviations: I_{K_r}, delayed rectifier potassium current; I_{K250}, delayed rectifier elicited by 250 msec depolarizing voltage steps; I_{K450}, delayed rectifier elicited by 450 msec depolarizing voltage steps; I_{K5000}, delayed rectifier elicited by 5000 msec depolarizing voltage steps; V_{test}, voltage steps; I_{Ca} or I_{K}, slow inward calcium current; I_{L}, long lasting inward calcium current; I_{K_r}, rapid component of the delayed rectifier; I_{K_s}, slow component of the delayed rectifier; I_{K_1}, inward rectifier potassium current; I_{Na}, outward sodium current; I-V, current-voltage relationship.
latsky, 1990; Wettwer et al., 1992). In contrast, I_Ks is selectively blocked by diuretics and has a more predominant role in repolarization during prolonged action potential duration or rapid heart rates during which I_Kr activation may accumulate (Courtney et al., 1992; Jurkiewicz and Sanguinetti, 1993; Turgeon et al., 1994; Daleau and Turgeon, 1994). Numerous studies assessing I_K properties in guinea pig ventricular myocytes have used either cadmium or nisoldipine to block I_Ca-L. Although these drugs became the gold standard blockers of I_Ca-L, no study carefully looked at their effects on I_K components in guinea pig ventricular myocytes.

Therefore, the objective of our study was to compare effects of cadmium and nisoldipine on the amplitude, kinetics and activation and deactivation current-voltage curves of I_Kr and I_Ks in guinea pig ventricular myocytes.

**Methods**

**Patch-Clamp Experiments**

**Cell preparation and solutions.** Experiments were performed on single ventricular myocytes obtained from adult guinea pig hearts by use of an enzymatic dissociation technique. All solutions used during the cell isolation procedure were oxygenated and maintained at 37°C. The hearts were mounted on a Langendorff apparatus and rinsed for 2 min with a calcium-free solution (solution A) containing (in mM): NaCl 132, KCl 4.8, MgCl_2 1.2, HEPES 10, glucose 5, pH was adjusted to 7.35 with NaOH. Then, hearts were perfused with a low sodium-high potassium HEPES-buffered solution (solution B, in mM): NaCl 17, HEPES 10, KCl 5.4, K-glutamate 128, MgCl_2 1) for a period of 2 min. At the end of this period, perfusion of solution B containing collagenase (final concentration 300 U/ml; Worthington Biochemical Corp., Freehold, NJ) and protease (0.7 U/ml; Sigma
Chemical Co., St. Louis, MO) was started and continued until the system pressure dropped to 15 mm Hg (approximately 15 min). Hearts were finally reperfused with a solution made of 60% solution B and 40% solution A containing 0.5 mM CaCl2. At this point, the ventricles were cut down and minced slightly to increase cell yield. After filtration through 200–μm nylon mesh, the dispersed cells were washed by centrifugation (200 rpm, 2 min), resuspended in solution A containing 1.8 mM CaCl2 and maintained at 30°C before use.

The normal Tyrode solution used to superfuse cells during recording of currents contained (in mM): NaCl 145, KCl 4, MgCl2 1, CaCl2 0.1, HEPES 10, glucose 5; pH was adjusted to 7.35 with NaOH. Either nisoldipine (Bayer Leverkusen) 0.2 μM or cadmium (Sigma) 0.1 mM were added to eliminate the slow inward calcium current (Ick). Calcium was lowered to 0.1 mM in the extracellular solution to reproduce experimental conditions generally used during study of Ick, to avoid any Ica contamination and to prevent leak current (Balser et al., 1990). Effects of cadmium or nisoldipine on IK were also assessed independently using a Tyrode solution containing 1.8 mM CaCl2 in the control and washout periods of these experiments. The pipette solution contained (in mM): MgCl2 2, CaCl2 1, EGTA 11, MgATP 5, K2ATP 5, HEPES 10; pH was adjusted to 7.2 with KOH and final potassium concentration was fixed at 130 mM with KCl.

**Electrophysiological Measurements**

A small aliquot of dissociated cells was placed in a 0.5-ml chamber mounted on the stage of an inverted microscope (model CK2, Olympus, Lake Success, NY). Cells were allowed to adhere to the coverslip at the bottom of the chamber and then superfused continuously with the external solution pre-warmed (30°C) by a Peltier device (Medical System Corp., Greenvale, NY). In our experiments, complete replacement of external solution contained in the chamber was achieved within 2 to 3 min when the superfusion rate was 2 ml/min.

All currents were recorded in the whole cell, voltage-clamp configuration of the patch-clamp technique using an Axopatch-1D amplifier (Axon Instruments Inc., Burlingame, CA). Voltage-clamp command pulses were generated by a 12-bit digital-to-analog converter (model TL/1, Axon Instruments Inc.) controlled by the pClamp software package (version 4.05b; Axon Instruments Inc.). Heat-polished patch-clamp pipette electrodes used (capillary glass from Radnoti, Starbore glass capillary tubing 1.2 mm O.D.) had a tip resistance of 1 to 5 MΩ when filled with the pipette solution. Series resistance was compensated 50 to 80% to improve fidelity of whole cell voltage-clamp measurements.

**Protocols**

Rod-shape cells with clear cross striations, resting potential of at least −78 mV and stable Ick and IK1 currents (as assessed during a baseline period of at least 4 min) were used. Effects of nisoldipine and cadmium on the Ick and IK1, activating components of IK, were studied in cells held at −40 mV (to inactivate INa) and depolarized by pulses lasting either 250 msec (IK250), 450 msec (IK450) or 5000 msec (IK5000). Test potentials of depolarizing pulses varied between −20 and +50 mV. IK was measured from the peak magnitude of tail current obtained upon repolarization to −40 mV.

**Data Storage and Analysis**

Currents were filtered at either 2 KHz (IK250 and IK450 protocols) or 100 Hz (IK5000 protocol) by a four-pole Bessel filter (−3 dB/octave). Currents were sampled at 4 KHz (IK250 and IK450) and 400 Hz (IK5000) by use of a 12-bit analog-to-digital converter (TL-1 DMA, Axon Instruments Inc.) and stored on hard disk for subsequent analysis. Data are presented as mean ± S.D. Statistically significant differences in IK activating curves for IK250 and IK5000 in the presence of cadmium and nisoldipine were compared by a Student’s paired t test. Best fit of data was established by comparison of χ2 analysis. Level of statistical significance was set at P < .05.

**Results**

Comparative effects of cadmium and nisoldipine on the delayed rectifier potassium current. Figure 1A illustrates outward currents elicited by a 5-sec pulse to various depolarizing potentials from a holding potential of −40 mV. Deactivating currents (tails) were recorded on repolarization to −40 mV. In these recordings, the same guinea pig ventricular myocyte was perfused with a low-calcium (0.1 mM) Tyrode solution containing either 0.1 mM cadmium (left panels) or nisoldipine 0.2 μM (right panels). When nisoldipine replaced cadmium in the perfusate, IK activating and tail currents elicited by depolarizing steps to voltages of more...
than 0 mV decreased although those elicited by lower depolarizing voltages increased. Figure 1B illustrates current-voltage relationships observed during the experiment shown in figure 1A for the deactivating currents. At a test potential of +50 mV, superfusion of the myocyte with the nisoldipine containing buffer reduced $I_{K5000}$ tail current by 90 pA (27%). In contrast, at a test potential of +20 mV, $I_{K5000}$ tail current was increased 25 pA (50%) by nisoldipine. A reversal of the effect was observed upon reperfusion of the myocyte with the cadmium containing buffer solution (fig. 1C).

To appreciate intercell reproducibility in these effects, the I-V relationship between $I_K$ tail current amplitudes and depolarizing voltage steps was constructed from 9 cells exposed first to cadmium and then to nisoldipine (fig. 2). In figure 2, tail currents amplitudes were normalized for cell capacitance. After short depolarizing pulses (250 msec; fig. 2A) $I_K$ tail current amplitude measured in the presence of cadmium tended to be reduced for depolarizing potential <0 mV but increased for depolarizing potentials >20 mV compared to current amplitudes measured during superfusion of nisoldipine. Differences in $I_K$ tail current amplitudes were statistically significant at test voltages of +10, +40 and +50 mV (P < .05). When stimulation lasted 5 sec (fig. 2B), a situation where outward current is largely $I_K$, a crossing over in the I-V curves was still observed although the crossing point was shift from +20 to 0 mV compared to $I_{K250}$. A significant difference in $I_K$ tail current amplitude was noticed at +20, +30, +40 and +50 mV (P < .05).

Kinetics of $I_{K5000}$ deactivating current were assessed in six cells using a biexponential curve fitting function. Table 1 summarizes changes in the faster time constant ($\tau_1$) caused by the exchange of cadmium by nisoldipine in the external bathing solution. Superfusion with nisoldipine increased $\tau_1$ by about 180% (P < .05) in a voltage-independent manner. On reperfusion with cadmium in the absence of nisoldipine (washout), $\tau_1$ almost returned to initial base-line values.

**Effects of cadmium on the delayed rectifier potassium current.** In this series of experiments, control record-
ings were obtained during superfusion of cells with normal Tyrode solution (containing 1.8 mM calcium). Test pulse duration was either 250 msec (\(I_{K250}\)), 450 msec (\(I_{K450}\)) or 5000 msec (\(I_{K5000}\)). Under control conditions, a large inward L-type calcium current was elicited (fig. 3). When the external solution was replaced by a solution containing cadmium 0.1 mM (calcium concentration lowered to 0.1 mM to reproduce experimental conditions used during study of \(I_K\)), the L-type calcium current was eliminated. However, the amplitude of \(I_K\) tail currents was increased for all pulse durations tested at +10 mV and for \(I_K\) tail current elicited by 5 sec pulses to −10 mV (fig. 4).

In contrast, the amplitudes of \(I_K\) tail current was reduced for the shorter pulses (i.e., 450 and 250 msec) after voltage steps to −10 mV. This effect was reproducibly observed in five cells tested (fig. 4, A and B). Amplitudes of \(I_{K250}\) and \(I_{K5000}\) tail current normalized by cell capacitance were determined to be decreased compared to control conditions but were increased for voltage pulses ≥ +20 mV (\(P < .05\)).

Table 2 summarizes changes in the fast time constant (\(\tau_1\)) of \(I_{K5000}\) tail current induced by cadmium 0.1 mM. Superfusion with cadmium decreased \(\tau_1\) by −30% (\(P < .05\)) compared to values determined in the presence of calcium 1.8 mM. This decrease was voltage-independent. Values of \(\tau_1\) returned toward base-line values on removal of cadmium and superfusion of cells with the normal Tyrode solution (containing 1.8 mM calcium).

Specific effects of cadmium on \(I_{Kr}\) and \(I_{Ks}\) were assessed using the specific \(I_{Kr}\) blocker E-4031. When 5 \(\mu\)M E-4031 was introduced in the extracellular solution, only \(I_{Ks}\) was present in the delayed rectifier. As in the previous experiments presented in figures 1 to 4, addition of cadmium to the control solution induced an increase in \(I_K\) amplitude at high voltages. These results were reproducible in three cells tested. Moreover, cadmium 0.1 mM modified the I-V curve at low voltages tested (fig. 5) inducing a positive shift of about +5 mV in \(I_K\) activation. Fast time constant of deactivation of \(I_K\) estimated in the presence of E-4031 was 310 ± 35 msec and 302 ± 38 msec after test pulses to +40 and +20 mV, respectively. Cadmium had no significant effects on \(I_{Ks}\) under the same conditions (i.e., 333 ± 37 msec at +40 mV and 326 ± 45 msec at +20 mV, respectively).

Another series of experiments were designed to determine the concentration-dependence of cadmium effects on \(I_K\). Figure 6 shows a typical example of \(I_K\) I-V curves obtained when cadmium concentration was increased sequentially from 20 to 100 \(\mu\)M and from 100 to 500 \(\mu\)M in the same cell. The increase in current amplitude elicited by +50 mV voltage tests was dependent on cadmium concentration in the range of the concentrations tested. Similar results were observed in four cells tested. In contrast, decrease in \(I_{Ks}\) observed at lower depolarizing potentials was almost unaffected by increasing concentration of cadmium of more than 20 \(\mu\)M.

Effects of nisoldipine on the delayed rectifier potassium current. Effects of nisoldipine 0.2 \(\mu\)M on \(I_K\) were determined and compared to recordings obtained using a normal Tyrode solution (containing 1.8 mM calcium) as the external solution. Traces in figure 7A show that this concentration of nisoldipine entirely blocks \(I_{Ca-L}\) but does not alter amplitude of \(I_{K250}\) tail current. As well no changes were observed on \(I_K\) activating and tail currents during long pulses (5000 msec; fig. 7B). These effects were reproducible in five cells tested (fig. 8, A and B). Finally, no significant changes in \(I_{K5000}\) deactivating constant (\(\tau_1\)) were observed by the superfusion of cells with nisoldipine 0.2 \(\mu\)M (table 3).

### Discussion

Our experiments examined the effects of cadmium and nisoldipine on the two components of \(I_K\), namely \(I_{Kr}\) and \(I_{Ks}\). Studies were conducted at concentrations of cadmium and nisoldipine usually used to block \(I_{Ca-L}\) in experiments designed to characterize modulation of \(I_K\) (Arena and Kass,

### Table 2

<table>
<thead>
<tr>
<th>Test Pulse Potential (mV)</th>
<th>Change in (\tau_1) (msec)(^{a, \circ})</th>
<th>Washout (Normal Tyrode)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>389 ± 191</td>
<td>443 ± 166</td>
</tr>
<tr>
<td>+20</td>
<td>346 ± 119</td>
<td>324 ± 101</td>
</tr>
<tr>
<td>+40</td>
<td>338 ± 126</td>
<td>358 ± 123</td>
</tr>
</tbody>
</table>

\(^{a}\) Mean ± S.D.

\(^{\circ}\) \(n = 8\) cells.

\(^{c}\) \(P < .05\) vs. baseline.

### Fig. 4

Current-voltage relationship of \(I_K\) tail current observed in five cells tested in the presence or absence of 0.1 mM cadmium (\(^* P < .05\)). Current was normalized for cell capacitance and obtained on repolarization to −40 mV after 250- (A) or 5000- (B) msec depolarizing pulse durations.
1988; Balser et al., 1990; Sanguinetti and Jurkiewicz, 1990a; Daleau and Turgeon, 1994). Our findings have shown a decrease in \( I_K \) amplitude at low voltage steps which is consistent with an induction of a shift in \( I_{Kr} \) activation previously reported (Follmer et al., 1992). In addition, our results also provide evidence that the inorganic calcium channel blocker cadmium also shifts to positive voltages the activation curve of \( I_{Ks} \) and increases its amplitude. However, the organic dihydropyridine derivative calcium channel antagonist nisoldipine, had no effects on \( I_K \) activation curve and had minimal effects on deactivation kinetics. These results suggest that nisoldipine is a better tool than cadmium for use in study of \( I_K \).

\( I_K \) recorded from guinea pig ventricular myocytes in the presence of 0.1 mM cadmium was decreased when elicited by voltage steps \( \leq 0 \) mV but increased when elicited by voltage steps \( \geq 20 \) mV compared to \( I_K \) obtained in the presence of nisoldipine or a 1.8 mM CaCl\(_2\)-containing Tyrode solution. Also, with E-4031 in the extracellular solution, cadmium increased \( I_K \) amplitude elicited at high voltage tested and positively shifts the \( I-V \) curve at low voltages. From these experiments, we cannot discriminate between a positive shift in \( I_{Kr} \) activation or a reduction in \( I_{Kr} \) amplitude. However, it has been described in cat ventricular myocytes, where \( I_K \) is mainly due to \( I_{Kr} \), that cadmium added directly to the bath solution containing 3 \( \mu \)M nitrendipine, decreased \( I_K \) at low depolarizing potentials but increased peak \( I_K \) tail current and shifted the voltage dependence of activation to more positive potentials by about 15 mV (Follmer et al., 1992). On the basis of our results and the results of Follmer et al. (1992), we propose that cadmium shifts toward positive potentials \( I_{Kr} \) and \( I_{Ks} \) \( I-V \) curves and increases \( I_{Ks} \) amplitude elicited at voltages \( \geq 120 \) mV.

In contrast, experiments conducted with nisoldipine were not associated with alteration in \( I_K \) characteristics which confirms previous results (Kass, 1982) obtained in Purkinje fibers with 10 \( \mu \)M nisoldipine (i.e., 50 times the concentration usually used to block \( I_{Ca} \)). From these experiments, we concluded that cadmium is almost exclusively responsible for the difference observed in \( I_K \) characteristics recorded in the presence of either cadmium or nisoldipine. In guinea pig ventricular myocytes, lanthanum, an inorganic calcium current blocker, blocks \( I_{Kr} \), and shifts positively the voltage dependency of \( I_{Ks} \) activation curve (Sanguinetti and Jurkiewicz, 1990b). Cobalt, another inorganic salt, alters \( I_K \) properties similar to lanthanum, with a more pronounced reduction of \( I_K \) at negative potentials and a positive shift of \( I_K \) \( I-V \) curve in the whole voltage range of \( I_{Ks} \) activation (Fan and Hiraoka, 1991).
Deactivation kinetics of \( I_K \) were faster in the presence of cadmium compared to nisoldipine. Recordings obtained showed that the fast time constant of \( I_K \) deactivation was increased by about 180% when 0.1 mM cadmium was replaced by 0.2 mM nisoldipine. This effect was voltage independent. However, when cadmium was added to a normal Tyrode solution, \( t_1 \) was decreased to 65 to 70% of control value, consistent with the increase of \( t_1 \) observed when cadmium was replaced by nisoldipine in the bath solution. In contrast, no changes in \( t_1 \) were observed when nisoldipine was added to a normal Tyrode solution. Modification in the kinetics of \( I_K \) produced by cadmium suggests a direct interaction of the compound with negatively charged gating particles. Follmer et al. (1992) also observed a faster decay of \( I_K \) tail currents in the presence of cadmium in cat ventricular myocytes. In contrast, such an effect was absent in frog heart cells (Duchatelle-Gourdon et al., 1989). In our experiments, cadmium had minimum effects on the fast time constant of deactivation in the presence of E-4031 which is consistent with a predominant effect on \( I_K \) deactivation kinetics.
Finally we have assessed effects of different concentrations of cadmium on $I_{K}$, i.e., 20, 100 and 500 $\mu$M (fig. 6). Changes in $I_{K}$ amplitude observed at lower depolarizing potentials were almost unaffected by cadmium concentration of more than 20 $\mu$M although increase in $I_{K}$ observed at positive depolarizing potentials remains concentration dependent.

In conclusion, we have shown that cadmium shifts the activation curve and increases the amplitude of the slow component of the delayed rectifier potassium current in guinea pig ventricular myocytes. These effects are complementary to the previously demonstrated shift in $I_{K}$-I-V curve caused by cadmium. These effects were observed at a concentration that overlaps with its effects on the L-type calcium current. Therefore, these effects must be considered in the interpretation of $I_{K}$-modulation studies where this cation is present. However, nisoldipine seems to be a more powerful and specific tool, at a concentration aimed to fully inhibit $I_{Ca}$ in $I_{K}$ studies.

Acknowledgments
The authors thank Michel Blouin and Carolle Bergeron for technical assistance.

References

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