Triamterene Inhibits the Delayed Rectifier Potassium Current (I\textsubscript{K}) in Guinea Pig Ventricular Myocytes

Pascal Daleau, Jacques Turgeon

Abstract In humans, proarrhythmia during therapy with action potential-prolonging drugs can be associated with hypokalemia often provoked by concomitant administration of diuretic agents. Consequently, therapy with class III antiarrhythmics and K\textsuperscript+}-sparring diuretics, such as triamterene, may be indicated. Triamterene, along with its K\textsuperscript+}-sparring properties, exhibits other pharmacological effects. In the heart, it can increase action potential duration (guinea pig atria and papillary muscles), protect against reperfusion-induced arrhythmias (rat), and increase the QT interval (humans). Therefore, studies were undertaken to assess effects of triamterene on cardiac K\textsuperscript+} repolarizing currents. Guinea pig ventricular myocytes were superfused at 30\degree C with Ca\textsuperscript{2+}-containing solution to block I\textsubscript{L} and held at −40 mV to inactivate I\textsubscript{K}. Currents were measured in the whole-cell configuration of the patch-clamp technique. The delayed rectifier outward current (I\textsubscript{K}) was elicited by short (250-millisecond) and long (5000-millisecond) depolarizing pulses, and time-independent currents were assessed by a rapid ramp test protocol. After high-voltage long pulses (+50 mV; 5000 milliseconds), tail current amplitude of the slow component of I\textsubscript{K} (I\textsubscript{K\textsubscript{slow}}) was decreased 36±6\% (n=6) and 51±8\% (n=6) by triamterene 10\textsuperscript{-3} and 10\textsuperscript{-4} mol/L, respectively. After low-voltage short pulses (−20 mV; 250 milliseconds), tail current amplitude corresponding essentially to the rapid component of I\textsubscript{K} (I\textsubscript{K\textsubscript{rapid}}) was decreased only 14±11\% (n=9) and 19±10\% (n=10) by triamterene 10\textsuperscript{-3} and 10\textsuperscript{-4} mol/L, respectively. These results were confirmed under conditions of pure I\textsubscript{K\textsubscript{slow}} (block of I\textsubscript{K\textsubscript{rapid}} by E-4031) and pure I\textsubscript{K\textsubscript{rapid}} (block of I\textsubscript{K\textsubscript{slow}} with nisoldipine, extracellular Ca\textsuperscript{2+} decreased to virtually 0 mmol/L, and 250-millisecond depolarizing pulse to −20 mV). In contrast, triamterene had no effects on time-independent currents. Thus, data obtained indicate that triamterene, at clinically relevant concentrations, inhibits I\textsubscript{K\textsubscript{rapid}} in a selective manner, although both components of I\textsubscript{K} (I\textsubscript{K\textsubscript{slow}} and I\textsubscript{K\textsubscript{rapid}}) were decreased. This block of I\textsubscript{K} may explain triamterene-related prolongation of cardiac repolarization and warn about potential drug interaction with action potential-prolonging agents. (Circ Res. 1994;74:1114-1120.)

Key Words • potassium channels • diuretics • cardiac repolarization • delayed rectifier

Recently, interest has been focused on the development of antiarrhythmic agents able to selectively prolong refractoriness through a lengthening of cardiac repolarization.\textsuperscript{1-3} Newly developed drugs with this so-called class III electrophysiological property have shown effectiveness in the prevention of ventricular arrhythmias in several animal models and in humans.\textsuperscript{4-11} However, previous electrophysiological studies have established that pronounced lengthening of cardiac repolarization can lead to arrhythmia aggravation and to proarrhythmic events.\textsuperscript{12,13}

Potassium currents responsible for limiting cardiac action potential duration vary depending on species and cell types. In guinea pig ventricular myocytes, potassium outward currents involved in the repolarization phase of the action potential are the delayed rectifier (I\textsubscript{K}), the inward rectifier (I\textsubscript{K\textsubscript{i}}), and the plateau (I\textsubscript{K\textsubscript{p}}) currents.\textsuperscript{14-17} The delayed rectifier potassium current of guinea pig ventricular myocytes is described by a rapidly (I\textsubscript{K\textsubscript{rapid}}) and a slowly (I\textsubscript{K\textsubscript{slow}}) activating component.\textsuperscript{18} Relative contributions of I\textsubscript{K\textsubscript{rapid}} and I\textsubscript{K\textsubscript{slow}} to an activating current elicited by a depolarizing pulse from a holding potential negative to −40 mV depend on both pulse duration and voltage. The ratio is proposed to favor I\textsubscript{K\textsubscript{rapid}} during short depolarizations at low voltages and to favor I\textsubscript{K\textsubscript{slow}} during long depolarizations to high voltages.

Triamterene is a potassium-sparing agent used concomitantly with thiazide diuretics to prevent excessive potassium losses.\textsuperscript{19} After oral administration in humans, peak plasma concentrations of triamterene reach 1 to 2 μg/mL (4 to 8 μmol/L).\textsuperscript{20} The exact mechanism of action of triamterene is not well understood, but it has been proposed that, in the kidneys, triamterene reduces electrical potential gradient across tubular epithelium (which represents the basis of renal potassium excretion) by interrupting electrogenic sodium transport.\textsuperscript{21} Studies have pointed out that triamterene also exhibits nonrenal pharmacological properties.\textsuperscript{22-24} In the heart, triamterene increases refractory periods of electrically driven isolated guinea pig atria and papillary muscles.\textsuperscript{23} Triamterene can also reduce the incidence and duration of arrhythmias induced by either cardiac glycoside toxicity\textsuperscript{25} or reperfusion of ligated rat coronary artery.\textsuperscript{26} Moreover, it has been reported that triamterene can increase the QT interval independently of plasma potassium concentration in humans.\textsuperscript{27}

The mechanisms by which triamterene exhibits cardiac electrophysiological effects are unknown. In this article, we present effects of triamterene on time- and voltage-dependent potassium currents involved in the.
repolarization phase of cardiac ventricular myocytes. Preliminary data obtained in this study have been reported in abstract form.28,29

Materials and Methods

Solutions

All solutions used during the cell isolation procedure were oxygenated and maintained at 37° C. Normal HEPES-buffered physiological solution contained (in mmol/L): NaCl 132, KCl 4, MgCl₂ 1.8, MgCl₂ 1.2, HEPES 10, glucose 5; pH was adjusted to 7.35 with NaOH. Nominally calcium-free solution was identical to this solution except that addition of CaCl₂ was omitted. The enzyme solution used for cell dispersion was prepared by dissolving collagenase (final concentration, 300 U/mL; Worthington Biochemical Corp) and protease (0.7 U/mL; Sigma Chemicals Inc) in the nominally calcium-free solution.

The external solution used to superfuse cells during recordings of currents contained (in mmol/L): NaCl 145, KCl 4, MgCl₂ 1, HEPES 10, glucose 5; CaCl₂ was lowered to 0.1 mmol/L, and CdCl₂ 0.1 mmol/L was added to eliminate the slow inward calcium current (I₅). In selected experiments, E-4031 (5 x 10⁻⁴ mol/L) was added to the external solution to eliminate IKᵣ, and to study more selectively the effects of triamterene on IKᵣ. In another series of experiments designed specifically to better dissect effects of the drug on IKᵣ, and IKᵦ, nisoldipine (0.2 μmol/L) was used instead of CdCl₂ to block ICD₉, since CdCl₂ has been reported to shift IKᵣ activation to more positive potentials.30 In addition, in this series of experiments, Ca²⁺ was removed from the extracellular solution to shift IKᵦ activation to positive potentials.31

The pipette solution contained (in mmol/L): 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 mmol/L with KCl.

Final concentrations (10⁻⁴, 10⁻³, or 10⁻² mol/L) of triamterene (Sigma Chemicals Inc) in the external solution were obtained by adding aliquots of a stock solution (10 mmol/L) prepared daily by dissolving 2.48 mg of triamterene in 1 mL of HCl 0.1 mol/L. Finally, the pH of control and drug-containing external solutions was adjusted to 7.35 with NaOH.

Cell Preparation

Experiments were performed on single ventricular myocytes obtained from adult guinea pig hearts by use of an enzymatic dissociation technique. The hearts were mounted on a Langendorff apparatus and rinsed for 5 minutes with the calcium-free solution. After this step, the heart was perfused with the same solution containing enzymes. Perfusion with the enzyme solution was continued until the system pressure dropped to 30 mm Hg (approximately 7 minutes). Then, the heart was reperfused with an enzyme-free solution containing CaCl₂ 200 μmol/L for 5 minutes. At this point, the ventricles were cut down, placed in HEPES-buffered physiological solution, and minced slightly to increase cell yield. After filtration through 200-μm nylon mesh, the dispersed cells were washed by centrifugation (500 rpm, 5 minutes), resuspended in HEPES-buffered physiological solution, and maintained at room temperature before use.

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). Cells were allowed to adhere to the coverslip at the bottom of the chamber and then superfused continuously with the external solution prewarmed (36°C) by a Peltier device (Medical System Corp). In our experiments, complete replacement of external solution contained in the chamber was achieved within 2 to 3 minutes when the superfusion rate was 2 mL/min.

All currents were recorded in the whole-cell, voltage-clamp configuration of the patch-clamp technique with an Axopatch-1D amplifier (Axon Instruments Inc). Voltage-clamp command pulses were generated by a 12-bit digital-to-analog converter (model TL/4, 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, Starebore glass capillary tubing, 1.2-mm outer diameter) had a tip resistance of 3 to 5 MΩ when filled with the pipette solution. Pipette capacitance and series resistance were compensated to minimize the duration of capacitive currents.

Protocols

Rod-shape cells with clear cross-striations, resting potential of at least −78 mV, and stable IKᵦ (during short and long pulses) and IKᵢ (as assessed during a baseline period of at least 4 minutes) were sampled at 4 kHz. Currents were recorded by using a four-pole Bessel filter (−3 dB of lower than 5 kHz) during depolarization pulses lasting either 250 (IKᵢ) or 5000 (IKᵦ) milliseconds. Test potentials of depolarizing pulses varied between −20 and +50 mV. IKᵦ was measured from the peak magnitude of tail current obtained on repolarization to −30 mV. Repolarizing currents were also assessed by the envelope-of-tails test of IKᵦ. In this protocol, pulse duration varied from 30 to 1230 milliseconds by increments of 200 milliseconds, and tail currents, measured on repolarization to −30 mV, were compared with corresponding activating currents elicited after depolarizing pulses to +40 mV. Finally, a voltage ramp was used to obtain the current-voltage (I-V) relation of the IKᵦ. In this protocol, cells were held at −40 mV before their membrane potential was changed from 0 to −100 mV in 500 milliseconds.

Data Storage and Analysis

Currents were filtered at either 2 kHz (IKᵢ) or 100 Hz (IKᵦ) protocols by a four-pole Bessel filter (−3 dB of lower than 5 kHz) protocol. Voltages were sampled at 4 kHz (IKᵢ) and 400 Hz (IKᵦ) by use of a 12-bit analog-to-digital converter (TL-1 DMA, Axon Instruments) and stored on hard disk for subsequent analysis. Data are presented as mean±SD, and statistically significant concentration-dependent block of IKᵦ and IKᵦ was tested by Hotelling's T² test.32 Statistically significant voltage dependency was tested by a conditional Hotelling's T² test.33 In this analysis, a Shapiro-Wilk test was used to assess normality. The level of statistical significance was set at P<.05.

Results

Fig 1A shows the membrane currents elicited by a 5000-millisecond voltage-clamp step from holding potential (−40 mV) to +40 mV followed by a 5000-millisecond repolarizing pulse to −30 mV. A time-dependent outward current activated during depolarization and complete deactivation of the tail current was observed during repolarization to −30 mV. Superfusion of the cell with triamterene 10⁻⁴ mol/L reduced the amplitude of IKSRO activating and tail currents.

In the same cell, a 250-millisecond voltage-clamp step from holding potential (−40 mV) to −10 mV activated a steadily increasing (time-dependent) outward current (Fig 1B). This current deactivates after repolarization to −30 mV. During superfusion of the cell with triamterene 10⁻⁴ mol/L, the amplitudes of IKSRO time-dependent activating and deactivating currents were almost unaffected.

Fig 2A illustrates the voltage-dependent and concentration-dependent decrease in tail currents induced by
superfusion of a total of 23 cells with triamterene after a 5000-millisecond depolarizing pulse (IK2500). Triamterene induced a concentration-dependent but voltage-independent decrease in tail currents. The three curves

Fig 1. A, Recording of membrane currents elicited in a cell by a 5000-millisecond depolarizing step to +40 mV followed by repolarization to −30 mV. Currents are shown at baseline and during superfusion with triamterene 10⁻⁴ M. Dashed arrow indicates zero current. B, Recording of membrane currents elicited by a short (250-millisecond) depolarizing step to −10 mV followed by repolarization to −30 mV during baseline and superfusion with triamterene 10⁻⁴ M/L. In A and B, holding potential was −40 mV.

(triamterene 10⁻⁴, 10⁻⁵, and 10⁻⁶ mol/L) had a parallel profile and were significantly different from one another at each potential (P = .001); however, at each concentration, inhibition was independent of depolarizing step voltage (P = .777). Mean decrease from baseline was 17% at 10⁻⁴ mol/L, 34% at 10⁻⁵ mol/L, and 52% at 10⁻⁶ mol/L.

In contrast to long depolarizing pulses (IK2500), the decrease in tail currents after short pulses (IK250) ap-
appeared to be both voltage and concentration dependent (Fig 2B). Curves obtained for triamterene $10^{-4}$ mol/L and $10^{-5}$ mol/L had a statistically significant parallel profile and were significantly different ($P=.002$). The voltage of the depolarizing pulse had a significant effect on both curves; the three first levels, i.e., $-20$, $-10$, and $0$ mV, showed smaller inhibition amplitude than that obtained for more positive depolarizations ($P=.011$). Conversely, the decrease in tail current induced by triamterene at depolarizing steps more positive than $+10$ mV was voltage independent.

Experiments were performed in the presence of E-4031 to eliminate $I_{K}$, and to assess more specifically the voltage dependence and extent of block of $I_{K}$ by triamterene. Records presented in Fig 3 clearly indicate that under these conditions, no time-dependent current was activated for pulses to voltages $\leq 0$ mV. During long pulses (5000 milliseconds), triamterene $10^{-4}$ mol/L caused a voltage-independent decrease in $I_{K}$ of about $50\%$ (Fig 4). This value is similar to that obtained in experiments performed without E-4031 (Fig 2A). During short pulses, the extent of inhibition of $I_{K}$ was similar ($\approx 50\%$; Fig 5) at voltages at which the current activates ($\geq 0$ mV). A summary of the results obtained in a series of four cells is presented in Fig 6A and 6B. These data suggest that block of $I_{K}$ by triamterene is voltage independent and time independent.

To better quantify the extent of block of triamterene on $I_{K}$, further experiments were performed using nisol
However, tested. Moreover, in these experiments, Ca²⁺ was virtually eliminated from the external solution. Under these conditions, triamterene 10⁻⁵ mol/L (n=6) caused a smaller decrease in activating (-6±22%) and tail (-11±27%) currents elicited at -20 mV compared with block observed in the activating (-46±14%) and tail (-43±14%) currents elicited at +50 mV. Typical records obtained under these conditions are presented in Fig 7. Again, these values are consistent with data obtained in the presence of Cd²⁺ at low depolarizing voltages during short pulses (Fig 2B).

The effects of triamterene on Iₖ were also assessed by an envelope-of-tails test (Fig 8). Neither under control nor in the presence of triamterene was the envelope-of-tails test verified. Nevertheless, triamterene caused an increase in the ratio of Iₖtail/Iₖact current for all pulse durations to +40 mV, consistent with a greater block of Iₖ by the drug.

Reversal of triamterene-induced decrease in Iₖ,E50 of Iₖ,500 was never complete; this was more apparent at higher concentrations of the drug (Table 1). Although some reversal of the effect was observed, no definite conclusions could be drawn on the magnitude of the drug-related decrease in Iₖ because of the potential rundown of Iₖ. To ascertain that the decrease of Iₖ induced by triamterene was not due to rundown of the currents, new series of experiments were undertaken. In these experiments, triamterene 10⁻⁵ mol/L was introduced, in a blinded manner, either in the first or in the second perfusion period. An increase in Iₖ induced by superfusion with the second solution was interpreted as a reversal of block, whereas a decrease in Iₖ was indicative of current block. In all cells tested (n=11), the solution containing triamterene was recognized by the blinded investigator because of noticeable increase or decrease in Iₖ on superfusion with the second solution.

Finally, triamterene (n=23, see Table 2) did not significantly alter the maximum positive amplitude of the background current, the voltage corresponding to this peak amplitude, or the reversing potential of this current, which corresponds to the resting potential of the cell. Fig 9 represents an example of time-independent currents recorded by a ramp protocol before and after application of 10⁻⁴ mol/L triamterene.

### Discussion

In this work, we have shown that triamterene inhibits Iₖ of guinea pig ventricular myocytes after (1) long-lasting depolarizations when Iₖ is almost fully activated and (2) to a lesser extent, after low-voltage depolarizing short pulses when Iₖ is the main activated component. Therefore, results obtained demonstrate selective blocking properties of triamterene for Iₖ.

Data obtained in this study showed that triamterene decreases Iₖ elicited during 5000-millisecond depolarizing pulses in a concentration-dependent but voltage-independent manner. For all voltages tested (-10 to +50 mV), inhibition reaches ~50% at 10⁻⁴ mol/L. Under conditions in which Iₖ was eliminated (E-4031), the time-dependent outward current elicited by long pulses to -10 mV and more positive potentials (at which Iₖ is activated) was also decreased =50% by triamterene 10⁻⁴ mol/L. During short pulses, virtually no time-dependent current could be measured at -20, -10, and 0 mV in the presence of E-4031. At more positive potentials (at which Iₖ is measurable), the decrease of Iₖ induced by triamterene was similar to that measured during long pulses. Therefore, our results indicate that triamterene inhibits Iₖ of guinea pig ventricular myocytes in a voltage- and time-independent but concentration-dependent manner (17% at 10⁻⁴ mol/L, 34% at 10⁻³ mol/L, and 52% at 10⁻² mol/L).

In initial experiments (cells exposed to Cd²⁺ in the absence of E-4031), triamterene 10⁻⁴ and 10⁻³ mol/L caused a concentration- and apparently voltage-dependent decrease of Iₖ,E50 in the voltage range of -20 to +10 mV. At higher voltages, the decrease in Iₖ,E50 was still concentration dependent but was voltage independent. The apparently voltage-dependent block may be explained either by a true voltage- and/or time-dependent block of Iₖ or by block to a different extent of the two

<table>
<thead>
<tr>
<th>Test Potential</th>
<th>Iₖ,E50  (n=10)</th>
<th>Iₖ,500  (n=10)</th>
<th>Iₖ,E50  (n=7)</th>
<th>Iₖ,500  (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+10 mV</td>
<td>48±33</td>
<td>24±22</td>
<td>58±46</td>
<td>22±15</td>
</tr>
<tr>
<td>+30 mV</td>
<td>39±29</td>
<td>12±15</td>
<td>36±27</td>
<td>25±24</td>
</tr>
<tr>
<td>+50 mV</td>
<td>42±26</td>
<td>16±18</td>
<td>34±29</td>
<td>21±13</td>
</tr>
</tbody>
</table>

Values are mean±SD.
components of $I_K$ ($I_{Kr}$ and $I_{Ks}$). Experiments performed with E-4031 (pure $I_{Kr}$) have already pointed out voltage- and time-independent inhibition of $I_{Kr}$ during long and short pulses. To verify the hypothesis that block of $I_{Kr}$ is more potent than that of $I_{Ks}$, we performed studies in conditions of pure $I_{Kr}$. Nisoldipine was used to block $I_{Ks}$ instead of Cd$^{2+}$, since Cd$^{2+}$ is known to shift the I-V curve of $I_{Kr}$ positively, and extracellular Ca$^{2+}$ was virtually abolished to cause a positive shift in $I_{Ks}$ I-V curve. Under these conditions, time-dependent outward current elicited by short depolarizing pulses to $-20$ or $-10$ mV (which corresponds to $I_{Ks}$) was blocked about 10% to 20% by triamterene $10^{-4}$ mol/L. Therefore, it was concluded that apparently voltage-dependent block of $I_{Ks}$ by triamterene in the presence of Cd$^{2+}$ was due to greater inhibition of $I_{Kr}$ (activated at positive potentials) than $I_{Ks}$ (activated in a relatively higher proportion than $I_{Kr}$ at negative potentials).

Other data obtained from the envelope-of-tails test also pointed out that triamterene inhibits more $I_{Kr}$ than $I_{Ks}$. During superfusion of cells with triamterene, activating currents elicited by depolarization to +40 mV were clearly more decreased than tail currents measured on repolarization to $-30$ mV. Since $I_{Kr}$ exhibits prominent rectification at this potential, activating currents elicited at +40 mV consist mainly of $I_{Kr}$, whereas the maximal amplitude of tail currents represents the sum of fully activated $I_{Kr}$ and $I_{Ks}$. Thus, the greater decrease observed in the activating than the tail current is indicative of a greater inhibition of $I_{Kr}$ than $I_{Ks}$.

Removal of triamterene from the cell bath was associated with a partial recovery of inhibited currents. Since rundown of $I_{Kr}$ is a well-described phenomenon, lack of washout data could preclude quantitative analysis of our results. Therefore, we performed blinded experiments in which triamterene was introduced either during the first (so-called "baseline") or the second perfusion period. We knew from experiments performed in the course of this study that triamterene does not alter the resting potential of guinea pig ventricular myocytes. Consequently, cells polarized below $-78$ mV during the first baseline period were discarded. In all experiments performed (11 of 11), the solution containing triamterene was recognized by the investigator because of a significant increase or decrease in $I_{Kr}$ on superfusion with the second solution. Thus, these additional blinded experiments confirmed results obtained in previous unblinded experiments and ascertained effects of triamterene on $I_{Kr}$. It appears, therefore, that like other lipophilic drugs such as amiodarone, triamterene could be trapped in the membrane because of its physicochemical properties.

In our experiments, the concentration-response curve for triamterene block of $I_{Kr}$ (and $I_{Ks}$) appears to be quite broad; i.e., a 10-fold increase in drug concentration increases block by a much smaller percentage than would be expected from a simple single binding site model. In our opinion, this is most likely explained by the poor solubility of triamterene at a $10^{-4}$ mol/L concentration. In fact, triamterene is a lipophilic drug that does not easily get into solution at pH 7.4. Therefore, it is possible that the final concentration was lower than that expected for the highest concentrations of the drug tested.

In summary, this report presents data demonstrating direct block of the cardiac $I_{Kr}$ current by the potassium-sparing diuretic triamterene. Block was selective for the $I_{Kr}$, a rare target of conventional class III antiarrhythmic drugs. Effects were observed at clinically relevant concentrations of triamterene and could explain, at least in part, antiarrhythmic and action potential–prolonging properties of the drug. We propose that administration of triamterene may modulate effects of other action potential–prolonging drugs.

Acknowledgments

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Simard is the Canada fellowship of (900114) and Marban E. Background potassium current active during the plateau of the action potential in guinea pig ventricular myocytes. Circ Res. 1993;72:890-900.


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P Daleau and J Turgeon

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