—Short Communications—

Modulation of the delayed rectifier potassium channel gating in guinea-pig ventricular myocytes by intracellular acidification

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It is well known that intracellular acidification following myocardial ischemia can inhibit background membrane conductance in mammalian ventricular myocytes. This is caused by the inhibition of the inwardly-rectifying potassium channel activity. In contrast, it is unclear what the effects of intracellular acidification on cardiac delayed rectifier potassium channel gating behavior are, despite the fact that the channel plays a significant role in the recovery phase of the action potential. We report here the effect of intracellular acidification on the delayed rectifier potassium channel under whole-cell and inside-out patch-clamp conditions.

Single guinea-pig ventricular myocytes were enzymatically dissociated according to the method described previously^{1,2}. The external normal Tyrode's solution used during whole-cell recordings contained (in mM): NaCl 142, KCl 5.4, CaCl₂ 1.8, MgCl₂ 1.0, glucose 10, HEPES 8 (pH=7.4). The pipette solution (standard internal solution) used during whole-cell recordings contained (in mM): KCl 150, MgCl₂ 1.0, EGTA 5; HEPES 8 (pH=7.3 with KOH). In excised inside-out patch recordings, the pipette solution and bath solution (cytosolic side of the membrane) contained (in mM): KCl 150, CaCl₂ 0.5, MgCl₂ 1.0, HEPES 8 (pH=7.3). Free Ca²⁺ concentration ([Ca²⁺]) was adjusted to 0.1 μ M (pCa=7) by adding CaCl₂. An acidic solution (pH=6.8 and 6/3) was prepared by changing the pH of the pipette solution in whole-cell and bath solution in inside-out patches. Membrane currents were recorded under

the condition of standard patch-clamp configurations³⁻⁵ using an Axopatch-1C amplifier (Axon Instruments, Inc., Foster City, CA). The single channel events were filtered off-line with a cut-off frequency of 2 KHz through an eight-pole low-pass Bessel filter (48 dB/octave, Model 902-LPF, Frequency Devices, Inc., Haverhill, MA), digitized with 14-bit resolution at a sample rate of 10 KHz in a manner similar to those described previously^{6,7}. Channel transitions were calculated using an averaging technique to determine channel amplitude. Leakage currents during voltage step recording were canceled by subtracting each trace from the average current traces without events. The data were analyzed on a computer (PC-9801, NEC, Tokyo, Japan) using locally written analysis programs that are based on the half-amplitude threshold analysis method^{8,9}. The temperature of the bath was monitored and maintained at 37 $\pm$ 1°C using a Peltier thermo-electrical device. The results are expressed as mean $\pm$ SD. Statistical analyses were performed using Student's t-test or one-way analysis of variance (ANOVA).

Fig. 1 (Panel A) shows examples of whole-cell currents recorded from guinea-pig ventricular myocytes after subtraction of linear leakage conductance at physiological temperature (37°C) in the control condition (pH=7.3, circle) and during intracellular perfusion with acidic solution (pH=6.8, square). A depolarizing voltage-clamp test pulse to + 50 mV was applied for 2 s from a holding

Fig. 1 Panel A: Effect of intracellular acidification on the whole-cell delayed rectifier potassium current evoked by 2-s duration test pulses to +50 mV from a holding potential of -50 mV at 37°C. Control trace (circle) was recorded in the standard internal solution (pH=7.3). The current (square) was recorded using the acidic internal solution (pH=6.8). The averaged current-voltage relationships of the current was measured in different internal solutions (pH=7.3 circles and pH=6.8 squares). The vertical bar through each point represents the SD (n=8 in both conditions). Panel B: pH-dependence of the unitary delayed rectifier potassium channel current in inside-out patch recordings. Sequential sweeps were sampled during 2-s voltage-clamp steps to +50 mV from a holding potential of 0 mV. Bath solutions (cytosolic side of the membrane) with various pH values were applied at 37°C. The pH of the bath solution is indicated on the left of each current sweep. The leak and capacitive currents have been subtracted in all sweeps. Averaged P_o in pH=7.3, 6.8 and 6.3 were measured in seven different cells. The vertical bar through each bar represents the SD. They were different from each other ($p < 0.01$ by ANOVA).

potential of -50 mV. The current magnitude was suppressed in the acidic condition. The averaged current amplitude at the end of the depolarizing clamp pulse was significantly smaller in the acidic condition (1.03 ± 0.17 nA, n=8) than in the control

(1.84 ± 0.26 nA, n=8, $p < 0.01$). The averaged current-voltage (I-V) relationships from eight different cells demonstrated significantly smaller conductance than the control at every depolarizing voltage. In contrast to these observations, perfusing myocytes with external acidic solution did not affect the current magnitude (n=5). These results indicate that the current may be modulated by intracellular H^+ . To examine these effects on the current in more detail, single channel currents were measured in excised inside-out patch configurations. Fig. 1 (Panel B) shows examples of unitary current activity obtained by applying 2-s durations test pulses to +50 mV from a holding potential of 0 mV. This patch contains only one channel. The pH of the bath solution (cytosolic side of the membrane) was lowered from the control at 7.3 to 6.8 and 6.3. Superfusion of the intracellular surface with acidic solution reversibly inhibited single channel activity with decreasing open state probability (P_o) without affecting unitary current amplitude. The averaged P_o was $71.4 \pm 12.0\%$ (n=7) at pH=7.3, $38.9 \pm 14.0\%$ (n=6) at pH=6.8, and $15.7 \pm 9.8\%$ (n=6) at pH=6.3. These values were significantly different from each other ($p < 0.01$). In contrast, perfusion of the patch with alkaline pH solution (pH=8.0) did not affect channel activity (n=6, not significant statistically).

Although intracellular pH may not be precisely controlled by intracellular perfusion because of the presence of a Na/H antiport on the plasma membrane in the whole-cell configuration, the results of single channel measurements with direct control of intracellular pH were similar to the whole-cell measurements. Because intracellular acidification, but not extracellular acidification, caused channel inhibition, H^+ may bind to the channel within a pore via the intracellular mouth. This H^+ binding may cause the alteration of the channel gating kinetics. Further study is indicated to define the mechanism of channel inhibition by intracellular acidification.

References

1. Koumi S-i, Wasserstrom JA, Ten Eick RE: J Physiol (Lond) 1995; 486: 647.
2. Koumi S-i, Wasserstrom JA, Ten Eick RE: J Physiol (Lond) 1995; 486: 661.
3. Koumi S-i, Arentzen CE, Backer CL, Wasserstrom JA: Circulation 1994; 90: 2213.

4. Koumi S-i, Backer CL, Arentzen CE: Circulation 1995; 92: 164.
 5. Koumi S-i, Wasserstrom JA: Am J Physiol 1994; 266: H 1812.
 6. Koumi S-i, Backer CL, Arentzen CE, Sato R: J Clin Invest 1995; 96: 5870.
 7. Koumi S-i, Sato R, Hayakawa H: J Membr Biol 1995; 145: 143.
 8. Koumi S-i, Martin RL, Sato R: Am J Physiol in press.
 9. Sato R, Koumi S-i: J Membr Biol 1995; 148: 185.
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