

Lactate Activates ATP-sensitive Potassium Channels in Guinea Pig Ventricular Myocytes

Edmund C. Keung and Qian Li

Cardiology Section, Veterans Affairs Medical Center, San Francisco, California 94121; and Department of Medicine, University of California San Francisco, San Francisco, California 94143

Abstract

The functional significance of cardiac ATP-sensitive potassium channels remains controversial because of the discrepancy between the low levels of ATP at which activation of the channels occurs and the much higher levels of ATP maintained during myocardial ischemia. We studied the effects of (+)-lactate, which accumulates in large quantity as a result of increased glycolysis during ischemia, on ATP-sensitive potassium channels in adult guinea pig ventricular myocytes using the whole-cell patch-clamp technique. Lactate at 20–40 mM in the internal solution activated ATP-sensitive potassium channels and shortened action potential duration. Activation of the channels occurred even in the presence of 2–5 mM ATP in the internal solution and was dependent on intracellular free magnesium levels. Our results suggest that intracellular lactate may play a significant role in activating cardiac ATP-sensitive potassium channels and shortening action potential duration even at ATP levels similar to those resulting from moderate to severe myocardial ischemia. (*J. Clin. Invest.* 1991. 88:1772–1777.) Key words: guinea-pig ventricular myocytes • ATP-sensitive K⁺ channel • ATP • lactate • myocardial ischemia • action potential

Introduction

It is well established that ATP-sensitive potassium (K_{ATP}) channels in cardiac cells are activated when cytosolic ATP concentrations fall below a critical level (1, 2). The opening of these potassium channels has been implicated as a potential cause for shortening of the action potential duration and the marked increase in K⁺ efflux observed during myocardial ischemia. However, a major problem with this hypothesis arises from the discrepancy between the ATP levels measured during myocardial ischemia and the level at which activation of K_{ATP} channels occurs. In excised cardiac membrane patches or whole-cell recordings, K_{ATP} channels open only when the ATP level is reduced to < 1 mM (1–3). During myocardial ischemia or metabolic inhibition, however, intracellular ATP decreases only to 2–5 mM from a normal level of ~ 8 mM (4, 5). One explanation for this discrepancy, among others (6, 7), is that metabo-

lites generated during ischemia or metabolic inhibition may markedly reduce the sensitivity of K_{ATP} channels to ATP (3, 6, 8). During myocardial ischemia increased glycolysis results in the accumulation of a large amount of intracellular lactate. The present study was undertaken to determine whether lactate activates K_{ATP} channels in the presence of relatively high concentrations of ATP.

We used the whole-cell patch-clamp technique to examine the effects of intracellular lactate on membrane currents in guinea pig ventricular myocytes. We found that intracellular application of lactate activated the ATP-sensitive potassium current (*I*_{KATP}) and shortened the action potential duration. Activation of *I*_{KATP} by lactate occurred even in the presence of 2–5 mM ATP in the patch-clamp pipettes and was highly dependent on intracellular free magnesium levels. These results suggest that lactate produced by glycolysis may contribute to the opening of K_{ATP} channels and the shortening of the action potential duration at ATP levels resulting from moderate to severe myocardial ischemia.

Methods

All experimental procedures were approved by the San Francisco Veterans Affairs Medical Center's Committee on Animal Research.

Cell preparation. Adult guinea pig ventricular cells were enzymatically isolated with collagenase as previously described (9).

Experimental solutions. The standard external solution contained (in millimolar): NaCl 137, KCl 5.4, MgCl₂ 1.0, CaCl₂ 1.8, Hepes 10, and glucose 10 at pH 7.4 (titrated with NaOH). For voltage clamp experiments, 2 mM CdCl₂ was added to the external solution to block the L-type calcium channels in voltage clamp experiments. The basic internal (pipette) solution contained (in millimolar): EGTA 5, Hepes 5, Na Hepes 5, K⁺ 150 (KCl, KOH, and K₂ATP, when present) at pH 7.2. Varying amounts of (+)-lactic acid (Sigma Chemical Co., St. Louis, MO), K₂ATP and MgCl₂ were added to obtain the concentrations needed. Glyburide (Sigma Chemical Co.) was added from a 10 mM stock solution (in DMSO) to the external solutions as required. The free Mg²⁺ concentration in the internal solution was calculated using a program provided by Fabiato (10).

Electrophysiological experiments. Membrane currents and potential were recorded in the whole-cell configuration (11). Pipette resistance ranged from 0.8 to 2.8 MΩ. 1–4 GΩ seals were usually achieved between the pipette tips and the cell membrane. Data acquisition and analysis were performed as previously described (9, 12). All experiments were performed at room temperature (22–24°C).

Results

Effects of lactate on transmembrane action potential and membrane holding current. Fig. 1 shows the effects of lactate on the action potentials and membrane holding current. Myocytes

Address correspondence and reprint requests to Edmund C. Keung, M.D., Cardiology Section (111C), Veterans Affairs Medical Center, 4150 Clement St., San Francisco, CA 94121.

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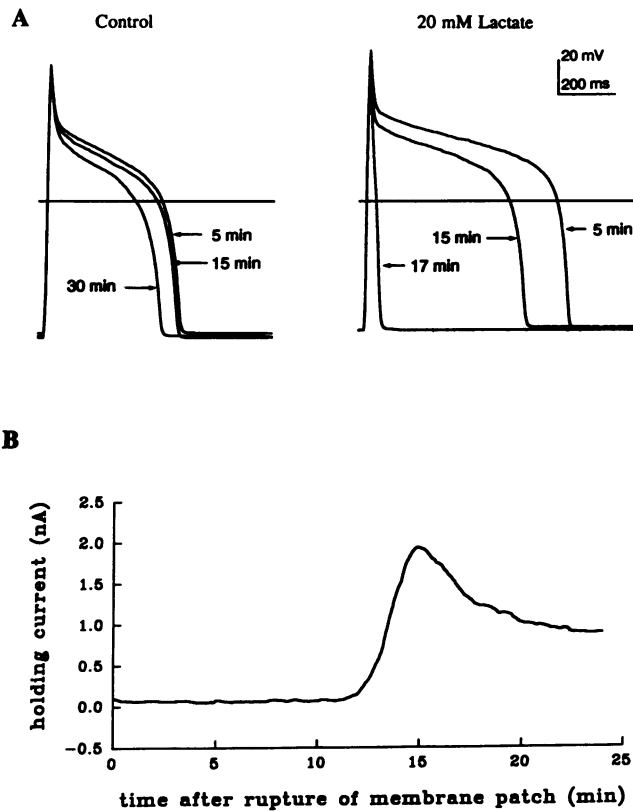


Figure 1. Effects of lactate on action potential and holding current. (A) Action potentials recorded from cells internally perfused with (right) and without (left) 20 mM lactate in the internal solution. Each trace was recorded at the time indicated after rupture of the membrane patch. Marked shortening of the action potential duration was observed 17 min after internal dialysis with lactate. (B) Change in holding current during internal dialysis with 20 mM lactate. Holding potential was -40 mV.

were internally dialyzed with and without lactate in the internal solution. Action potentials were elicited using the current-clamp mode. When 20 mM lactate was present in the internal solution, an obvious shortening of the action potential duration was evident 15 min after rupture of the membrane patch, followed by a dramatic decrease at 17 min (Fig. 1 A, right). The cell became inexcitable within 30 s after the recording at 17 min. A similar phenomenon was observed in all four cells internally perfused with lactate. In contrast, when lactate was absent in the internal solution, the action potential essentially remained unchanged between 5 and 15 min (Fig. 1 A, left). A slight reduction in the action potential duration was observed at 30 min. This reduction in the action potential duration was probably due to L-type calcium current rundown and loss of some intracellular constituents. Fig. 1 B shows changes in the holding current induced by internal dialysis with 20 mM lactate. When the membrane potential was clamped at -40 mV, a small outward holding current (I_h) was recorded after rupture of the membrane patch. A gradual outward shift in the holding current was evident within 10 min and was followed by a rapid increase to ~ 2 nA. Soon after I_h reached a maximum, a slow decrease of I_h began. The average time for the increase in I_h to occur was 16.2 ± 1.6 min (mean \pm SE, $n = 16$). In the absence of

lactate, I_h remained unchanged for up to 100 min (data not shown).

Activation of K_{ATP} channels by internal perfusion with lactate. The effects of lactate on K_{ATP} channels are shown in Fig. 2. Fig. 2 A depicts current traces in the presence of 20 mM lactate in response to depolarization voltage clamp steps from a holding potential of -40 mV. Small time-dependent outward current traces, which represent the delayed rectifying potassium currents, were recorded in response to depolarization clamp steps 1 min after rupture of the membrane patch. 10 min after the cell was internally perfused with 20 mM lactate, the magnitude of the outward current increased markedly as the membrane holding current shifted from 0.1 nA at 1 min to 3.5 nA at 15 min. The current eventually became time independent. Fig. 2 B shows the current-voltage (I-V) relations obtained from myocytes internally perfused with and without lactate. In the absence of lactate and ATP in the internal solution, the I-V relation maintained the characteristic inward rectification throughout the recording, which ended at 40 min (Fig. 2 B, left). In five out of six cells tested, activation of $I_{K_{ATP}}$ was not observed during recording durations of 40–100 min. However, when 20 mM lactate and 0 mM ATP were present in the internal solution, K_{ATP} channels were activated rapidly (Fig. 2 B, right, and Table I). The inward rectification was abolished at 10 min. At 12 min, the current magnitude at 60 mV increased to > 10 nA. The reversal potential for the current remained at -80 mV until the I-V relationship became ohmic 15 min after rupture of the membrane patch. In all experiments in which I_h increased, activation of ATP-dependent potassium current as characterized by the loss of inward rectification and an increase in the magnitude of the outward current at the end of 380-ms step pulses always occurred. Furthermore, in association with a partial recovery of the holding current after it reached a maximum level, a reduction in the outward current was observed.

Effect of glyburide on the lactate-induced outward current. Cardiac K_{ATP} channels have been shown to be blocked by sul-

Table I. Effects of Intracellular Lactate, ATP and Free Mg^{2+} on K_{ATP} Channels

(+)-lactate	ATP	free Mg^{2+}	No. positive/ No. cells tested	Time
mM	mM	mM		min
0	2	0.3	0/3	40–100 [‡]
0	0	1.1	1/6	40–95 [‡]
20	0	1.1	16/16	$16.2 \pm 1.6^{\S}$
40	0	1.1	6/6	$12.3 \pm 1.6^{\S}$
40	2	0.3	9/9	$24.9 \pm 3.3^{\S}$
		0.5	2/5	
40	5	0.1	5/5	$26.8 \pm 3.7^{\S}$
		0.3	2/3	
		0.5	2/4	
40	5	1.1	0/4	40–95 [‡]

* Number of cells in which activation of K_{ATP} channels was observed. Cells were considered negative if no activation of K_{ATP} channels was observed during recording duration ≥ 40 min. [‡] Total time of whole-cell recording, terminated either by experimenter or cell death. [§] Time to activation of K_{ATP} channels in cells show positive response (mean \pm SE).

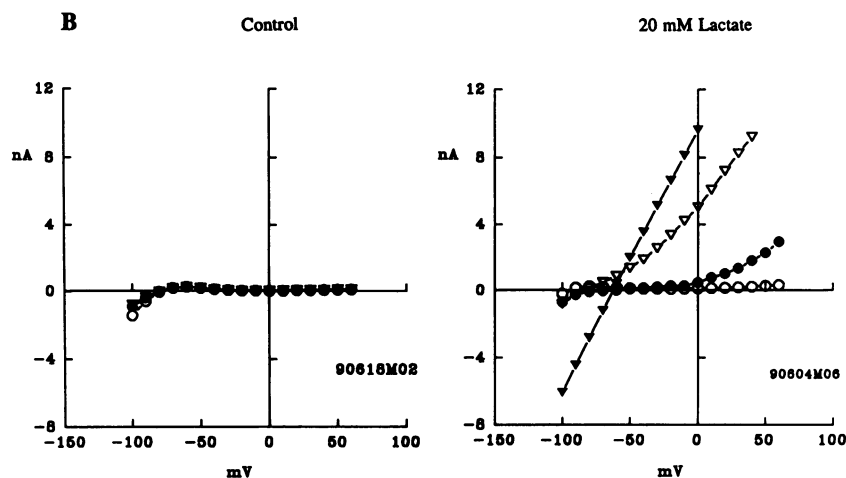
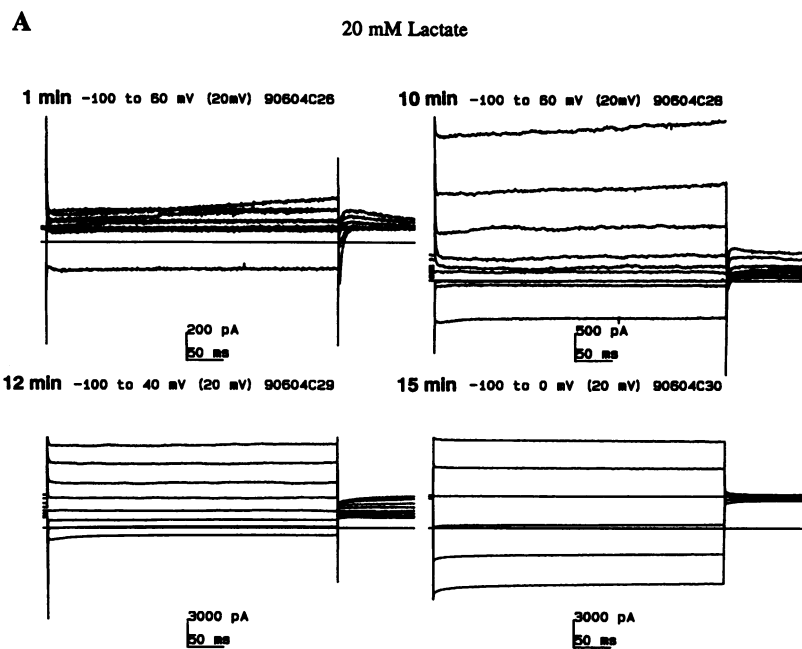


Figure 2. Activation of K_{ATP} channels by internal dialysis with lactate. (A) Current traces recorded at times indicated during internal perfusion with ATP-free internal solution containing 20 mM lactate. The membrane potential was held at -40 mV and voltage steps were from -100 mV to the levels indicated in increments of 20 mV. Zero current levels are indicated by horizontal lines. (B) Current-voltage relations of K_{ATP} current obtained from cells dialyzed with (right) and without (left) 20 mM lactate. The current was measured at the end of 380-ms clamp pulses from a holding potential of -40 mV. The free Mg^{2+} concentration in the pipettes was 1.1 mM. Symbols: Left (control): 10 min, open circles; 20 min, solid circles; 40 min, open triangles. Right (20 mM lactate): 1 min, open circles; 10 min, solid circles; 12 min, open triangles; 15 min, solid triangles. The time indicated represents minutes after rupture of the membrane patches.

fonylureas such as tolbutamide (13) and glyburide (14). Accordingly, we examined the effect of glyburide on the outward current induced by intracellular lactate. Cells were internally perfused with 40 mM lactate with or without 100 μ M glyburide. The mean magnitude of the outward current elicited by a 380-ms clamp step from -40 to 20 mV is shown in Fig. 3. In cells internally dialyzed with 40 mM lactate, a very large outward current was activated in < 10 min. Glyburide significantly reduced the magnitude of the outward current at 10 and 15 min of recording ($P < 0.05$).

Lactate-induced activation of the K_{ATP} current in the presence of ATP. Although lactate production via glycolysis is increased during myocardial ischemia, ATP content at the time of action potential shortening is maintained at relatively high levels (4, 5). In an attempt to determine the functional significance of K_{ATP} channel activation by lactate, we examined the effects of lactate on K_{ATP} channels in the presence of ATP. Fig. 4 shows examples of current-voltage relations of K_{ATP} channels obtained from cells internally perfused with varying concentra-

tions of lactate and ATP. As demonstrated in Fig. 4, B and C, lactate-activated K_{ATP} channels even in the presence of 2–5 mM ATP in the internal solution. Similar to I_{KATP} induced by lowering intracellular ATP (1, 2), the outward current induced by lactate clearly saturated at positive membrane potentials (Fig. 4 B).

Intracellular free magnesium (Mg^{2+}) has been shown to block I_{KATP} induced by lowering ATP in excised membrane patches (15, 16). To investigate if Mg^{2+} modulates the lactate-activated outward current in a similar fashion, we examined the effects of lactate on K_{ATP} channels in the presence of varying concentrations of ATP and Mg^{2+} in the internal solution. The results are summarized in Table I and examples are illustrated in Fig. 5. Activation of K_{ATP} channels by lactate appeared to be sensitive to Mg^{2+} concentration. In the presence of 2 mM ATP and 0.3 mM Mg^{2+} , 40 mM lactate activated K_{ATP} channels in all nine cells tested (Fig. 4 B, Table I). When Mg^{2+} was increased to 0.5 mM, lactate activated K_{ATP} channels in two out of five cells. A similar inhibitory effect was observed in

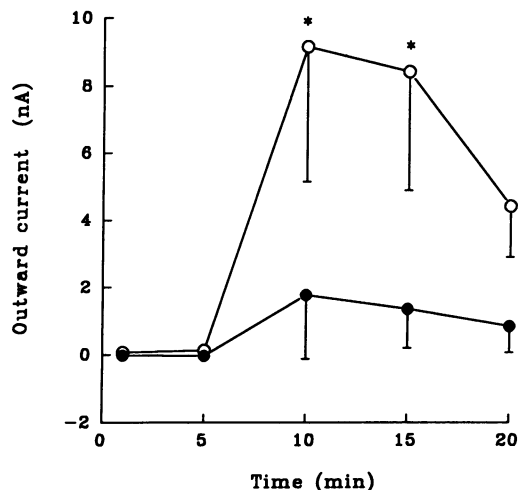


Figure 3. Effect of glyburide on the outward current activated by 40 mM lactate in the internal solution. The mean magnitude of the outward current at the end of a 380-ms pulse in the presence (solid circles, $n = 6$) and in the absence (open circles, $n = 5$) of 100 μM glyburide was plotted against the duration of whole-cell recording. Glyburide was added to the bath solution at the beginning of the experiments. Glyburide significantly suppressed the magnitude of the outward current ($P < 0.05$, represented by the asterisks) at 10 and 15 min of recording. Statistical analysis was performed with two-way ANOVA and a posteriori comparisons using the Newman-Keuls method. Error bars represent SE.

the presence of 5 mM ATP and 40 mM lactate in the internal solution (Fig. 4 C). A further increase of Mg^{2+} to 1.1 mM resulted in complete blockade of the I_{KATP} in all four cells tested (Fig. 4 D). In the absence of ATP in the internal solution, however, Mg^{2+} even at a concentration of 1.1 mM failed to inhibit activation of K_{ATP} channels by lactate (Fig. 2). Data shown in Table I indicate that increasing ATP and Mg^{2+} not only prolonged the time to activation of K_{ATP} channels, but could also completely suppress the K_{ATP} channels in some cells.

Discussion

The functional significance of cardiac ATP-sensitive potassium channels remains controversial because of the discrepancy between the low levels of ATP at which activation of the channels occurs and the much higher levels of ATP maintained during myocardial ischemia. Based on results obtained from inside-out membrane patches isolated from rat ventricular myocytes, several mechanisms have been advanced to account for the observed discrepancy. A reduction of intracellular pH from 7.25 to 6.25, or an increase in intracellular ADP level, both occurring during ischemia, increased half-maximal inhibition of K_{ATP} channels (K_{IATP}) from 25 to 50–100 μM ATP (3). Moreover, it is estimated that activation of only 5% of K_{ATP} channels will result in changes in action potential duration (17).

However, in the whole-cell configuration, Noma and Shibasaki failed to observe activation of K_{ATP} current (with the metabolic inhibitor, cyanide) in ventricular myocytes in the presence of 2 mM ATP in the internal solution (1). Our whole-cell voltage clamp experiments clearly show that lactate, a bypro-

duct of myocardial ischemia, activates an outward current in guinea pig myocytes in the presence of 2–5 mM ATP. This outward current shares many characteristic features of the ATP-sensitive potassium current in cardiac cells (1, 2, 15, 16): (a) It is time independent. (b) The apparent reversal equilibrium potential is ~ -80 mV. (c) It shows saturation at positive membrane potentials. (d) It is modulated by Mg^{2+} and ATP. (e) It is suppressed by glyburide. The outward current does not represent a chloride current under our experimental conditions (18). In the experiment shown in Fig. 1 B, for example, the external and internal Cl^- concentrations were 148 and 126 mM, respectively. The equilibrium potential for chloride was therefore 4 mV. At a holding potential of -40 mV, activation of chloride channels by lactate would result in an inward shift of the holding current, as opposed to the marked outward shift shown in Fig. 1 B.

Belles et al. have shown that K_{ATP} channels are activated during prolonged whole-cell recordings in the absence of ATP in the internal solution (13). Moreover, they noted that inclusion of 3 or 20 mM ATP markedly increased the time to activation of K_{ATP} current (from 19 min without ATP to 48 and 127 min with 3 and 20 mM ATP at 34–36°C, respectively) (13). The reason for this spontaneous activation of K_{ATP} current is probably due to the washout of some intracellular components and/or ATP consumption during prolonged whole-cell recordings. With 0 or 2 mM ATP in the internal solutions we did not observe any K_{ATP} current in eight out of nine cells during the 40–100 min of recording at 22–24°C. By contrast, inclusion of lactate in the internal solution resulted in rapid appearance of the K_{ATP} current even when ATP was present in the pipette, suggesting a direct role of lactate in the activation of K_{ATP} channels (Table I).

During myocardial ischemia tissue ATP content decreases as lactate content increases. Subendocardial ATP decreases from a control value of 25 to 9 and 7 $\mu\text{mol/g}$ dry weight after 10 and 20 min of sustained ischemia, respectively (4). Tissue lactate content increases from 12 to 101 and 175 $\mu\text{mol/g}$ dry weight at the same time intervals (4). We asked whether the ATP and lactate concentrations we used are similar to those measured in experimental ischemia. The concentration of ATP and lactate in millimolar can be estimated as follows (5): (ATP or lactate $\mu\text{mol/g}$ dry wt) \times (1 g dry wt/5 g wet wt) \times 0.58 μl myoplasmic volume/g wet wt. Accordingly, after 10–20 min sustained ischemia, subendocardial ATP decreases from 8 to 2–3 mM while lactate increases from 4 to 35–60 mM. These values are virtually identical to those used in our experiments.

At present, the mechanisms underlying the lactate activation of the K_{ATP} channel have yet to be defined. There are several possibilities. First, diffusion of lactate into the cytosol may interfere with ATP production via glycolysis, thereby depleting the ATP pool. The basal metabolic rate of quiescent myocytes is very low and glycolysis, as measured by the rate of lactate formation, is negligible (19). Therefore, under our experimental conditions, inhibition of glycolysis by lactate, if present, would minimally affect ATP production. Moreover, incubation of isolated rat cardiac myocytes under anoxic conditions with 50 mM lactate fails to affect cell ATP content, which suggests that the effect of lactate on glycolytic rates is minimal (20). Second, lactate, like ADP (3, 8), may reduce the sensitivity of K_{ATP} channels to ATP. Third, lactate may act like cromakalim (21) and pinacidil (22) as a channel opener. Lastly, activa-

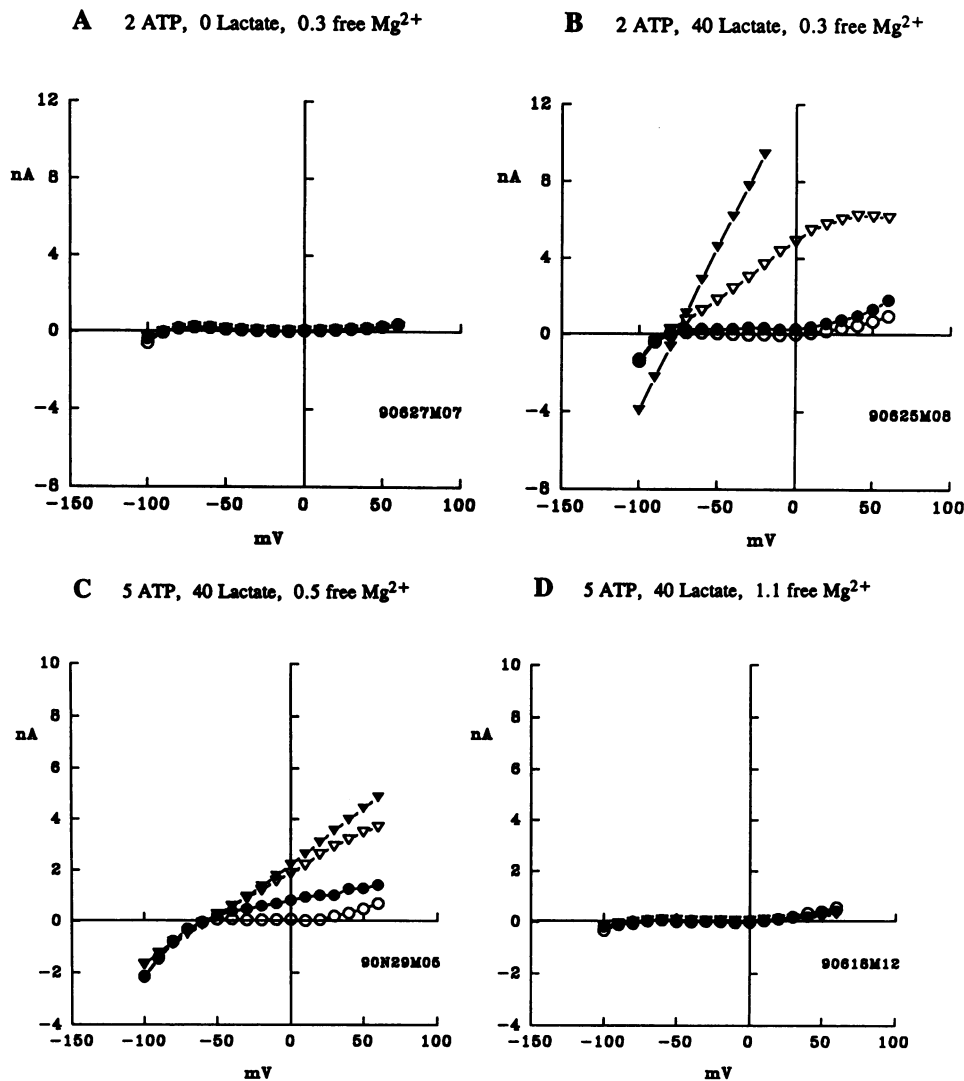


Figure 4. Effects of lactate and free Mg^{2+} on K_{ATP} channels in the presence of ATP. Current-voltage relations were obtained from myocytes perfused internally with internal solution containing lactate, ATP and free Mg^{2+} as indicated (in millimolar). (A) Without lactate, activation of K_{ATP} channels was not observed after 60 min of perfusion. Symbols: 1 min, open circles; 30 min, solid circles; 60 min, open triangles. (B and C) At 0.3 and 0.5 mM free Mg^{2+} , lactate-activated K_{ATP} channels in the presence of 2 and 5 mM ATP. Symbols for B: 5 min, open circles; 25 min, solid circles; 30 min, open triangles; 33 min, solid triangles. Symbols for C: 15 min, open circles; 30 min, solid circles; 40 min, open triangles; 50 min, solid triangles. (D) 1.1 mM free Mg^{2+} suppressed activation of K_{ATP} channels by lactate. Symbols: 15 min, open circles; 30 min, solid circles; 50 min, open triangles. The time indicated represents minutes after rupture of the membrane patches.

tion of K_{ATP} channel by lactate may require certain cytosolic components which may not be present in excised membrane patches. This may explain the apparent failure of lactate to activate K_{ATP} channel in excised rat heart cell membranes (3).

An interesting question arising from the present study is the functional significance of the magnesium sensitivity of K_{ATP} channels. Inhibition of K_{ATP} channels by Mg^{2+} has been reported (15, 16). In the present study, increasing Mg^{2+} to 1.1 mM in the presence of 5 mM ATP completely blocked lactate activation of K_{ATP} channels. It is generally agreed that intracellular free Mg^{2+} increases as cytosolic $MgATP$ concentration decreases during ischemia (23–25). Thus, lactate activation of K_{ATP} channels may be blunted by increased Mg^{2+} . On the other hand, as suggested by Wilde et al. (26), high extracellular K^+ during ischemia may partially remove the blockade produced by the increase in intracellular Mg^{2+} concentration (15). Indeed, when we increased extracellular K^+ to 20 mM, the inhibitory effect of Mg^{2+} on K_{ATP} channels was partially removed. In the presence of 2 mM ATP and 1.1 mM Mg^{2+} , 40 mM lactate activated K_{ATP} channels at 18 and 30 min in two out of two cells tested. Even at 5 mM ATP, K_{ATP} current occurred at 40 min in one out of three cells tested.

In conclusion, our results demonstrate that activation of K_{ATP} channels by intracellular lactate occurred in the presence of ATP levels similar to those maintained during moderate to severe myocardial ischemia. The ability of lactate to activate K_{ATP} channels under these conditions may provide some additional insight in the pathophysiological relevance of cardiac K_{ATP} channels.

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