ATP-Sensitive Potassium Channel Modulation of the Guinea Pig Ventricular Action Potential and Contraction

C.G. Nichols, C. Ripoll, and W.J. Lederer

The role of ATP-sensitive potassium (K\textsubscript{ATP}) channels in modulating the action potential and contraction of guinea pig ventricular myocytes was investigated. Under voltage clamp, the maximum whole-cell K\textsubscript{ATP} channel conductance was estimated (195±10 nS, n=6) by exposing the cells to complete metabolic blockade (2 mM cyanide in the presence of 10 mM 2-deoxy-glucose). In isolated inside-out membrane patches, the ATP dependence of K\textsubscript{ATP} channel activity under relevant conditions was measured (half-maximal inhibition at 114 μM). Under current clamp (with intracellular ATP concentration = 5 mM), the effect of graded K\textsubscript{ATP} channel activation on the action potential and the twitch was estimated by injection of a current (proportional to voltage) that simulated the K\textsubscript{ATP} conductance. As this “conductance” was increased, the action potential was shortened, and contractile amplitude declined, as expected. From the results of these experiments, the quantitative dependence of the action potential duration on intracellular ATP concentration was estimated, without relying on a mathematical model of the cell membrane. The results imply that K\textsubscript{ATP}-dependent action potential shortening is likely to occur if ATP concentration falls below normal levels (~5 mM), as may happen regionally, or globally, during myocardial ischemia. (Circulation Research 1991;68:280–287)

The shortening of the cardiac action potential that results from metabolic blockade has been attributed to the activation of ATP-sensitive potassium (K\textsubscript{ATP}) channels. However, the mechanism by which the current is activated is a matter of controversy since the measured ATP concentration ([ATP]) at the time of action potential shortening is still at, or near, normal levels. The density of K\textsubscript{ATP} channels in the cardiac membrane is high; estimates of channel density range from 0.5 to 10/μm\textsuperscript{2}. Consequently, the effects of channel opening on the action potential might occur with the activation of only a few channels, raising the possibility that, even though the reported \(k_{1/2}([\text{ATP}])\) causing half-maximal inhibition of channel activity for channel inhibition by ATP is in the micromolar range (17–100 μM), changes of [ATP] in the millimolar (i.e., physiological) range could affect action potential duration (APD) and contractile behavior. Indeed, Faivre and Findlay\textsuperscript{7} have recently reported that tolbutamide, a blocker of K\textsubscript{ATP} channels, prolongs the normal action potential in rat ventricle. Thus, the role of these channels in the pathophysiological regulation of APD and of cardiac excitability may have been underestimated (see reviews by Fozzard and Makielski,\textsuperscript{8} Ashcroft,\textsuperscript{9} and Janse and Wit\textsuperscript{10}). Ideally, one would like to simultaneously set the level of intracellular nucleotides (or measure them) and measure the action potential and the twitch, but such experiments have proved unsatisfactory in single cells.\textsuperscript{2,3,11} In whole-heart studies, such as those of Elliott et al.,\textsuperscript{3} intercellular chemical and electrical inhomogeneity could be a major problem, especially during metabolic inhibition. In the present study, we have adopted an alternative strategy to estimate the relation between K\textsubscript{ATP} conductance and APD in the single cell. We have used an active feedback circuit to inject current that is proportional to the membrane potential, thereby simulating the effects of the addition of a linear K\textsuperscript{+} conductance on APD and contraction. The results suggest that, even though the K\textsubscript{ATP} channel is half-maximally closed at micromolar levels of ATP, K\textsubscript{ATP}-dependent shortening of the action potential may occur if [ATP] falls at all below normal levels.
edg of the cell along one horizontal video line. The cell length is reported as a voltage signal that is proportional to the distance between the ends of the cell. The second chamber, based on a design by Qin and Noma,14 is described by Lederer and Nichols;6 this chamber allows very rapid changes of the solution bathing the intracellular surface of an isolated inside-out membrane patch. Microelectrodes (1–2 MΩ for current clamp, 2–5 MΩ for isolated membrane patches) were pulled from filaminted borosilicate glass (1.5-mm o.d., No. TW150F-6, World Precision Instruments, New Haven, Conn.) on a horizontal puller (BB-CH Mechanex, Geneva). Cells, or membrane patches, were current- or voltage-clamped using a whole-cell clamp (model 8900, Dagan Corp., Minneapolis, Minn.). Micropipettes were sealed onto cells by applying suction to the rear of the pipette. Low-resistance access to the cell interior (break-in) was achieved by applying greater suction to rupture the patch of cell membrane under the tip of the pipette.15

**Bathing Solutions**

*Whole-cell experiments.* The cells were dialyzed with pipettes containing the following (mM): potassium glutamate 140, KCl 5 or 10, HEPES 5 or 10 (titrated to pH 7.1 with NaOH), and MgATP 5, pH 7.1. In the metabolic blockade experiments, ATP was omitted from the dialyzing solution, and 1 mM MgCl2 was included. In the experiments to measure the maximum conductance in metabolic blockade, 1 mM potassium EGTA was also included in the dialyzing solution. The cells were bathed in a solution of the following composition (mM): NaCl 140, KCl 4, MgCl2 1, CaCl2 1, sodium HEPES 10, and glucose 10. The solution pH was 7.4. In metabolic blockade experiments, glucose was replaced by 10 mM 2-deoxyglucose at least 20 minutes before exposure to cyanide. NaCN (2 mM) was added from a concentrated solution (200 mM NaCN and 670 mM HEPES) immediately before use. This minimized the loss of HCN gas from the solution before use. In the experiments to measure the maximum conductance in metabolic blockade, CaCl2 was excluded from the bathing solution.

*Inside-out patch experiments.* Pipettes were filled with an extracellular solution of the following composition (mM): NaCl 140, KCl 4, CaCl2 1, and sodium HEPES 5, buffered to pH 7.25. The “intracellular” (bath) solution contained (mM) KCl 140, potassium HEPES 10, free Mg2+ 0.5, K2ADP 0.2, K2GDP 0.05, and potassium EGTA 1. Solution pH was 7.25. [ATP] was varied as described in “Results.”

**Results**

*The Effects of KATP Channel Activation on the Action Potential and the Twitch*

Current-clamped action potentials were elicited by brief depolarizing pulses (5 msec). In control conditions, APD at −60 mV (APD60) was 302±24 msec.

**Materials and Methods**

*General*

Isolated ventricular myocytes were obtained from adult guinea pig hearts by established enzymatic dissociation techniques.12 The experiments were performed at room temperature in one of two experimental chambers mounted on the stage of an inverted microscope (Nikon Diaphot, Nikon Inc., Garden City, N.Y.). The first chamber, used in whole-cell voltage-clamp experiments, allowed rapid solution changes.13 Video images of the cell were obtained using a video camera and recorded for subsequent analysis. Continuous recordings of cell length were obtained from a video dimension analyzer (Crescent Electronics, Utah), which tracks both
Figure 1 illustrates the effect of metabolic inhibition on action potential shape and twitch shortening. The action potential shortening develops slowly at first and then accelerates until the only remnant depolarization is the stimulus pulse itself (Figure 1A). The action potential shortens at all potentials. It is possible that decreased calcium currents or sodium currents may also contribute to the decline in APD. Decreased calcium currents\(^ {16} \) and sodium currents (C.G. Nichols, unpublished results) are observed in metabolic blockade, although they are temporally preceded by increased outward K\(^ + \) current.\(^ {2} \) Since the changes in APD can be reversed by glibenclamide (a blocker of the K\(_{ATP} \) channel without effect on calcium currents) in many experiments, these changes in APD may be attributed to the activation of K\(_{ATP} \) channels,\(^ {17} \) and the changes in twitch shortening are attributed to the changes in APD.\(^ {12} \)

The Maximum Conductance Available Through K\(_{ATP} \) Channels

Figure 2 shows the results of an experiment designed to measure the maximal conductance available through K\(_{ATP} \) channels. On exposure to metabolic blockade, there was a very large increase in conductance that was quasi-linear; this increase reversed at approximately -75 mV (Figure 2B). On removal of metabolic blockade, partial reversal of the current changes is observed. In these experiments, series resistance compensation was used. The uncompensated series resistance was less than 1 M\( \Omega \).\(^ * \) A linear fit was obtained, and in six cells, the maximum conductance through K\(_{ATP} \) channels was 195±10 nS. In four experiments, the maximum conductance may have been underestimated, since current run-down (i.e., a slow decline in conductance\(^ {11,18} \) was evident as soon as the conductance maximum was reached.

The ATP Dependence of Guinea Pig Ventricular K\(_{ATP} \) Channels

The ATP dependence of guinea pig K\(_{ATP} \) channels has been measured\(^ {4} \) in the absence of other nucleotides. In rat ventricular myocytes, MgADP and MgGDP both increase K\(_{ATP} \) channel activity at a given level of ATP and shift the ATP dependence of channel opening to higher [ATP].\(^ {6,19} \) We have therefore measured channel activity in likely physiological concentrations of 200 \( \mu \)M ADP,\(^ {20} \) 50 \( \mu \)M GDP, and 0.5 mM free Mg\(^ {2+} \).\(^ {21} \) Figure 3A shows K\(_{ATP} \) channel activity (approximately 25 channels are present) in an isolated inside-out membrane patch on exposure to different [ATP]. The channel open probability (P\(_{o} \)) approaches one in zero ATP and is close to zero in 4 mM ATP. The activity of a single ATP-independent K\(^ + \) channel is present in the exposure to 1 mM ATP and the second exposure to 4 mM ATP. Such channels were occasionally

\( * \) Nevertheless, even with a series resistance of 1 M\( \Omega \), a current of 13 nA (mean current observed at 0 mV) would still cause a 13-mV voltage error. It is imperative to account for series resistance as fully as possible in experiments measuring such large conductances. However, we expect that the measured conductance will be underestimated somewhat and therefore provides only a lower-limit estimate of the maximum conductance during metabolic blockade.
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3. Activity (I)

The ATP dependence of ATP-sensitive potassium channel activity in inside-out membrane patches in the presence of 50 μM GDP, 200 μM ADP, and 1 mM free Mg²⁺ concentration. Panel A: Current recording illustrating the protocol used to obtain the data. The patch was moved from a solution containing 4 mM ATP (zero current) into one containing zero ATP (maximum current) and then into two test solutions (in this case, containing 0.1 and 1 mM ATP before moving back to 0 and 4 mM ATP solutions). ATP concentration ([ATP]) is given above the recording. The current in each solution (ignoring the first 3 seconds) was averaged. Panel B: Graph showing dose–response relation for ATP inhibition of ATP-sensitive potassium channels. Ordinate shows mean patch current in test [ATP] as a fraction of that in zero [ATP]. Data show mean±SEM (where SEM is greater than the symbol); n=4 to 8 in each case. The solid line is fitted according to Equation 1 in the text.

observed in patches. Their contribution to patch current was insignificant (<1%), and their contamination was ignored in analysis. Under these conditions (Figure 3), the ATP dependence of channel activity (I) could be described by a sigmoid relation (Figure 3B):

I=I_{\text{max}}[1+([\text{ATP}]k_{12})^H] \tag{1} where $k_{12}=114$ μM, $H=2$, and $I_{\text{max}}$ is the maximum current observed in zero ATP. $I_{\text{max}}$ corresponds to $P_o$ close to one in these experiments. In the absence of ADP, GDP, or magnesium, the channel activity was fit by $k_{12}=79$ μM.²²

The Effect of $K_{\text{ATP}}$ Current on the Action Potential and Twitch: Estimation by Current Injection

APD declines rapidly during metabolic inhibition, too fast to switch between current- and voltage-clamp to measure the $K_{\text{ATP}}$ conductance responsible for a given amount of action potential shortening. It is also possible that other membrane currents are being affected in this or other pharmacological maneuvers used to activate $K_{\text{ATP}}$ conductance. For this reason and because we want to know the effect of the activity of the $K_{\text{ATP}}$ channel alone on APD and twitch, we have carried out the following series of experiments. APD and twitch shortening were measured under current-clamped conditions. Intracellular ATP (ATP) was unchanged so that none of the metabolic consequences of altered ATP were observed, but by injecting current equivalent to the $K_{\text{ATP}}$-dependent current ($I_{\text{ATP}}$), the effects of $I_{\text{ATP}}$ on APD could be assessed. The actual current injected was linearly related to the membrane potential ($V_m$), with reversal at −75 mV, close to the $K_{\text{ATP}}$ current reversal potential (EATP), and was scaled by the conductance factor ($G_{\text{ATP}}$):

$$I_{\text{ATP}}=G_{\text{ATP}}(V_m-E_{\text{ATP}}) \tag{2}$$

$G_{\text{ATP}}$ was proportional to the $P_o$ of the “simulated” ATP:

$$G_{\text{ATP}}=g \cdot n \cdot P_o \tag{3}$$

where $g$ is single channel conductance, $n$ is the maximum number of channels, and $P_o$ is equal to the relative current (I) in Equation 1, since $I_{\text{max}}$ corresponds to $P_o=1$.

As the $G_{\text{ATP}}$ was increased, the action potential shortened (Figures 4A and 4B) in a manner similar to the action potential shortening produced by the activation of $K_{\text{ATP}}$ channels after metabolic blockade (Figure 1). As $G_{\text{ATP}}$ increased and the action potential shortened, the twitch amplitude declined (Figures 4A and 4B), again resembling the decline of the twitch amplitude seen with metabolic blockade. This demonstrates that the action potential shortening is indeed adequate to explain the decline of the twitch in metabolic blockade, as proposed by Lederer et al.¹² and Stern et al.²³ Figure 5A shows plots of APD and twitch shortening for two experiments in which $K_{\text{ATP}}$ conductance was activated by metabolic blockade and for three experiments in which simulated $K_{\text{ATP}}$ current was injected. Both sets of data could be described by the same linear relation. Action potential failure and the cessation of mechanical activity typically occurred at injected conductance of more than 10 nS. Figure 4C shows the injected conductance plotted versus the membrane potential for the experiment shown in Figures 4A and 4B. A linear fit to the current–voltage relation was used to determine the conductance ($G_{\text{ATP}}$) responsible for a given action potential shortening and decline in twitch shortening. Where necessary, the error in the measured membrane potential (due to the drop in potential across the electrode tip as current is injected) was corrected. However, care was taken to minimize series resistance after break-in, and in the worst cases, the voltage error (before correction) was always less than.
FIGURE 4. The effect of injection of simulated ATP-sensitive potassium channel current on the electrical and mechanical behavior of isolated guinea pig ventricular myocytes. Panel A: Slow-time base recording of injected current (top recording), membrane potential (second recording), action potential duration (APD) at −60 mV (third recording), and cell shortening (bottom recording). The simulated ATP-sensitive potassium channel conductance was increased stepwise. This caused the APD to shorten and twitch amplitude to decrease. Panel B: Superimposed fast-time base recordings of injected current (top recording), action potentials (middle recording), and twitches (bottom recording) from the times (i-xi) indicated in panel A. Panel C: Plots of injected current versus membrane potential (during the plateau of the action potential) from the recordings in panel B above. A linear fit to each curve gives the simulated ATP-sensitive potassium channel conductance in each case.
5 mV. Figure 5B shows that a monotonically declining relation existed between APD and $G_{\text{ATP}}$ in five experiments. The empirical curve was a least-squares fit according to Equation 3:

$$\text{APD}=1/[1+(G_{\text{ATP}}/G_{1/2})^S]$$  \hspace{1cm} (4)

where $G_{1/2}=1.36$ nS and $S=1.2$. By combining Equations 1, 2, and 4 and assuming a maximum $G_{\text{ATP}}$ (195 nS), we may predict a relation between APD and $[\text{ATP}]$ (Figure 5C).

**Discussion**

*What is the ATP Dependence of the APD?*

When metabolism is inhibited in cardiac cells, APD and the twitch amplitude decline to undetectable levels (Figure 1). Potentially, the changes in APD could result from the activation of any outward current or the decline of any inward current. Voltage-clamp studies indicate that an increase in outward current is primarily responsible.\(^2\) Reversal of the action potential shortening and increased outward current by injection of ATP\(^{12,16}\) or application of glybenclamide\(^1\) provides reasonable evidence that these effects occur because of the activation of $K_{\text{ATP}}$ channels, although whether or not decreased ATP is responsible for channel activation is a matter of controversy.\(^1\) We propose that a fall in ATP is responsible for channel activation under these conditions, since it has been shown that injection of ATP can reverse the action potential shortening and contractile failure that result from metabolic inhibition.\(^12,16\) It is clearly important to answer the following question: How does APD depend on $K_{\text{ATP}}$ channel activity and $[\text{ATP}]$? However, at the present time it is not possible to measure simultaneously intracellular nucleotide levels and electrical or mechanical parameters in single cells. It has also proven very difficult to control the level of nucleotides; therefore, there is no direct way to correlate the $[\text{ATP}]$, and APD at the single cell level. We have previously used a computer simulation (oxisoft, D. Noble, Oxford, England) to model the effects of $K_{\text{ATP}}$ channel activation on the rat heart action potential.\(^3\) There are many adjustable parameters in such a mathematical model, and a guinea pig version of the model is currently available. In the present study, we have used a more direct approach to determine the effects of $K_{\text{ATP}}$ channel activation on the guinea pig ventricular action potential and contraction. The current through $K_{\text{ATP}}$ channels over the physiological membrane voltage range (−80 to +20 mV; Figure 2) is quite well described by a time-independent, linear conductance. We have used these features to mimic the effect of current activation on APD in the intact cell. This approach uses the real cell and therefore has obvious advantages over the use of a computer simulation of current activation. A previous study\(^24\) investigating the effects of current injection on the action potential has used constant-current pulses. By feeding-back an appropriately scaled and offset

![Figure 5. Panel A: Graph showing the relation between twitch shortening (relative to control) and action potential duration (APD) at −60 mV (relative to control) from experiments in which ATP-sensitive potassium channel activity was increased by metabolic blockade (open symbols) and from experiments in which simulated ATP-sensitive potassium conductance was used to shorten the action potential (filled symbols). Different symbols correspond to different experiments. Panel B: Graph showing the relation between APD at −60 mV (relative to control) and simulated ATP-sensitive potassium conductance from experiments of the type shown in Figure 3. Different symbols correspond to different experiments. The curve was a least-squares fit according to Equation 3 in the text. Panel C: Graph showing 1) the relation between APD at −60 mV and the ATP concentration ([ATP]) inferred from combining the relations in Figures 3B and 5B (solid line) as discussed in the text and 2) the relation between fractional channel closure (i.e., 1 minus relative patch current) and [ATP] (dashed line) from Figure 3B.](http://circres.ahajournals.org/)

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membrane potential to the current command input, our experiments have been able to assess directly the contribution of a linear conductance to APD. With appropriate circuitry, one could mimic the effect of more complex, nonlinear conductances.

**At What [ATP] Will the APD Begin to Shorten?**

Given the ATP dependence of \( K_{ATP} \) channel activity in inside-out membrane patches under physiological conditions (Figure 3, \( k_{1/2}=114 \) μM, \( H=2 \)) and the maximum current available (\( G_{ATP}=195 \) nS) through K\(_{ATP}\) channels (Figure 2), our results permit the estimation of the ATP dependence of APD and twitch amplitude in guinea pig ventricular myocytes. We measured 50% shortening of the APD at an injected conductance of 1.3 nS, which was 0.70% of our measured estimate of the maximum available conductance. Given the measured ATP dependence of channel activity (Figure 3), 0.7% of maximum channel activity would occur at 1.36 mM ATP, more than an order of magnitude higher than the \( k_{1/2} \) for channel activity. Ten percent shortening of the APD would occur at 0.13% of maximum channel activity, corresponding to 3.1 mM ATP* (Figure 5C). The values obtained in this analysis depend on many different parameters measured with varying accuracy. Nevertheless, we can place confidence in the prediction that even at normal [ATP] (≈5 mM)\(^+\) changes in [ATP] will begin to alter channel activity and affect the action potential (Figure 5C). Consistent with this argument, Faivre and Findlay\(^7\) have recently demonstrated that tolbutamide (a blocker of K\(_{ATP}\) channels) prolongs the action potential under normal metabolic conditions. The contractile activity (Figure 5A) and, hence, energy consumption (by Ca\(^++\) pumps and actomyosin ATPase) are very sensitive to APD. Our results argue that the K\(_{ATP}\) channel system, far from being a vestigial one with an ATP sensitivity that is too high for the channel to be of physiological importance, may be part of a powerful negative-feedback system that will protect the cell against transient compromise of energy supply as may occur in pathophysiological and clinical conditions of metabolic impairment.

Our results, although predicting action potential shortening with decreased [ATP] in the millimolar range, do not fully account for the observation that action potential shortening can be observed with [ATP] changes of approximately 20%.\(^1\) We hypothesize that cell-to-cell or regional inhomogeneity of [ATP] may provide the remaining explanation. The possibility that the cell membrane senses a different pool of ATP than the rest of the cytoplasm has been suggested, or implied, by many authors.\(^26\)–\(^31\) Noma and Shibasaki\(^2\) have considered the possibility that, after depletion of creatine phosphate, the ATP that is hydrolyzed in support of contraction may not be phosphorylated within each cardiac beat.\(^32\) It has also been suggested that [ATP] concentrations may not be homogeneous between cells in the heart, especially under conditions of ischemia or hypoxia (see review by Allen and Orchard\(^33\)). We have previously discussed in depth\(^3\) the implications of intercellular or intracellular inhomogeneity of [ATP] or electrical activity on the kinds of relations discussed above. In a multicellular preparation, any of these inhomogeneities will tend to lead to K\(_{ATP}\) channel activity at higher mean tissue [ATP] than it would in the case of uniform distribution of ATP. Thus, it seems entirely likely that small reductions in [ATP], measured in intact hearts\(^1\) could cause action potential shortening through activation of K\(_{ATP}\) channels.

**Conclusions**

The results of this study suggest that even very small increases in the \( P_o \) of K\(_{ATP}\) channels (<1%), which therefore require small decreases of [ATP], will result in significant shortening of the guinea pig cardiac APD and reduction of the twitch amplitude. This finding has important implications for the role of K\(_{ATP}\) channel activity in the electrical and chemical derangements resulting from metabolic compromise in mild or severe myocardial ischemia.

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**References**


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