Activation of ATP-Sensitive K⁺ Channels by Cromakalim
Effects on Cellular K⁺ Loss and Cardiac Function in Ischemic and Reperfused Mammalian Ventricle

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Pharmacological modulation of [K⁺], accumulation and action potential changes during acute myocardial ischemia is under evaluation as a promising new antiarrhythmic and cardioprotective strategy during myocardial ischemia and reperfusion. We studied the effects of cromakalim, a K⁺ channel opener that activates ATP-sensitive K⁺ channels, in isolated arterially perfused rabbit interventricular septa subjected to ischemia and reperfusion and, through use of the patch clamp technique, in inside-out membrane patches excised from guinea pig ventricular myocytes. During aerobic perfusion, 5 μM cromakalim shortened action potential duration (APD) from 217±7 to 201±10 msec, had no effect on [K⁺], and reduced tension by 17±3% (n=11). During ischemia, pretreatment with 5 μM cromakalim resulted in 1) more rapid APD shortening (71±9 versus 166±7 msec at 10 minutes and 63±12 versus 122±8 msec at 30 minutes), 2) similar [K⁺], accumulation after 10 minutes (8.9±0.3 versus 9.6±0.5 mM) but a trend toward increased [K⁺], accumulation after 30 minutes (11.0±1.7 versus 9.6±1.0 mM), and 3) similar times for tension to decline to 50% of control (2.14±0.16 versus 2.14±0.19 minutes) but shorter time to fall to 20% of control (4.34±0.33 versus 4.90±0.22 minutes; p=0.003). After 60 minutes of reperfusion following 30 minutes of ischemia, recovery of function was similar, with a trend toward better recovery of developed tension (to 58±9% versus 39±10% of control; p=0.18) and tissue levels in cromakalim-treated hearts but no differences in APD or rest tension. Thus, 5 μM cromakalim had mild effects in normal heart but greatly accelerated APD shortening during ischemia without markedly increasing [K⁺], accumulation, possibly because the more rapid APD shortening reduced the time-averaged driving force for K⁺ efflux through ATP-sensitive K⁺ channels. A significant cardioprotective effect during 30 minutes of ischemia plus 60 minutes of reperfusion could not be demonstrated in this model. In excised membrane patches studied at room temperature, the ability of cromakalim to activate ATP-sensitive K⁺ channels was significantly potentiated by 100 μM but not 15 μM cytosolic ADP, suggesting that in addition to the modest fall in cytosolic ATP during early ischemia, the rapid increases in cytosolic ADP may further sensitize cardiac ATP-sensitive K⁺ channels to activation by cromakalim. This factor may contribute to the marked acceleration of APD shortening during ischemia produced by concentrations of cromakalim that have minimal effects on APD in aerobic perfused tissue. Activation of ATP-sensitive K⁺ channels by cromakalim was not significantly affected by other ischemic factors such as lactate (20 mM), Pi (10 mM), or acidosis (pH 6.5). (Circulation Research 1992;71:1324–1333)

Key Words • myocardial ischemia • ATP-sensitive potassium channels • K⁺ channel openers • cromakalim • patch clamp

Increasing evidence suggests that activation of ATP-sensitive K⁺ (K₂ATP) channels plays an important role in extracellular K⁺ concentration ([K⁺]₀) accumulation and electrophysiological changes during early ischemia.⁠¹⁻⁵ The resulting cellular depolarization, slow conduction, and altered refractoriness are major factors predisposing the ischemic heart to lethal reentrant arrhythmias—the leading cause of death from coronary artery disease.⁠⁶⁻⁷ However, whether activation of K₂ATP channels has only deleterious effects during ischemia is not clear. Inhibition of the Na⁺ pump as cytosolic ATP (ATP) is progressively depleted would eventually lead to cellular K⁺ loss, membrane depolarization, and other electrophysiological changes predisposing the heart to the development of similar reentrant arrhythmias, even in the absence of K₂ATP channel activation. It could be argued that by accelerating the development of inexcitability, activation of K₂ATP chan-

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nels may shorten the time window during which the ischemic heart is susceptible to ischemic arrhythmias. Also, the more rapid rate of action potential duration (APD) shortening and earlier onset of inexcitability has an energy-sparing effect by reducing contractile force and cytosolic Ca2+ cycling during ischemia. Consistent with this cardioprotective action, the KATP channel agonists have been reported to exert cardioprotective effects during ischemia.8-12 The purpose of the present study was to characterize in greater detail the effects of cromakalim, a KATP channel agonist, on [K+]o accumulation, action potential configuration, and contractile and metabolic performance during total global ischemia and reperfusion in isolated arterially perfused rabbit interventricular septa and to evaluate its cardioprotective potential in this model. Factors potentially influencing KATP channel activation by cromakalim during ischemia were also studied through the use of patch-clamp techniques in excised membrane patches from guinea pig ventricular myocytes.

**Materials and Methods**

**Isolated Intact Heart Experiments**

**Preparation and experimental setup.** New Zealand White rabbits of either sex (weight, 2–3 kg) were anesthetized with pentobarbital sodium after heparinization, and the hearts were removed through a thoracotomy incision. The septal branch of the left coronary artery was cannulated, and interventricular septum was isolated and mounted in a nitrogen-filled chamber maintained at 37°C, as described previously.13 For tension measurements, a ligature placed at the apex of the septum was tied to a tension transducer. The hearts were paced at 75 beats per minute through a bipolar platinum electrode. Arterial perfusion was maintained at a rate of 1.75 ml/min at 37°C with a perfusion pump. Venous effluent samples were collected through polyethylene tubing anchored at the base of the septum. The arterial perfusate consisted of (mM) NaCl 120, KCl 4, CaCl2 1.5, NaHCO3 25, NaH2PO4 0.44, MgCl2 1, dextrose 5.6, and 10 units/l insulin. pH was maintained at 7.3–7.4 by gassing the perfusate with 5% CO2 and 95% O2. Total global ischemia was produced by turning off the perfusion pump. Each preparation was allowed to equilibrate for at least 1 hour before any experimental intervention.

**Electrodes.** Intracellular potential was monitored using floating glass microelectrodes filled with 3 M KCl.14 [K+]o was monitored continuously with intramyocardial valinomycin K+-selective minielectrodes.15 K+-selective electrodes were calibrated in vitro before and after removal from the heart at 37°C in the standard arterial perfusate with different K+ concentrations (4, 8, 10, and 12 mM with KCl replacing NaCl isotonically) and also in situ by transiently increasing the perfusate K+ concentration to 10 mM after insertion of the electrode in the muscle. The results were discarded if the slope per 10-fold change in K+ was less than 56 mV at 37°C or if the in situ response differed by more than 10% from that obtained during in vitro calibration. For convenience the results are reported in concentration rather than activity units, assuming the activity coefficient of the calibration solutions did not differ significantly from that of the extracellular fluid surrounding the tip of the K+ electrode. The K+ electrode was typically positioned with 2–3 mm of the floating microelectrode.

**Metabolic assays.** To measure the tissue high-energy phosphates the septum was quickly smash-frozen between copper tongs precooled in liquid N2 and then immersed in liquid N2. Tissue content of ATP, ADP, and AMP were determined by high-performance liquid chromatography from ≈200-mg tissue samples extracted with 3 M perchloric acid after pulverization.15 The lactate content was determined by spectrophotometric techniques.15 Lactate content was measured in samples of venous effluent collected for 30-second intervals.

**Isolated Ventricular Myocyte Experiments**

**Cell isolation.** Guinea pigs (weight, 200–300 g) were anesthetized with pentobarbital sodium and the hearts removed through a thoracotomy incision. Hearts were mounted on a Langendorff perfusion apparatus, and enzymatic digestion was performed by retrograde perfusion of collagenase and protease through the aorta as described by Mitra and Morad.16

**Patch clamp methods.** Cells were placed in a shallow, 0.5-ml capacity chamber mounted on the stage of an inverted microscope and were superfused at a rate of 1–4 ml/min with modified Tyrode’s solution consisting of (mM) KCl+KOH 150, CaCl2 0.5, EGTA 2, MgCl2 2, MgATP 2, and HEPES 5, pH 7.1. All experiments were performed at room temperature (21–25°C). Patch electrodes (tip diameter, 1–2 μm; resistance, 2–4 MΩ) were pulled from Corning 8161 glass and filled with an internal solution consisting of (mM) KCl 4, NaCl+NaOH 145, and HEPES 5, pH 7.35. Patch electrodes were mounted to the headstage of an Axopatch 1B or 200 patch-clamp amplifier. Membrane current and voltage signals were recorded on a chart recorder and videocassette recorder and later digitized and analyzed by computer using Axotape software (Axon Instruments). A 10-channel rapid perfusion device4 was used for quick solution changes (half-time <100 msec) at the intracellular side of the patch (facing the bath). The patch electrode potential was held at 0 mV to record outward currents through KATP channels and to avoid contamination by the inwardly rectifying K+ channels. Patches in general contained 10–40 channels. KATP channels were activated maximally before and after each intervention by exposure to ATP-free solution. Because rundown of KATP channels is a common problem, only experiments in which the control level of current in ATP-free solution after an intervention returned to >80% of the preintervention value were accepted for data analysis.

**Drugs and Chemicals**

Chemicals were obtained from Sigma Chemical Co., St Louis, Mo., and cromakalim was generously provided by Beecham Pharmaceuticals, Bristol, UK. Cromakalim was dissolved in dimethyl sulfoxide to make a stock solution of 100-mM concentration in the isolated myocyte experiments and in ethanol to make a stock solution of 10 mM in the isolated heart experiments and was added immediately before the experiment at the appropriate dilution to achieve the desired concentration in the perfusate.
**Data Analysis**

Student’s t test was used to compare groups with the Bonferroni correction when appropriate. Variance between groups was tested and compensated for when necessary. Differences were considered significant at \( p<0.05 \).

**Results**

**Effects of Cromakalim During Early Ischemia (First 10 Minutes)**

To examine the effects of cromakalim on the early phase of \([K^+]_o\) accumulation, APD shortening, and contractile performance during ischemia, five rabbit interventricular septa paced at 75 beats per minute were subjected to three successive 10-minute episodes of total global ischemia separated by 30-minute recovery periods (Figures 1 and 2). The middle ischemic episode was preceded by perfusion with cromakalim for 10 minutes. During aerobic perfusion, 10 \( \mu M \) cromakalim markedly shortened the APD and decreased developed tension by >75\%. However, 5 \( \mu M \) cromakalim only mildly shortened APD by 7\% from 217±7 to 201±10 msec, reduced developed tension by 17±3\%, and had no detectable effect on \([K^+]_o\). Therefore, we chose 5 \( \mu M \) as the concentration to examine during ischemia. During ischemia, APD shortening was significantly more rapid in the presence of 5 \( \mu M \) cromakalim, declining to 71±9 versus 166±7 msec after 10 minutes (\( p<0.001 \)). However, cromakalim did not significantly alter the magnitude of \([K^+]_o\) accumulation after 10 minutes of ischemia (8.9±0.3 versus 9.6±0.5 mM; \( p=0.20 \)). Cromakalim had no effect on the initial rate of decline of developed tension, with time for tension to fall to 50\% occurring after 2.14±0.19 and 2.14±0.16 minutes in the presence and absence of the drug, respectively (Figure 2). However, the time for tension to fall to 20\% of the preischemic value was significantly shorter in the cromakalim-treated hearts (4.34±0.33 versus 4.90±0.22 minutes; \( p=0.003 \)).

**Effects of Cromakalim During Prolonged Ischemia Followed by Reperfusion**

To assess the effects of cromakalim on recovery of function after a prolonged ischemic episode, hearts were subjected to a single 30-minute period of total global ischemia, either in the absence (\( n=6 \)) or presence (\( n=6 \)) of a 10-minute pretreatment with 5 \( \mu M \) cromakalim. Both groups were subsequently reperfused for 60 minutes in the absence of cromakalim to assess recovery of function (Figure 3).

**Effects during ischemia.** As in the hearts subjected to 10 minutes of ischemia, cromakalim caused more rapid APD shortening during ischemia, to 63±12 versus 122±8 msec at 30 minutes (\( p<0.01 \)). Cromakalim had no significant effect on the initial rate of \([K^+]_o\) accumulation during the first 10 minutes of ischemia (8.4±0.4 versus 8.5±0.4 mM with and without cromakalim, respectively) but tended to accelerate the later rise in \([K^+]_o\) (11.0±1.7 versus 9.6±1.0 mM; \( p>0.05 \)). Although this difference did not reach statistical significance, the pattern of \([K^+]_o\) accumulation was different in the cromakalim-treated hearts. \([K^+]_o\) accumulation during...
ischemia in this and other preparations is typically triphasic,\textsuperscript{1,13,17} having an early and late rise separated by a plateau phase. In the control hearts, $[K^+]_o$ was still in the plateau phase after 30 minutes of ischemia. In the cromakalim-treated hearts, however, $[K^+]_o$ increased progressively by 30 minutes of ischemia, due to a marked abbreviation of the plateau phase and early onset of the late rise. The decline in developed tension during early ischemia was more rapid in the cromakalim-treated hearts, but by 30 minutes developed tension was completely abolished in both cases. The increase in rest tension during ischemia was similar in both groups (4.1±0.7 versus 3.4±0.6 g after 30 minutes of ischemia in the cromakalim-treated and untreated hearts respectively; $p>0.05$).

Reperfusion. After 60 minutes of reperfusion, APD was restored to a similar degree in the cromakalim-treated and control hearts (227±8 versus 215±14 msec), and $[K^+]_o$ remained elevated to a similar extent (5.1±1.0 versus 4.7±0.3 mM with and without cromakalim). Cromakalim-treated hearts showed a tendency toward better recovery of developed tension (to 58±9% versus 39±10% of the preischemic level; $p=0.18$), whereas the return of rest tension toward the preischemic level was similar in both groups (increase in rest tension over the preischemic value of 1.96±0.81 versus 1.69±0.82 g, respectively; $p>0.05$). Cromakalim had no effect on venous effluent lactate content during control aerobic perfusion, but during the first 2 minutes of reperfusion, venous lactate content was 30% higher in the cromakalim-treated group (0.77±0.05 versus 0.58±0.03 $\mu$mol/g per minute; $p<0.01$), suggesting that cromakalim increased lactate accumulation during ischemia and/or increased lactate production or transmembrane transport during the first few minutes of reperfusion. The effects of cromakalim on tissue content of high energy phosphates after 60 minutes of

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Summary of the effects of 5 $\mu$M cromakalim during ischemia on action potential duration at 90% repolarization (APD$_{90}$), $[K^+]_o$, and developed tension (DT) normalized to the preischemic control value (DT$_o$). Results are mean±SEM for five hearts subjected to the protocol in Figure 1, with the open squares representing the values during ischemia in the presence of cromakalim and the filled symbols before (triangles) and after (squares) exposure to the drug. Temperature, 37°C; heart rate, 75 beats per minute.

![Figure 3](http://circres.ahajournals.org/)

**Figure 3.** Effects of 5 $\mu$M cromakalim on action potential duration at 90% repolarization (APD$_{90}$), $[K^+]_o$, developed tension (DT) normalized to the preischemic control value (DT$_o$), rest tension normalized similarly (RT/RT$_o$), and effluent lactate content in hearts subjected to 30 minutes of ischemia plus 60 minutes of reperfusion. Values are mean±1 SEM for six hearts in the absence (control, open triangles) and six hearts in the presence of cromakalim (filled dots). Cromakalim was not present during reperfusion. Temperature, 37°C; heart rate, 75 beats per minute.
reperfusion are summarized in Figure 4. Tissue ATP content was not significantly different, although there was a trend toward improvement in the cromakalim-treated hearts (0.80±0.09 versus 0.52±0.10 μmol/g wet wt.; p>0.05). There were no significant differences in tissue ADP or AMP levels.

Effects of Cromakalim on K\textsubscript{ATP} Channels in Excised Membrane Patches

The observation that cromakalim had little effect on APD under aerobic perfused conditions but considerably accelerated the rate of APD shortening during ischemia is consistent with observations in excised membrane patches that cromakalim more effectively activated K\textsubscript{ATP} channels at low [ATP], than at high [ATP].

It is also possible that other factors in the ischemic environment may facilitate activation of K\textsubscript{ATP} channels by cromakalim. [ADP] increases substantially during ischemia to the range of ≈100 μM,\textsuperscript{5,19} and we previously found that ADP, in the presence of Mg\textsuperscript{2+} markedly interfered with the ability of sulfonylurea antagonists to block K\textsubscript{ATP} channels.\textsuperscript{4} We therefore investigated whether physiological (≈15 μM) or ischemic (≈100 μM) [ADP], (in the presence of 2 mM Mg\textsuperscript{2+}) altered the ability of cromakalim to activate K\textsubscript{ATP} channels. We also studied whether other components of the ischemic environment (lactate, \textit{P}, and low pH) affect the activation of K\textsubscript{ATP} channels by cromakalim.

Dose-response of K\textsubscript{ATP} channels to cromakalim. Inside-out patches were excised from guinea pig ventricular myocytes. Experiments were performed at room temperature with physiological 4 mM [K\textsuperscript{+}], 150 mM [K\textsuperscript{+}], and membrane potential held at 0 mV to generate outward currents through the K\textsubscript{ATP} channels. Upon removal of ATP from the bath (perfusing the cytoplasmic surface of the membrane patch), K\textsubscript{ATP} channels opened and had a unitary current amplitude of ≈1 pA at 0 mV in the presence of 2 mM Mg\textsuperscript{2+}. Addition of 100 μM ATP to the bath decreased the current through K\textsubscript{ATP} channels (I) significantly relative to the current in ATP-free solution (I\textsubscript{max}), with I/I\textsubscript{max} averaging 0.33±0.04 (Figure 5). In the presence of 100 μM ATP, 5 and 30 μM cromakalim were only mildly effective at stimulating K\textsubscript{ATP} channels, consistent with the previously reported reduced sensitivity of K\textsubscript{ATP} channels to the drug at room temperature compared with 37°C.\textsuperscript{20} Cromakalim at 100 μM, however, increased I/I\textsubscript{max} more consistently, from 0.33±0.04 to 0.66±0.06 in the presence of 100 μM ATP, (p<0.05). When ATP was further increased to 300 or 1,000 μM, 100 μM cromakalim also significantly increased I/I\textsubscript{max} (Figures 6 and 7), from 0.08±0.02 to 0.23±0.05 (p<0.05) for 300 μM ATP, and from 0.02±0.004 to 0.06±0.005 (p<0.005) for 1,000 μM ATP.

Effects of ADP on cromakalim-induced activation of K\textsubscript{ATP} channels. Figures 6 and 7 illustrate the effects of 100 μM ADP, (with 2 mM Mg\textsuperscript{2+}) on cromakalim-induced activation of K\textsubscript{ATP} channels. In the presence of 100 μM ATP\textsubscript{1}, either 100 μM ADP\textsubscript{1} or cromakalim alone resulted in a near maximal activation of K\textsubscript{ATP} channels (with I/I\textsubscript{max} increasing from 0.33±0.04 to 0.76±0.06 and 0.66±0.07, respectively), and in combination no consistent additive effect was observed (0.73±0.17) (Figure 7). However, a small additive effect (as seen in the example in Figure 6) could have been obscured by mild channel rundown. At 300 μM ATP\textsubscript{1}, 100 μM ADP\textsubscript{1} or cromakalim alone activated K\textsubscript{ATP} channels to a lesser extent (from I/I\textsubscript{max}=0.07±0.02 to 0.20±0.11 and 0.22±0.05, respectively), but in combination a marked synergistic effect was observed (0.56±0.08; p<0.05). At 1,000 μM ATP\textsubscript{1}, 100 μM ADP\textsubscript{1} had no effect on I/I\textsubscript{max} (from 0.03±0.005 to 0.02±0.004) while 100 μM cromakalim modestly activated K\textsubscript{ATP} channels (0.06±0.005). Again, the combination was markedly synergistic (0.15±0.036).

Figure 8 shows that 15 μM ADP (also with 2 mM Mg\textsuperscript{2+}) was much less effective at potentiating the effects of cromakalim. At 300 μM ATP\textsubscript{1}, 100 μM cromakalim+15 μM ADP, increased I/I\textsubscript{max} from 0.07±0.02.
and the effects of both blocking and facilitating activation of \(K_{\text{ATP}}\) channels during ischemia have been investigated. Sulfonylurea antagonists of \(K_{\text{ATP}}\) channels such as glibenclamide and tolbutamide have been reported to modestly reduce ischemic \([K^+]_i\) accumulation or net cellular \(K^+\) loss\(^{3,11,12}\) and to have antiarrhythmic effects during early ischemia\(^{21,22}\) but generally had deleterious effects on recovery of cardiac function after reperfusion\(^{3,11,12}\). In contrast, \(K^+\) channel openers which activate \(K_{\text{ATP}}\) channels have often been reported to reduce the severity of reperfusion injury\(^{9,11,12}\) although they may be proarrhythmic\(^{23,24}\). In this study we found that 5 \(\mu M\) cromakalim had no significant cardioprotective effect in isolated arterially perfused rabbit interventricular septa subjected to 30 minutes of total global ischemia followed by 60 minutes of reperfusion. Although there was improved recovery of tissue ATP levels and a tendency toward better recovery of developed tension (but not rest tension) following reperfusion in the cromakalim-treated hearts, these changes were marginal. However, a number of limitations should be recognized. We did not evaluate other indices of the severity of ischemic injury such as enzyme release or tissue histology. Also, we examined only one duration of ischemia (30 min-

![Figure 6](http://circres.ahajournals.org/)

**Figure 6.** Single-channel recordings from three excised inside-out patches illustrating the interaction between 100 \(\mu M\) ADP, and 100 \(\mu M\) cromakalim at different ATP concentrations (100, 300, and 1,000 \(\mu M\)). At the first bar in each trace, 2 mM ATP\(_i\) was removed, activating outward current through multiple ATP-sensitive \(K^+\) (\(K_{\text{ATP}}\)) channels. Application of 100 (top trace), 300 (middle trace), or 1,000 \(\mu M\) ATP\(_i\) (bottom trace) suppressed channel activity to an increasing extent. In the continued presence of same concentration of ATP\(_i\), patches were then exposed to 100 \(\mu M\) cromakalim (CROM), 100 \(\mu M\) cromakalim+100 \(\mu M\) ADP\(_i\), and 100 \(\mu M\) ADP\(_i\), as indicated by the bars. At the last arrowhead, the patches were again exposed to 2 mM ATP\(_i\). Stimulation of \(K_{\text{ATP}}\) channels by ADP\(_i\) and cromakalim were additive at 300 and 1,000 \(\mu M\) ATP\(_i\), but not at 100 \(\mu M\) ATP\(_i\). Asterisks indicate zero current level. \([K^+]_i\) was 4 mM, \([K^+]_o\) 150 mM, and free Mg\(^{2+}\) 2 mM throughout, membrane potential 0 mV, and temperature 23°C.

*Discussion*

**\(K^+\) Channel Openers and Cardioprotection During Ischemia/Reperfusion**

Mounting evidence suggests that \(K_{\text{ATP}}\) channels play an important role in both \([K^+]_o\) accumulation and APD shortening during myocardial ischemia and hypoxia\(^{1-5}\) and the effects of both blocking and facilitating activation of \(K_{\text{ATP}}\) channels during ischemia have been investigated. Sulfonylurea antagonists of \(K_{\text{ATP}}\) channels such as glibenclamide and tolbutamide have been reported to modestly reduce ischemic \([K^+]_o\) accumulation or net cellular \(K^+\) loss\(^{3,11,12}\) and to have antiarrhythmic effects during early ischemia\(^{21,22}\) but generally had deleterious effects on recovery of cardiac function after reperfusion\(^{3,11,12}\). In contrast, \(K^+\) channel openers which activate \(K_{\text{ATP}}\) channels have often been reported to reduce the severity of reperfusion injury\(^{9,11,12}\) although they may be proarrhythmic\(^{23,24}\). In this study we found that 5 \(\mu M\) cromakalim had no significant cardioprotective effect in isolated arterially perfused rabbit interventricular septa subjected to 30 minutes of total global ischemia followed by 60 minutes of reperfusion. Although there was improved recovery of tissue ATP levels and a tendency toward better recovery of developed tension (but not rest tension) following reperfusion in the cromakalim-treated hearts, these changes were marginal. However, a number of limitations should be recognized. We did not evaluate other indices of the severity of ischemic injury such as enzyme release or tissue histology. Also, we examined only one duration of ischemia (30 min-

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utes), and we assessed the extent of recovery at a single time point after reperfusion (60 minutes). We do not know the extent to which 30 minutes of ischemia in this model produces stunning versus irreversible injury and cannot exclude the possibility that cromakalim may have been more beneficial after longer or shorter durations of ischemia or if recovery had been assessed at a different time point. We cannot exclude the possibility that a higher concentration of cromakalim would have been cardioprotective. However, 5 μM cromakalim already caused mild APD shortening and negative inotropy under aerobic perfused conditions. We did not consider it useful to test a higher dose that markedly altered baseline cardiac function under aerobic conditions (such as 10 μM), since this would be useless clinically. Finally, despite the many advantages of the rabbit interventricular septum for making electrophysiological measurements during genuine ischemia, the validity of extrapolating the findings in this low-work crystalloid-perfused preparation to more clinically relevant models of myocardial ischemia may be questioned. However, many other interventions that are cardioprotective in more physiological models of ischemia/reperfusion are well-documented to be cardioprotective in this preparation.25–27 Considering these various factors, one may anticipate that the effects of K⁺ channel opener recovery of postischemic function may be variable depending on the particular model and experimental conditions. This may explain why, in contrast to our results, Grover et al.8–10 found that 7 μM cromakalim had cardioprotective effects in globally ischemic rat hearts. Similarly, Cole et al.,11 using a different K⁺ channel opener, found that 1–10 μM pinacidil enhanced mechanical recovery of isolated arterially perfused guinea pig right ventricle subjected to 30 minutes of ischemia. Aside from the species difference, our experimental conditions were similar, raising the possibility that the specific K⁺ channel opener may also be important. Mitani et al.,12 using 100–200 μM nicorandil, had results similar to ours, and did not find any statistically significant improvement in recovery of mechanical function or tissue high energy phosphates in Langendorff rat hearts subjected to 30 minutes of ischemia plus 30 minutes reperfusion.

**Effects of Cromakalim on [K⁺]o Accumulation and APD Shortening During Ischemia**

The present study provides further insights into the relation between [K⁺]o, accumulation and APD shortening during myocardial ischemia. It may seem paradoxical that whereas cromakalim markedly accelerated the rate of APD shortening during the first 10 minutes of ischemia, presumably by activating K_{ATP} channels, it had no effect on the rate of [K⁺]o accumulation over the same period. This raises an important question about the relation between activation of K_{ATP} channels and cellular K⁺ efflux. Since K_{ATP} channels are relatively voltage-independent over the range of membrane potentials relevant to the action potential,5,28 most of the increase in K⁺ efflux rate resulting purely from activation of these channels will occur during systole, when the driving force for K⁺ efflux is much greater. However, by increasing membrane K⁺ conductance cromakalim will also drive the membrane potential (E_m) toward the K⁺ equilibrium potential (E_K) during both systole (shortening APD) and diastole. Thus, the APD shortening and decrease in the driving force (E_m – E_K) for K⁺

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**Figure 7.** Summary of the interaction between 100 μM ADP, and cromakalim (CROM) on the activity of ATP-sensitive K⁺ channels in excised inside-out patches at various ATP concentrations (100, 300, and 1,000 μM). For each patch the time-averaged current (I) was normalized to that in the absence of ATP, (I_{max}). The values represent mean ±1 SEM for the number of patches indicated in parentheses. See text for further details. Conditions are the same as in Figure 6.

**Figure 8.** Effects of physiological (15 μM) versus ischemic (100 μM) ADP, on activation of ATP-sensitive K⁺ channels by 100 μM cromakalim (CROM) in the presence of 300 μM ATP, in excised inside-out patches. For each patch the time-averaged current (I) was normalized to that in the absence of ATP, (I_{max}). Values are mean ±1 SEM for the number of patches indicated in parentheses. Conditions are the same as in Figure 6.
efflux over the entire cardiac cycle brought about by cromakalim will tend to counteract the drug’s effect at promoting increased $K^+$ loss, resulting in a negligible overall effect on $K^+$ efflux and ischemic $[K^+]_o$ accumulation. In our previous study examining the relation between $K_{ATP}$ channel activation and $K^+$ efflux, it was necessary to postulate an increase in inward currents (possibly due to facilitation of $Ca^{2+}, Cl^-$, or other cationic or anionic conductances) coupled with an increase in $K^+$ conductance due to activation of $K_{ATP}$ channels to explain the majority of increased $K^+$ efflux during hypoxia and ischemia. Because activation of $K_{ATP}$ channels alone without a concomitant increase in inward currents may not greatly augment cellular $K^+$ efflux for the reasons discussed above, this may account for the failure of cromakalim to significantly increase the rate of $[K^+]_o$ accumulation despite accelerating APD shortening during early ischemia. In contrast, Mitani et al.\textsuperscript{12} found that in rat ventricle nicorandil did increase the initial rate of ischemic $[K^+]_o$ accumulation. APD was not recorded in these experiments, but it is possible that the much shorter action potential in rat ventricle may lead to a different relation between APD shortening and $[K^+]_o$ accumulation during ischemia in this preparation.

Alternatively, other mechanisms of $[K^+]_o$ accumulation may also be important during ischemia, including anion-coupled $K^+$ loss,\textsuperscript{20} activation of $Na^+$-activated\textsuperscript{30} or arachidonic acid-activated $K^+$ channels,\textsuperscript{31} suppression of the $Na^+$ pump\textsuperscript{32} (also see Reference 33), or shrinkage of the extracellular space.\textsuperscript{34} Cromakalim would not be expected a priori to affect these mechanisms, although for unclear reasons cromakalim did cause an increase in venous lactate content during the first few minutes of reperfusion. We cannot exclude the possibility that this or other nonspecific effects of cromakalim were responsible for the apparent dissociation between the drug’s effects on APD shortening and $[K^+]_o$ accumulation during early ischemia.

Despite the similar magnitude of ischemic $[K^+]_o$ accumulation in control and cromakalim-treated hearts, the pattern was different, with a marked abbreviation of the plateau phase of $[K^+]_o$ accumulation in the presence of cromakalim (Figure 3). This may be related to the fact that the APD did not shorten much further during the last 20 minutes of ischemia in the cromakalim-treated hearts (Figure 3), so that net $K^+$ efflux rate and hence the rate of $[K^+]_o$ accumulation remained nearly constant. In the control hearts, on the other hand, APD continued to shorten progressively over the last 20 minutes of ischemia, which has been suggested to be an important factor contributing to the development of the plateau phase of $[K^+]_o$ accumulation by progressively reducing the time-averaged driving force for $K^+$ efflux.\textsuperscript{35}

**Effects of Cromakalim on Contractile Function During Ischemia**

In intact heart subjected to ischemia, most of the early decline in developed tension occurs before the APD has shortened significantly, and has been attributed primarily to altered responsiveness of the myofilaments to $Ca^{2+}$, by accumulation of intracellular $P_i$ and $H^+$, as well as mechanical factors related to collapse of the vascular space.\textsuperscript{36} APD shortening probably has a major effect on the decline of tension during hypoxia or ischemia only under conditions in which the consequences of altered myofilament responsiveness to $Ca^{2+}$ are minimized, such as when unloaded shortening is used as an index of contractile performance in isolated myocytes subjected to hypoxia.\textsuperscript{37} This may explain why cromakalim did not significantly shorten the time required for developed tension to decline to 50% during ischemia, since the half-time for tension decline was less than 2 minutes, and even in the cromakalim-treated hearts APD had only shortened by $\approx 10\%$ after 2.5 minutes of ischemia (Figure 2). However, cromakalim did modestly but significantly shorten the time for tension to fall to 20% during ischemia, presumably by accelerating the rate of APD shortening during the later phase of tension decline. The acceleration of the mechanical failure during ischemia by $K_{ATP}$ channel agonists is consistent with previous reports\textsuperscript{11,12} and may have contributed to an energy-sparing effect after the first several minutes of ischemia. Accelerated APD shortening may have had a further energy-sparing effect, even after active tension development was completely suppressed, by reducing the energy costs of $Ca^{2+}$ cycling. However, despite these potentially beneficial cardioprotective effects, cromakalim did not result in significantly improved contractile or metabolic recovery after reperfusion in this experimental model.
Interaction of Cromakalim With ADP,

It has been shown previously that the effectiveness of K⁺ channel openers such as cromakalim and pinacidil at activating K<sub>ATP</sub> channels increases as ATP decreases. This observation may explain why cromakalim was more effective at shortening the APD during hypoxia than during aerobic perfusion, which we have also found during ischemia in the septal preparation. However, the decline in ATP during ischemia or hypoxia is not very rapid and typically falls by only 25–50% after 10 minutes. With estimated [ATP], remaining still well within the millimolar range, it is not clear from the available data whether modest declines in ATP, of this magnitude would be expected to significantly increase the ability of K⁺ channel openers to activate K<sub>ATP</sub> channels. We tested whether other factors may be important in sensitizing the ischemic or hypoxic myocardium to the effects of K⁺ channel openers. We examined ADP, and its interaction with cromakalim in this context for two reasons: in the presence of Mg<sup>2+</sup>, ADP is known to interfere with the ability of both ATP<sup>39,40</sup> and sulfonylureas<sup>1</sup> to block K<sub>ATP</sub> channels; and free [ADP], normally estimated in heart muscle at 10–30 μM from the creatine kinase equilibrium reaction,<sup>41</sup> increases rapidly during ischemia and hypoxia to the ≈ 100 μM level. At a concentration of ATP, fixed at either 300 or 1,000 μM, we found that 100 μM ADP + 2 mM Mg<sup>2+</sup> facilitated the activation of cromakalim to activate K<sub>ATP</sub> channels, whereas no significant facilitation was observed with a physiological ADP concentration (15 μM ADP + 2 mM Mg<sup>2+</sup>). These results suggest that the rapid increase in ADP, in ischemic or hypoxic heart may significantly potentiate cromakalim-induced activation of K<sub>ATP</sub> channels and APD shortening. In contrast to ADP,<sup>42</sup> other components of the ischemic environment such as elevated H<sup>+</sup>, lactate, and Pi did not appear to significantly affect the sensitivity of K<sub>ATP</sub> channels to cromakalim. However, additional factors such as Ca<sup>2+</sup>, amphiphiles, or free radicals were not tested.

The observation that ADP, modulates the interaction of K<sub>ATP</sub> channels with cromakalim is generally consistent with the emerging hypothesis that the ADP/ATP, ratio, rather than ATP, alone, is the important physiological regulator of K<sub>ATP</sub> channel activity in hearts<sup>39,40</sup> and other organ sites, such as pancreatic beta islet cells.<sup>42</sup> Further studies will be needed to clarify the mechanisms underlying interactions between ADP, and K⁺ channel openers on K<sub>ATP</sub> channels.

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Activation of ATP-sensitive K+ channels by cromakalim. Effects on cellular K+ loss and cardiac function in ischemic and reperfused mammalian ventricle.

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