Specific Block of the Anti-Ischemic Actions of Cromakalim by Sodium 5-Hydroxydecanoate

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The potassium channel activators cromakalim and pinacidil were recently shown to have anti-ischemic properties in isolated globally ischemic rat hearts. The effects of two reported blockers of ATP-sensitive potassium channels, glibenclamide (glyburide) and sodium 5-hydroxydecanoate, on the anti-ischemic efficacy of cromakalim were determined in this model. Buffer-perfused rat hearts were subjected to 25 minutes of ischemia followed by 30 minutes of reperfusion. Pretreatment of these hearts with 60 μM cromakalim significantly decreased indexes of contractile function but caused a significant improvement of postreperfusion function and a significant decrease in release of lactate dehydrogenase and in end-diastolic pressure. Pretreatment with glibenclamide at concentrations that reversed the presischemic effects of cromakalim (0.05 and 1.0 μM) also significantly reversed its postsischemic protective effects. Sodium 5-hydroxydecanoate (100 and 300 μM) had no effect on the presischemic (negative inotropic) effects of cromakalim but completely reversed its cardioprotective effects. Sodium 5-hydroxydecanoate did not reverse the cardioprotective effects of the calcium entry blocker diltiazem. In phenylephrine-contracted rat aorta, glibenclamide (0.1–10 μM) inhibited cromakalim-induced relaxation, whereas sodium 5-hydroxydecanoate (10–1,000 μM) had no effect. Similarly, the ability of cromakalim to shorten cardiac action potential duration in guinea pig papillary muscle and to increase outward whole-cell potassium currents in isolated myocytes was inhibited by glibenclamide, whereas sodium 5-hydroxydecanoate was without effect. Thus, both glibenclamide and sodium 5-hydroxydecanoate inhibited the effects of cromakalim after reperfusion; however, sodium 5-hydroxydecanoate, unlike glibenclamide, had no effect in nonsischemic preparations. These results suggest that sodium 5-hydroxydecanoate is an ischemia-selective inhibitor of ATP-sensitive potassium channels. (Circulation Research 1991;69:949–958)

Potassium channel activators (KCAs) such as pinacidil and cromakalim are a chemically diverse group of compounds that are thought to open potassium channels, thereby relaxing smooth muscle and shortening cardiac action potentials. These effects of KCAs can be reversed with glibenclamide (glyburide), a relatively selective blocker of ATP-sensitive K⁺ channels in a number of tissue types. Recently, pinacidil and cromakalim were shown to improve postreperfusion function and to reduce release of lactate dehydrogenase (LDH) in an in vitro model of global ischemia. A potassium channel mechanism was implicated for the anti-ischemic effects of these compounds, since their protective actions were reversed by glibenclamide.

Recently, sodium 5-hydroxydecanoate (5-HD, Figure 1), a proposed class III antiarrhythmic agent, which is structurally unrelated to glibenclamide, has been shown to inhibit cardiac ATP-sensitive potassium channels. A previous study indicated that 5-HD completely reversed the postsischemic cardioprotective effects of cromakalim, yet 5-HD did not inhibit the presischemic coronary dilator effects of cromakalim. Glibenclamide completely inhibited both the presischemic coronary dilator effect and the postsischemic cardioprotective effects of cromakalim. These preliminary observations led us to believe that 5-HD may possess some degree of ischemia selectivity. The use of coronary flow as an indicator of presischemic activity is imperfect, however, because the negative inotropic effects of cromakalim would complicate the interpretation of the coronary flow data. Therefore, to investigate the possibility of isch-
For isolated ischemic rat hearts, we compared the ability of 5-HD and glibenclamide to reverse both the preischemic and postdrug cardiodepressant effects and the postschismic reperfusion cardioprotective effects of cromakalim. In addition, to further characterize the ischemia selectivity of the actions of 5-HD, we also compared the actions of 5-HD and glibenclamide in modulating the vasorelaxant and cardiac electrophysiological effects of cromakalim. The results show that 5-HD, unlike glibenclamide, specifically inhibits the postreperfusion anti-ischemic actions of cromakalim.

### Materials and Methods

#### Isolated Ischemic Rat Hearts

Male Sprague-Dawley rats (450–550 g) were anesthetized using 100 mg/kg i.p. pentobarbital sodium. They were intubated and then treated with heparin (1,000 units/kg i.v.). While being mechanically ventilated, their hearts were perfused in situ via retrograde cannulation of the aorta. The hearts were then excised and quickly moved to a Langendorff apparatus, where they were perfused with Krebs-Henseleit bicarbonate buffer containing (mM) NaCl 112, NaHCO3 25, KCl 5.0, MgSO4 1.2, KH2PO4 1.0, CaCl2 1.25, dextrose 11.5, and pyruvate 2.0 aerated with 95% O2–5% CO2 at a constant perfusion pressure (75 mm Hg) such that pH was 7.4. A water-filled latex balloon attached to a metal cannula was then inserted into the left ventricle and connected to a Statham pressure transducer (Gould Instruments, Cleveland, Ohio) for measurement of left ventricular pressure. The hearts were allowed to equilibrate for 15 minutes, at which time end-diastolic pressure (EDP) was adjusted to 5 mm Hg. This balloon volume was maintained for the duration of the experiment. Preischemia, predrug function, and heart rate were then measured. Cardiac function was determined using the double product of heart rate (HR) and left ventricular developed pressure (LVDP) divided by 1,000.13 LVDP was calculated from the difference between left ventricular peak systolic pressure and EDP. Cardiac temperature was maintained throughout the experiment by submerging the hearts in 37°C buffer that was allowed to accumulate in a stoppered, heated chamber in which the buffer solution was exchanged regularly.

The hearts were then treated with vehicle, 60 μM cromakalim (E.R. Squibb & Sons, Inc., Princeton, N.J.), 60 μM cromakalim plus 100–300 μM 5-HD (Squibb), or 0.01–1.00 μM glibenclamide (Sigma Chemical Co., St. Louis, Mo.) (n = 4–8 each). The drug treatment was begun 10 minutes before the onset of ischemia. Immediately before the institution of ischemia, cardiac function was determined. Global ischemia was initiated by completely shutting off the perfusate flow, and this was maintained for 25 minutes. Reperfusion was then begun without drug, and the hearts were allowed to recover for 30 minutes. Reperfusion cardiac function and flow were then measured. At this time, the reperfusion effluent was sampled for cumulative LDH release as previously described14; LDH release is a sensitive index of cell viability.15 Concentrations were determined using a kit supplied by Boehringer Mannheim Corp., Indianapolis, Ind., based on the technique of Wroblewski and LaDue16; LDH was expressed as units per gram preischemia heart weight.

In a separate series of experiments, the effects of 1 μM diltiazem (n = 5), and 1 μM diltiazem plus 100 μM 5-HD (n = 5) versus vehicle (n = 5) were studied to determine if 5-HD could also inhibit the cardiodepressant and cardioprotective actions of diltiazem, a blocker of voltage-operated calcium channels.

#### Vascular Smooth Muscle

Male Wistar Kyoto rats (~14 weeks old) were killed with CO2. The thoracic aorta was removed and placed into a modified Krebs’ solution (20–22°C). After removal of blood and adherent connective tissue, rings (~4 mm in width) were cut from each aorta. The endothelium was mechanically removed, and the tissues were mounted for isometric force recording in individual 20-ml organ chambers between a micrometer for control of tissue length and a force transducer (model FT.03, Grass Instrument Co., Quincy, Mass.). Mechanical responses were recorded with polygraphs (model 7D, Grass Instrument). Aortic rings were gradually stretched over a 2-hour equilibration period to a preload of 2 g, which was maintained throughout the experiment.

The modified Krebs-Henseleit solution contained (mM) NaCl 118.4, NaHCO3 25.0, KCl 4.7, KH2PO4 1.2, MgSO4 1.2, CaCl2 2.5, and dextrose 11.7. Calcium-free Krebs’ solution was prepared by deletion of CaCl2 and addition of 1.0 mM EGTA. All solutions were aerated with 95% O2–5% CO2 such that the pH was 7.4 and were maintained at 37°C. 5-HD (10–1,000 μM) or glibenclamide (0.1–10 μM) was added to the bath 10 minutes before phenylephrine (PE) to determine effects, if any, on basal force. In the continued presence of 5-HD or glibenclamide, the aortic rings were contracted with 0.01 μM PE and allowed to reach a steady-state level of force before a cumulative concentration-relaxation curve was obtained for cromakalim. A steady-state level of relaxation was always reached before addition of the next higher concentration of cromakalim. If no relaxation was apparent after 10 minutes, the next higher concentration was added. When the rings attained their maximum relaxation, the relaxation was reversed by the addition of a 4 M KCl (in the presence of test compound) to give a final bath concentration of 60 mM. The rings were then washed with calcium-free

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**Figure 1.** Chemical structures of glibenclamide (glyburide) and sodium 5-hydroxydecanoate (5-HD).
Krebs’ solution and allowed to relax completely. Paired tissues, in the absence and presence of test compounds, were run in parallel.

All force determinations for relaxation in the aortic rings were made by assuming that the level of force attained in calcium-free Krebs’ solution was zero (or 100% relaxation) and that the maximal force attained in the presence of PE alone was 100% (or 0% relaxation). Force is reported relative to these levels. All active force measurements were determined by assuming that the preload was only passive force. IC50 values were determined as the concentration where 50% of the maximal relaxation was attained. The effects of the test compounds on baseline and on both the PE- and potassium-induced contractions were determined relative to control tissues from the same animals.

**Cardiac Electrophysiology**

**Guinea pig papillary muscles.** Standard microelectrode techniques were used to study the electrophysiological effects of 5-HD and glibenclamide in isolated guinea pig papillary muscles. Male Hartley guinea pigs (250–350 g) were killed by cervical dislocation, and the hearts were rapidly removed. Papillary muscles were obtained from either ventricle and were rapidly fixed to the waxed bottom of a tissue chamber using insect pins. Preparations were superfused with a modified Tyrode’s solution containing (mM) NaCl 140.0, KCl 4.0, CaCl2 5.0, MgCl2 2.0, HEPES 5.0, and glucose 10.0, pH 7.4. The solution was aerated with 100% O2 and was maintained at 35–36°C. Two wire electrodes, insulated except at the ends, were placed over one end of the preparation, and stimulating pulses of 1-msec duration and −2× threshold were applied at a frequency of 1 Hz. Preparations were allowed to recover for 30 minutes before impalements were begun.

Determinations of intracellular resting and action potentials were made using glass microelectrodes filled with 3 M KCl (8–30 mΩ) and referenced to ground with an Ag/AgCl wire placed in the tissue chamber. For each protocol, impalements were made to establish values under control conditions. To determine the ability of test compounds to block the shortening of action potential duration induced by cromakalim, the preparation was then exposed to concentrations of test compound (100 μM 5-HD and 0.1 μM glibenclamide). Superfusion with each test compound was maintained for 25–30 minutes to achieve steady-state effects. After exposure to the antagonist, the ability of the test compound to block cromakalim-induced action potential shortening was determined by the addition of 100 μM cromakalim in the continued presence of the test compound. Superfusion was continued until a steady-state level of action potential shortening induced by cromakalim was attained. After a washout period of 25–60 minutes with Tyrode’s solution, the effects of 100 μM cromakalim alone were again tested.

For the reversal protocol, the order of drug addition was cromakalim (100 μM) alone superfused for 25 minutes, followed by superfusion with a solution containing cromakalim (100 μM) in the presence of variable concentrations of the test compound.

A WPI amplifier (model S-7071, World Precision Instruments, Sarasota, Fla.) was used to record the intracellular signal. These were digitized for analysis by an IBM/PC-type computer system and stored digitally on 3.5-inch diskettes by a digital oscilloscope (model 310, Nicolet Instrument Corp., Madison, Wis.). Resting and action potential characteristics were analyzed using a computerized data acquisition system. Action potential duration was measured at 90% repolarization (APD90), and the effects of the various agents were compared with the action potential of the same papillary muscle under control conditions. Data were expressed as percent change from control APD90.

**Guinea pig ventricular myocytes.** The procedures for cell dissociation and whole-cell voltage clamping have recently been described in detail. Briefly, a collagenase digestion followed by recovery of isolated single myocytes in a high K+–containing recovery solution was used. After further recovery in Tyrode’s solution, myocytes were voltage-clamped using an Axoclamp 2A (Axon Instruments, Foster City, Calif.) or a List L/M-EPC-7 (List Electronics, Darmstadt, FRG) amplifier and fire-polished glass micropipettes (1–3 MΩ) containing (mM) KCl 125, MgCl2 4, CaCl2 2, KOH 30, NaCl 10, EGTA 10, HEPES 5, and glucose 10, pH 7.2. Steady-state current–voltage relations were measured using a ramp command voltage (6 mV/sec). This ramp was generated by and data were acquired on a Compaq 386 computer (Compaq Computer Corp., Houston, Tex.) running PCLAMP software (Axon Instruments). Experiments were done at 33–35°C.

The guinea pig ventricular myocytes were also used to study the effects of 5-HD on single ATP-sensitive K+ channels recorded from inside-out patches. Pipettes, similar to those used to record whole-cell currents, but with resistances of 2–8 MΩ, were filled with an external solution containing (mM) KCl 150, CaCl2 2, MgCl2 4, and HEPES 5, pH 7.3. The bath (internal) solution contained (mM) KCl 140, KH2PO4 0.5, EGTA 3, HEPES 10, and K2ATP 0.1, pH 7.25. A List L/M-EPC-7 patch-clamp amplifier was used in these experiments, and single-channel currents were recorded on videotape using a VR-10 PCM (Instrutect Corp., Mineola, N.Y.) and an RCA video recorder (RCA 670, Indianapolis, Ind.). PCLAMP software was used to analyze single-channel currents. Current recordings were displayed on a two-channel recorder (Dash II MT, Astro-Med, Inc., West Warwick, R.I.) with a frequency response of −3 dB at 500 Hz. Experiments were done at 23–25°C.

When appropriate, statistical differences were determined using either Student’s t tests for paired data or analyses of variance. Significance was set at
$p<0.05$ using a two-tailed test. All data are expressed as mean±SEM.

**Results**

**Ischemic Rat Hearts**

Before ischemia, 60 μM cromakalim had no effect on HR (285±6 beats/min in control versus 269±5 beats/min in the presence of cromakalim, $n=4$), although it significantly reduced LVDP (from 141±9 mm Hg in control to 93±4 mm Hg in cromakalim, $p<0.05$, $n=4$), indicating a negative inotropic effect. This negative inotropic effect was reflected as a decrease in double product (HR×LVDP/1,000, Figure 2). As illustrated in Figure 2, 5-HD did not reverse the postdrug preischemic negative inotropic effect of cromakalim. After global ischemia, the postreperfusion double product was significantly reduced in vehicle-treated hearts (Figure 2), indicating severe ischemic damage. Although cromakalim depressed the preischemic double product, it significantly improved postreperfusion function compared with vehicle-treated hearts. Despite its lack of preischemic effects, 5-HD significantly reversed the protective effects of cromakalim on the reperfusion double product, in what appeared to be a concentration-dependent manner (Figure 2). Postreperfusion EDP and LDH release are shown in Figure 3. Severe contracture, observed in vehicle-treated hearts, was significantly reduced by cromakalim. LDH release was also significantly reduced by cromakalim. 5-HD completely reversed the protective effects of cromakalim and, at the 300 μM concentration, actually increased LDH release compared with vehicle-treated hearts.

The effects of 5-HD on the cardioprotective effects of diltiazem were also determined to test whether its actions were specific for KCAs and not a general antagonistic action of compounds with other antiischemic mechanisms (i.e., calcium entry blockade). Diltiazem (1 μM) significantly reduced the preischemic double product from 39.6±1.8 to 16.9±1.9 ($p<0.05$), consistent with its calcium entry–blocking activity, but improved the postreperfusion double product to 26.1±1.2 ($n=5$). As shown in Figure 4, diltiazem also reduced the severity of ischemic dam-

![Figure 2](image_url)  
**Figure 2.** Bar graph showing the effect of cromakalim (C) with or without concomitant treatment with sodium 5-hydroxydecanoate (HD) on the double product: heart rate (HR)×left ventricular developed pressure (LVDP)/1,000. Clear bars represent preischemia, postdrug values, and cross hatched bars represent postreperfusion values. For vehicle, $n=8$; for 60 μM C, $n=4$; for 100 μM HD+C, $n=6$; and for 300 μM HD+C, $n=6$. *$p<0.05$ compared with respective vehicle group value; †$p<0.05$ compared with respective value at 60 μM C.

![Figure 3](image_url)  
**Figure 3.** The effect of cromakalim (C) with or without concomitant treatment with sodium 5-hydroxydecanoate (HD) on postreperfusion end-diastolic pressure (EDP) and lactate dehydrogenase (LDH) release. Cromakalim significantly reduced EDP and LDH release compared with vehicle. HD reversed the beneficial effects of C on EDP; for LDH, HD not only reversed the beneficial effects of C but also significantly worsened ischemia relative to vehicle. For vehicle, $n=8$; for 60 μM C, $n=4$; for 100 μM HD+C, $n=6$; and for 300 μM HD+C, $n=6$. *$p<0.05$ compared with respective vehicle group value; †$p<0.05$ compared with respective value at 60 μM C.
on diltiazem’s postreperfusion improvement in the double product (26.3 ± 1.9, n = 5).

Glibenclamide, at concentrations ≥0.05 μM, significantly reversed the preischemic decrease in LVDP induced by 60 μM cromakalim (LVDP was 93 ± 7 mm Hg in the cromakalim-treated group versus 134 ± 7 mm Hg in the cromakalim plus 0.05 μM glibenclamide group). The reversal by glibenclamide of the preischemic cardiodepressant effects of cromakalim was also reflected in the double product data presented in Figure 5. Glibenclamide also reversed the protective effect of cromakalim on the postreperfusion double product (Figure 5). The reversal by glibenclamide both of the preischemic cardiodepressant effects of cromakalim and of the cardioprotective effect of cromakalim on postreperfusion function was concentration dependent, but there appeared to be little separation between preischemic and postreperfusion activity of glibenclamide. Postreperfusion LDH and EDP data for the glibenclamide study are shown in Figure 6. Cromakalim significantly reduced LDH release and EDP, whereas glibenclamide reversed these protective effects. At 1 μM, glibenclamide not only reversed the protective effects of cromakalim but significantly worsened the severity of ischemic injury relative to vehicle-treated hearts.

Vascular Smooth Muscle

The actions of 5-HD as a modulator of the cromakalim-induced relaxation of PE contractions are shown in Figure 7A. 5-HD (10 μM–1 mM) had no effect on baseline tension or on the relaxation response induced by increasing concentrations of cromakalim. 5-HD also did not affect the contractile responses to either PE or KCl. Data for the effects of glibenclamide on cromakalim-induced relaxation of rat aorta are shown in Figure 7B. At all concentrations tested (0.1–10 μM), glibenclamide shifted the concentration–relaxation response curves of cromakalim to the right. The dissociation constant (Kd) for glibenclamide was 0.057 μM. Glibenclamide

![Figure 4](image-url)  
**Figure 4.** Bar graphs showing the effect of diltiazem with and without concomitant treatment of sodium 5-hydroxydecanoate (5-HD) on postreperfusion end-diastolic pressure (EDP) and lactate dehydrogenase (LDH) release. Diltiazem (1 μM) significantly reduced EDP and LDH release compared with vehicle (*p<0.05). 5-HD (100 μM) failed to reverse the protective effect of diltiazem. For each group, n = 5.

![Figure 5](image-url)  
**Figure 5.** Bar graph showing the effect of cromakalim (C) with or without concomitant treatment with glibenclamide (G) on the double product: heart rate (HR) × left ventricular developed pressure (LVDP)/1,000. Clear bars represent preischemia, postdrug values, and cross-hatched bars represent postreperfusion values. For vehicle, n = 4; for 60 μM C, n = 4; for 0.01 μM G+C, n = 6; for 0.05 G+C, n = 4; and for 1.00 μM G+C, n = 4. *p<0.05 compared with value for respective vehicle group; †p<0.05 compared with respective value at 60 μM C.
Effects of 5-HD and glibenclamide (0.1 μM) had no effect on the resting potential or on action potential amplitude. The effects of these compounds on action potential duration are shown in Figure 8. At 100 μM, 5-HD prolonged the action potential slightly (~7%), but neither blocked (Figure 8A, left columns) nor reversed (Figure 8B, left columns) the shortening of APD <sub>90</sub> induced by 100 μM cromakalim. Glibenclamide (0.1 μM) alone had no effect on APD <sub>90</sub> but inhibited the shortening of APD <sub>90</sub> induced by 100 μM cromakalim (Figure 8A, right columns). Treatment with 1 μM glibenclamide completely inhibited the cromakalim-induced shortening of action potential duration (data not shown). The shortening of APD <sub>90</sub> induced by cromakalim was consistently reversed by increasing concentrations of glibenclamide (≥1 μM, Figure 8B, right columns).

In isolated guinea pig ventricular myocytes, cromakalim (100 μM) induces a large and linear increase in outward current in outward current in outward current in outward current in outward current in outward current (3,4,6,17) (Figure 9). 5-HD (100 μM) failed to inhibit (not shown) or to reverse this cromakalim-induced increase in current (Figure 9A). Similar results were obtained with 100 μM 5-HD in three myocytes and with 300 μM 5-HD in five myocytes. For example, in the five myocytes treated with 300 μM 5-HD, 0.58±0.14 nA was observed at +20 mV under control conditions. Addition of cromakalim significantly increased this current to 5.01±1.50 nA (p<0.02 compared with control), and the current was unchanged (4.63±1.01 nA) by the subsequent addition of 300 μM 5-HD in the continued presence of cromakalim. Glibenclamide (0.1 μM) was able to completely reverse this cromakalim-induced current (Figure 9B). In six myocytes, control current at +20 mV was 0.23±0.05 nA. Cromakalim significantly increased the current to 4.07±0.61 nA (p<0.002 compared with control), and addition of glibenclamide significantly reduced the current to 0.31±0.08 nA (p<0.005 compared with cromakalim).

The effects of 5-HD on single ATP-sensitive potassium channels were studied in inside-out membrane patches from ventricular myocytes. This channel had a conductance of 80–85 pS in symmetrical K<sup>+</sup>-recording conditions, and its probability of opening was inhibited by intracellular ATP. When the intracellular side of the membrane was exposed to 100 μM ATP, bursts of channel openings were evident. Addition of 100 μM 5-HD reduced the number of bursts, and long closed periods were observed. In four patches, 5-HD significantly reduced open probability to 0.14±0.07 from a control mean value of 0.24±0.07 (p<0.03). No effect of 5-HD on single-channel conductance was observed. The effects of 5-HD were reversed on washout.

**Discussion**

Previous findings have shown that several KCAs can protect ischemic myocardial tissue in what appears to be a direct cardioprotective effect. This protective effect was reversed by glibenclamide, a reported blocker of ATP-sensitive potassium chan-
In the present study we examined the effects of 5-HD, an agent also reported to block ATP-sensitive potassium channels in cardiac ventricular myocytes, and compared the effects of 5-HD with those of glibenclamide. The results in the rat heart model of global ischemia clearly show that 5-HD and glibenclamide had similar effects on the postreperfusion cardioprotective actions of cromakalim. While 5-HD and glibenclamide alone had no effects on the severity of ischemia, they not only reversed the protective effects of cromakalim but also became proischemic. Similar proischemic effects of glibenclamide previously were reported for the reversal by glibenclamide of the protective effects of KCAs. Although the postischemic effects of glibenclamide and 5-HD were similar, their effects on the normal or preischemic actions of cromakalim differed markedly. Glibenclamide completely reversed the preischemic coronary vasorelaxant effect of cromakalim, whereas 5-HD had no effect, even at concentrations that reversed the anti-ischemic actions of cromakalim. These data indicate that some degree of ischemia selectivity may be observed for potassium channel blockers.

We wished to investigate this concept further, although we chose not to use coronary flow as an index of preischemic activity because, in heart preparations, changes in cardiac work can alter coronary resistance and complicate interpretation of the data. Therefore, we used the cardiodepressant effect of cromakalim as our index of preischemic activity and the postreperfusion function as our index of ischemic activity. High concentrations of cromakalim have been shown to depress preischemic function but also to reduce ischemic/reperfusion damage. Thus, cromakalim has preischemic and postischemic effects on contractile function that are opposite to one another. We found that 60 μM cromakalim caused a 30–40% drop in contractility without any effect on heart rate before ischemia. Cromakalim also significantly improved reperfusion function, indicating anti-ischemic activity. Although 5-HD did not reverse the preischemic cardiodepressant effect of cromakalim, it completely reversed the postreperfusion protective effects of cromakalim. Both 5-HD and glibenclamide (at sufficiently high concentrations) not only reversed the beneficial effects of cromakalim but also worsened the severity of ischemia compared with control, an effect not seen when these agents were given alone. Although the ischemic effects of the two blockers appeared to be similar, only glibenclamide was found to reverse the preischemic cardiodepressant effects of cromakalim.

Previous studies have suggested that the cardioprotective effects of cromakalim were related to its ability to activate potassium channels. The reversal of these cardioprotective effects by glibenclamide apparently was related to a specific blockade of ATP-sensitive potassium channels and not to some nonspecific, proischemic effect, because glibenclamide failed to reverse either the preischemic cardiodepression or the postreperfusion cardioprotective actions of the calcium entry blocker diltiazem. We found that 5-HD was also unable to reverse either the
cardiodepressive or the cardioprotective effects of diltiazem. These results suggest that 5-HD, like glibenclamide, lacks nonspecific proischemic actions. Furthermore, the ability of 5-HD to reverse the postischemic improvement in cardiac function of cromakalim and other KCAs9 without affecting the preischemic actions of the KCAs suggests that the actions of 5-HD were ischemia selective. The results of the present study clearly show that 5-HD had little or no pharmacological activity in vascular smooth muscle or normal cardiac muscle. Furthermore, we found that 5-HD was unable to modify the actions of cromakalim in either vascular smooth muscle or normal cardiac muscle. Glibenclamide alone also had little or no activity in vascular smooth muscle or normal cardiac muscle. On the other hand, glibenclamide antagonized the vasorelaxant actions of cromakalim in rat aorta.20 Additionally, glibenclamide blocked and/or reversed the shortening of the action potential and the increase in outward current induced by cromakalim in normal cardiac preparations, as previously reported.3,4,21 These findings of differences between the effects of glibenclamide and 5-HD on the actions of cromakalim in normal tissues are interesting in light of recent reports that glibenclamide is a potent blocker of the ATP-sensitive potassium channel in many cell types7,8 and that 5-HD blocked this channel in cardiac cells.10,11

Using single-channel patch-clamping techniques, we confirmed that 5-HD can inhibit cardiac ATP-sensitive potassium channels: channel open probability was reduced in the presence of 5-HD. Relatively low concentrations of ATP (≤100 μM) at the intracellular face of the membrane must be used to obtain openings of this channel,22 and it is under conditions of low ATP (or increased internal ADP) that the inhibition of the channel by glibenclamide7,8 or 5-HD10,11 was observed. It has also been observed that both glibenclamide and 5-HD have no effect on action potential duration of guinea pig papillary muscles under normoxic conditions but that both compounds reversed the shortening of the action potential induced by hypoxia (Dr. Jeffrey E. Byrne, Bristol-Myers Squibb, personal communication, May 1991). The relevance of the low levels of ATP used or obtained in electrophysiological experiments and the intracellular concentrations actually attained during ischemia is not known, but low ATP may be one of the factors involved in the ischemia selectivity of the actions of 5-HD. Other mechanisms may be involved in the ischemic process, and these may also contribute to the ischemic actions of 5-HD.

The potent block of ATP-sensitive potassium channels by glibenclamide has led many investigators to suggest that its ability to inhibit the actions of KCAs is evidence that the ATP-sensitive potassium channel is the site of action of KCAs (e.g., see
Reference 20). There is electrophysiological evidence that KCAs open these channels in cardiac muscle, but the identity of the potassium channel opened by KCAs in smooth muscle is less certain. Gelband and coworkers have found that cromakalim and pinacidil increase the opening probability of high conductance Ca2+-activated potassium channels isolated from rabbit aorta. Glibenclamide alone has no effect on this channel but reverses the increase in open probability induced by cromakalim or pinacidil. These results suggest that, in addition to its reported channel-blocking activity, glibenclamide may be a competitive receptor antagonist of the KCAs. These observations may help explain some of the differences in the actions of glibenclamide and 5-HD we observed: 5-HD may have no affinity for the smooth muscle KCA "receptor." Furthermore, even if both agents block ATP-sensitive potassium channels, the divergent actions of glibenclamide and 5-HD on vascular and cardiac actions of cromakalim may suggest that the two agents are active at different receptors or at different sites on the same receptor in normal and ischemic tissues. This notion is supported by the findings that glibenclamide inhibited the preischemic cardiodepression induced by cromakalim but 5-HD failed to do so and that both agents inhibited the cardioprotective effect of cromakalim.

We presently do not know the mechanism for the ischemia selectivity of 5-HD. Information about the binding of 5-HD to glibenclamide sites in the myocardium and/or the identification of a 5-HD binding site may be useful in working out the mechanism of ischemia selectivity. Additional information about the mechanisms of cardioprotection of the KCAs may also be gained from greater understanding of the ischemia selectivity of 5-HD.

References

5. Smallwood JK, Steinberg MI: Cardiac electrophysiological effects of pinacidil and related pyridlycyanoguanidines: Rela-
9. Grover GJ, McCullough JR, Henry DE, Conder ML, Sleph PG: Anti-ischemic effects of the potassium channel activators pinacidil and cromakalim and the reversal of these effects with the potassium channel blocker glyburide. J Pharmacol Exp Ther 1989;251:98–104
15. Van der Laarse A, Holaar L, Van der Volk LJM: Release of alpha hydroxybutyrate from neonatal rat heart cell cultures exposed to anoxia and reoxygenation: Comparison with impairment of structure and function of damaged cardiac cells. Circ Res 1979;13:345–353
18. Grover GJ, Sleph PG, Dzwonczyk S, Parham CS: Anti-ischemic and antifibrillatory effects of the K⁺ channel activators cromakalim and pinacidil in dogs and isolated rat hearts (abstract). Circulation 1989;80(suppl II):II-499

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