Agents that modulate cardiac and smooth muscle K⁺ channels have stimulated considerable interest in recent years because of their therapeutic potential in a number of cardiovascular diseases. Foremost among these drugs are the so-called Class III antiarrhythmic agents, which act by prolonging cardiac action potentials, and K⁺ channel openers, which hyperpolarize and thereby relax smooth muscle cells. Many of the newly developed Class III antiarrhythmic agents probably act by specific block of one subtype of delayed rectifier K⁺ current, I_Kr, whereas other agents block more than one type of cardiac K⁺ current. Much controversy exists over the specific type of K⁺ channel (or channels) in smooth muscle that are activated by the K⁺ channel openers. Both groups of K⁺ channel modulators have great therapeutic promise, but the Class III antiarrhythmic agents may suffer from a side-effect that is directly linked to their specific mechanism of action. (Hypertension 1992;19:228–236)

**KEY WORDS** • potassium • ion channels • antihypertensive therapy • antiarrhythmia agents

**Class III Antiarrhythmic Agents**

Cardiac cells are characterized by the presence of a dizzying array of different K⁺ channel subtypes. Inward rectifier K⁺ channels (I_K) are primarily responsible for maintaining the resting potential near the equilibrium potential for K⁺ (E_K) and for terminal repolarization of the action potential (from about -20 to -90 mV). I_K channels open (activate) almost instantly in response to a change in membrane potential and allow inward K⁺ current to pass more easily than outward K⁺ current ("inward rectification"). The major role of the numerous other cardiac K⁺ currents is to initiate or at least participate in repolarization of the action potential. The major currents responsible for repolarization of heart cells are the transient outward (I_TO) and delayed rectifier (I_K) K⁺ currents. I_TO channels are so named because they are open only transiently, that is, these channels close (inactivate) soon after opening in response to a positive change in membrane potential (depolarization) and thus only contribute to the initial phase of repolarization. Delayed rectifier channels are so named because they open in response to a depolarization only after a distinct delay, and a plot of current through these channels versus membrane potential (current-voltage plot) is not linear (the current exhibits rectification). As discussed below, there are at least two types of I_K channels (I_Ks and I_Kr) in ventricular and atrial cells. In addition, cardiac cells have channels that are either inhibited by intracellular ATP (K-ATP channels) or activated by intracellular sodium or by external acetylcholine (K_ACh channels). Not all channel types are found in a given cell type. For example, guinea pig ventricular cells have little or no I_TO and K_ACh channels are restricted to atrial cells. The hallmark of cardiac action potentials, a long plateau phase, results from either a very slow rate of activation (e.g., I_Ks) or intense inward rectification (e.g., I_Kr, I_Kr) of repolarizing K⁺ currents.
Antiarrhythmic Effects of C3A Drugs: Mechanism of Action

Antiarrhythmic effects of the C3A agents result from prolongation of cardiac action potentials (Figure 1A) and thus refractory period of the myocardium. Lengthening of refractory period by these drugs is associated with a decrease in the incidence of reentrant ventricular arrhythmias. Reentrant arrhythmias are believed to be the major cause of ventricular fibrillation, the lethal rhythm disorder associated with sudden cardiac death.

In addition to prolonging action potential duration, most C3A drugs also enhance contractility, decrease defibrillation threshold, and slightly slow heart rate.

The prototypical C3A drugs are amiodarone, clofilium, and sotalol. Although amiodarone and sotalol have been studied more than any other C3A agents, none are "pure" in that they also have significant Class I activity (block Na+ current: amiodarone and clofilium), Class II activity (block β-adrenergic receptors: sotalol), or Class IV activity (block Ca2+ current: amiodarone). Amiodarone is usually administered to patients for extended periods before antiarrhythmic effects are noted, and probably acts by blocking a variety of cardiac channels, but a voltage-dependent block of IK may be responsible for its acute C3A activity. Clofilium has potent (EC50=13 nM) C3A activity in canine cardiac Purkinje fibers but has no effect on atrial muscle at 100 nM. Even higher concentrations (more than 10 μM) are required to prolong action potential duration of guinea pig ventricular cells. Clofilium blocks Ik in isolated guinea pig ventricular myocytes with an IC50 of approximately 50 μM and blocks Ik in rat myocytes in a frequency-dependent manner with an IC50 less than 1 μM. The basis of its potent action on cardiac Purkinje cells has yet to be adequately explained but could possibly result from a more potent block of Ik in these cells or from high affinity block of a large conductance, Ca2+-activated K+ channel present in these cells, but not in other cardiac cell types.

Sotalol is a β-adrenergic receptor blocker that also has C3A activity in humans. The (-) enantiomer is more potent as a β-adrenergic receptor blocker than the (+) enantiomer, but both are equipotential as C3A drugs. Contrary to widely held beliefs, (+)-sotalol has significant β-adrenergic receptor blocking activity (pKd=5.0, determined by inhibition of 125I-pindolol binding) relative to its potency as a C3A in isolated guinea pig hearts: pEC50=4.9. Carmeliet was the first to demonstrate the cellular mechanism of action of racemic sotalol and both its enantiomers in isolated rabbit Purkinje fibers. Ik, measured as deactivating tail currents, was blocked about 50% by 10 μM sotalol. This concentration had no significant effect on Ito, IK1, or sodium current in this preparation. Greater than 90% block of Ik was observed at 100 μM. In contrast, Berger et al. reported that (+)-sotalol or (±)-sotalol blocked Ito and IK1 by 23% and 7%, respectively, at 10 μM but had no effect on Ik at 100 μM in isolated sheep cardiac Purkinje fibers. Ik was decreased by only 16% at 1 M in the sheep fibers. The species-dependent effect of sotalol on Ik is well correlated with its ability to prolong action potential duration. Sotalol (10 μM) lengthened action potential duration by 41% in rat Purkinje fibers but only by 5% in sheep Purkinje fibers. This raises the obvious possibility that Ik represents the activation of different channel types in these two species. Guinea pig ventricular muscle is similar to sheep Purkinje fibers with respect to its sensitivity to sotalol. However, as discussed below, Ik of guinea pig myocytes actually represents the sum of two distinct outward K+ currents.

Two Components of Delayed Rectifier K+ Current

The first voltage clamp analysis of Ik in cardiac tissue was performed by Noble and Tsien. They proposed that Ik in sheep Purkinje fibers was the composite of two distinct currents, called Ik1 and Ik2. These currents were separable on the basis of kinetics, reversal potential, and degree of rectification. Subsequent studies concluded that quantitative analysis of Ik in such a multicellular preparation was hampered by accumulation of K+ in intracellular clefts, and that the multieponential onset of Ik activation and subsequent deactivation could be adequately described in guinea pig myocytes assuming either n Hodgkin-Huxley-like activation ki-
voltages, Figure 2). The current blocked by sotalol and E-4031 is methanesulfonanilides, but note that $I_{Kr}$ is plotted on an expanded current scale relative to the raw records of panel b. Similar to $I_{K1}$, $I_{Kr}$ shows marked rectification (nearly linear between $-30$ and $0\text{ mV}$, then decreasing at more positive voltages).

Panel c: Digital subtraction of the two tracings in panel b was performed to obtain the current-voltage relation for the E-4031-sensitive current $I_{Kr}$. Note that $I_{Kr}$ is plotted on an expanded current scale relative to the raw records of panel b. Similar to $I_{K1}$, $I_{Kr}$ shows marked rectification (nearly linear between $-30$ and $0\text{ mV}$, then decreasing at more positive voltages).

FIGURE 2. Representative tracings show block of specific $K^+$ current ($I_{Kr}$) by the Class III antiarrhythmic agent E-4031. $K^+$ currents were recorded from an isolated guinea pig ventricular myocyte during voltage ramps from $-90\text{ mV}$ to $+40\text{ mV}$ (panel a). Currents recorded during control (con) conditions include inward rectifier $K^+$ current, $I_{Ki}$ ($-90$ to $-20\text{ mV}$) and delayed rectifier $K^+$ currents (both $I_{Ks}$ and $I_{Kt}$, $-30$ to $+40\text{ mV}$). Na$^+$ and Ca$^{2+}$ currents were eliminated by replacement of extracellular NaCl with equimolar TrisCl and by removal of extracellular Ca$^{2+}$ and addition of 0.4 $\mu\text{M}$ nisoldipine (35°C). After exposure of cell to 5 $\mu\text{M}$ E-4031 (E), which specifically blocks $I_{Kr}$, only $I_{Ks}$ and $I_{Kt}$ remain. Panel c: Digital subtraction of the two tracings in panel b was performed to obtain the current-voltage relation for the E-4031-sensitive current $I_{Kr}$. Note that $I_{Kr}$ is plotted on an expanded current scale relative to the raw records of panel b. Similar to $I_{K1}$, $I_{Kr}$ shows marked rectification (nearly linear between $-30$ and $0\text{ mV}$, then decreasing at more positive voltages).

The ability of sotalol to completely block $I_{Kr}$ in whole Purkinje fibers is quite plausible that $I_{Kr}$ of rabbit Purkinje cells is equivalent to $I_{Kr}$ of guinea pig myocytes, thus explaining the ability of sotalol to completely block $I_{Kr}$ in whole Purkinje fibers. $I_{Ks}$ and $I_{Kr}$ are also present in guinea pig atrial cells. In these cells, Horie et al recorded two distinct types of single channel currents that may correspond to $I_{Ks}$ and $I_{Kr}$ of whole cell currents. In inside-out patch recordings the conductance of the two channels were 3 and 10 picoamperes (pS) when measured in symmetrical $K^+$ (150 mM) conditions. The 3-pS channel deactivated more slowly than the 10-pS channel, suggesting that the two channels represent the unit activity underlying $I_{Ks}$ and $I_{Kr}$, respectively. In rabbit nodal cells, $I_{Kr}$ is almost indistinguishable from $I_{K2}$ recorded from guinea pig myocytes. The conductance of single $I_{Kr}$ channels in these nodal cells was 11 pS, in good agreement with the similar channel ($I_{Kr}$) recorded from guinea pig atrial myocytes. Single channel currents corresponding to $I_{Kr}$ were not resolvable in patches of guinea pig ventricular cells, suggesting that the channels have an extremely low conductance (less than 1 pS in physiological $K^+$ gradient) and are present at high density in cardiac sarcolemmal membrane. Two types of $I_{Kr}$ have also been described in embryonic chick atrial cells, but the effect of C3A drugs has yet to be evaluated in this preparation.

A $K^+$ channel with very slow activation kinetics was recently isolated by expression cloning from rat kidney. Complementary DNAs (cDNAs) encoding this channel protein ("$I_{Kr}$") have since been cloned and expressed from neonatal rat heart and human heart and may be the protein that forms $I_{Kr}$ channels, presumably as homooligomers. The $I_{Kr}$ protein is unlike any other $K^+$ channel protein both in primary sequence and its unusually small size, only 130 amino acids.

**Not All C3A Agents Are Specific $I_{Kr}$ Blockers**

Several other newly developed C3A agents that are structurally related to sotalol and E-4031 may also act by selectively blocking $I_{Kr}$. Examples include UK-68,798, UK-66,914, and sematilide. The selective action of these drugs is true only with respect to other cardiac channels. UK-68,798 was also reported to block a Ca$^{2+}$-activated K$^+$ channel recorded from CA1 hippocampal neurons. Several drugs have C3A properties in addition to other activities. For example, OPC-8490 is an inotropic agent and quinidine is a Class I antiar-
rhythmic agent (blocks sodium current), but both drugs also have C3A activity. OPC-8490 was reported to block $I_K$ of guinea pig atrial myocytes in a voltage-dependent and time-independent manner, with block increasing as the membrane potential was made more negative. $^{38}$ OPC-8490 blocked both components (fast and slow) of $I_K$ deactivation (closure of channels from the open state) with a greater effect on the fast component. The apparent voltage-dependent block of $I_K$ by OPC-8490 could just as easily be explained as relatively specific block of $I_{K_{S}}$. Quinidine has also been reported to block $I_K$ in a voltage-dependent fashion. $^{39,40}$ Although quinidine clearly blocks both components of $I_K$, $I_{K_{S}}$ is blocked at lower concentrations than is $I_{K_{L}}$. This suggests that the clinically relevant C3A action of quinidine may be block of $I_{K_{L}}$ and not $I_{K_{S}}$. Pirmenol, a pyridine methanol derivative, blocks both $I_K$ and $I_{Na}$ in rabbit Purkinje fibers, similar to quinidine, and is therefore best classified as a class Ia antiarrhythmic agent. $^{41}$ Tedisamil is a diazobicyclic C3A and bradycardic agent that blocks $I_{Na}$ and $I_K$ in rodent cardiac cells and mouse astrocytes. $^{42}$ Tedisamil has no effect on $I_{Na}$ or $I_{K_{S}}$ at concentrations that cause a voltage-independent, open channel block of $I_{Na}$ and $I_K$ with apparent $K_{D}$'s of 2.5 and 4.5 $\mu$M, respectively. Tedisamil did not alter the steady-state voltage dependence of $I_{K_{S}}$ inactivation nor the use-dependent decrease in $I_{K_{S}}$ magnitude. $^{42}$ This compound is a C3A agent in rat cardiac muscle due to its block of $I_{Na}$ but acts by blocking $I_K$ in guinea pig ventricular myocytes that have little or no $I_{Na}$. The methanesulfonanilide UK-68,798 has no effect in adult rat heart, consistent with the apparent absence of $I_K$ in this species. $^{4}$ RP 58866 is a benzopyran derivative that was reported to decrease outward $I_{K_{S}}$ but had little effect on inward $I_{K_{L}}$. At concentrations (0.1–30 $\mu$M) required for C3A activity, RP 58866 had no effect on $I_{Na}$, $I_{K_{S}}$, or $I_{K_{L}}$. Although several agents with C3A activity block $I_{K_{S}}$ in addition to other actions (e.g., quinidine), RP 58866 is the first pure C3A drug that apparently acts by selective block of $I_{K_{S}}$. The mechanisms of action of C3A drugs that act at least in part by blocking one or more types of cardiac $K^+$ channels are summarized in Table 1.

### Table 1. Mechanisms of Action of Class III Antiarrhythmic Drugs

<table>
<thead>
<tr>
<th>Proposed mechanism of action</th>
<th>Examples</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific $I_{K_{S}}$ block</td>
<td>E-4031</td>
<td>2,3</td>
</tr>
<tr>
<td>$I_K$ block ($I_{K_{L}}, I_{K_{S}}$ not specified)</td>
<td>UK-68,798</td>
<td>34</td>
</tr>
<tr>
<td>$I_{Na}$ block</td>
<td></td>
<td>35</td>
</tr>
<tr>
<td>$I_K$ and $I_{Na}$ block</td>
<td>risotilide</td>
<td>22</td>
</tr>
<tr>
<td>$I_{Na}$ channel &amp; $\beta$-adrenergic receptor block</td>
<td>sematilide</td>
<td>36</td>
</tr>
<tr>
<td>$I_{K_{L}}$, $Na^+$ channel block</td>
<td>RP 58866</td>
<td>43</td>
</tr>
<tr>
<td>$I_{K_{S}}$, $Ca^{2+}$ channel block</td>
<td>tedisamil</td>
<td>42</td>
</tr>
<tr>
<td>$K^+$ channel block</td>
<td>clofilium</td>
<td>10,11</td>
</tr>
</tbody>
</table>

Mixed activity

- $K^+$ and $Na^+$ channel block
  - ("Class Ia")
  - quinidine
  - pirmenol
  - sotalol
  - amiodarone

- $K^+$ channel & $\beta$-adrenergic receptor block
  - sotalol
  - amiodarone

Only those agents for which voltage clamp data are published are included in this list.

$I_{K_{L}}$, delayed rectifier $K^+$ current ($I_{K_{L}}+I_{K_{S}}$); $I_{K_{S}}$, rapidly activating, rectifying $I_K$; $I_{K_{L}}$, slowly activating $I_K$; $I_{K_{S}}$, inward rectifier $K^+$ current; $I_{Na}$ transient outward $K^+$ current.

### Rate-Dependence and Proarrhythmic Effects of C3A Drugs

An unfortunate side effect of almost all C3A agents is their propensity toward inducing a distinct ventricular tachyarrhythmia, torsades de pointes. $^{44}$ Torsades de pointes as defined here is associated with a long QT interval and is characterized by a sinusoidal-like pattern of the surface electrocardiogram. It is widely believed that the cellular basis of this arrhythmia is the development of early after depolarizations (EADs) (Figure 1B) resulting from incomplete repolarization of the cardiac action potential. $^{44}$ EADs have been recorded from isolated cardiac tissue treated with almost all C3A drugs and were recently recorded from patients with torsades de pointes. $^{45}$ An in vivo model of torsades de pointes revealed that the doses of cesium, sematilide, clofilium, LY 97119, amperozide, or UK-68,798 required to prolong the QT interval in anesthetized rabbits was extremely well correlated with the dose that produced ventricular tachycardia. $^{46}$ Theoretically, induction of torsades de pointes is not an unavoidable consequence of C3A drug therapy. This arrhythmia is usually preceded by a long diastolic interval immediately before initiation of the tachyarrhythmia. C3A drugs are all more effective at prolonging action potential duration at slow, relative to fast, heart rates. $^{4,21}$ C3A drugs are all more effective at prolonging action potential duration at slow, relative to fast, heart rates. $^{4,27}$ Therefore, C3A drugs are more likely to induce EADs, and thereby torsades de pointes, at slow heart rates or after an unusually long diastolic pause. As discussed by Hondeghem and Snyder, $^{47}$ an agent that prolonged action potential duration preferentially at fast heart rates with little effect at slow rates would have less propensity toward precipitation of torsades de pointes. Unfortunately, a C3A agent with such a profile does not exist. The newly developed methanesulfonanilide C3A drugs (e.g., E-4031 and UK-68,798) apparently do not induce torsades de pointes in dogs, the species most used for drug efficacy studies. $^{21,25}$ Susceptibility of a given species to drug-induced torsades de pointes by these specific $I_K$ blockers is probably determined by the relative importance of $I_{K_{S}}$ to phase 3 repolarization of the...
cardiac action potential. In rabbit cardiac myocytes there is little or no \( I_{Kr} \)-like current, whereas dog and guinea pig myocytes have a relatively large \( I_{Kr} \) in addition to \( I_{Ks} \). Virtually nothing is known about the types of \( K^+ \) channels that control repolarization in human ventricular cells. However, C3A drugs can also induce torsades de pointes in humans, especially in patients who are hypokalemic or have unusually slow heart rates. Sudden cardiac death resulting from ventricular fibrillation can also be caused by a rare inherited disease called long QT syndrome. The exact nature of the inherited defect is unknown, but genetic analysis suggests a highly probable link with the Harvey-ras-1 gene, which codes for a GTP-binding protein that may in turn modulate \( K^+ \) channel activity.

The mechanism of rate-dependent lengthening of action potentials by C3A drugs is not well understood. It has been proposed that agents such as E-4031 preferentially block open \( I_Kr \) channels; however, this mode of block would be expected to result in the opposite effect: greater lengthening at fast rates. In guinea pig myocytes, the magnitude of \( I_Kr \) was not altered by applying depolarizing pulses at frequencies of either 0.5 or 3 Hz (NK Jurkiewicz and MC Sanguinetti, unpublished observations). Although UK-68,798 (1 \( \mu \)M) completely blocked \( I_Kr \) in these cells following trains of pulses delivered at either frequency, action potential lengthening is much greater at the slower pacing frequency. The rate-dependent prolongation of action potentials by C3A drugs may reflect the greater contribution of \( I_Kr \) and \( I_{Ks} \) to total outward, repolarizing current that occurs at higher pacing frequencies in these cells. The increase in \( I_Kr \) (and perhaps increased rate of \( I_Kr \) inactivation) more than offsets the block of \( I_Kr \) at higher pacing rates, limiting the effectiveness of these drugs to prolong action potential duration and refractory period.

The ability of C3A drugs to prolong action potential duration is also influenced by the presence of \( \beta \)-adrenergic receptor agonists. Lengthening of refractory period by E-4031 in isolated papillary muscles was nearly abolished if muscles were pretreated with isoproterenol. Isoproterenol increased the conductance of \( I_Kr \) in these cells, in contrast to the expected decrease in \( I_Kr \) conductance by E-4031. The augmentation of these other currents by isoproterenol has the same functional effect as rapid heart rates on the ability of C3A agents such as E-4031 to prolong action potentials. Thus, C3A drugs would be expected to have decreased efficacy in the presence of high sympathetic tone, high heart rates, or both.

**Potassium Channel Openers**

Several PCOs are currently in clinical trials for treatment of hypertension and may be useful as cardioprotectants. Cardiac PCOs may represent potential therapies for other disorders such as angina pectoris, asthma, irritable bladder syndrome, and perhaps even epilepsy. The PCOs represent a very diverse group of chemical structures that most likely do not share a single common mechanism. Seven distinct classes of PCOs are recognized: the benzenopyrans (e.g., cromakalim and EMD 52692), cyanoguanidines (e.g., pinacidil), nicotinamides (e.g., nicorandil), thiofranomides (e.g., RP 49356), pyrimidine oxides (e.g., minoxidil), benzo thiadi azines (e.g., diazoxide), and a dihydropyridine (nigul.

**Mechanism of Action in Cardiac Cells**

The most likely clinical use of PCOs will be as smooth muscle relaxants; however, the cellular mechanisms of action of diazoxide and cromakalim were first determined in \( \beta \)-pancreatic cells and cardiac cells. In all studies of cardiac cells, the PCOs have been demonstrated to be specific activators of \( K_{ATP} \) channels. Cardiac \( K_{ATP} \) channels have a unit conductance of 25 and 80 pS in the presence of 5 or 140 mM intracellular \( K^+ \), respectively, and are inhibited by intracellular ATP (ATP) with a \( K_i \) of 17–100 \( \mu \)M, being dependent on many factors such as the ATP/ADP, ratio, pH, and intracellular Mg. The open probability (\( P_o \)) of \( K_{ATP} \) channels in cardiac cells under normal conditions is extremely low, such that exposure of cells to antidiabetic sulfonylureas (e.g., glibenclamide and tolbutamide) that block these channels have no measurable effect on action potentials or net membrane currents. The \( P_o \) of \( K_{ATP} \) channels is increased when intracellular ATP concentration ([ATP]) is lowered, either artificially in excised patches or in whole cells exposed to hypoxic conditions or metabolic inhibitors. However, the physiological importance of \( K_{ATP} \) channels during ischemia, hypoxia, or metabolic poisoning has long been in doubt, since a profound shortening of cardiac action potentials can be measured at times when [ATP] is only slightly decreased. Recent studies have shed light on the apparent discrepancy between the measured decrease in [ATP], (a drop from 5.4 to 4.3 mM) and the \( K_i \) of 17–100 \( \mu \)M for block of the channels by ATP. It has been estimated that opening of less than 1% of the total number of \( K_{ATP} \) channels activated by either a PCO (SR 44866) or complete metabolic blockade is required to shorten action potential duration by 50%. Less than 10% of the \( K_{ATP} \) current that could be activated by SR 44866 was sufficient to make an isolated guinea pig myocyte electrically inexcitable. Thus, very small increases in the \( P_o \) of \( K_{ATP} \) channels by PCOs, even in the presence of near normal [ATP], will result in considerable shortening of action potential duration by cromakalim. Action potentials were recorded from isolated guinea pig papillary muscle before (panel a) and after (panel b) exposure to 10 \( \mu \)M and 30 \( \mu \)M (panel c) cromakalim. Effect of cromakalim was reversed by 0.3 \( \mu \)M glibenclamide, a \( K_{ATP} \) channel blocker (panel d).
cardiac action potentials (Figure 3). The effect of PCOs on K$_{ATP}$ activity depends on [ATP]; less of a stimulatory effect occurs at higher internal ATP levels. In inside-out patches of guinea pig myocytes, 30 µM RP 49356 shifted the concentration–response for ATP closure of K$_{ATP}$ channels by one order of magnitude. 

PCOs have either arrhythmogenic or antiarrhythmic properties, depending on the experimental model in which they are examined. Glibenclamide prevents ventricular fibrillation in the ischemic rat heart, but not in the dog. Block of K$_{ATP}$ channels during ischemia may even have deleterious effects, exacerbating myocardial stunning during reperfusion. Such an undesirable effect is not expected considering the suggested physiological role of cardiac K$_{ATP}$ channels. Noma proposed that during ischemia, activation of these channels accompanied by action potential shortening might act to protect the contractile cells from intracellular Ca$^{2+}$ overload. A high concentration of RP 49356 (100 µM) had no effect on contractile activity of isolated rat myocytes when cells were bathed in a solution containing 10 mM glucose but had profound negative inotropic effects if glucose was replaced with 2-deoxyglucose. This may explain why many of the PCOs have cardioprotective effects in ischemia models at doses that do not have measurable effects on coronary flow or cardiac function under control conditions. The proposed mechanisms of action for the various reported effects of the PCOs on cardiac and smooth muscle are summarized in Figure 4.

**Mechanism of Action in Smooth Muscle Cells**

The target molecule of PCOs in smooth muscle cells is a matter of considerable controversy. Pharmacological evidence and efflux studies support the idea that all PCOs relax smooth muscle by increasing K$^+$ efflux, causing hyperpolarization that, in turn, limits the entry of extracellular Ca$^{2+}$ required for sustained contraction (Figure 5). The general pharmacology of PCOs is reviewed elsewhere. However, there is not a consensus regarding the specific channel type (or types) that are opened by these drugs. Glibenclamide is a specific blocker of K$_{ATP}$ current in β-pancreatic and cardiac cells and is therefore often used as a pharmacological probe to determine if a given PCO acts by activation of K$_{ATP}$ channels. The vasorelaxant action of cromakalim, minoxidil sulfate, and diazoxide in rat portal venous strips was inhibited by glibenclamide (0.1–30 µM) but not by toxins that block Ca$^{2+}$-activated K$^+$ channels, amrinone, or charybdotoxin. The decrease in coronary perfusion pressure caused by cromakalim, hypoxia, or ischemia in isolated guinea pig hearts could be prevented by pretreatment with glibenclamide. The increase in $^{36}$K$^+$ efflux induced by cromakalim or ATP$_i$ depletion in rabbit mesenteric arteries was greatest in Ca$^{2+}$-free solutions and antagonized by glibenclamide. Together these findings provide strong pharmacological evidence that PCOs open K$_{ATP}$ channels but not Ca$^{2+}$-activated K$^+$ channels. The use of glibenclamide as a specific probe may not be valid in smooth muscle preparations, since this agent also blocks the large conductance, Ca$^{2+}$-activated K$^+$ channel (BK$_{Ca}$) activated by PCOs.
Voltage clamp experiments performed in the last 3 years have only added to the apparent complexity regarding the type of channel (or channels) that are activated by the PCOs. Cromakalim was demonstrated to activate a large conductance (135 pS in 60 KCl/120 K+), Ca2+-insensitive, ATP-inhibited K+ channel in rat mesenteric artery cells.93 The conductance of these channels is about twofold to threefold greater than that of KATP channels of cardiac or pancreatic B-cells but almost identical to BKCa channels under similar ionic conditions. However, unlike BKCa channels, the activity of these channels was recorded in a Ca2+-free solution (5 EGTA in pipette), unaffected by charybdotoxin (100 nM), and blocked by glibenclamide.93 In the same preparation, BKCa channels were shown to be unaffected by 1 mM ATP, 1 μM gliburide, or 10 μM cromakalim. In contrast, BKCa channels that are regulated by ATP and activated by PCOs have been recorded from other smooth muscle cell types94-99 and from aortic microsomes incorporated into planar lipid bilayers.97 Cromakalim at 50 nM increased P0 of BKCa channels incorporated into bilayers from 0.1 to 0.16 (at -40 mV, pCa=6), equivalent to a ~15 mV shift in the P0-voltage curve.97 In porcine coronary artery cells a channel similar to BKCa with respect to conductance and Ca2+ sensitivity but inhibited by ATP, (K0=0.3-0.5 mM at 0 nV, pCa=6) was given the name KATP,94 KATPCa channels were also recorded from rabbit aortic and tracheal smooth muscle cells.96,98 Cromakalim reversed the inhibitory effects of ATP, (increased P0) on these channels.95,97 A possible role for other Ca2+-activated K+ channels in the action of nicorandil has also been suggested. The outward K+ current induced by nicorandil in isolated rat portal vein cells was suppressed in nominally Ca2+-free solutions.90 In these cells, nicorandil increased P0 of a 10 pS Ca2+-activated K+ channel but not the 132 pS BKCa channel. Another proposed target for cromakalim is the delayed rectifier K+ channel in rabbit portal vein.99 In the same preparation, pinacidil inhibits an oscillatory outward K+ current that is activated by intracellular Ca2+ released from the sarcoplasmic reticulum.100 Block of this K+ current by pinacidil would presumably increase the excitability of portal vein cells if not offset by greater activation of other K+ channel types. Interestingly, glibenclamide prevented the ability of pinacidil to inhibit the oscillatory K+ current.100

Mechanism of Action in Other Cell Types

In insulin-secreting cells (RINm5F cells), cromakalim, pinacidil, RP 49356, nicorandil, and diazoxide activate KATP channels but only at concentrations greater than 100 μM.101,102 When ATP facing the inside of the membrane patch was replaced with ATPγS, diazoxide and cromakalim no longer were capable of activating KATP channels, leading to the proposal that these channel activators act via protein phosphorylation.103 In mouse skeletal muscle the activation of KATP channels by cromakalim and pinacidil but not RP 49356 required the presence of ATP on the internal side of the membrane patch.103 These PCOs had no effect on BKCa channels in this preparation. In membrane blebs of human skeletal muscle, several PCOs (EMD 52692, RP 49356, and cromakalim) were reported to activate both an ATP-sensitive and an ATP-insensitive K+ channel.104 Neither channel type was Ca2+-dependent, since the recordings were obtained in a Ca2+-free medium. Activation of PCOs of either channel type was reversed by glibenclamide. This result once again emphasizes the uncertainty in using this blocker to prove a role for KATP channels in the action of specific PCOs.

Summary

C3A agents prolong action potential duration by blocking one or more types of K+ channels. The most recently introduced and potent compounds, exemplified by E-4031 and UK-68,798, are specific blockers of IKr, a rapidly activating delayed rectifier K+ current. The potency and arrhythmogenic potential of these compounds depends on the species-dependent importance of IKr in repolarization of cardiac myocytes.

PCOs are reported to open a variety of different K+ channel subtypes in smooth muscle cells. The most important, yet unanswered, question is whether all the channels activated by PCOs in membrane patches play a role in the action of these drugs under physiological conditions and at clinically relevant doses. The resting input resistance of smooth muscle cells is 1-10 GΩ, and therefore only a very small increase in P0 of any type of K+ channel is required to hyperpolarize the resting membrane potential sufficiently to cause relaxation.105 In general, evidence from pharmacology studies using isolated smooth muscle strips supports the view that PCOs open a glibenclamide-sensitive, Ca2+-insensitive K+ channel. It remains to be determined whether PCOs activate one or a combination of large conductance KATP, BKCa, or KATP, channels in small caliber resistance vessels, which are the most relevant vascular tissues with respect to the antihypertensive properties of the PCOs.

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