Inhibition of ATP-Sensitive Potassium Channels of Adult Rat Heart Cells by Antiarrhythmic Drugs

Robert A. Haworth, Atilla B. Goknur, and Herbert A. Berkoff

We have investigated the effect of antiarrhythmic drugs on the increased potassium conductance induced in isolated adult rat heart cells by ATP depletion. The rate of ^60Rb uptake in the presence of ouabain was used as a measure of potassium conductance. Treatment of cells with rotenone plus p-trifluoromethoxyphenylhydrazone (FCCP) rapidly depleted ATP levels and strongly stimulated the rate of ^60Rb uptake. The stimulated uptake and the ATP depletion were inhibited by oligomycin; thus, the uptake was not induced by rotenone plus FCCP directly. The stimulated uptake, but not the ATP depletion, was inhibited potently by glyburide (IC50, 38.3 nM), quinidine (IC50, 2.7 /xM), verapamil (IC50, 4.5 /xM), and amiodarone (IC50, 19.1 /xM). The stimulated uptake was also inhibited by tetraethylammonium ion and by 4-aminopyridine but not by tetrodotoxin or manganese. We conclude that 1) the stimulated Rb uptake is measuring ATP-sensitive potassium channel activity, 2) the ATP-sensitive potassium channel is strongly inhibited by quinidine, verapamil, and amiodarone, and 3) this inhibition may contribute to the antiarrhythmic action of these drugs. (Circulation Research 1989;65:1157-1160)

Antiarrhythmic drugs exhibit a wide variety of electrophysiological effects. Some drugs act by prolonging the inactivation of sodium channels, either by directly binding to the sodium channel or by delaying repolarization.1 It is not yet clear what role may be played by other channels in the generation of arrhythmias. Physiologically important conditions that are arrhythmogenic include hypoxia and ischemia. Under these conditions, ATP levels drop, and a shortening of the action potential is observed.2 The latter appears to stem from an increase in time-independent outward potassium current.3 Therefore, using a novel assay with ^60Rb, we have investigated the effect of antiarrhythmic drugs on the increased potassium conductance induced by ATP depletion.

Materials and Methods

Cells were isolated from female retired breeder rat hearts as previously described4 and resuspended in experimental medium containing (mM) NaCl 118, KCl 4.8, N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid (HEPES) 25, KH2PO4 1.2, MgSO4 1.2, CaCl2 1, glucose 11, sodium pyruvate 5, and insulin 1 (juM), pH adjusted to 7.4 with NaOH at 37° C. Guinea pig heart cells were isolated by an identical procedure. At the times shown on the graphs in Figures 1 and 2, 0.5 ml aliquots were centrifuged through bromododecane for measurement of ^60Rb uptake, as previously described for other isotopes.5 Other 0.1 ml aliquots were added to 0.1 ml cold 16% perchloric acid, and ATP was measured by high-performance liquid chromatography.6 The following drugs were generously supplied by these laboratories: nitrendipine by Miles Laboratories, New Haven, Connecticut, amiodarone by Wyeth Laboratories, Philadelphia, Pennsylvania, and glyburide by Hoechst-Roussel Pharmaceuticals, Somerville, New Jersey.

Results

Potassium conductance of isolated adult rat heart cells was measured on cells in suspension from the rate of ^60Rb uptake in the presence of ouabain to inhibit uptake by the sodium pump. Rubidium, a potassium analogue, is a good choice for measuring potassium channel activity since its specificity for potassium channels over the sodium channel exceeds that of potassium itself.7 Cells treated with 1 mM ouabain alone showed a low linear rate of ^60Rb uptake, and this rate was unaffected by the addition of 100 /uM quinidine (Figure 1A). Higher levels of ouabain caused no further measurable inhibition of
Stimulation of quinidine-sensitive $^{86}$Rb uptake by ATP depletion.

**Figure 1.** Graphs showing stimulation of quinidine-sensitive $^{86}$Rb uptake by ATP depletion. Cells in experimental medium with 2 μCi/ml tritiated water were given 1 mM ouabain (C, □, ○, ⧫, and △), followed after 15 seconds by 100 μM quinidine (■ and ●) or 40 μM oligomycin (△), followed after 15 seconds by 3 μM rotenone plus 0.3 μM p-trifluoromethoxyphenylhydrazone (○, ●, and △), followed after 15 seconds by 0.1 μCi/ml $^{86}$Rb, the latter at time 0 on the graph. Panel A: $^{86}$Rb uptake. Values are measured values minus 2.9 nmol/mg, an intercept arising from extracellular $^{86}$Rb. Panel B: ATP decline. Data is from a single experiment.

$^{86}$Rb uptake; this result indicated that pump inhibition was complete (data not shown). Uptake of $^{86}$Rb is expressed as nanomole potassium equivalents per milligram protein, as though the $^{86}$Rb behaved just like potassium. To rapidly induce the ATP-sensitive potassium conductance, we treated cells with metabolic inhibitors rotenone, an inhibitor of mitochondrial nicotinamide adenine dinucleotide-linked respiration, and p-trifluoromethoxyphenylhydrazone (FCCP), an uncoupler of oxidative phosphorylation, which induces a large mitochondrial ATPase activity. When cells were given rotenone and FCCP 15 seconds before $^{86}$Rb, the level of ATP was rapidly depleted (Figure 1B), and the uptake of isotope was massively accelerated. The accelerated uptake rate was inhibited by quinidine (Figure 1A) although quinidine had no effect on the ATP depletion (Figure 1B). Oligomycin, an inhibitor of mitochondrial ATPase activity, prevented the ATP depletion induced by rotenone plus FCCP (Figure 1B) and also prevented the increase in conductance (Figure 1A). This shows that the conductance change was not induced by rotenone plus FCCP directly. Measurements with $^{14}$C sucrose showed, as we have found previously, that no increase in sucrose-permeable space occurred in the course of the experiment even though the cells were ATP-depleted (data not shown). There was, therefore, no evidence for rupture of the sarcolemma.

No significant effect of 25 μM tetrodotoxin (TTX) on $^{86}$Rb uptake was observed; after 3 minutes, uptake was 70.6±5.1 nmol/mg with TTX and 72.4±5.9 nmol/mg without TTX (n=3 measurements). Also, neither 0.1 μM isoproterenol nor 0.1 μM isoproterenol plus 1 μM propranolol had any effect on $^{86}$Rb uptake induced by rotenone plus FCCP (data not shown).

To determine the IC$_{50}$ for quinidine, the $^{86}$Rb uptake after 3 minutes was measured, under the conditions of Figure 1, in the presence of various levels of quinidine (Figure 2). The IC$_{50}$ was 3.8 μM. The inhibitory action of a number of antiarrhythmic drugs and potassium channel inhibitors was tested in the same way, and the results are shown in Table 1. Since verapamil inhibited (Table 1), we checked for calcium channel participation by looking for the verapamil effect in a medium with 10 mM MnCl$_2$ in place of 1 mM CaCl$_2$. Manganese replacement had no effect on stimulated $^{86}$Rb uptake, and the verapamil effect remained with a similar IC$_{50}$. Nitrendipine, a potent dihydropyridine calcium channel blocker, was threefold less effective than verapamil at inhibiting $^{86}$Rb uptake. $^{86}$Rb uptake was also...
Amiodarone* and verapamil (-Mn) were also isolated and tested. We found that this IC50 is equivalent to 0.0045% solution.

Table 1. Inhibition of 86Rb Uptake by Antiarrhythmic Drugs and Potassium Channel Inhibitors

<table>
<thead>
<tr>
<th>Drug</th>
<th>IC50 (nM)</th>
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<tbody>
<tr>
<td>Quinidine</td>
<td>2.7±0.2</td>
</tr>
<tr>
<td>Quinidine (guinea pig)</td>
<td>17.0±4.0</td>
</tr>
<tr>
<td>Verapamil (−Mn)</td>
<td>4.5±0.4</td>
</tr>
<tr>
<td>Nitrendipine</td>
<td>2.8±1.0</td>
</tr>
<tr>
<td>Amiodarone*</td>
<td>13.4±4.7</td>
</tr>
<tr>
<td>Tween 80f</td>
<td>19.1±5.9</td>
</tr>
<tr>
<td>Diphenylhydantoin</td>
<td>32.0±4.7</td>
</tr>
<tr>
<td>Lidoxycline</td>
<td>102.3±4.0</td>
</tr>
<tr>
<td>4-Aminopyridine</td>
<td>98.8±19.5</td>
</tr>
<tr>
<td>4-Aminopyridine</td>
<td>10.7±1.4</td>
</tr>
<tr>
<td>Tetraethylammonium chloride</td>
<td>38.3±9.7</td>
</tr>
<tr>
<td>Glyburide</td>
<td>85.3±8.6</td>
</tr>
</tbody>
</table>

Values are mean±SD obtained on three preparations of heart cells from rat unless otherwise indicated. The IC50 value was taken as the concentration of drug needed to inhibit the quinidine-sensitive 86Rb uptake in 3 minutes by 50%. Thus, the uptake obtained in the presence of quinidine (100 nM) was subtracted from all values before IC50 was calculated.

Table 2. Comparison of the Magnitude of 86Rb Uptake in the Presence of Rotenone Plus p-Trifluoromethoxyphenyldiazon in Rat and Guinea Pig Heart Cells

<table>
<thead>
<tr>
<th></th>
<th>86Rb uptake (nmeq K/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−Quinidine</td>
</tr>
<tr>
<td>Rat</td>
<td>87.2±13.9</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>104.8±25.0</td>
</tr>
</tbody>
</table>

Values are mean±SD of measurements on three preparations taken 3 minutes after 86Rb addition under the conditions of Figure 1. Quinidine concentration was 100 μM.

### Discussion

Glyburide is a specific inhibitor of ATP-sensitive potassium channels. A potassium channel has been described by Noma in heart that is activated at low ATP levels. Since 86Rb uptake stimulated by rotenone plus FCCP was inhibited completely by low levels of glyburide, we conclude that this uptake is a measure of ATP-sensitive potassium channel activity. The ATP-sensitive potassium channel in heart has also been found to be inhibited by TEA and 4-aminopyridine; this finding is consistent with our observation (Table 1).

The insensitivity of the stimulated 86Rb uptake to TTX shows that 86Rb uptake did not occur through the sodium channel, as expected from the specificity of this channel, and that sodium channel activity was not necessary for the 86Rb uptake observed. Likewise, since 10 mM manganese will block calcium channels, we conclude from the insensitivity of 86Rb uptake to manganese that calcium channels do not participate in the 86Rb uptake and that verapamil truly inhibits the ATP-sensitive potassium conductance.

Quinidine has been reported to inhibit several channels in isolated ventricular myocytes: sodium channels, delayed outward potassium currents in rabbit, and 4-aminopyridine (Table 1). Like quinidine, none of these drugs prevented the depletion of ATP induced by rotenone plus FCCP, and the inhibition of the 86Rb uptake stimulated by rotenone plus FCCP was complete (data not shown).

To determine whether or not these results were unique to rat heart cells, guinea pig heart cells were also isolated and tested. We found that guinea pig heart cells showed a large quinidine-sensitive 86Rb uptake induced by rotenone plus FCCP similar to that of rat although the quinidine-insensitive uptake was larger (Table 2). This larger quinidine-insensitive uptake was matched by a similar large basal rate of 86Rb uptake in the presence of ouabain alone (data not shown). The 86Rb uptake induced by rotenone plus FCCP was also inhibited by glyburide. The IC50 values for inhibition by quinidine and glyburide were larger than those for rat but were still within a therapeutically relevant range (Table 1).
potential duration. Lidocaine and diphenylhydantoin, known inhibitors of the sodium channel, were poor inhibitors of MRb uptake (Table 1). Of all the drugs tested, these are the only antiarrhythmic agents whose effect on ATP-sensitive potassium conductance was negligible at therapeutic concentrations. Therefore, their action may be restricted to a direct effect on sodium channels, an effect that has led to their classification as class I agents. How might inhibition of ATP-sensitive potassium channels have an antiarrhythmic effect? One possibility is by extending action potential duration and, hence, the refractory period in ATP-depleted cells. Exposing cells to metabolic stress, such as anoxia or ischemia or uncouplers of oxidative phosphorylation, results in a shortening of action potential duration and an increase in potassium conductance, probably mediated by ATP-sensitive potassium channels. Such conditions are arrhythmogenic. Blocking these channels would be expected to relengthen the action potential duration, which itself could have an antiarrhythmic effect. Another possibility, not exclusive of the first, is that potassium efflux through ATP-sensitive potassium channels causes extracellular potassium buildup. Such buildup is observed during ischemia, and the resulting depolarization may tend to be arrhythmogenic because of decreases in conduction velocity. Thus, blocking these channels could have an antiarrhythmic effect through reducing potassium efflux during ischemia. Both of these possibilities are supported by recent data showing that glyburide prevented hypoxia-induced shortening of the effective refractory period in ferret papillary muscle and that glyburide reduced potassium loss during global ischemia and abolished irreversible ventricular fibrillation during regional and global ischemia in rat. Finally, the effect of antiarrhythmic drugs on tissue excitability is known to be enhanced during ischemia. The latter may be explained by a voltage dependence of drug activity on sodium channels. Our results suggest that an action on ATP-sensitive potassium channels could also contribute to this effect.

References

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