Anti-Cholinergic Effects of Quinidine, Disopyramide, and Procainamide in Isolated Atrial Myocytes: Mediation by Different Molecular Mechanisms

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Effects of quinidine, disopyramide, and procainamide on the acetylcholine (ACh)-induced K⁺ channel current were examined in single atrial cells, using the tight-seal, whole-cell clamp technique. The pipette solution contained guanosine-5'-triphosphate (GTP) or guanosine-5'-O-(3-thiotriphosphate) (GTP-γS, a nonhydrolysable GTP analogue). In GTP-loaded cells, not only ACh but also adenosine induced a specific K⁺ channel current via GTP-binding proteins (G) by activating muscarinic ACh or adenosine receptors. Quinidine and disopyramide depressed the ACh-induced K⁺ current quite effectively. Procainamide had a weak inhibitory effect. Quinidine also depressed adenosine-induced K⁺ current, while the effect of disopyramide on adenosine-induced current was much smaller than that on ACh-induced current. In GTP-γS-loaded cells, the K⁺ channel was uncoupled from the receptors and was activated irreversibly, probably due to direct activation of G proteins by GTP-γS. Quinidine depressed the GTP-γS-induced K⁺ current just as in the cases of ACh- and adenosine-induced currents of GTP-loaded cells. Disopyramide had only a weak inhibitory effect and procainamide showed no effect. From these results, it is strongly suggested that the major mechanisms underlying the anti-cholinergic effects of quinidine, disopyramide, and procainamide are different; quinidine may inhibit the muscarinic K⁺ channel itself and/or G proteins, while disopyramide and high doses of procainamide may mainly block functions of muscarinic ACh receptors in atrial myocytes. (Circulation Research 1989;64:297-303)

It is well known that some antiarrhythmic agents, such as quinidine and disopyramide, have an anti-cholinergic action in addition to their direct depressant effects on the cardiac membrane excitability.¹,² Probably because of their anti-cholinergic action, effects of these drugs on the heart rate and the atrioventricular conduction are variable, depending on whether the heart is under the control of autonomic nerves.³-⁵ Both drugs slow the heart rate and the atrioventricular conduction in the absence of autonomic nerve control and accelerate heart rate and atrioventricular conduction in the innervated condition. Thus, the anti-cholinergic action is one important property of the drugs that determines their net effects on the heart, especially in intact animals or humans.

Mirro et al⁶ showed that both quinidine and disopyramide are competitive antagonists for cardiac muscarinic acetylcholine (ACh) receptors, leading them to conclude that anti-cholinergic effects of these agents result from blockade of muscarinic receptors. Their concept has been widely accepted. However, Nawrath et al⁷ suggested that quinidine may also have effects on the potassium conductance of the cardiac cell membrane that are opposite to the effects of ACh and proposed a possibility that receptor blockade by quinidine is not the only mechanism underlying the anti-cholinergic action of the drug. In the present study, to elucidate the postsynaptic mechanisms underlying the anti-cholinergic action of various antiarrhythmic agents, we examined effects of quinidine, disopyramide,
and procainamide on the ACh-induced K⁺ channel current in single atrial cells. The cells were loaded with GTP or guanosine-5'-O-(3-thiotriphosphate) (GTP-γS), a nonhydrolysable GTP analogue. In GTP-loaded atrial cells, the K⁺ channels were regulated by muscarinic ACh and adenosine receptors, but in GTP-γS–loaded cells, the K⁺ channels were functionally uncoupled from the membrane receptors and were irreversibly activated.8–12 Thus, we could examine effects of these drugs on the muscarinic K⁺ channel current with and without regulation of the membrane receptors. Some of the quinidine results have been published previously.13

**Materials and Methods**

**Preparations**

Single atrial cells of the guinea pig heart were isolated by an enzymatic dissociation method as described elsewhere.14,15 Briefly, collagenase (0.04% wt/vol; Type I, Sigma Chemical, St. Louis, Missouri) in nominally calcium-free Tyrode's solution was perfused through the coronary artery with Langendorff apparatus for 40–60 minutes (37°). The heart was then stored in high potassium/low chloride solution at 4° C for later experiments. A small piece of the atrial tissue was disected and was gently agitated in the recording chamber filled with control Tyrode's solution. Spindle-like relaxed atrial cells that showed clear striations were used for the experiments. All experiments were performed at 35–37° C.

**Current Measurements**

The tight-seal, whole-cell voltage clamp technique was used.16 The electrode resistance (3–5 MΩ) in series to the cell membrane was compensated for to minimize the duration of capacitive surges on the current trace in response to voltage steps. A junctional potential of −13 mV was added to the measured potential. Although high concentrations of the antiarrhythmic drugs examined in the present study (quinidine, disopyramide, and procainamide) depressed various ionic currents, i.e., high-threshold (L type) calcium current, the delayed-rectifying K⁺ current and the inward-rectifying background iK₁ current (Figure 1 in the case of quinidine; see also References 17 and 18), these agents did not affect the steady-state current at the holding potential of about −50 mV appreciably in single atrial cells. The cells were, therefore, held at −53 mV to examine specific effects of these agents on the muscarinic ACh receptor–regulated K⁺ channel.

**Solution**

The control Tyrode’s solution contained (mM) NaCl 136.5, KCl 5.4, CaCl₂ 1.8, MgCl₂ 0.53, glucose 5.5, and HEPES-NaOH buffer 5.5 (pH 7.4). The millimolar composition of the high potassium/low chloride solution was taurine 10, oxalic acid 10, glutamic acid 70, KCl 25, KH₂PO₄ 10, glucose 11, EGTA 0.5, and HEPES-KOH buffer 10 (pH 7.3–

![Figure 1](http://circres.ahajournals.org/)

**Figure 1.** Effects of quinidine on the membrane currents of single atrial myocytes. The cell was held at −50 mV and command pulses (500 msec) to various potentials were applied every 7 seconds. In the control, the cell had the high-threshold (L type) Ca²⁺ current, the delayed-rectifier K⁺ current, and the inward-rectifying background iK₁ current. Quinidine (100 μM) depressed all three kinds of ionic current, but the holding current was not affected appreciably by quinidine. Disopyramide and procainamide also did not depress the holding current at around −50 mV in single atrial cells. Arrows indicate zero current level.

7.4). The pipettes were filled with the following solution (mM): K aspartate 130, KCl 20, KH₂PO₄ 1, MgCl₂ 1, EGTA-KOH 5, Na₂ATP (Sigma) 3, and HEPES-KOH buffer 5 (pH 7.3). GTP (sodium salt; Sigma; 100 μM) or GTP-γS (tetralithium salt; Boehringer; 100 μM) was also added to the solution. Various concentrations of ACh chloride (Sigma), adenosine (Sigma), quinidine-sulfate (Sigma), and procainamide-hydrochloride (Sigma) were used. Disopyramide-phosphate was a gift from Chugai Pharmaceuticals, Ltd, Japan.

**Data Analysis**

On calculating percent inhibition of the muscarinic K⁺ channel current by various drugs, the difference
between the steady-state current in the ACh (1.1 μM) or adenosine (10 μM) containing solution, and the current level in the absence of agonists was taken as 100% in GTP-loaded cells. In GTP-γS-loaded cells, the difference between the persistent outward current in the absence of agonists and the initial current level just after the break of the patch membrane in the pipette was taken as 100%. Student’s t test was used for statistical analysis of the data (Table 1). A value of p<0.05 was considered significant.

## Results

### Effects of Quinidine, Disopyramide, and Procainamide on Muscarinic ACh Receptor–Regulated K⁺ Channel Current in GTP-Loaded Atrial Cells

Figure 2 shows effects of quinidine, disopyramide, and procainamide on the ACh-induced K⁺ channel current in atrial cells loaded with GTP through the pipettes. On application of ACh (1.1 μM) to the bath, an outward current flowing through a specific K⁺ channel was induced.11 After rapid activation, the ACh-induced current desensitized to a steady level in the continuous presence of ACh.19,20 After the current had settled to a steady level, quinidine, disopyramide, or procainamide was added to the bathing solution. Quinidine and disopyramide depressed the ACh-induced outward current effectively in a concentration-dependent manner, but the effects of procainamide were much smaller than those of quinidine and disopyramide (Figure 2A).

The extent of inhibition of the ACh-induced K⁺ current was plotted in Figure 2B for each concentration of the drug. Disopyramide was the most effective inhibitor of the current. The threshold concentration of the drug to inhibit the current was 0.01 μM. The current was depressed completely at doses of more than 100 μM. The concentration of disopyramide that produced half-maximal inhibition of the current (EC₅₀) was about 1.5 μM. Quinidine was slightly less effective than disopyramide, with an EC₅₀ of 10 μM. At 100 μM quinidine, about 95%
of the current was inhibited. Complete inhibition of the current was observed with more than 500 µM quinidine. The effect of procainamide on the ACh-induced current was much smaller than those of disopyramide and quinidine. Only about 35% of the ACh-induced current was depressed even with 100 µM procainamide. This order of potency of the drugs on inhibition of the ACh-induced current agrees with previous reports on the anti-cholinergic effects of these drugs.

Effects of Drugs on the Muscarinic K⁺ Channel Current Uncoupled From Receptors

Recently, it was shown that GTP-binding proteins (G), whose functions are inhibited by pertussis toxin, couple muscarinic receptors to a specific inward-rectifying K⁺ channel in atrial myocytes. Intracellularly applied nonhydrolysable GTP analogues can directly activate the G proteins and cause irreversible activation of the muscarinic K⁺ channel. Thus, we examined effects of quinidine, disopyramide, and procainamide on the muscarinic K⁺ channel current, uncoupled from the membrane receptors in GTP-γS–loaded cells (Figure 3).

In GTP-γS–loaded cells, the K⁺ current was induced gradually after a delay of 10–20 seconds, even with ACh absent from the bath solution. ACh accelerated the activation and evoked a large outward current. After rapid activation, the current also decreased gradually to a steady level. In contrast to the GTP-loaded cells, the ACh-induced current in GTP-γS–loaded cells persisted even after ACh was washed out of the bath solution. Atropine did not affect the remaining current in GTP-γS–loaded cells in the absence of ACh. These observations confirm the hypothesis that muscarinic K⁺ channels were functionally uncoupled from the muscarinic ACh receptors, being irreversibly activated in GTP-γS–loaded cells.

In GTP-γS–loaded cells, quinidine, added to the bath solution, inhibited the ACh-induced K⁺ current in a concentration-dependent fashion, just as in the case of GTP-loaded cells (top current trace, Figure 3A): The threshold concentration of quinidine to inhibit the current was 0.1 µM. The K⁺ current was blocked completely with 500 µM quinidine. The EC₅₀ of quinidine for depressing the ACh-induced current in GTP-γS–loaded cells was approximately 10 µM. The percent inhibition curve for quinidine block of the ACh-induced current did not differ significantly between GTP-loaded and GTP-γS–loaded atrial cells (Figures 2B and 3B; see also Table 1).

Disopyramide, on the other hand, inhibited the muscarinic K⁺ channel current to a much smaller extent in GTP-γS–loaded cells than in GTP-loaded cells. As shown in the middle current trace of Figure 3A, the inhibition of the current by disopyramide was much smaller and slower than that observed in...
Depression of muscarinic K⁺ channel current in GTP-γS-loaded cells. A: Original current traces. Protocols of superfusing with acetylcholine (ACh), quinidine (qui), disopyramide (diso), and procainamide (pro) are shown by the bars above each current trace. Concentrations of the substances are indicated in micromoles. Note that an outward current increased gradually in the absence of ACh in the bath solution. Spontaneous increase of outward K⁺ current occurred with a delay of 10–20 seconds after the rupture of the patch membrane in the pipette. The cells were held at −53 mV. Continuous lines are the zero current level. B: Relation between percent inhibition of the muscarinic K⁺ channel current and the drug concentration in GTP-γS-loaded cells. Quinidine (•••••), disopyramide (○○○○○), and procainamide (●●●●●). The difference between the remaining outward current in the absence of ACh and the initial current level just after break of the patch membrane in the pipette was taken as 100%. Symbols are mean values (±SD). See Table 1 for details.

Effects of the Drugs on Adenosine Receptor-Regulated Muscarinic K⁺ Channel Current

We have shown that adenosine activates the same K⁺ channels with ACh via G proteins in atrial myocytes. Activation of the K⁺ channel by ACh and adenosine are achieved by different membrane receptors, that is, muscarinic ACh and P₂-purinergic receptors. We examined effects of quinidine, disopyramide, and procainamide on adenosine-regulated muscarinic K⁺ channel current in GTP-loaded cells.

On application of adenosine (10 μM), an outward current through the K⁺ channel was induced by activation of P₂-purinergic receptors. The adenosine-induced outward K⁺ current also decreased time-dependently to a steady level in the continuous presence of adenosine. After the current had settled to a steady level, quinidine, disopyramide, and procainamide were applied to the bath solution (Figure 4A). Quinidine inhibited the adenosine-induced current concentration dependently. Both the time-course and the magnitude of quinidine inhibition of the current were very similar to those observed in ACh-induced currents in GTP-loaded and GTP-γS-loaded cells (top current trace, Figure 4A). The percent inhibition curves of quinidine on adenosine- and ACh-induced currents in GTP-loaded cells and in GTP-γS-loaded cells did not differ significantly from one another (Figure 4B, Table 1; see also Figures 2B and 3B).

Disopyramide did not inhibit the adenosine-induced K⁺ current effectively (middle current trace, Figure 4B): Inhibition of the current was observed with more than 10 μM disopyramide, and only 30% of the current was depressed with 100 μM disopyramide. The percent inhibition curve of disopyramide on the adenosine-induced current in GTP-loaded cells was similar to that obtained on the ACh-induced current in GTP-γS-loaded cells (Figures 3B and 4B, Table 1).

Procainamide (100 μM) did not affect the adenosine-induced current significantly (bottom trace, Figure 4A).

Discussion

In the present study, we have examined the postsynaptic mechanisms underlying the anti-cholinergic effects of various antiarrhythmic drugs, quinidine, disopyramide, and procainamide, by comparing effects of these drugs on the muscarinic K⁺ channel current in GTP-loaded and GTP-γS-loaded cells. Recently, it was shown that muscarinic regulation of cardiac K⁺ channel is composed of at least
three components: the muscarinic ACh receptors, pertussis toxin-sensitive G proteins, and the specific inward-rectifying K⁺ channel.⁸⁻¹¹ Nonhydrolysable GTP analogues such as GTP-γS and Gpp(NH)p cause irreversible activation of the G proteins and result in persistent increase of the current flow through the K⁺ channel.⁹⁻¹¹ Therefore, ACh-induced current in GTP-γS-loaded cells can be depressed by inhibition of the steps involved between GTP-γS-activated G proteins and the muscarinic K⁺ channel, but it is expected that blockade of the functions of the ACh receptor will not affect the K⁺ current in GTP-γS-loaded cells.

Although it has been proposed that anti-cholinergic effects of quinidine and disopyramide are both caused by blockade of the muscarinic ACh receptors by these drugs,⁶ we have found that effects of these drugs on the ACh-induced K⁺ channel current are completely different in GTP-γS-loaded cells. Quinidine inhibited the ACh-induced K⁺ current in GTP-γS-loaded cells as effectively as in GTP-loaded cells, while disopyramide only slightly depressed the current. Since depression of ACh-induced current in GTP-γS-loaded cells is not attributable to receptor blockade by the drug, it is concluded that quinidine may effectively depress the ACh-induced K⁺ current by blocking the muscarinic K⁺ channel itself and/or functions of activated G proteins. Consistent with this idea, the adenosine-induced K⁺ current in GTP-loaded cells was also depressed by quinidine in a similar concentration-dependent manner. Since it is well known that quinidine blocks various K⁺ channels, such as the calcium-activated K⁺ channel,²¹,²² the delayed outward K⁺ channel,²³,²⁴ (see Figure 1), and the inward-rectifier iK₁ current,²³,²⁴ direct inhibition of the K⁺ channel by the drug may be the most likely mechanism for quinidine depression of the GTP-γS-induced K⁺ channel current. Relations between percent inhibition of the K⁺ current and the quinidine concentration in GTP-loaded and GTP-γS-loaded cells did not differ significantly from one another, suggesting that blockade of the muscarinic ACh receptors by quinidine does not contribute much to the depression of the K⁺ current even in GTP-loaded cells in the present experimental conditions.

Disopyramide depressed the ACh-induced K⁺ current in GTP-loaded cells most effectively among the three kinds of drugs examined in the present study. However, the inhibitory effect of disopyramide on the muscarinic K⁺ channel current decoupled from the receptors in GTP-γS-loaded cells and on the current induced by adenosine in GTP-loaded cells were much smaller than that on ACh-induced current in GTP-loaded cells. We interpret these results as indications that disopyramide may inhibit selectively such functions of the muscarinic ACh receptors as agonist-binding to the receptors and the signal transduction from the receptor to G proteins. Because Mirro et al.⁶ showed that disopyramide is a competitive inhibitor for muscarinic ACh-receptors with a Kᵢ of about 1 μM, the most likely mechanism for the anti-cholinergic effect of disopyramide may be a specific blockade of agonist-binding to the muscarinic ACh receptors. The inhib-

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**FIGURE 4.** Depression of adenosine receptor-regulated muscarinic K⁺ channel current in GTP-loaded cells. A: Original current traces. Protocols of superfusing with adenosine (Ado), quinidine (qui), disopyramide (diso), and procainamide (pro) are shown by the bars above each current trace. Concentrations of the substances are indicated in micromoles. The cells were held at −53 mV. Continuous lines are the zero current level. B: Relation between percent inhibition of the Ado-regulated channel current and the concentration of drugs in GTP-loaded cells. Quinidine ( ●●●●), disopyramide (●●●●), and procainamide (●●●●). The difference between the steady-state current in the Ado-containing solution and the current level in the absence of Ado was taken as 100%. Symbols are mean values (±SD). See Table 1 for details.
litory effect of disopyramide on the K⁺ channel current in GTP-γS–loaded cells and on the current induced by adenosine in GTP-loaded cells did not differ significantly, suggesting that disopyramide does not affect the P₂X-purinergic receptors.

The present results also indicate that a high concentration of procainamide may selectively block the muscarinic ACh receptors, although the drug did not significantly affect the ACh-induced current in the concentration range of clinical use (1–10 μM). 23

Clinical and experimental studies have previously documented cardiac and extracardiac anti-cholinergic effects of quinidine and disopyramide. 1–5 These actions of the drugs occur at concentrations achievable in humans. The present study is the first to show that different molecular mechanisms may underlie the cardiac anti-cholinergic effects of these agents, which may provide a novel insight into understanding the action of these drugs.

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Key Words • anti-cholinergic effect • antiarrhythmic drug • acetylcholine • K⁺ channel • atrial cells
Anti-cholinergic effects of quinidine, disopyramide, and procainamide in isolated atrial myocytes: mediation by different molecular mechanisms.
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doi: 10.1161/01.RES.64.2.297

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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http://circres.ahajournals.org/content/64/2/297

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