Quinidine is a Competitive Antagonist at $\alpha_1$- and $\alpha_2$-Adrenergic Receptors

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SUMMARY. Although quinidine is known to have antiadrenergic effects in the cardiovascular system, the precise mechanism by which it exerts these effects is not well defined. We asked whether quinidine binds directly to adrenergic receptors. Radioligand-binding assays were used to identify $\alpha_1$-adrenergic receptors ($[^3]H$prazosin-binding sites) on membranes prepared from rat heart and kidney, $\alpha_2$-adrenergic receptor ($[^3]H$yohimbine-binding sites) on human platelets and rat kidney membranes, and $\beta$-adrenergic receptors ($[^125]I$iodocyanopindolol-binding sites) on rat heart and kidney membranes. Although it did not effectively compete for binding to $\beta$-adrenergic receptors, quinidine competed for binding to $\alpha_1$- and $\alpha_2$-adrenergic receptors and yielded equilibrium dissociation constants of 0.3–3 $\mu$M. Two other antiarrhythmic agents, lidocaine and procainamide, did not compete for binding to $\alpha$-adrenergic receptors. Further experiments demonstrated that the interactions of quinidine with the cardiac $\alpha_1$- and platelet $\alpha_2$-adrenergic receptors were competitive and reversible. We conclude that the antiadrenergic actions of quinidine can be explained by occupancy and competitive blockade of $\alpha_1$- and $\alpha_2$-adrenergic receptors. (Circ Res 55: 376–381, 1984)

THE antiarrhythmic drug quinidine appears to act by multiple mechanisms. In addition to its well-known direct effects on myocardial excitability, conduction velocity, and contractility, quinidine also has indirect effects on the cardiovascular system, including anticholinergic and antiadrenergic actions (c.f. Nelson, 1928; Hiatt, 1950; James and Nadeau, 1967, Schmid et al., 1974; Mirro et al., 1980; Toda et al., 1981, Caldwell et al., 1983). The anticholinergic effects on the heart appear to result from blockade of muscarinic receptors (Mirro et al., 1980), but the mechanism by which quinidine exerts its antiadrenergic actions has not been clearly defined. There are many possibilities, including a nonspecific "local anesthetic" or "membrane-stabilizing" effect, an interaction with receptor-linked ion channels, or a direct blockade at adrenergic receptors. With the availability of radioligand-binding techniques, one can test whether quinidine acts to block adrenergic response by binding to receptors. We report here results of radioligand-binding studies examining the interaction of quinidine and other antiarrhythmic agents with adrenergic receptors in heart, kidney, and platelets.

Methods

Tissue Preparation

Heart

Left ventricles were excised from adult Sprague-Dawley rats and were homogenized using a Brinkman Polytron at setting 8 for 15 seconds. After filtering through 200 $\mu$m Nitex mesh, the homogenate was centrifuged at 8000 g for 10 minutes. The supernatant extract was discarded, and the pellet was washed once and stored at $-70^\circ$ until used. The final suspension was 0.5–2.0 mg protein/ml. The buffer used for washing membranes, as well as in the binding experiments, consisted of 50 mM Tris HCl, 8 mM MgCl$_2$, 0.5 mM EGTA at pH 7.5.

Kidney

Renal membranes were prepared as described previously (Snavely and Insel, 1982). Briefly, the renal cortices of adult rats were homogenized, debris and nuclei were removed by centrifugation at 50,000 g for 5 minutes, the membranes were pelleted and washed once by centrifugation at 30,000 g for 25 minutes, and resuspended to a concentration of ~2 mg protein per ml. The buffer consisted of 150 mM NaCl, 50 mM Tris HCl, 10 mM MgCl$_2$, at pH 7.5.

Platelets

Blood was drawn from healthy volunteers who had taken no medication for 2 weeks. Platelets were isolated and washed as previously reported (Motulsky et al., 1983a), and intact platelets were suspended in 100 mM NaCl, 50 mM Tris HCl, 5 mM EDTA at pH 7.4. For preparation of membranes, the platelets were suspended in 5 mM Tris HCl, 5 mM EDTA at pH 7.5, and were ruptured by three freeze-thaw cycles using liquid nitrogen. The disrupted platelets were then suspended in ice-cold 50 mM Tris-HCl, 8 mM MgCl$_2$, 2 mM dithiothreitol, and 0.5 mM EGTA at pH 7.5, and this buffer was used in all subsequent steps. The membranes were washed twice by centrifugation at 30,000 g for 15 minutes at 4°C with intervening resuspension using a Tekmar homogenizer, and the final pellet was stored at $-70^\circ$. On the day of the experiment, the platelets were thawed, washed once, and were suspended at 0.5–1.0 mg protein/ml.
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Protein Determination

The protein content of the membrane preparations was determined by the method of Lowry et al. (1951) using bovine serum albumin standards.

Radioligand Binding

Tissue, radioligand, and competing drug were incubated at 25°C. The buffer used, the incubation time, and the total volume are mentioned in each figure legend. After the binding reached equilibrium (45–60 minutes), 10 ml of the appropriate buffer were added to the tube and the contents were filtered through Whatman GF/C fiberglass filters. The tubes and filters were then washed with a further 10 ml of buffer, and the radioactivity trapped in the filter was determined with a liquid scintillation or γ counter. Nonspecific binding to α-adrenergic receptors was defined as binding that occurred in the presence of 10 μM phentolamine; nonspecific binding to β-adrenergic receptors was defined as binding that occurred in the presence of 1 μM (∼-)propranolol. Specific binding was defined as the total binding minus the nonspecific binding. We have previously validated all radioligand assays used in this study (Karliner et al. 1982; Snively and Insel, 1982, Snively et al., 1982, 1983; Motulsky et al., 1980).

Data Analysis

Competitive radioligand-binding experiments were analyzed by nonlinear regression assuming a single class of homogeneous receptors, and the computer drew the curves shown in the figures using this model (Munson and Rodbard, 1980). Saturation-binding isotherms were analyzed by Scatchard plots. We present the mean and standard error of results obtained in replicate experiments. Unless stated otherwise, all data shown in figures are representative of those obtained in at least two separate experiments, and all experimental points were determined in triplicate.

Results

Quinidine Competes for Binding to α1- and α2-Adrenergic Receptors

Each of the two subtypes of α-adrenergic receptors can be readily identified by selective radioligands. [3H]prazosin is a radioligand that selectively identifies α1-adrenergic receptors in several tissues. As shown in Figure 1, quinidine competes for [3H]-
prazosin binding in membranes prepared from both rat heart and rat kidney. From these competitive binding experiments [and knowing the dissociation constant (K_D) for the binding of [H]prazosin to these receptors], one can calculate the dissociation constant (K_i) for the binding of the inhibitor, quinidine, to the receptors. From the data in Figure 1, the K_i of α_1-adrenergic receptors for quinidine was 1.3 μM in rat kidney membranes and 2.2 μM in rat heart membranes. Two other antiarrhythmic drugs, lidocaine and procainamide, were tested at concentrations up to 100 μM and did not compete at the cardiac α_1-receptors.

α_2-Adrenergic receptors were identified with the selective radioligand [H]yohimbine. Because rat myocardium does not have α_2-adrenergic receptors (Williams et al., 1981) that are detectable in radioligand-binding studies, we identified these receptors on rat kidney membranes and on human platelets. The K_i of α_2-adrenergic receptors for quinidine was 1.4 μM using kidney membranes and 2.5 μM using intact platelets (Fig. 2). The interaction of agonists and antagonists at the platelet α_2-adrenergic receptor can be differentiated by the addition of GTP to the incubation (Michel et al., 1980; Motulsky et al., 1980). This addition substantially decreases the affinity of agonists but not antagonists in competition for radioligand-binding sites. The interaction of quinidine at α_2-adrenergic receptors on washed platelet membranes (K_i = 0.1-0.5 μM) was not al-

**Table 1**

<table>
<thead>
<tr>
<th>Quinidine Interacts Competitively with α-Adrenergic Receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absence of quinidine</td>
</tr>
<tr>
<td>----------------------</td>
</tr>
<tr>
<td>B_max (fmol/mg)</td>
</tr>
<tr>
<td>α_1-Receptors on cardiac membranes ([H]prazosin)†</td>
</tr>
<tr>
<td>58</td>
</tr>
<tr>
<td>37</td>
</tr>
<tr>
<td>α_2-Receptors platelet membranes ([H]yohimbine)‡</td>
</tr>
<tr>
<td>461</td>
</tr>
<tr>
<td>428</td>
</tr>
<tr>
<td>α_2-Receptors on intact platelets‡</td>
</tr>
<tr>
<td>319</td>
</tr>
<tr>
<td>238</td>
</tr>
</tbody>
</table>

Each row represents data obtained in a single experiment performed as in Figure 4.

* B_max and K_D measured in the presence of quinidine.
† B_max expressed as fmol bound/mg protein.
‡ B_max expressed as sites/intact platelet.
Competition of Quinidine for \( \alpha_1 \) - and \( \alpha_2 \)-Adrenergic Receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Tissue</th>
<th>Ki (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha_1 )</td>
<td>Rat left ventricle (7)</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>( \alpha_1 )</td>
<td>Rat renal cortex (4)</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>( \alpha_2 )</td>
<td>Rat renal cortex (4)</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>( \alpha_2 )</td>
<td>Intact platelets (5)</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>( \alpha_2 )</td>
<td>Platelet membranes (5)</td>
<td>0.3 ± 0.1</td>
</tr>
</tbody>
</table>

Ki values were determined either by competitive binding curves or by saturation-binding isotherms performed in the presence and absence of quinidine. Mean values ± SEM are shown for the number of experiments indicated in parentheses for each tissue.

Table 2

\[ K_i = \frac{[\text{quinidine}]}{K_D^*} - 1 \]

Here \( K_D^* \) is the \( K_D \) measured in the presence of quinidine. Using this formula, the \( K_i \) of quinidine at the cardiac \( \alpha_1 \)-receptors was 1.5–3.6 pM, and the \( K_i \) of quinidine at the platelet \( \alpha_2 \)-receptors was 0.3–0.4 pM when platelet membranes were used and 2–4 pM when intact platelets were used (Table 1). These values are quite close to those determined in competition experiments (Fig. 1–3), and the results from both types of experiments are pooled in Table 2. In both types of experiments, quinidine had a 8- to 10-fold lower \( K_i \) in platelet membranes than in intact platelets. This discrepancy may be due to Na+, which was present in experiments with intact platelets but not platelet membranes. We found that the \( K_i \) of quinidine for \( \alpha_2 \)-adrenergic receptors in platelet (and kidney) membranes was 4- to 5-fold higher in the presence of 100 mM NaCl than in its absence.

The following experiments were performed to determine whether quinidine interacted reversibly with the receptors. We incubated the membranes...
with quinidine, washed the membranes to remove quinidine, and then measured radioligand binding as described above. The effect of quinidine on radioligand binding to both platelet \(\alpha_2\)- and cardiac \(\alpha_1\)-adrenergic receptors was reversed by washing the membranes (Fig. 4). Thus quinidine interacts with \(\alpha_1\)- and \(\alpha_2\)-adrenergic receptors in a competitive and reversible manner.

**Discussion**

Although it has been known for over 50 years that quinidine can block some adrenergic-mediated responses (Nelson, 1928), the mechanism has not been clearly defined. Our results demonstrate that quinidine is a competitive antagonist of \(\alpha_1\)-, and \(\alpha_2\)-receptors in several tissues, with a dissociation constant (concentration required to bind half the receptors) of 1–3 \(\mu\)M. The therapeutic concentration of quinidine is 2–7 \(\mu\)g/ml (Kessler et al., 1974; Hirschfeld et al., 1977), equal to 1–2 \(\mu\)M. Thus, concentrations of quinidine achieved clinically are likely to block \(\alpha_1\)-adrenergic receptors on important cardiovascular tissues. Other recent work (de Zoeten et al., 1983; Ciofolo, 1980) demonstrated that quinidine also blocks \(\alpha_1\)- and \(\alpha_2\)-adrenergic receptors in the brain. Earlier studies using another radioligand ([\(^{3}H\)]WB4101, 2-N-[2,6-dimethoxyphenoxyethyl]-aminomethyl 1,4-benzodioxane) had also suggested that quinidine competes for \(\alpha_1\)-adrenergic receptors in rat heart membranes (Yamada, et al., 1980). Thus, direct receptor blockade can explain the adrenergic blocking properties of quinidine that have been previously noted in physiological experiments.

As noted above, quinidine also has anticholinergic properties, and Mirro et al. (1980) have shown that quinidine competes for radioligand binding to muscarinic cholinergic receptors on guinea pig right atria and canine ventricles with a \(K_i\) of 2–3 \(\mu\)M, values quite close to those we found for blockade of cardiac \(\alpha_1\)-adrenergic receptors. Using rat left ventricular membranes, we have confirmed that quinidine blocks cardiac muscarinic cholinergic receptors (data not shown, and Motulsky, 1983b).

It is intriguing that similar concentrations of quinidine are able to block muscarinic cholinergic, \(\alpha_1\)-adrenergic, and \(\alpha_2\)-adrenergic receptors. Nearly identical results have been observed for another antiarrhythmic agent, verapamil (Cavey et al., 1977; Glossman and Hornung, 1980; Fairhurst et al., 1980; Atlas and Adler, 1981; Barnathan et al., 1982; Karlner et al., 1982; Motulsky et al., 1983a). Conceivably, quinidine and verapamil may bind to similar regions on the distinct receptors. Alternatively, they may bind to another membrane component that intimately interacts with all three. Additional work will be needed to define the mechanism by which quinidine and verapamil block many types of autonomic receptors.

The clinical implications of these results are speculative. It is not clear to what degree adrenergic blockade contributes to many of the therapeutic or toxic effect of quinidine. Quinidine can produce hypotension secondary to peripheral vasodilation, which responds to norepinephrine (Gilman et al., 1980). This hypotension may be explained by the ability of quinidine to antagonize the effects of catecholamines at \(\alpha\)-adrenergic receptors. The consequences of adrenergic blockade by quinidine would be expected to be most pronounced in patients who have increased adrenergic tone, high plasma levels of quinidine, or who are receiving other drugs such as prazosin, phentolamine, phenoxybenzamine, or verapamil that block \(\alpha\)-adrenergic receptors.

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**References**


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