BRIEF COMMUNICATIONS

Interaction of Verapamil and Other Calcium Channel Blockers with \( \alpha_1 \)- and \( \alpha_2 \)-Adrenergic Receptors

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SUMMARY. To determine the specificity of the previously demonstrated competition of verapamil with radioligand binding to \( \alpha \)-adrenergic receptors, we examined the interaction of calcium channel blockers with \( \alpha_1 \)- and \( \alpha_2 \)-adrenergic receptors on several tissues. Verapamil competed for \( [3H] \) prazosin binding to \( \alpha_1 \)-adrenergic receptors and for \( [3H] \) yohimbine binding to \( \alpha_2 \)-adrenergic receptors in several tissues (human platelets, rat kidney and heart, and cultured muscle cells) with dissociation constants of 0.6-6 \( \mu \)M. The calcium channel blockers D600, D591, fendiline, and prenylamine—which are structural analogues of verapamil—also competed for \( [3H] \) yohimbine binding to human platelets. Two other calcium channel blockers, diltiazem and nifedipine, did not compete for \( [3H] \) yohimbine binding to human platelets or \( [3H] \) prazosin binding to membranes prepared from rat ventricles. We used \( [3H] \) nitrendipine binding to identify putative calcium channels on rat myocardial membranes. Nifedipine and verapamil blocked these \( [3H] \) nitrendipine-binding sites on ventricular membranes, but epinephrine and prazosin did not, indicating that the ventricular \( \alpha_1 \) receptors and calcium channels are distinct. We found no specific \( [3H] \) nitrendipine binding to human platelets. We conclude that the interaction of verapamil with \( \alpha \)-adrenergic receptors is not receptor subtype or tissue specific, that interaction with \( \alpha \)-adrenergic receptors is not a property of all calcium channel blockers, and that the interaction of verapamil with \( \alpha \)-adrenergic receptors and its interaction with calcium channels occur at at least two distinct sites. (Circ Res 52: 226-231, 1983)

Methods

Drug Sources

\( [3H] \) Prazosin was from Amersham; \( [3H] \) yohimbine and \( [3H] \) nitrendipine were from New England Nuclear. The following drugs were kind gifts from their manufacturers: verapamil, D600, and D591 from Knoll, nifedipine from Pfizer, nitrendipine from Bayer, prenylamine from Hoechst, fendiline from Thieman, diltiazem from Marion, and phenotolamine from Ciba-Geigy.

Tissue Preparation

Platelets were obtained from healthy volunteers, as previously described (Motulsky et al., 1980). Briefly, platelet-rich plasma was centrifuged at 2500 g for 10 minutes, and the platelets were washed twice and finally resuspended in 50 mM Tris-HCl, 100 mM NaCl, 5 mM EDTA (pH 7.5) at \( 2 \times 10^8/\text{ml} \). Platelet membranes were prepared as described (Motulsky et al., 1980).

Ventricular membranes were prepared from the left ventricles of Sprague-Dawley rats. The ventricles were minced and homogenized in ice cold 50 mM Tris-HCl at pH 7.5 with six 10-second bursts, using a Brinkmann Polytron at maximum setting. After filtering through five layers of cheesecloth, the homogenate was centrifuged at 200 g for 10 minutes to remove debris. The supernatant was then centrifuged at 30,000 g for 10 minutes to obtain the membrane pellet, which was washed twice and stored at \( -70^\circ \)C.

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used. One preparation of pooled membranes from several animals was used for all the experiments shown.

We prepared the renal membranes as described (Snively et al., 1982). Briefly, the renal cortex of Sprague-Dawley rats was homogenized with a Potter-Elvejem Teflon glass tissue homogenizer. Debris was removed by centrifugation at 30,000 g for 25 minutes in a buffer containing 50 mM Tris-HCl and 10 mM MgCl₂ at pH 7.5.

Membranes from BC3H-1 muscle cells were prepared as previously described by a method essentially identical to that used for the renal membranes (Hughes et al., 1982).

Radioligand Binding

Tissue, radioligand, and competing drug were incubated at 25°C in 0.25 ml of the buffers noted above in a shaking water bath. Once the binding had reached equilibrium (times noted in figure legends), 10 ml of the appropriate buffer were added to the tube and the contents filtered through Whatman GF/C filters. The tubes and filters then were washed with a further 10 ml of buffer and the radioactivity on the filters was determined by liquid scintillation counting. Specific binding to a-adrenergic receptors was defined as that binding which could be competed by 10 μM phentolamine. Specific [³H]nitrendipine binding was defined as that competed by 10 μM nifedipine. Tubes containing [³H]nitrendipine or nifedipine were incubated in the dark in order to prevent photolysis of the compounds. Radioligand-binding data shown in figures represent mean values of samples run in duplicate or triplicate; replicate samples generally differed from each other by <8%.

Data Analysis

To analyze experiments in which unlabeled drugs competed with radioligand binding to a₁, and a₂-adrenergic receptors, we used a computer program that performs non-linear regression analysis using the law of mass action for one class of binding sites (Munson and Rodbard, 1980).

[³H]Nitrendipine-binding data were not subjected to this analysis, because the interaction of the unlabeled drugs with [³H]nitrendipine binding is complex and the method used above, which assumes that labeled and unlabeled drugs compete for a single set of binding sites, is therefore not appropriate.

Platelet Aggregation

Platelet-rich plasma (0.5 ml) was stirred at 37°C with various drugs, and light transmittance was monitored. The transmittance of PRP was defined to be 0%; that of plasma alone as 100%. As the platelets aggregate, the transmittance approaches that of plasma.

Results

The Interaction of Verapamil with α₁- and α₂-Adrenergic Receptors in Various Tissues

Each of the two types of α-adrenergic receptors, α₁ and α₂, can be readily identified, using the selective radioligands [³H]Prazosin and [³H]Yohimbine, respectively (reviewed in Hoffman and Lefkowitz, 1980, and Motulsky and Insel, 1982). In order to determine whether previously observed blockade of α-adrenergic receptors by verapamil was tissue-specific, we studied several tissues (Table 1). Verapamil competed similarly (at μM concentrations) for [³H]Prazosin binding to the α₁-adrenergic receptors on membranes prepared from cultured BC3H-1 muscle cells and rat renal cortex and for [³H]Yohimbine binding to the α₂-adrenergic receptors on human platelets and rat renal cortical membranes (Fig. 1). Thus, the ability to interact with verapamil seems to be a general property of α-adrenergic receptors; it is not tissue- or receptor subtype-specific.

The Interaction of Calcium Channel Blockers with the α₂-Adrenergic Receptors on Human Platelets

We examined the interaction of verapamil and other calcium channel blockers with the α₂-adrenergic receptors of human platelets in more detail. The competition between [³H]Yohimbine and various concentrations of verapamil for binding to the α₂-adrenergic receptors on membranes prepared from human

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td><strong>The Interaction of Verapamil with α-Adrenergic Receptors</strong></td>
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<tr>
<td><strong>K_D (μM)</strong></td>
</tr>
<tr>
<td><strong>Tissue</strong></td>
</tr>
<tr>
<td>Rat left ventricle</td>
</tr>
<tr>
<td>Rat renal cortex</td>
</tr>
<tr>
<td>BC3H-1 cells</td>
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<tr>
<td>Human platelets</td>
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</table>

* From Karliner et al., 1982.
† Data are mean of 2-3 experiments. In individual experiments, the values were: rat renal cortical α₁-receptors, 0.9, 2.1, 3.0 μM; rat renal cortical α₂ receptors, 3.7, 6.1, and 7.3 μM; BC3H-1 cells, 1.0 and 1.1 μM; and human platelets, 1.3, 1.7, and 1.8 μM.
Verapamil competes for [3H]yohimbine binding to membranes prepared from human platelets. Platelet membranes, 4.7 nM [3H]yohimbine, and various concentrations of verapamil were incubated for 30 minutes and the specific [3H]yohimbine binding determined. The experiment was performed in parallel in the presence and absence of 100 μM GTP and 100 mM NaCl. The Kd of [3H]yohimbine is 2.7 nM. The data shown are representative of those obtained in two similar experiments. In the two experiments, the Kd's for verapamil were 1.3 and 1.0 μM in the absence of GTP and NaCl and 1.2 and 1.8 μM in the presence of GTP and NaCl.

The interaction of agonists and antagonists at α2-adrenergic receptors on human platelet membranes can be differentiated by the addition of Na+ and GTP. Although antagonist binding is relatively unaffected, the affinity of agonist binding is decreased in the presence of GTP and Na+ (Tsai and Lefkowitz, 1979; Motulsky et al., 1980; Michel et al., 1980; Limbird et al., 1982). Verapamil competed for [3H]yohimbine binding to platelet membranes identically in the presence and absence of Na+ and GTP, indicating that it is an antagonist (Fig. 2).

To determine whether other calcium channel blockers can compete for α2-adrenergic receptor-binding sites, we examined the competition of a number of calcium channel-blocking drugs for [3H]yohimbine binding to intact platelets. The compounds D591, D600, fendiline, and prenylamine are structural analogues of verapamil, and they competed for [3H]yohimbine binding to platelets as verapamil did (Table 2). The calcium antagonists diltiazem, nifedipine, and nitrendipine, however, bear no structural resemblance to verapamil, and they did not compete for [3H]yohimbine binding even at high concentrations (100 μM). Similarly, with renal cortical membranes, nifedipine binding to intact platelets. The compounds D591, D600, fendiline, and prenylamine are structural analogues of verapamil, and they competed for [3H]yohimbine binding to platelets as verapamil did (Table 2). The calcium antagonists diltiazem, nifedipine, and nitrendipine, however, bear no structural resemblance to verapamil, and they did not compete for [3H]yohimbine binding even at high concentrations (100 μM).

**Table 2**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Kd (μM)</th>
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<tbody>
<tr>
<td>Verapamil</td>
<td>1.6</td>
</tr>
<tr>
<td>D591</td>
<td>34</td>
</tr>
<tr>
<td>D600</td>
<td>2.2</td>
</tr>
<tr>
<td>Fendiline</td>
<td>2.6</td>
</tr>
<tr>
<td>Prenylamine</td>
<td>1.6</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>†</td>
</tr>
<tr>
<td>Nitrendipine</td>
<td>†</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>†</td>
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</tbody>
</table>

* Each value is the mean of at least two separate determinations. In individual experiments, the values were: verapamil, 1.3, 1.7, 1.8 μM, D591, 25 and 43 μM, D600, 2.2 and 2.2 μM, fendiline, 1.8 and 3.4 μM, and prenylamine, 0.6 and 2.7 μM.
† No competition at 100 μM.
CL 

LJ O

C_> UJ 

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FIGURE 5. Verapamil blocks [3H]prazosin binding to membranes prepared from left ventricles of rats. Membranes, 0.2 nm [3H] prazosin and various concentrations of verapamil, nifedipine, or diltiazem were incubated for 60 minutes and the specific binding determined. This experiment was performed in parallel with that of Figure 4.

(up to 100 μM) did not compete for [3H]prazosin or [3H]yohimbine-binding sites, and although high concentrations of diltiazem competed completely for binding of both ligands, its KD was >50 μM.

To correlate our radioligand-binding data with platelet function, we measured epinephrine-induced platelet aggregation in the presence of the calcium channel-blocking drugs. Verapamil prevented epinephrine-induced aggregation, but nifedipine and diltiazem did not (Fig. 3). None of the three compounds induced aggregation.

[3H]Nitrendipine Binding to Ventricular Membranes

[3H]Nitrendipine, a structural analogue of nifedipine, binds with high affinity to putative calcium channels in membranes prepared from rat heart and other tissues (Ehlert et al., 1982; Murphy and Snyder, 1982; Bolger et al., 1982). We found that nifedipine competed for all the specific [3H]nitrendipine binding in ventricular membranes. Verapamil also blocked [3H]nitrendipine binding, but prazosin and epinephrine did not (Fig. 4). In a parallel experiment, nifedipine did not block [3H]prazosin binding although verapamil did (Fig. 5). Thus, the sites at which verapamil blocks α-adrenergic receptors and at which it blocks calcium channels appear to be distinct.

We also attempted to measure specific [3H]nitrrendipine binding to human platelets. However, we found no specific binding (defined as binding competed for either by 10 μM verapamil or 10 μM nifedipine) to intact platelets or to platelet membranes incubated either in the presence or absence of 8 mM MgCl2 (not shown).

Discussion

The terms, "calcium antagonists," "calcium channel blockers," and "calcium entry blockers," are used interchangeably and are deceptively simple. These drugs have diverse structures and have heterogeneous physiological and clinical effects. They all block calcium entry at the "slow" voltage-sensitive channel in myocardial tissue, but the precise mechanisms are unknown (reviewed in Fleckenstein, 1977; Henry, 1980; Winquist et al., 1981).

Previous studies have demonstrated that verapamil (and its methoxy derivative D600) competitively blocks radioligand binding to α-adrenergic and muscarinic receptors (Katliner et al., 1982; Nayler et al., 1982; Barnatham et al., 1982; Atlas and Adler, 1981; Cavey et al., 1977; Fairhurst et al., 1980; Glossman and Hornung, 1980). These effects occur at concentrations of ~1 μM, somewhat higher than concentrations achieved clinically in the plasma (~0.1 μM) but similar to those achieved in the human (Kates et al., 1981) and canine (Keefe and Kates, 1982) myocardium. Thus, these studies have suggested that some of the clinical and experimental effects of verapamil may be due to its blockade α-adrenergic receptors. These studies have also raised the question of whether all calcium channel blockers also block α-adrenergic receptors and whether calcium channels and α-adrenergic receptors are closely linked.

Our results demonstrate that verapamil competes similarly for [3H]prazosin (α1-selective) and [3H]yohimbine (α2-selective) binding to several tissues. Thus, the interaction of verapamil with α-adrenergic receptors is not tissue- or α-receptor subtype-specific. However, nifedipine and diltiazem (which are not structurally related to verapamil) do not compete for either [3H]prazosin binding to α1-adrenergic receptors on rat heart or for [3H]yohimbine binding to α2-adrenergic receptors on human platelets. Thus, the ability to interact with α-adrenergic receptors is not a property of all drugs that block calcium channels; rather, it is a particular property of verapamil and its analogues. This is not surprising, because the structure of verapamil bears far greater resemblance to that of epinephrine than to those of nifedipine and diltiazem.

Our results with GTP (Fig. 2) indicate that verapamil acts as an antagonist. Other data (not shown) indicate that verapamil binds competitively and reversibly to the platelet α1-adrenergic receptor. It is therefore quite likely that verapamil binds directly to the receptor-binding site and does not influence radioligand binding by an interaction at an adjacent site.

As anticipated from the radioligand-binding data, we found that verapamil inhibited epinephrine-induced aggregation. This corresponds with previous in vitro and in vivo data (Barnatham et al., 1982; Owen et al., 1980; Ikeda et al., 1981). In contrast, nifedipine and diltiazem had negligible effects on epinephrine-induced platelet aggregation.

[3H]Nitrendipine can be used to directly identify putative calcium channels in myocardial tissue (Ehlert et al., 1982; Murphy and Snyder, 1982; Bolger et al., 1982; Williams and Tremble, 1982). [3H]Nitrendipine binds with high affinity (KD = 0.1 nm) to these sites.
Verapamil interacts at these [3H]nitrendipine-binding sites, although the interaction is complex and cannot be explained by simple competition between a labeled and unlabeled drug for the same set of sites (Ehler et al., 1982). Our data agreed with those of Ehler et al. (1982): verapamil did compete for [3H]nitrendipine-binding sites, but even high concentrations of verapamil did not completely block the specific binding. In contrast, nifedipine competed for all the specific [3H]nitrendipine-binding sites, whereas epinephrine and prazosin blocked none. Thus, verapamil appears to interact with two or more distinct sites in the heart; the a-adrenergic receptor identified with [3H]prazosin and competed for by epinephrine but not nifedipine, and the putative calcium channel identified by [3H]nitrendipine and competed for by nifedipine but not prazosin or epinephrine. The fact that verapamil competes for radioligand binding to a-adrenergic receptors had suggested the possibility (Glossman and Hornung, 1980; Karlner et al., 1982) that verapamil exerts both its effect on calcium channels and its effects on a-adrenergic receptors by binding to one site. Our current results, however, suggest that verapamil binds to two distinct sites.

The physiological and clinical implications of our findings are unclear. Whereas it has been shown that verapamil blocks some a-adrenergic responses (e.g., Endoh et al., 1975; Van Meel et al., 1981; Nghiem et al., 1982; Hultén et al., 1982), the detailed experiments to discriminate critically between the effects due to blockade of a-adrenergic receptors and those due to blockade of calcium channels have not been reported. Our results suggest that some of the differences between verapamil and nifedipine or diltiazem may be due to its blockade of a-adrenergic receptors. For example, verapamil, but neither nifedipine nor diltiazem, can be used to treat supraventricular arrhythmias. It is tempting to speculate that this effect of verapamil may be due to a-adrenergic blockade.

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Portions of these data were presented at the 1981 meeting of the American Heart Association (Motulsky et al., 1981).

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