BRIEF COMMUNICATIONS

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

Harvey J. Motulsky, Marshall D. Snavely, Richard J. Hughes, and Paul A. Insel
From the Division of Pharmacology, University of California, San Diego, La Jolla, California

SUMMARY. To determine the specificity of the previously demonstrated competition of verapamil with radioligand binding to \( \alpha_1 \)-adrenergic receptors, we examined the interaction of calcium channel blockers with \( \alpha_1 \)- and \( \alpha_2 \)-adrenergic receptors on several tissues. Verapamil competed for \([H] \) prazosin binding to \( \alpha_1 \)-adrenergic receptors and for \([H] \) 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 \([H] \) yohimbine binding to human platelets. Two other calcium channel blockers, diltiazem and nifedipine, did not compete for \([H] \) yohimbine binding to human platelets or \([H] \) prazosin binding to membranes prepared from rat ventricles. We used \([H] \) nitrendipine binding to identify putative calcium channels on rat myocardial membranes. Nifedipine and verapamil blocked these \([H] \) 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 \([H] \) 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 least two distinct sites. (Circ Res 52: 226-231, 1983)

RADIOLIGAND binding allows one to identify cell surface receptors directly, and to examine indirectly the interaction of unlabeled compounds with those receptors, using competitive binding studies. In a number of laboratories, such experiments have led to the surprising result that verapamil, commonly thought to be exclusively a calcium channel blocker, also blocks radioligand binding to several types of membrane receptors. For example, verapamil is a competitive inhibitor of radioligand binding to \( \alpha \)-adrenergic receptors in rat ventricular membranes (Glossman and Hornung, 1980; Karlimer et al., 1982) and in human platelets (Barnathan et al., 1982). However, several questions have not been addressed. (1) Does verapamil interact with all \( \alpha \)-adrenergic receptors? (2) Do all calcium channel-blocking drugs block binding to \( \alpha \)-adrenergic receptors? (3) Does verapamil act at the same site to inhibit calcium entry and block binding to adrenergic receptors? In this paper, we examine the interaction of verapamil and other calcium channel blockers with \( \alpha_1 \)- and \( \alpha_2 \)-adrenergic receptors on several tissues. We show that verapamil and its analogues block radioligand binding to both subtypes of \( \alpha \)-adrenergic receptors, that the structurally dissimilar calcium antagonists nifedipine and diltiazem do not, and that verapamil interacts with at least two distinct sites on rat ventricular membranes.

Methods

Drug Sources
\([H] \) Prazosin was from Amersham; \([H] \) Yohimbine and \([H] \) 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 phentolamine 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 \( \approx2 \times 10^9/ml \). 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 \( -70^\circ C \). After thawing, the membranes were washed once before being...
used. One preparation of pooled membranes from several animals was used for all of the experiments shown.

We prepared the renal membranes as described (Snavely et al., 1982). Briefly, the renal cortex of Sprague-Dawley rats was homogenized with a Potter-Elvehjem Teflon glass tissue homogenizer. Debris was removed by centrifugation at 500 g for 5 minutes, and the remaining particulate suspension was washed once 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 α-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 α₁- and α₂-adrenergic receptors, we used a computer program that performs nonlinear 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 decreases.

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 platelets is shown in Figure 1.
FIGURE 2. Verapamil competes for \(^{3}H\)yohimbine binding to membranes prepared from human platelets. Platelet membranes, 4.7 nM \(^{3}H\)yohimbine, and various concentrations of verapamil were incubated for 30 minutes and the specific \(^{3}H\)yohimbine binding determined. The experiment was performed in parallel in the presence and absence of 100 \(\mu M\) GTP and 100 \(\mu M\) NaCl. The \(K_d\) of \(^{3}H\)yohimbine is 2.7 nM. The data shown are representative of those obtained in two similar experiments. In the two experiments, the \(K_d\)'s for verapamil were 1.3 and 1.0 \(\mu M\) in the absence of GTP and NaCl and 1.2 and 1.8 \(\mu M\) in the presence of GTP and NaCl.

binding to intact platelets. The compounds D591, D600, fendiline, and prenylamine are structural analogues of verapamil, and they competed for \(^{3}H\)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 \(^{3}H\)yohimbine binding even at high concentrations (100 \(\mu M\)). Similarly, with renal cortical membranes, nifedipine

platelets is shown in Figure 2. Similar results were obtained when intact platelets were used (Table 1). The interaction of agonists and antagonists at \(\alpha\)-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 \(^{3}H\)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 \(\alpha\)-adrenergic receptor-binding sites, we examined the competition of a number of calcium channel-blocking drugs for \(^{3}H\)yohimbine binding to intact platelets. The compounds D591, D600, fendiline, and prenylamine are structural analogues of verapamil, and they competed for \(^{3}H\)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 \(^{3}H\)yohimbine binding even at high concentrations (100 \(\mu M\)). Similarly, with renal cortical membranes, nifedipine

In individual experiments, the values were: verapamil, 1.3, 1.7, 1.8 \(\mu M\), D591, 25 and 43 \(\mu M\), D600, 2.2 and 2.2 \(\mu M\), fendiline, 1.8 and 3.4 \(\mu M\), and prenylamine, 0.6 and 2.7 \(\mu M\).

† No competition at 100 \(\mu M\).

<table>
<thead>
<tr>
<th>Compound</th>
<th>(K_d) ((\mu M)) (\ast)</th>
</tr>
</thead>
<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>
</tr>
</tbody>
</table>

\(\ast\) Each value is the mean of at least two separate determinations.

FIGURE 3. Verapamil blocks epinephrine-induced platelet aggregation. Platelet-rich plasma was incubated at 37\(^{\circ}\)C with 10 \(\mu M\) of verapamil, diltiazem, or nifedipine for 5 minutes. Aggregation induced by 1 \(\mu M\) epinephrine was measured. The final incubation included 0.5 ml of BPP, 0.005 ml of calcium channel blocker, and 0.005 ml of epinephrine in 1 mg/ml ascorbic acid. The data shown are similar to those obtained in two separate experiments, in that 10 \(\mu M\) verapamil blocked epinephrine-induced aggregation in both experiments, whereas 10 \(\mu M\) diltiazem and nifedipine were ineffective in blocking aggregation.

FIGURE 4. Verapamil blocks \(^{3}H\)nitrendipine binding to membranes prepared from the left ventricles of rats. Membranes, 0.05 \(\mu M\) \(^{3}H\)nitrendipine, and various concentrations of verapamil, nifedipine, epinephrine, or prazosin were incubated for 90 minutes and the specific radioligand binding determined. \(0\%\) specific binding is defined as that binding occurring in the presence of 10 \(\mu M\) nifedipine; it was 89\% of the total binding. Each tube contained 1 ml of tissue suspension (final) containing 0.5 mg protein. Without competition, there was 25 fmol/mg specific \(^{3}H\)nitrendipine binding. The data shown are similar to those obtained in two separate experiments, in that epinephrine and prazosin (up to 0.1 mM) had limited ability to compete for \(^{3}H\)nitrendipine-binding sites, whereas nifedipine competed fully for \(^{3}H\)nitrendipine sites (EC\(_{50}\) 5\(\mu M\)) and up to 10 \(\mu M\) verapamil competed for only about 80\% of the \(^{3}H\)nitrendipine sites.
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 binding to α-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 do 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 α2-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 aggregation. 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 α2-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 do those of nifedipine and diltiazem.

Discussion

The terms, "calcium antagonists," "calcium channel blockers," and "calcium entry blockers," are used interchangeably and are deceptively simple. These

![Graph](https://example.com/graph.png)
Verapamil interacts at these \[^{3}H\]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 (Ehlert et al., 1982). Our data agree with those of Ehlerl et al. (1982): verapamil did compete for \[^{3}H\]nitrendipine-binding sites, but even high concentrations of verapamil did not completely block the specific binding. In contrast, nifedipine competed for all the specific \[^{3}H\]nitrendipine-binding sites, whereas epinephrine and prazosin blocked none. Thus, verapamil appears to interact with two or more distinct sites in the heart; the \(\alpha\)-adrenergic receptor identified with \[^{3}H\]prazosin and competed for by epinephrine but not nifedipine, and the putative calcium channel identified by \[^{3}H\]nitrendipine and competed for by nifedipine but not prazosin or epinephrine. The fact that verapamil competes for radioligand binding to \(\alpha\)-adrenergic receptors has suggested the possibility (Glossman and Hornung, 1980; Karliner et al., 1982) that verapamil exerts both its effect on calcium channels and its effects on \(\alpha\)-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 \(\alpha\)-adrenergic responses (e.g., Endoh et al., 1975; Van Meel et al., 1981; Ngheim et al., 1982; Hulthen et al., 1982), the detailed experiments to discriminate critically between the effects due to blockade of \(\alpha\)-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 dilazem may be due to its blockade of \(\alpha\)-adrenergic receptors. For example, verapamil, but neither nifedipine nor dilazem, can be used to treat supraventricular arrhythmias. It is tempting to speculate that this effect of verapamil may be due to \(\alpha\)-adrenergic blockade.

We thank Joan Burns for technical assistance and Sandra Dutky for assistance in preparing this manuscript.

This work was supported by grants from the American Heart Association (79-680), California Heart Association (81-5115) and National Institutes of Health (HL 25457). HJM is the recipient of a National Science Foundation Predoctoral Fellowship, R7H of a California Heart Association Post doctoral Fellowship, and PAI of an Established Investigatorship of the American Heart Association.

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

Addres for reprints: Dr. Paul A. Insel, Division of Pharmacology, M 013 H, University of California, San Diego, La Jolla, California 92033.

Received June 25, 1982, accepted for publication November 11, 1982.

References


Endoh M, Wagner J, Shumann HJ (1975) Influence of temperature on the positive inotropic effects mediated by \(\alpha\)- and \(\alpha\)-adrenoreceptors in the isolated rabbit papillary muscle. Naunyn Schmiedebergs Arch Pharmakol 282: 61-72


Murphy KMM, Snyder SH (1982) Calcium antagonist receptor binding sites labelled with \[^{3}H\]nitrendipine. Eur J Pharmacol 77: 201-202

Naylor WG, Thompson JE, Jarrott B (1982) The interaction of

Circulation Research / Vol. 52, No. 2, February 1983
calcium antagonists (slow channel blockers) with myocardial alpha adrenoceptors. J Mol Cell Cardiol 14: 185-188


INDEX TERMS: Adrenergic receptors • Calcium channel blockers • [3H]Nitrendipine • Verapamil • Radioligand binding • Alpha-adrenergic receptors
Interaction of verapamil and other calcium channel blockers with alpha 1- and alpha 2-adrenergic receptors.
H J Motulsky, M D Snively, R J Hughes and P A Insel

Circ Res. 1983;52:226-231
doi: 10.1161/01.RES.52.2.226

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/52/2/226