The interaction of verapamil and norverapamil with \( \beta \)-adrenergic receptors

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ABSTRACT  To determine the effect of calcium-channel blockers on \( \beta \)-adrenergic receptors, we studied the interactions of verapamil, diltiazem, and nifedipine with both human lymphocyte \( \beta_2 \)-adrenergic receptors and rat myocardial \( \beta_1 \)-adrenergic receptors by means of radioligand binding assays. We also determined the functional consequences of these interactions by measuring adenylyl cyclase activity. Radioligand binding studies in vitro demonstrated a \( K_I \) of verapamil for the lymphocyte \( \beta_2 \)-receptor of 32 ± 4 \( \mu \)M. Diltiazem and nifedipine were much less potent. In studies of adenylyl cyclase activity, verapamil was shown to act as a competitive \( \beta \)-receptor antagonist. Also, norverapamil, the active metabolite of verapamil, had the highest affinity for the \( \beta \)-receptor of any of the calcium-channel blockers studied (\( K_I = 4.2 \pm 0.8 \mu \)M). After 1 week of verapamil administration in six normal subjects, isoproterenol-stimulated adenylyl cyclase activity in lymphocytes was increased from 60 ± 4% to 83 ± 10% over basal activity (p < .05). This was associated with an increase in lymphocyte \( \beta \)-receptor affinity for agonist as represented by the decrease in the IC\textsubscript{50} for isoproterenol inhibition of \([\text{\text{\textsuperscript{125I}}]}\) iodocyanopindolol binding from 240 ± 20 to 170 ± 10 nM (p < .05). Additionally, plasma norepinephrine levels were reduced from 206 ± 58 to 92 ± 18 pg/ml with 1 week of verapamil treatment (p < .05). Our data suggest that verapamil affects lymphocyte \( \beta \)-receptor function either directly or indirectly via a reduction in plasma catecholamine levels.


IMPORTANT differences in clinical effects are apparent between the Food and Drug Administration–approved calcium-channel blockers verapamil, diltiazem, and nifedipine. This difference is evident in the pharmacologic effects of these agents. For example, both nifedipine and verapamil reduce blood pressure in hypertensive subjects. However, nifedipine is associated with an increase in heart rate and stroke index,\(^1\) whereas verapamil may cause a reduction in heart rate and stroke index.\(^2\) The reason for these differences is not clear but is not surprising considering the marked structural differences between these agents. It has been suggested that these agents may act at different calcium channels (reviewed in ref. 3). Additionally, it has been appreciated that verapamil and related compounds may interact at sites other than calcium channels, including opiate and \( \alpha \)-adrenergic receptors,\(^3,4\) and this interaction may be of therapeutic significance.\(^5\)

Because many of the effects of verapamil are similar to those seen with \( \beta \)-adrenergic receptor blockers, we have studied the possible interactions of verapamil with \( \beta_2 \)- and \( \beta_1 \)-adrenergic receptor systems by means of radioligand binding techniques and assays of \( \beta \)-adrenergic–mediated adenylyl cyclase activity. We have also compared the effects of verapamil in vitro with those of diltiazem, nifedipine, and norverapamil, the primary active metabolite of verapamil. Additionally, to determine the effect of verapamil on \( \beta \)-receptor regulation in vivo, we have studied human leukocyte \( \beta \)-receptors from normal subjects before, during, and after long-term oral administration of verapamil.

Methods

Materials. The following drugs were gifts from the manufacturers: verapamil hydrochloride and norverapamil bixalate from Dr. Siebeneck, Knoll Pharmaceutical Co.; diltiazem hydrochloride from Marion Laboratories; nifedipine hydrochloride from Pfizer Laboratories. [\( \text{\text{\textsuperscript{32P}}} \)]ATP, [\( \text{\text{\textsuperscript{3H}}} \)]cyclic AMP, and [\( \text{\text{\textsuperscript{125I}}} \)]iodocyanopindolol (ICYP) were purchased from New England Nuclear, Ltd. All other compounds were obtained from Sigma Chemical Co.
Tissue preparation. Whole blood was taken from healthy volunteers according to a modification of previously described methods.7 Lymphocytes were isolated from ethylenediamine tetra-acetate acid (EDTA)-anticoagulated blood according to the method of Boyum.8 Fresh blood (100 ml) was centrifuged at 300 g for 14 min at room temperature, the platelet-rich plasma was aspirated, and theuffy coats were collected into two 50 ml tubes and diluted approximately fourfold with 30 ml of phosphate-buffered saline (pH 7.6). A Ficoll (6%) Hypaque (10%) solution was carefully subfused and the samples centrifuged at 400 g for 40 min at room temperature. After careful removal of the saline/plasma layer, the lymphocyte band was harvested by aspiration. The mononuclear cells isolated by this procedure were at least 80% small lymphocytes.

The lymphocytes were washed with ice-cold buffer containing 100 mM NaCl, 15 mM MgCl2, 3 mM EDTA, 6 mM sucrose, and 20 mM Tris-HCl (pH 7.9 at room temperature) and centrifuged at 37,000 g for 10 min. The supernatant was discarded and the pellet was resuspended in an ice-cold "lysing" buffer of 3 mM EDTA and 2 mM Tris-HCl (pH 7.9 at room temperature) and homogenized with a Polytron, setting 7, for 10 sec. The homogenate was centrifuged at 37,000 g for 10 min. The pellet from this centrifugation was demonstrated morphologically to contain no intact cells. For binding studies, the preparations were resuspended in 20 mM Tris-HCl, 15 mM MgCl2, 3 mM EDTA, and 100 mM NaCl (pH 7.9 at 25°C) at a concentration of 200 to 300 μg protein/ml and frozen and stored at −80°C. Storage for up to 10 days did not alter binding characteristics. For adenylyl cyclase studies, preparations were suspended in 75 mM Tris-HCl and 25 mM MgCl2 (pH 7.9 at 25°C) and assayed immediately.

Rat ventricular cell homogenates were prepared from primary cell cultures of heart cells from neonatal rats according to the methods of Marvin et al.9 Membranes were prepared from cell cultures as follows: growth medium was removed and adherent cells were washed twice with a cold lysis buffer containing 5 mM Tris-HCl (pH 7.4 at 4°C), 2 mM EDTA, and 2 mM MgCl2. Cell lysis was achieved by incubating the cells in lysis buffer for 15 min on ice. This resulted in cell swelling. The cells then lysed when the plates were scraped with a rubber policeman.

The resulting lysate was pelleted at 37,000 g for 10 min at 4°C, then resuspended in 20 mM Tris-HCl (pH 7.5 at 37°C), 100 mM NaCl, 15 mM MgCl2, 6 mM sucrose, and 3 mM EDTA. Radioligand binding studies. β-Receptor binding studies were performed according to modifications of previously described methods,7 with use of ICYP (2.2 Ci/μmol, New England Nuclear Corp.). ICYP and other drugs were prepared in 1.25 mM ascorbic acid as an antioxidant with 10 μg/ml bovine serum albumin.

Aliquots of the lymphocyte or cardiac cell preparations containing 10 to 20 μg of protein were incubated with various concentrations of ICYP in a final volume of 250 μl containing 0.5 mM ascorbic acid, 1.0 μg bovine serum albumin, 12 mM Tris-HCl (pH 7.9, 24°C), 60 mM NaCl, 9 mM MgCl2, 1.8 mM EDTA, and 3.6 mM sucrose. Samples were incubated in disposable polypropylene tubes at 37°C for 100 min, at which time steady state had been reached at all concentrations. Incubations were stopped by the addition of 10 ml of 0.9% NaCl with 10 mM Tris-HCl and 15 mM MgCl2 (pH 7.9 at 25°C) to each assay tube followed by sepa-ration of membrane-bound and "free" ICYP by rapid filtration through Whatman GF/C filters. Each filter was washed with an additional 10 ml of buffer and radioactivity was determined in a gamma counter. Protein concentration was determined by the method of Lowry et al.,10 with bovine serum albumin as a standard.

ICYP bound selectively to β-adrenergic receptors in both cardiac and lymphocyte membrane preparations. Specific binding of ICYP was reversible and saturable, and linear Scatchard plots were obtained consistent with antagonist binding to a single class of receptors. Nonspecific binding was determined by the amount of ICYP bound in the presence of 100 μM isoproterenol and accounted for less than 20% of total binding at concentrations of ICYP approaching its Ki. By means of competition binding techniques, binding was shown to be stereoselective. The order of potency for agonists (determined by comparing the concentrations required to inhibit 50% of specific ICYP binding [IC50]) in lymphocyte membranes was isoproterenol > epinephrine > norepinephrine, consistent with binding to a β2-receptor subtype. In cardiac membranes the order of potency was isoproterenol > epinephrine = norepinephrine, consistent with binding to a β1-receptor subtype.

Receptor affinity for calcium-channel blockers was derived from the inhibition of ICYP competition binding with varying concentrations of verapamil, norterapamil, dilutazem, or nifedi- pine. The former three drugs were dissolved in ethanol to a final concentration of 0.2%. Nifedipine was dissolved in 2% ethanol. Studies in which nifedipine was used were conducted under dim light to prevent the drug from degrading. Concentrations of ethanol up to 2% did not alter binding.

In studies of the effect of verapamil treatment on β-receptors in vivo, affinity for the agonist isoproterenol was determined from competition curves in the absence and presence of guanyli-midodiphosphate [Gpp(NH)p, a hydrolysis-resistant analog of GTP], as previously described.7 For each competition curve, specific binding was analyzed by a nonlinear curve-fitting procedure with a generalized model for complex ligand receptor systems.11 This procedure, based on the law of mass action, provides estimates of the dissociation constants for the competitor. Fitted estimates of receptor affinity for the competitors were obtained assuming one and two affinity states. The two affinity state model was accepted only if it significantly improved (p < .05) the "fit" of the data over the one affinity state model. Additionally, for isoproterenol competition curves, the IC50 for isoproterenol was calculated by logit analysis to determine a measure of β-receptor affinity for agonist, independent of a particular model for agonist-receptor interactions.

We demonstrated that competition curves for the agonist isoproterenol in the absence of added guanine nucleotides were "shallow," with a slope factor less than 1, and were best fit to a two affinity state model. These data suggest heterogeneous binding of agonists to the leukocyte β-receptor. This is not caused by the presence of multiple β-receptor subtypes, since the lymphocyte β-receptor population is primarily of the β2 subtype. These data are consistent with the ternary complex model of the β-receptor, i.e., that the receptor binds agonists with two affinities, high and low.12,13 According to this model, formation of the "high affinity complex" is a prerequisite for β-receptor–mediated adenylyl cyclase activity. An impaired ability to form the high-affinity complex has been correlated with reduced β-adrenergic adenylyl cyclase activity (so-called uncoupling of the β-receptor complex) as in the phenomenon of desensitization.14 Changes in the ability to form the high-affinity complex can be inferred from changes in the competition curves for agonists (e.g., isoproterenol).13 By means of computerized fitting of isoproterenol competition curves, an impaired ability to form the high-affinity state has been correlated with reductions in the computer-derived estimates for the proportion of receptors binding agonist with a high affinity (%RHi) and for the ratio of the dissociation constants of the high- and low-affinity states (Kf/Kfh).13 Therefore, to study the effect of verap- amil treatment on the ability to form the high-affinity complex of the β-receptor, we performed agonist competition curves with lymphocyte membranes from subjects given verapamil.
With the addition of Gpp(NH)p the fit of isoproterenol competition curves was not significantly improved by assuming a two-affinity state model. This is consistent with the results of previous studies, suggesting that addition of guanine nucleotides in vitro converts the receptor population to a homogeneous low-affinity state. Therefore the IC50 for isoproterenol competition in the presence of added guanine nucleotides solely represents the low-affinity state of the receptor for agonists.

In initial studies we showed that competition curves for the antagonist propranolol were steeper (slope factor approaching 1) and were best explained by a one affinity site model. This suggests that for the lymphocyte ß-receptor, as in other model systems, an antagonist binds with one homogeneous affinity, not dependent on guanine nucleotides.

Receptor density and Kd for the antagonist radioligand ICYP were determined from saturation curves of specific ICYP binding at six concentrations of ICYP from 5 to 30 pM. Receptor density and Kd were determined by computerized nonlinear curve fitting analysis.

**Adenylate cyclase assay.** Adenylate cyclase activity was measured in lymphocyte membrane preparations by a modification of the method of Salomon et al. Enzyme activity was inferred by the ability of membranes to mediate conversion of [α32P]ATP to [c32P] cyclic AMP (cAMP). One hundred to 200 μg of membranes suspended in 75 mM Tris-HCl and 25 mM MgCl2 (pH 7.9, 25° C) was added to give a final incubation volume of 150 μl with 1 μCi [α32P] ATP (New England Nuclear), 0.3 mM ATP, 10 mM MgCl2, 0.1 mM CAMP, 5 mM phosphoenolpyruvate, 40 μg/ml pyruvate kinase, and 20 μg/ml myokinase. GTP (100 μM) was added to samples incubated with isoproterenol. Maximal activity of adenylate cyclase (non-ß-receptor mediated) was determined by the addition of NaF (3 mM) in the absence of GTP. Incubations were carried out at 37° C for 8 min and were terminated by the addition of 1 ml of a solution containing 100 μg ATP, 50 μg CAMP, and 15,000 counts/min [3H] cAMP (New England Nuclear). CAMP was isolated by sequential Dowex and Alumina chromatography as described by Salomon et al. and corrected for recovery with [3H] CAMP as the internal standard. NaF and isoproterenol-stimulated adenylate cyclase activity were expressed as the percentage increase in activity compared with basal and GTP stimulated activity, respectively. Adenylate cyclase activity was linear with time and protein concentration over the range studied.

Schild plots for verapamil and norverapamil inhibition of isoproterenol-stimulated adenylate cyclase activity were constructed from concentration-response curves, each with six concentrations of isoproterenol either with no inhibitor or at three concentrations of each inhibitor. The EC50 was determined from logit analysis of the dose-response curves of isoproterenol-stimulated adenylate cyclase activity. The dose ratio was determined at each concentration of verapamil or norverapamil, i.e., the EC50 for isoproterenol-stimulated adenylate cyclase activity at each concentration of the inhibitor was divided by the EC50 for isoproterenol in the absence of inhibitor. From the plot of log dose ratio-1 vs log inhibitor concentration, the antilog of the x-intercept is the A1, a measure of how potently the drug inhibits isoproterenol-stimulated adenylate-cyclase activity.

All experiments described were performed on three to six different occasions.

**Subject protocol.** To determine the effect of verapamil on leukocyte ß-receptor regulation in man, we studied six healthy male subjects before, during, and after treatment with verapamil.

Subjects were between 20 and 33 years of age and had no abnormality on routine history and physical examination and were normotensive. All were drug free for at least 3 days before study. Subjects were studied on three occasions: before treatment, after 1 week of verapamil treatment (120 mg three times daily), and at least 4 days after discontinuing verapamil. All subjects remained in bed overnight in the University of Iowa Clinical Research Center. Before 7 A.M. and before rising, an indwelling catheter was inserted; 20 min later a 100 ml blood sample was drawn.

Blood samples were analyzed for plasma norepinephrine by high-performance liquid chromatography–electrochemical detection and for verapamil and norverapamil by high-performance liquid chromatography–fluorescence detection (Bio-Science, Bellwood, IL). Radioligand binding studies and assays for adenylate cyclase activity were performed as described above. Maximal ß-adrenergic–mediated adenylate cyclase activity was determined at a 100 μM concentration of isoproterenol. Maximal nonreceptor-stimulated activity was determined with 3 mM NaF.

The statistical significance of differences between study periods was determined by analysis of variance followed by Student's t test for paired data or Wilcoxon signed ranks test (where appropriate); p < .05 in a two-sided test was taken as the minimum level of significance.

**Results**

In the lymphocyte broken cell preparation, verapamil inhibited specific ICYP binding with a Kd of 32 ± 4 μM. In contrast, diltiazem was considerably less potent and nifedipine showed essentially no affinity for ß-adrenergic receptors (table 1).

To further characterize the nature of verapamil competition for ICYP binding, saturation binding studies were performed in the presence and absence of 100 μM verapamil. The addition of verapamil resulted in a 155 ± 2% increase in the Kd of ICYP with virtually no change in receptor concentration (Bmax) consistent with the effect of a competitive inhibitor (figure 1).

To determine by radioligand binding techniques whether verapamil might be a ß-receptor agonist or antagonist, we analyzed verapamil competition curves. In the absence of Gpp(NH)p, competition curves were best fit by a model of verapamil binding to

<table>
<thead>
<tr>
<th>Drug</th>
<th>Lymphocyte Kd (μM)</th>
<th>Cardiac Kd (μM)</th>
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<tr>
<td>Norverapamil</td>
<td>4.2 ± 0.8</td>
<td>26 ± 11</td>
</tr>
<tr>
<td>Verapamil</td>
<td>32 ± 4</td>
<td>115 ± 15</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>137 ± 21</td>
<td>131 ± 14</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>&gt; 300</td>
<td>&gt; 300</td>
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The Kd (mean ± SEM) for each drug was determined from competition curve experiments performed on three separate occasions except for the Kd of verapamil for the ß2-receptor, which was determined from six experiments.
FIGURE 1. Alterations in ICYP saturation binding curves by verapamil in the lymphocyte preparation: Scatchard analysis. The addition of 100 μM verapamil (○) resulted in a reduction in apparent Kd for ICYP binding. This is demonstrated by the more shallow slope as compared with control (●). However, total binding (Bmax) was unaltered, as shown by the virtually identical x-intercepts of the plots. Actual Bmax and Kd were determined by computer curve fitting analysis of saturation binding curves. Each point is the mean of triplicate determinations (representative of three identical experiments).

The β-receptor with a single affinity. Also, verapamil competition curves were not altered by the addition of Gpp(NH)p. The homogeneous binding of verapamil and the lack of effect of guanine nucleotides are characteristic of a β-receptor antagonist.

The possibility that verapamil inhibited isoproterenol-stimulated adenylate cyclase activity by competitive interactions at the β-receptor was studied using a Schild plot analysis. The EC50 of dose-response curves for isoproterenol-stimulated adenylate cyclase activity with each of three concentrations of verapamil were determined by logit analysis and dose ratios were calculated. Verapamil inhibited isoproterenol-stimulated adenylate cyclase activity in a dose-dependent fashion. The calculated A2 value from the Schild plot for verapamil inhibition of isoproterenol-stimulated adenylate cyclase activity was 48 μM (figure 2). This closely approximated the affinity derived from radioligand binding assays. Verapamil demonstrated no effect on basal or NaF-stimulated activity.

The effects of calcium-channel blockers on ICYP binding were measured in rat myocyte β1-receptors. The Ki of verapamil for the myocyte β1-receptor was almost 4 fold higher (115 ± 15 μM) than that in the lymphocyte β2-receptor and closely approximated that for diltiazem (table 1).

Effects of norverapamil. We studied the effects of norverapamil (the major metabolite of verapamil) in both lymphocyte β2-adrenergic and myocyte β1-adrenergic receptor systems and compared the effects of norverapamil with those of the parent compound. In the lym-
phocyte $\beta_2$-adrenergic receptor system, norverapamil was approximately eightfold more potent than verapamil in competing for ICYP binding ($K_i$ norverapamil = $4.2 \pm 0.8$ µM) (figure 3). The addition of norverapamil to saturation binding studies resulted in an increase in apparent $K_d$ for ICYP ($188 \pm 25\%$ of control) with no change in $B_{max}$ ($107 \pm 4\%$ of control). Norverapamil inhibited isoproterenol-stimulated adenylate cyclase activity with an $A_2$ of 3.6 µM (figure 2) but had no effect on basal or NaF-stimulated activity. To rule out the possibility that the oxalate salt independently affected adenylate cyclase activity we studied the effect of sodium oxalate. The addition of sodium oxalate up to a concentration of 1 mM had no demonstrable effect on basal or isoproterenol-stimulated adenylate cyclase activity.

In the cardiac $\beta_2$-receptor system, the potency of norverapamil in inhibiting ICYP binding was lower ($K_i$ = $26 \pm 11$ µM) than that in the $\beta_2$-receptor system. However, norverapamil still demonstrated a higher potency for the cardiac $\beta$-receptor than verapamil ($K_i$ = $115 \pm 15$ µM).

**Effect of oral verapamil treatment on lymphocyte $\beta$-receptors.** Our study of the effect of oral verapamil treatment on lymphocyte $\beta$-receptor regulation was based on the following hypothesis: if verapamil or norverapamil were acting as $\beta$-receptor antagonists in vivo, we would expect that long-term administration of verapamil would result in $\beta$-receptor "up-regulation" analogous to that observed with long-term administration of propranolol.15-17 Oral therapy with verapamil in human subjects resulted in plasma concentrations of verapamil and norverapamil comparable to those previously reported.18, 19 Plasma concentrations were 128 ± 21 ng/ml ($0.28 \pm 0.05$ µM) for verapamil and 155 ± 21 ng/ml ($0.35 \pm 0.05$ µM) for norverapamil 5 to 7 hr after drug administration after 1 week of treatment.

Verapamil treatment was associated with significant alterations in leukocyte $\beta$-receptor properties as determined by assays of adenylate cyclase activity and by radioligand binding studies. Isoproterenol-stimulated adenylate cyclase activity increased from 60 ± 4% to 83 ± 10% (over basal activity) after 1 week of verapamil treatment ($p < .05$, see figure 4). After withdrawal of verapamil, isoproterenol-stimulated adenylate cyclase activity decreased to a level not different from the initial control values (figure 4). Neither basal adenylate cyclase activity nor NaF-stimulated activity was altered by treatment with verapamil (table 2).

The increase in $\beta$-adrenergic-mediated adenylate

![Figure 3](http://circ.ahajournals.org/)

**Figure 3.** Verapamil and norverapamil competition for ICYP binding in the lymphocyte $\beta_2$-receptor system. Each point is the mean of triplicate determinations. The verapamil (●) and norverapamil (△) curves are representative of three experiments.

![Figure 4](http://circ.ahajournals.org/)

**Figure 4.** Alterations in $\beta$-adrenergic–mediated adenylate cyclase activity with verapamil administration. Isoproterenol-stimulated adenylate cyclase activity was expressed as the percentage increase in activity compared to GTP-stimulated activity. + = mean in six subjects. *p < .05 compared with pretreatment control and posttreatment washout periods.
Table 2

Alterations in adenylate cyclase activity with verapamil treatment

<table>
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<tr>
<th>Period</th>
<th>NaF-stimulated activity (%)</th>
<th>Basal activity (pmol cAMP/min/mg)</th>
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<tr>
<td>Control</td>
<td>367 ± 47</td>
<td>83 ± 11</td>
</tr>
<tr>
<td>Treatment</td>
<td>393 ± 34</td>
<td>89 ± 8</td>
</tr>
<tr>
<td>Washout</td>
<td>314 ± 34</td>
<td>96 ± 11</td>
</tr>
</tbody>
</table>

Adenylate cyclase activity is represented as the mean ± SEM of triplicates from each of six subjects studied before, during, and after treatment with verapamil 120 mg three times daily for 1 week.

cyclase activity after verapamil treatment was associated with an increase in β-receptor affinity for the agonist isoproterenol as determined in radioligand binding studies. Thus the IC₅₀ of isoproterenol in competing for ICYP binding in the absence of Gpp(NH)p was reduced from 240 ± 20 to 170 ± 10 nM (p < .05), representing a 41% increase in affinity (figure 5). Withdrawal of verapamil was associated with a return of the IC₅₀ of isoproterenol to pretreatment levels (270 ± 50 nM). Computer analysis of competition curves with a two-site model for agonist binding demonstrated no significant changes with treatment in either the ratio of high-affinity sites/low-affinity sites (R₉/R₀) or the ratio of dissociation constants for the high- and low-affinity sites (Kₑ/Kᵦ). A reduction in the IC₅₀ for isoproterenol in the presence of Gpp(NH)p was also demonstrated with treatment (control, 719 ± 169 nM; treatment, 373 ± 63 nM; washout, 680 ± 142 nM; p < .05 between treatment and either control or washout periods) (figure 5). However, neither receptor density (Bₘₐₓ) nor affinity for the radioligand (Kᵦ of ICYP) was altered after 1 week of verapamil treatment.

For all six subjects, plasma norepinephrine levels from supine blood samples were decreased from 206 ± 58 to 92 ± 18 pg/ml (p < .05) during treatment and returned to baseline levels after treatment (236 ± 58 pg/ml). In contrast, plasma epinephrine and dopamine levels were unaltered with treatment. No significant correlations were demonstrated between plasma norepinephrine levels and either isoproterenol-stimulated adenylate cyclase activity or β-receptor affinity for agonist.

Discussion

Initial studies in the 1960s suggested verapamil acted as a β-adrenergic antagonist based on its hemodynamic effects and antagonism of β-agonist stimulated effects.²⁰ Subsequently, its effect as a calcium-channel blocker was elucidated and its primary pharmacologic action was attributed to this mechanism (for reviews see refs. 3 and 21 to 23).

Our radioligand binding data demonstrate that verapamil in vitro acts competitively to inhibit β-receptor-specific ICYP binding. Verapamil’s effect as a β-adrenergic antagonist is shown by the insensitivity of verapamil to the guanine nucleotide Gpp(NH)p in radioligand competition binding curves and by verapamil inhibition of isoproterenol-stimulated adenylate cyclase activity. It should be pointed out, however, the potency (1/Kᵦ) of verapamil at the human lymphocyte β-receptor is more than 100 times lower than its reported potency at calcium channels in other tissues.²¹²²

In the two β-receptor systems studied, verapamil showed a much higher potency at the lymphocyte β₂-receptor than at the cardiac β₁-receptor, where verapamil and diltiazem were virtually identical in their potency for β-receptor interactions. Although the actual Kᵦ estimates between different tissue preparations are not necessarily comparable, it is interesting that nifedipine and diltiazem exhibit similar Kᵦ values in both leukocyte and myocardial β-receptor systems. Of the three drugs, only verapamil showed a difference in potency between β₁- and β₂-receptor systems. Therefore these data suggest some degree of β₂-adrenergic selectivity for verapamil. This is consistent with the results of previous studies by Karliner et al.,³ which demonstrated that cardiac β₁-receptor affinity for verapamil was very low.

We also found that norverapamil, the major metabo-
lite of verapamil, had a much higher affinity for the β-receptor than the parent compound. Norverapamil is partially active as a calcium-channel blocker,24 appears rapidly in the plasma after oral administration of verapamil, and achieves comparable plasma concentrations to the parent drug (up to 1 μM).18,19 Our data obtained in vitro demonstrate that norverapamil is a competitive β₂-receptor antagonist with a Kᵢ in the low micromolar range. In the studies in vivo, plasma norverapamil levels 5 to 7 hr after oral administration of verapamil were 0.35 ± 0.05 μM, much lower than the Kᵢ of norverapamil for the β-receptor as determined from studies in vitro. This might suggest that norverapamil could not act as a β-blocker in vivo. However, tissue levels for verapamil and norverapamil have been found to be up to 10-fold higher than corresponding plasma levels.22 Assuming that the effect of verapamil on the lymphocyte β-receptor was representative of its effect on β₂-receptors in other tissues (e.g., vascular smooth muscle), at the dosage of verapamil studied (360 mg/day), tissue concentrations of norverapamil could be within the range where we would expect significant β₂-receptor blockade.

In vivo, long-term verapamil administration was associated with increased lymphocyte β-adrenergic-mediated adenylate cyclase activity. This appears to be related to an increase in receptor affinity for agonists without a corresponding increase in antagonist binding (that is, an increase in the Bₘₐₓ or a decrease in the Kᵢ for ICYP). It is notable that the increase in β-receptor affinity for isoproterenol was not associated with an increase in Rₙ or in Kᵢ/Kᵢₚ and was also demonstrated in the presence of Gpp(NH)p. Thus the increase in affinity of the recognition site of the receptor for isoproterenol is caused by a shift of both the high- and low-affinity states of the receptor for agonist. Therefore the associated increase in β-adrenergic-mediated adenylate cyclase activity cannot be caused simply by an increased ability to form the high-affinity complex (according to the ternary complex model12,13). Alterations in the low-affinity state have been reported in other studies of human leukocyte β-receptor regulation.26,27 However, the molecular explanation for these changes is unclear. The increase in receptor affinity for agonist with verapamil treatment is not caused by persistent verapamil in the membrane preparation, since this would have caused an artifactual reduction in receptor affinity for isoproterenol.

The reduction in plasma norepinephrine levels seen with verapamil administration was unexpected. Theoretically, verapamil might have been expected to mediate an increase in plasma norepinephrine through its effect as a presynaptic α₂-adrenergic receptor antagonist.4,28 However, we have found the reverse situation. Assuming the reduction in plasma norepinephrine levels does represent a decrease in central sympathetic outflow, this observation may explain why verapamil treatment does not cause the reflex increase in heart rate and contractility seen with other vasodilators.

One interpretation of our data is that the increase in lymphocyte β-receptor responsiveness with verapamil administration is related to the β-receptor–blocking effect of verapamil (or norverapamil) in vivo. This interpretation is made by analogy to the effect of propranolol on lymphocyte β-receptor regulation. Long-term administration of propranolol has been shown to result in up-regulation of lymphocyte β-receptors presumably by blocking the long-term desensitizing effects of circulating catecholamine hormones.15–17 In fact, the proportional increase in leukocyte β-receptor responsiveness seen with verapamil is comparable to that seen after treatment with the β-blocker propranolol.15,17 However, it is notable that the increase in lymphocyte β-adrenergic responsiveness with propranolol therapy is primarily thought to be caused by increased receptor density.16,17 In contrast, we saw no changes in lymphocyte β-receptor density with verapamil but instead noted an increase in β-receptor affinity for agonists.

Alternatively, enhanced β-receptor responsiveness with verapamil administration could be secondary to the reduction in plasma norepinephrine levels. As we have previously demonstrated, small changes in plasma catecholamines within the physiologic range have been associated with regulation of human lymphocyte β-adrenergic responsiveness and β-receptor affinity for agonist.7 The reduction in plasma norepinephrine levels associated with verapamil treatment is within the range over which reciprocal increases in β-adrenergic responsiveness might be anticipated. Hopefully future studies will resolve which of these mechanisms, i.e., β-blockade vs reduction in plasma catecholamine levels, is primarily responsible for the effect of verapamil treatment on human lymphocyte β-receptors in vivo.

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References


Erratum

There is a discrepancy between the values given in table 1 and figure 2 in the above article. The correct values are found in table 1.
The interaction of verapamil and norverapamil with beta-adrenergic receptors.
R D Feldman, G D Park and C Y Lai

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