Hemodynamic and β-Adrenergic Receptor Adaptations During Long-term β-Adrenoceptor Blockade

Studies With Acebutolol, Atenolol, Pindolol, and Propranolol in Hypertensive Patients

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In an attempt to further clarify the mechanism of the maintenance of the antihypertensive effect of β-adrenoceptor antagonists, the effects of four antagonists with different ancillary properties (acebutolol, atenolol, pindolol, and propranolol) on systemic and renal hemodynamics, body fluid volumes, hormones, and lymphocyte β-adrenoceptor density were studied in four groups of 10 hypertensive patients. The patients were observed for 3 weeks during active treatment and for 2 weeks after withdrawal of treatment. At the end of the 3-week treatment period, the four drugs had an equal antihypertensive effect (fall in mean arterial pressure, 10–13%). Although renin activity was suppressed (60–70%) by all four drugs, changes in renin or pretreatment values of renin levels were not correlated with the fall in blood pressure. The drugs had no effect on plasma catecholamine concentrations or body fluid volumes. Despite similar antihypertensive effects among the four drugs, the changes in flow and resistance underlying the fall in blood pressure differed considerably. With pindolol, the fall in blood pressure was associated with a fall in vascular resistance (26±6%), whereas with propranolol, it was predominantly associated with a fall in cardiac output (11±7%). No significant changes in vascular resistance or cardiac output occurred with atenolol or acebutolol. The changes in renal blood flow and renal vascular resistance occurred in parallel with the changes in cardiac output and systemic vascular resistance. Plasma epinephrine concentration and pretreatment cardiac chronotropic responsiveness to isoproterenol appeared to be inversely correlated with lymphocyte β-adrenoceptor density (B_max) (r = -0.41 and -0.43, respectively). With pindolol, B_max decreased maximally by 39±6%, and with propranolol, it increased by 51±17%. With both drugs, significant changes in B_max were already present 24 hours after treatment. Furthermore, 1 week after withdrawal of treatment with pindolol, B_max was still down-regulated, and cardiac chronotropic responsiveness was still decreased, whereas 1 week after withdrawal of propranolol, B_max was still up-regulated, and cardiac chronotropic responsiveness was still increased. No changes in B_max occurred with the β₁-selective antagonists acebutolol and atenolol. Thus, despite an equal antihypertensive effect, the four β-adrenoceptor antagonists appear to have dissimilar effects on cardiac output, renal blood flow, and lymphocyte β-adrenoceptors. Changes in cardiac output, the circulating blood volume, or angiotensin-mediated vasoconstriction are factors unlikely to be crucial for the antihypertensive effect of β-adrenoceptor antagonists. Therefore, interference with vasoconstrictor nerve activity through blockade of either central or peripheral prejunctional β-adrenoceptors could be an alternative explanation of their blood pressure–lowering potential. (Circulation 1989;80:903–914)

We recently reported the acute hemodynamic effects of four β-adrenoceptor antagonists studied for 24 hours after they had been administered orally for the first time.
to four groups of 10 patients with essential hypertension. The antagonists were acebutolol, which is β₁ selective with a moderate degree of partial agonist activity, atenolol, which is β₁ selective, highly hydrophilic, and devoid of partial agonist activity, pindolol, which is nonselective with strong partial agonist activity, and propranolol, which is nonselective, highly lipophilic, and devoid of partial agonist activity. The experimental conditions in that study were rigidly standardized by restricting the patients to bed for 36 hours. The results led us to conclude that despite the different ancillary properties the four drugs exerted a similar antihypertensive effect, which was mediated by a vasodilator mechanism. Autoregulatory adjustment of the vasculature to changes in tissue perfusion or suppression of renin activity were found to be unlikely candidates to explain this vasodilator mechanism.

The present study is an extension of our initial report. The patients were observed for 3 weeks during active treatment and for 2 weeks after withdrawal of treatment. In this period, repeated measurements of cardiac output, renal perfusion, plasma catecholamine levels, renin activity, aldosterone levels, body fluid volumes, lymphocyte β-adrenoceptors, and cardiac chronotropic responsiveness to isoproterenol were performed. In this way, by searching for similarities, dissimilarities, and eventually common denominators of the antihypertensive effect of the four different β-adrenoceptor antagonists, we hoped to gain additional information on the mechanism that maintains the antihypertensive effect during prolonged treatment.

Methods

Patients

Forty male patients (age range, 27–64 years; mean age, 46 years) with mild-to-moderate essential hypertension were studied. They were recruited from the outpatient hypertension clinic if their untreated sitting diastolic blood pressure was over 95 mm Hg (phase V of Korotkoff sounds) at three separate occasions. Routine clinical and laboratory evaluation did not reveal causes of their hypertension. A history of or clinical signs of coronary or valvular heart disease, congestive heart failure, cerebrovascular disease, or chronic obstructive lung disease were all negative. After the purpose and the procedures of the study had been explained, all patients gave their consent to participate. The protocol was approved by the local hospital ethical review committee.

Study Protocol

This was a single-blind, placebo-controlled study, and it lasted for 7 weeks. Antihypertensive and other types of medication, if any, were discontinued at least 3 weeks before the study. A moderate dietary salt restriction was advised (approximately 8 g/day NaCl). After the washout period, placebo was given for 2 weeks. The placebo tablets were matched with the active medication with regard to the appearance and the number of tablets taken each day. After the placebo period, the patients were treated with one of the four β-adrenoceptor antagonists for 3 weeks. During the first week of the active treatment, propranolol was given 40 mg three times daily, atenolol 50 mg once daily, acebutolol 200 mg twice daily, and pindolol 5 mg twice daily. Depending on the blood pressure response, the dose of the β-adrenoceptor antagonists was doubled during each of the two subsequent weeks until blood pressures below 140/90 mm Hg were obtained. After 3 weeks of active treatment, the patient was given placebo again for 2 weeks.

During the entire study, patients were seen at weekly intervals in the outpatient clinic. At each visit, supine blood pressure and heart rate were measured for 1 hour. Cardiac output, glomerular filtration rate, effective renal plasma flow, and the plasma and extracellular volumes were measured at the end of the initial placebo period and after 3 weeks of active treatment. Blood was collected while the patients were in the supine position for determination of plasma levels of catecholamines, renin levels, and aldosterone levels, two times, one week apart, during the placebo period and at the end of the active treatment period. The density of β-adrenoceptors on lymphocyte membranes (Bmax) was determined twice during the placebo period, 24 hours after the first dose of the β-adrenoceptor antagonists, at the end of the second and third weeks of active treatment, and at the end of the first and second weeks after withdrawal of active treatment. Blood was always sampled at the end of the dosing interval of the respective drugs. The chronotropic responsiveness of cardiac β-adrenoceptors to isoproterenol (CD50) was assessed at the end of the initial placebo period and 1 week after withdrawal of the β-adrenoceptor antagonists.

Hemodynamic Measurements

Supine blood pressure and heart rate were measured at 5-minute intervals for 1 hour by means of an automatic oscillometric device (Datascope, Accutorr I, Datascope, Paramus, New Jersey). Blood pressure readings with this device agree well with intra-arterial blood pressure measurements.2 The values of systolic, diastolic, and mean arterial blood pressures and heart rate obtained during 1 hour were averaged.

Cardiac output was measured by an isotope dilution technique3,4 after 2 hours of supine rest. 99mTc-Labeled human serum albumin (Technescan HSA, Mallinckrodt Diagnostica, Petten, The Netherlands) at 100–200 μCi was used as the indicator. After a rapid intravenous injection of the isotope, the time-concentration curve was recorded for 2 minutes by precordial counting of radioactivity with the use of a single probe. Additional recordings were made after 5 and 10 minutes when blood was
sampled for measurement of radioactivity. Cardiac output was calculated according to the formula of Hamilton. In a previous study,\(^3\) we showed that this method correlates well with the dye-dilution technique (n=57, r=0.92). The coefficient of variation for duplicate measurements with this technique is 6% (n=38).\(^3\) During the measurement of cardiac output, heart rate was derived from a simultaneously recorded electrocardiogram.

Systemic vascular resistance index (dynes·sec/cm\(^2\)/m\(^2\)) was calculated as 80 multiplied by mean arterial pressure (mm Hg) divided by cardiac index (l/min/m\(^2\)), and stroke index (ml/m\(^2\)) was calculated as cardiac index divided by heart rate (beats/min).

**Glomerular Filtration Rate and Renal Plasma Flow**

For renal function studies, a constant infusion technique was used.\(^5,6\) Effective renal plasma flow and glomerular filtration rate were estimated by means of the clearance of \(^{131}\)I-hippuran and \(^{125}\)I-thalamate (Amersham, UK). The priming dose for hippuran was 0.3–0.4 \(\mu Ci/kg\) body wt, and for thalamate, it was 0.08–0.1 \(\mu Ci/kg\) body wt. The sustaining infusion rates were 0.2 and 0.05 \(\mu Ci/min\), respectively. The clearance of the isotopes was determined at steady state after 90 and 105 minutes. Renal blood flow was calculated by means of central venous packed cell volume and by assuming 75% renal extraction of hippuran.\(^6\) Glomerular filtration rate and renal blood flow were corrected for body surface area and were expressed per meter squared. Renal vascular resistance index (dynes·sec/cm\(^2\)/m\(^2\)) was calculated as mean arterial pressure multiplied by 80 divided by renal blood flow (l/min/m\(^2\)).

**Body Fluid Volumes**

The same indicator used for measuring cardiac output (i.e., \(^{99m}\)Tc human serum albumin) was also used for determining plasma volume. Plasma samples were taken 10, 20, and 30 minutes after the intravenous injection. By linear extrapolation of the time-log-concentration curve, the radioactivity at time zero was calculated.\(^3\) Extracellular fluid volume was estimated by measuring the distribution volume of intravenously injected Na\(^{25}\)S sulphate (50–60 \(\mu Ci\)) with blood sampling at 0, 30, 60, 80, 100, and 120 minutes.\(^7\)

**Isoproterenol Infusions**

For determining the chronotropic responsiveness of the heart, increasing infusion rates of isoproterenol were used. Heart rate was derived from a continuously recorded electrocardiogram. Isoproterenol was infused through an indwelling cannula (Venflon, 18G, Viggo, Helsingborg, Sweden) in a forearm vein by means of an infusion pump (Perfusor VI, B Braun Melsungen, FRG). Baseline values of heart rate were obtained during a continuous infusion of saline at a flow rate of 22 ml/hr for 20 minutes. The infusion was then switched to isoproterenol, 3.5, 7, 14, 35, and 70 ng/kg/min. The infusion rate was increased every 10 minutes until a rise in heart rate of at least 25 beats/min was obtained. The rise in heart rate during each dose step was used for constructing a log dose-response curve. From this curve, the dose of isoproterenol required to increase the heart rate by 25 beats/min (CD\(_{25}\), ng/kg/min) was calculated.

\(\beta\)-Adrenergic Receptor Density on Lymphocyte Membranes

**Preparation of Lymphocytes.** Blood samples for the preparation of lymphocyte membranes were always taken after 1 hour of supine rest at the end of the dosing interval before isoproterenol was given and before isotopes were used for hemodynamic studies.

Lymphocytes were isolated from fresh heparinized blood by a modification of the technique described by Boyum.\(^8\) Fresh heparinized blood (50 ml) was diluted with an equal volume of 0.154 M NaCl and was divided into four equal fractions. An aliquot (15 ml) of Isolymp (Gallard-Schlesinger Chemical Manufacturing, Carle Place, New York) was carefully layered under 25 ml diluted blood with an 18-gauge spinal tap needle. Tubes were centrifuged at 400g for 40 minutes at 20\(^\circ\) C. After careful removal of the plasma, the lymphocyte band (at least 90% small lymphocytes, <8% monocytes, <2% polymorphonuclear leukocytes) was harvested by vacuum aspiration. Lymphocytes prepared from 50 ml whole blood were subdivided into four equal fractions, each of which was diluted with 20 ml 20 mM Tris-isosaline (pH 7.5, 20\(^\circ\) C). After centrifugation at 400g for 40 minutes at 20\(^\circ\) C, the supernatant was carefully removed by vacuum suction, and the pellet was gently resuspended in 20 ml solution I (anhydrous D-glucose 1.0 g/l, CaCl\(_2\) 0.0056 g/l, MgCl\(_2\), 6H\(_2\)O 0.4249 g/l, KCl 0.4026 g/l, and Tris 17.565 g/l, pH 7.6) with a rubber policeman. Homogenates were centrifuged at 20,000g for 10 minutes at 4\(^\circ\) C, and supernatants were discarded. Pellets resuspended in 10 ml ice-cold distilled H\(_2\)O were then homogenized (Polytron, Kinematica, Kriens/Luzern, Switzerland, setting 6 for 10 seconds), and samples were centrifuged at 20,000g for 10 minutes at 4\(^\circ\) C. The supernatants were discarded, and the four pellets were resuspended in two tubes containing 4 ml solution I (4\(^\circ\) C) with a Polytron (setting 5.5 for 10 seconds). The membranes originating from 50 ml whole blood were stored at -70\(^\circ\) C until assay.

\(\beta\)-Adrenergic Receptor Binding Assay

(-)-Pindolol was iodinated with \(^{125}\)I, and \(^{125}\)I-pindolol was purified to theoretical specific activity (2.2 Ci/\(\mu\)mol) as described previously.\(^9\) Samples were thawed and rehomogenized (Polytron, at setting 6 for 8 seconds). Aliquots (100 \(\mu\)l) of each homogenate containing 15–25 \(\mu\)g protein were incubated with the indicated concentrations of \(^{125}\)I-
pindolol and GTP (100 μM) in a final volume of 0.250 ml. All assays were carried out in 0.01% ascorbic acid, 0.0004% bovine serum albumin, 0.154 M NaCl, and 20 mM Tris at pH 7.4 at 25°C.

Binding assays were routinely carried out in new disposable polypropylene tubes (Sarstedt No. 538). Samples were incubated for 25 minutes at 37°C. Reactions were stopped by the addition of 10 ml Tris-isosaline (20°C), and samples were filtered through Schleicher and Schuell glass fiber filters (ZE21). Each filter was washed with an additional 10 ml 10 mM Tris-isosaline (20°C), and radioactivity remaining on the filter was determined in a gamma counter.

Specific binding of $^{125}\text{I}$-pindolol was defined as the amount of $^{125}\text{I}$-pindolol bound in the absence of a competing ligand minus the amount bound in the presence of 50 μM of (-)-isoproterenol. This concentration is 100 times the $K_d$ of isoproterenol, and with an observed Hill coefficient of 1, it corresponds to 99% occupancy of the receptors. The amount of $^{125}\text{I}$-pindolol bound was always less than 10% of the total amount of $^{125}\text{I}$-pindolol in the incubation. Thus, the presence of competing drug did not affect the concentration of free $^{125}\text{I}$-pindolol. The density of specific binding sites for $^{125}\text{I}$-pindolol was determined by Scatchard analysis of the specific binding of $^{125}\text{I}$-pindolol. Duplicate samples were incubated with increasing concentrations of $^{125}\text{I}$-pindolol (10,000–250,000 cpm, 11–288 pM) with or without isoproterenol to define specific binding.

**Protein Determinations.** Protein concentrations were determined according to the method of Lowry et al, using bovine serum albumin as a standard.

### Catecholamine, Active Renin, and Aldosterone Concentrations

For determination of plasma catecholamine levels, 10 ml venous blood was collected in chilled tubes containing 19 mg ethyleneglycol-bis-(β-aminoethyl-ether)-N,N,N',N'-tetraacetic acid and 12 mg gluthathione. After centrifugation at 0°C, samples were stored at −70°C until assay. Plasma norepinephrine and epinephrine levels were measured by a high-performance liquid chromatography system with electrochemical detection. For measurement of active plasma renin concentration, 10 ml blood was sampled in chilled tubes containing ethylenediaminetetraacetic acid in a final concentration of 2 mg/ml blood. Samples were centrifuged immediately at 0°C and stored at −20°C until assay. Active plasma renin concentration was measured indirectly by a radioimmunooassay of formed angiotensin I in the presence of saturating concentrations of sheep plasma renin substrate as described previously. For determination of plasma aldosterone, 5 ml blood was collected in heparinized tubes. Samples were centrifuged immediately and stored at −20°C until assay. Plasma aldosterone levels were measured by a radioimmunooassay with a commercial kit (Coat-A-Count, Diagnostic Products, Los Angeles, California).

### Statistical Analysis

Data are mean value±SEM. Plasma values of renin were not distributed normally, but after logarithmic transformation, values were distributed normally and mean values were calculated after such transformation. Linear regression analysis was performed by the method of least squares. Differences between groups were analyzed by analysis of variance, and differences within each group were analyzed by Student’s paired t test. A p value less than 0.05 was considered to indicate a significant difference.

### Results

**Systemic Hemodynamics**

With regard to the clinical characteristics of the four groups of patients at the end of the washout period, there were no significant differences (Table 1). The changes in blood pressure and heart rate during administration and after withdrawal of the four β-adrenoceptor blocking agents were very similar (Figure 1). After 3 weeks of treatment, the four

<table>
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<tr>
<th>Characteristics</th>
<th>Propranolol</th>
<th>Atenolol</th>
<th>Acebutolol</th>
<th>Pindolol</th>
</tr>
</thead>
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<tr>
<td>Patients (n)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
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<tr>
<td>Age (yr)</td>
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<td>42±3</td>
<td>48±3</td>
<td>47±3</td>
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<td>Body weight (kg)</td>
<td>78.3±4.5</td>
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<td>86.1±2.6</td>
<td>82.8±3.3</td>
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<td>Height (cm)</td>
<td>172±3</td>
<td>171±2</td>
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<td>179±2</td>
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<td>Body surface area (m²)</td>
<td>1.95±0.07</td>
<td>1.90±0.04</td>
<td>2.09±0.04</td>
<td>2.04±0.05</td>
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<td>Systolic arterial pressure (mm Hg)</td>
<td>166±4</td>
<td>162±4</td>
<td>161±4</td>
<td>165±2</td>
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<tr>
<td>Diastolic arterial pressure (mm Hg)</td>
<td>108±3</td>
<td>109±3</td>
<td>106±2</td>
<td>105±2</td>
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<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>126±4</td>
<td>127±3</td>
<td>124±3</td>
<td>125±3</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>67±2</td>
<td>66±3</td>
<td>76±4</td>
<td>71±3</td>
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</table>

Data are mean±SEM, measured at the end of the washout period.

Mean arterial pressure is diastolic arterial pressure plus (systolic minus diastolic arterial pressure) divided by three.
Van den Meiracker et al

Ancillary Properties of β-Adrenoceptor Antagonists

ARTERIAL PRESSURE
mmHg

160
140
120
100
80

HEART RATE
beats/min

80
65
50

FIGURE 1. Plot of changes in blood pressure and heart rate before, during, and after propranolol (○), atenolol (▲), acebutolol (□), and pindolol (●).

drugs shared an equal antihypertensive effect. Doses at this time were acebutolol 480±60 mg b.i.d., atenolol 95±12.5 mg once daily, pindolol 15±5 mg b.i.d., and propranolol 112±13 mg t.i.d. Systolic and diastolic arterial pressures were reduced by 10±2% (p<0.01) and 9±2% (p<0.001) with acebutolol, 11±3% (p<0.01) and 12±3.1% (p<0.01) with atenolol, 9±1% (p<0.01) and 11±2% (p<0.01) with pindolol, and 13±3% (p<0.01) and 12±3% (p<0.01) with propranolol, respectively. Systolic and diastolic arterial pressures 1 week after cessation of active treatment did not differ from values observed during the initial placebo period. Heart rate decreased during administration of acebutolol, atenolol, and propranolol. In contrast, no change in heart rate occurred with pindolol (Table 2). After 3 weeks of treatment, the heart rate during acebutolol (64±2 beats/min) was slightly higher (p<0.05) than the heart rates during atenolol (58±3 beats/min) or propranolol (57±2 beats/min). After withdrawal from active treatment, heart rates did not differ from the values of the initial placebo period in any of the four

TABLE 2. Effects of Propranolol, Atenolol, Acebutolol, and Pindolol on Systemic and Renal Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Propranolol</th>
<th>p</th>
<th>Placebo</th>
<th>Atenolol</th>
<th>p</th>
<th>Placebo</th>
<th>Acebutolol</th>
<th>p</th>
<th>Placebo</th>
<th>Pindolol</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>123±5</td>
<td>107±4</td>
<td>&lt;0.01</td>
<td>117±3</td>
<td>104±3</td>
<td>&lt;0.001</td>
<td>115±3</td>
<td>104±3</td>
<td>&lt;0.01</td>
<td>122±3</td>
<td>106±2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>65±2</td>
<td>53±2</td>
<td>&lt;0.001</td>
<td>64±3</td>
<td>57±3</td>
<td>&lt;0.001</td>
<td>71±3</td>
<td>61±2</td>
<td>&lt;0.01</td>
<td>68±2</td>
<td>63±3</td>
<td>NS</td>
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<tr>
<td>CI (l/min/m²)</td>
<td>3.0±0.1</td>
<td>2.6±0.2</td>
<td>NS</td>
<td>2.6±0.2</td>
<td>2.6±0.2</td>
<td>NS</td>
<td>3.3±0.3</td>
<td>3.1±0.2</td>
<td>NS</td>
<td>2.7±0.2</td>
<td>3.1±0.2</td>
<td>&lt;0.01</td>
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<td>SI (ml/m²)</td>
<td>46±2</td>
<td>50±3</td>
<td>NS</td>
<td>41±2</td>
<td>46±3</td>
<td>&lt;0.05</td>
<td>47±3</td>
<td>51±3</td>
<td>NS</td>
<td>40±3</td>
<td>49±3</td>
<td>&lt;0.01</td>
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<tr>
<td>SVRI (dynes/sec/cm²/m²)</td>
<td>3,440±280</td>
<td>3,538±260</td>
<td>NS</td>
<td>3,746±230</td>
<td>3,299±223</td>
<td>NS</td>
<td>2,924±193</td>
<td>2,776±198</td>
<td>NS</td>
<td>3,809±316</td>
<td>2,859±228</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GFR (ml/m²)</td>
<td>58±3</td>
<td>54±1</td>
<td>NS</td>
<td>52±2</td>
<td>57±3</td>
<td>NS</td>
<td>56±2</td>
<td>56±2</td>
<td>NS</td>
<td>56±2</td>
<td>59±2</td>
<td>NS</td>
</tr>
<tr>
<td>RBF (ml/m²)</td>
<td>630±38</td>
<td>549±28</td>
<td>&lt;0.01</td>
<td>539±25</td>
<td>563±33</td>
<td>NS</td>
<td>592±36</td>
<td>600±35</td>
<td>NS</td>
<td>558±23</td>
<td>596±30</td>
<td>NS</td>
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<tr>
<td>RVR (dynes/sec/cm²/m²)</td>
<td>16,362±1,498</td>
<td>18,207±635</td>
<td>NS</td>
<td>17,726±938</td>
<td>15,170±1,000</td>
<td>&lt;0.01</td>
<td>16,106±1,287</td>
<td>14,134±917</td>
<td>NS</td>
<td>17,741±1,213</td>
<td>1,455±962</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Data are mean±SEM.

MAP, mean arterial pressure; HR, heart rate; CI, cardiac index; SI, stroke index; SVRI, systemic vascular resistance index; GFR, glomerular filtration rate; RBF, renal blood flow; RVR, renal vascular resistance.
groups (Figure 1). Mean arterial pressure fell by 10±3% (p<0.01) with acebutolol, 11±2% (p<0.001) with atenolol, 12±2% (p<0.01) with pindolol, and 13±3% (p<0.01) with propranolol after 3 weeks of treatment (Figure 2). With pindolol, the fall in blood pressure was entirely due to a fall in vascular resistance index (−26±6%, p<0.001), and cardiac index was slightly increased (16±6%, p<0.01) (Table 2, Figure 2). With the other three drugs, the fall in mean arterial pressure was not associated with significant changes in either vascular resistance index or cardiac index. Changes in cardiac output were not correlated to changes in blood pressure. Stroke index did not change with either acebutolol or propranolol, whereas it was increased with atenolol (14±7%, p<0.01) and pindolol (26±6%, p<0.01).

Renal Hemodynamics

Changes in renal blood flow and renal vascular resistance occurred in parallel with the changes in systemic hemodynamics (Table 2, Figure 2). With pindolol, renal vascular resistance decreased (−17±5%, p<0.01), whereas with propranolol, renal blood flow decreased (−12±4%, p<0.01). Renal blood flow did not change with acebutolol or atenolol, and renal vascular resistance decreased (14±4%, p<0.01) in patients on atenolol. The percent changes in cardiac index and renal blood flow were positively correlated (r=0.44, p<0.01). As a consequence, the percent changes in systemic vascular resistance index and renal vascular resistance index were also strongly correlated (r=0.90, p<0.001). As expected, patients with a higher blood pressure had a lower renal blood flow (r=−0.36, p<0.05). Changes in blood pressure and renal blood flow during treatment were not correlated.

Plasma Catecholamines, Active Renin, and Aldosterone Concentrations

Before treatment, plasma norepinephrine and epinephrine concentrations did not differ between the four groups (Table 3). Plasma catecholamine concentrations did not change with any of the four β-adrenoceptor antagonists. Renin levels were decreased by all four drugs. At the end of the 3-week treatment period, active renin was decreased by 65±9% (p<0.01) with acebutolol, 66±11% (p<0.01)
with atenolol, 62±7% (p<0.001) with pindolol, and 70±5% (p<0.001) with propranolol. Pretreatment levels of active renin or the decrease in active renin and the blood pressure responses to the four drugs after 3 weeks of treatment were not correlated. Pretreatment levels of renin and aldosterone were correlated (r=0.32, p<0.05). Plasma aldosterone was lowered by all four drugs (Table 3). The percent decrements in aldosterone and renin during β-adrenoceptor blockade were not correlated.

**Body Fluid Volumes**

Before treatment, plasma volumes and extracellular fluid volumes were not different between the four groups. No significant changes in these variables occurred during treatment (Table 3).

**Lymphocyte β-Adrenoceptor Density, Plasma Epinephrine Concentration, and Chronotropic Responsiveness to Isoproterenol**

During placebo administration, the density of β-adrenoceptors on lymphocyte membranes (Bmax) and the chronotropic responsiveness to isoproterenol (CD25) did not differ between the four groups (Tables 4 and 5). During placebo administration, Bmax and CD25 were inversely correlated (r=-0.43, p<0.01). Interestingly, Bmax and plasma epinephrine concentration were also inversely correlated (r=-0.41, p<0.01), but plasma epinephrine concentration and CD25 were not. Bmax was not correlated with plasma norepinephrine level, age, or systolic, diastolic, or mean arterial pressures.

Bmax increased by 51±17% (p<0.01) after 3 weeks of treatment with propranolol (Figure 2). By 24 hours after starting the treatment, Bmax was increased by 30±10% (p<0.05). Furthermore, 1 week, but not 2 weeks, after propranolol withdrawal, lymphocyte β-adrenergic receptor density was still increased. With pindolol, Bmax decreased maximally by 39±6% (p<0.01) after 3 weeks of treatment (Figure 3). As was the initial increase in Bmax after propranolol administration, the fall in Bmax after pindolol was also seen by 24 hours after beginning of treatment (−35±5%, p<0.01). One week after withdrawal of treatment, Bmax was still decreased by 33±9% (p<0.05). No significant changes in Bmax were seen with the β-adrenoceptor antagonists acebutolol and atenolol. One week after withdrawal of propranolol and atenolol, CD25 was significantly decreased (p<0.05). Conversely, 1 week after withdrawal of pindolol, CD25 was increased (p<0.01) (Table 5). Separate analysis of the pindolol and propranolol group showed that before (n=18, r=-0.80, p<0.01) and 1 week after withdrawal (n=15, r=-0.63, p<0.05), Bmax and CD25 were inversely correlated (r=-0.41, p<0.01), but plasma epinephrine concentration and CD25 were not. Bmax was not correlated with plasma norepinephrine level, age, or systolic, diastolic, or mean arterial pressures.

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

### Table 3. Effects of Active renin and B-Adrenoceptor blockade on Plasma Concentrations of Norepinephrine, Epinephrine, Active Renin, and Aldosterone and on Body Fluid Volumes

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Propranolol</th>
<th>Placebo</th>
<th>Atenolol</th>
<th>Placebo</th>
<th>Acebutolol</th>
<th>Placebo</th>
<th>Pindolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>216±19</td>
<td>281±33</td>
<td>264±32</td>
<td>282±85</td>
<td>221±25</td>
<td>189±35</td>
<td>254±31</td>
<td>257±27</td>
</tr>
<tr>
<td>Epinephrine (pg/ml)</td>
<td>81±15</td>
<td>73±14</td>
<td>38±6</td>
<td>33±6</td>
<td>63±11</td>
<td>36±8</td>
<td>86±16</td>
<td>74±11</td>
</tr>
<tr>
<td>Active renin (µU/ml)</td>
<td>14.2±3.9</td>
<td>3.5±0.9±</td>
<td>11.3±2.8</td>
<td>2.4±0.6±</td>
<td>8.5±2.5</td>
<td>1.8±0.9±</td>
<td>10.9±2.0</td>
<td>3.4±0.8±</td>
</tr>
<tr>
<td>Aldosterone (pg/ml)</td>
<td>109±21</td>
<td>45±8†</td>
<td>88±8</td>
<td>61±5†</td>
<td>106±22</td>
<td>81±13*</td>
<td>96±13</td>
<td>71±14*</td>
</tr>
<tr>
<td>Plasma volume (l)</td>
<td>2.9±0.1</td>
<td>3.1±0.2</td>
<td>2.8±0.1</td>
<td>2.9±0.2</td>
<td>3.5±0.1</td>
<td>3.6±0.2</td>
<td>3.4±0.1</td>
<td>3.5±0.2</td>
</tr>
<tr>
<td>Extracellular fluid volume (l)</td>
<td>13.0±0.6</td>
<td>13.5±0.6</td>
<td>14.3±0.7</td>
<td>14.8±0.4</td>
<td>15.5±0.9</td>
<td>15.7±0.9</td>
<td>15.4±0.4</td>
<td>14.9±0.5</td>
</tr>
</tbody>
</table>

Data are mean±SEM.

*p<0.05, †p<0.01, ‡p<0.001 3 weeks treatment vs. placebo.

### Table 4. Effects of Propranolol, Atenolol, Acebutolol, and Pindolol on Density of β-Adrenergic Receptors on Lymphocyte Membranes and on the Affinity Constant for Specific 131I-Pindolol Binding to Lymphocyte Membranes

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>β-Adrenoceptor blockade</th>
<th>Withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bmax (fmol/mg protein)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propranolol</td>
<td>23±3</td>
<td>22±3</td>
<td>27±3†</td>
</tr>
<tr>
<td>Atenolol</td>
<td>26±3</td>
<td>24±3</td>
<td>26±3</td>
</tr>
<tr>
<td>Acebutolol</td>
<td>29±3</td>
<td>26±2</td>
<td>28±2</td>
</tr>
<tr>
<td>Pindolol</td>
<td>17±2</td>
<td>17±2</td>
<td>13±1†</td>
</tr>
<tr>
<td>Kd (pM)</td>
<td>82±22</td>
<td>87±18</td>
<td>73±19</td>
</tr>
<tr>
<td>Propranolol</td>
<td>57±12</td>
<td>64±12</td>
<td>68±14</td>
</tr>
<tr>
<td>Atenolol</td>
<td>51±5</td>
<td>58±9</td>
<td>54±8</td>
</tr>
<tr>
<td>Acebutolol</td>
<td>50±6</td>
<td>56±11</td>
<td>59±13</td>
</tr>
</tbody>
</table>

Data are mean±SEM.

Bmax, density of β-adrenergic receptors on lymphocyte membranes; Kd, affinity constant.

*p Twenty-four hours after administration of the first dose of the β-adrenergic antagonist.

†p<0.05; ‡p<0.01 active treatment or withdrawal vs. placebo.
were inversely correlated (Figure 4). In agreement with a previous report, the apparent $K_d$ for specific $^{125}$I-pindolol binding to lymphocyte membranes was increased after 3 weeks of treatment with propranolol (Table 4). No change in $K_d$ values was seen with the other $\beta$-adrenoceptor antagonists.

**Discussion**

**Systemic Hemodynamics**

In this study, we confirmed previous findings that $\beta$-adrenoceptor antagonists, irrespective of their different ancillary pharmacologic properties, lower an elevated blood pressure to a similar extent when doses are adequate. Also, in relation to these different ancillary properties, the systemic hemodynamic effects of the antagonists, other than their antihypertensive effects, are markedly different. With propranolol, which is a nonselective antagonist devoid of partial agonist activity, the fall in blood pressure was predominantly associated with a decrease in cardiac output (Figure 2). Conversely, during treatment with pindolol, which is a nonselective antagonist with a relatively high degree of partial agonist activity, the antihypertensive effect was entirely due to a fall in vascular resistance. At variance with some previous studies, the antihypertensive effect of the $\beta$-selective antagonist atenolol was not associated with a decrease in cardiac output. The alterations in systemic hemodynamics caused by acebutolol did not differ from those of atenolol. This reflects the relatively low degree of partial agonist activity of acebutolol.

In comparing the present data with those of previous studies, the fall in cardiac output, if present at all, during administration of $\beta$-adrenoceptor antagonists devoid of partial agonist activity was relatively small. Moreover, after pindolol with its pronounced partial agonist activity, cardiac output even moderately increased. Hemodynamic measurements in this study were performed after 2 hours of supine rest. The patients were therefore in a baseline state, and as reflected by their low heart rates, cardiac sympathetic drive was low. This may explain why on average relatively little cardiodepression was exerted by the $\beta$-adrenoceptor antagonists and why the cardiodystimulant action of pindolol could be demonstrated.

The hemodynamic data after 3 weeks of treatment with equipotent doses of the four $\beta$-adrenoceptor agonists are in close agreement with the hemodynamic profiles of these drugs during the onset of their blood pressure-lowering effect within the first 24 hours after their administration.

**Figure 3.** Plot of percent changes in lymphocyte $\beta$-adrenoceptor density during administration and after withdrawal of propranolol (○), atenolol (▲), acebutolol (□), and pindolol (●).
blockade, we suggested that withdrawal of vagal tone, afterload reduction, and an increase in venous return to the heart were the factors most likely to be involved in the adaptive changes of cardiac output.

**Renal Function**

Although deterioration of renal function during administration of \( \beta \)-adrenoceptor antagonists has sporadically been reported,\(^{20,21} \) the adverse effects of these drugs on renal function usually appear to be small and reversible.\(^{22,23} \) This could be confirmed in the present study: glomerular filtration rate did not change significantly after any of the four drugs, although it tended to decrease on propranolol and to increase on pindolol.

Reduction in cardiac output, suppression of renin, blockade of vasodilating \( \beta \)-receptors in the kidney, and alterations in plasma volume are all possible factors, which may adversely affect renal perfusion during \( \beta \)-adrenoceptor blockade.\(^{22,23} \) In the present study, renin was suppressed to a similar degree, and plasma volume was unchanged by any of the four drugs, whereas changes in renal perfusion occurred in parallel with the changes in cardiac output. In our view, these findings suggest that the changes in renal perfusion, which may occur during \( \beta \)-adrenoceptor antagonism, are at least in part a consequence of the changes induced by \( \beta \)-adrenoceptor antagonists in systemic hemodynamics.

**Plasma Catecholamine Concentrations**

The literature is not unanimous concerning the effects of \( \beta \)-adrenoceptor antagonism on plasma levels of norepinephrine and epinephrine. Increments, decrements, and no changes in plasma catecholamine levels during administration of \( \beta \)-receptor antagonists have all been reported.\(^{24} \) The interpretation of plasma levels of norepinephrine during administration of \( \beta \)-blockers is complicated by the fact that these drugs may affect not only the release of this neurotransmitter into plasma but also its clearance rate from plasma. Norepinephrine is mainly cleared from the circulation by the liver and the lung.\(^{25} \) A decreased flow through these organs, concomitant with a fall in cardiac output, impairs the clearance of norepinephrine and as a consequence may result in increased plasma levels of the neurotransmitter.\(^{26-28} \) In the present study, this may explain why norepinephrine tended to increase with propranolol, but not with the other three drugs, because cardiac output tended to decrease only on this drug. Increments in plasma norepinephrine concentration during \( \beta \)-adrenoceptor antagonism therefore do not necessarily imply that sympathetic tone is increased. In other words, reduced sympathetic activity and diminished release of norepinephrine can easily be masked by this phenomenon. The absence of a rise in norepinephrine levels during the "vasodilator" and antihypertensive action of \( \beta \)-adrenoceptor antagonists can be considered inappropriate and could therefore suggest interfer-
ence of these drugs with the sympathetic nervous system.

**Renin Suppression**

Suppression of active renin levels during administration of \( \beta \)-adrenoceptor antagonists has been well documented.\(^{16,24} \) The \( \beta \)-adrenoceptor mediating renin release is probably of the \( \beta_1 \)-subtype because \( \beta_1 \)-selective antagonists cause a similar degree of renin suppression as nonselective \( \beta \)-adrenoceptor antagonists.\(^{29} \) Whether or not renin release is also suppressed by pindolol is still a matter of controversy.\(^{30,31} \) In the present study, renin levels were markedly suppressed by all four drugs. Moreover, the degree of renin suppression on pindolol was not different from that of the other three drugs. This finding seems paradoxical in light of the effects of pindolol on heart rate and cardiac output, in which the partial agonist activity of the drug came clearly to expression. This differential effect of pindolol on renin on the one side and on hemodynamics on the other side supports in vitro studies, which suggest that pindolol’s agonist activity is predominantly restricted to the \( \beta_1 \)-receptor.\(^{9,32} \) This subtype \( \beta \)-receptor coexists with the \( \beta_1 \)-receptor in the human heart.\(^{33-35} \) An alternative explanation for our findings is that under resting conditions renal juxtaglomerular \( \beta \)-receptors are subjected to a higher sympathetic tone than the cardiac \( \beta \)-receptors.

The renin suppressant effect of \( \beta \)-adrenoceptor blocking agents has been linked to the anti hypertensive mechanism of these drugs.\(^{36} \) Indeed, evidence indicates that in patients with high-renin forms of hypertension renin suppression contributes to the blood pressure–lowering effect of these drugs, but this renin suppressant effect probably does not also contribute to the blood pressure–lowering effect of these drugs in most patients with normal or low-renin forms of hypertension.\(^{37,38} \) If, in the present study, the fall in blood pressure was due to renin suppression, a relation between pretreatment values of renin and the antihypertensive effect has been expected; however, such a correlation was not found. Furthermore, in our short-term study, renin was already near maximum suppression 2 hours after administration of acebutolol, atenolol, and propranolol, whereas blood pressure had not fallen at all.\(^1 \) Moreover, in that study, pindolol exerted its maximal antihypertensive effect 24 hours after administration, whereas renin was no longer reduced at that time.

**Lymphocyte \( \beta \)-Adrenoceptor Density and Isoproterenol Sensitivity**

Studies in humans and in vitro studies indicate that the changes in the density and responsiveness of \( \beta \)-adrenoceptors on lymphocytes can be used as a model to study the alterations of these receptors in less accessible tissues such as the heart and the lung.\(^{39,40} \) To some extent, our study supports these data. First, during placebo, an inverse correlation between the density of lymphocyte \( \beta \)-adrenoceptors and the chronotropic responsiveness of the heart to isoproterenol was established. An inverse correlation between these two parameters has also been reported by Fraser et al.\(^{41} \) However, in their study, this correlation was found after repeated measurements of these parameters in the same group of normotensive subjects under different sodium balances. Second, and more important, the down-regulation of lymphocyte \( \beta \)-adrenoceptors 1 week after withdrawal of pindolol coincided with a decrease in the cardiac chronotropic responsiveness to isoproterenol, whereas the up-regulation of lymphocyte \( \beta \)-adrenoceptors 1 week after withdrawal of propranolol coincided with an increased cardiac chronotropic responsiveness.

Lymphocyte \( \beta \)-adrenoceptors are of a homologous \( \beta_1 \)-subtype.\(^{42} \) No change in these receptors would therefore be expected during administration of \( \beta_1 \)-selective \( \beta \)-adrenoceptor antagonists, as shown in the present study with atenolol. During administration of acebutolol, lymphocyte \( \beta \)-adrenoceptors also did not change. Once again, this may be related to the \( \beta_1 \)-selectivity of this drug. On the other hand, the partial agonist activity of at least some \( \beta \)-adrenoceptor blocking agents, regardless of \( \beta_1 \) selectivity, was recently shown to possess a \( \beta_2 \) component.\(^43 \) Absence of any effect of acebutolol on lymphocyte \( \beta \)-adrenoceptors may therefore indicate that the partial agonist activity of this drug, at least compared with that of pindolol, is rather weak, which fits well with the hemodynamic effects of this drug. The dose of isoproterenol to increase the heart rate by 25 beats/min 1 week after withdrawal of atenolol was also significantly decreased, indicating an increased cardiac chronotopic responsiveness of the heart. This increased cardiac chronotropic responsiveness might be explained by an up-regulation of cardiac \( \beta_1 \)-receptors.

Another notable finding of this study was the inverse correlation between plasma concentrations of epinephrine and the density of \( \beta \)-adrenoceptors on lymphocytes. This finding suggests that at least under basal conditions, endogenous epinephrine, which is the natural \( \beta_1 \)-agonist in humans, is capable of regulating these receptors. In agreement with previous reports, plasma concentrations of norepinephrine and lymphocyte \( \beta \)-adrenoceptor density were not correlated.\(^{44} \) This is not surprising because norepinephrine has no \( \beta_1 \)-adrenoceptors activity.

In summary, our results indicate that lymphocyte \( \beta \)-adrenoceptors are a suitable model for studying drug-induced changes in cardiovascular \( \beta \)-adrenoceptors when these changes are caused by \( \beta \)-adrenergic agents. Studies on lymphocyte \( \beta \)-adrenoceptors may aid in explaining the so-called “\( \beta \)-blocker withdrawal syndrome,” which has been reported after discontinuation of propranolol and other \( \beta \)-adrenoceptor antagonists but never after discontinuation of pindolol.\(^{45} \) Studies on lympho-
cyte β-adrenoceptors may also be helpful for determining the degree of partial agonist activity or receptor selectivity of β-adrenoceptor antagonists.

Conclusions

The β-adrenoceptor antagonists propranolol, pindolol, acebutolol, and atenolol appear to exert, with appropriate doses, equal antihypertensive effects. Despite this similarity among the four drugs in antihypertensive effect, the drugs have dissimilar effects on cardiac output, renal blood flow, and lymphocyte β-adrenoceptors. In contrast, their effects on catecholamine concentrations, the renin-angiotensin-aldosterone system, and body fluid volumes are not different. Changes in cardiac output, the circulating blood volume, or angiotensin-mediated vasoconstriction are unlikely to be the crucial mechanism for the antihypertensive effect of β-adrenoceptor antagonists. By exclusion of the above possibilities, we suggest that interference with sympathetic vasoconstrictor nerve activity through blockade of either central or peripheral prejunctional β-adrenoceptors could be an alternate explanation of antihypertensive effect of these drugs. The absence of a fall in plasma norepinephrine levels does not necessarily contradict such a contention because concomitant changes in the clearance of plasma norepinephrine have to be taken into account.

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**KEY WORDS** • $\beta$-adrenergic receptor blockers • partial agonist activity • cardiac output • vascular resistance • renal circulation • body fluid volumes • renin • catecholamines • aldosterone • $\beta$-adrenergic receptors • isoproterenol • acebutol • atenolol • pindolol • propranolol
Hemodynamic and beta-adrenergic receptor adaptations during long-term beta-adrenoceptor blockade. Studies with acebutolol, atenolol, pindolol, and propranolol in hypertensive patients.
A H van den Meiracker, A J Man in't Veld, F Boomsma, D J Fischberg, P B Molinoff and M A Schalekamp

Circulation. 1989;80:903-914
doi: 10.1161/01.CIR.80.4.903

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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