Regulation of Myocardial and Vascular \( \alpha \)-Adrenergic Receptor Affinity

Effects of Guanine Nucleotides, Cations, Estrogen, and Catecholamine Depletion

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SUMMARY. The threshold sensitivity of cardiovascular tissues to \( \alpha \)-adrenergic stimulation is determined largely by the affinity of \( \alpha \)-adrenergic receptors for agonists. To determine whether changes in \( \alpha \)-adrenergic receptor affinity could contribute to the regulation of cardiac and vascular responsiveness, we used the \( \alpha \)-adrenergic-selective radioligands, \([\text{H}]\text{prazosin}, [\text{H}]\text{-WB-4101}, \) and \([\text{H}]\text{rauwalscine}, \) to study and contrast the determinants of \( \alpha \)-adrenergic receptor affinity in myocardium and vascular smooth muscle from the rat. In both tissues, \( \text{I-epinephrine} \) binding to the \( \alpha \)-adrenergic receptor describes a shallow curve suggesting more than one affinity state. Computer analysis of binding to myocardial \( \alpha \)-receptors indicates that 15% are of high affinity (\( K_d = 11 \text{ nM} \)) and 85% are of low affinity (\( K_d = 400 \text{ nM} \)). Expression of high affinity sites is magnesium dependent (maximum effect, 5–10 \text{ mm}), and suppressed by the guanosine 5‘-triphosphate analogue Gpp(NH)p (maximum effect, 1 \text{ mm}) and sodium (maximum effect, 100–200 \text{ mm}). In vascular smooth muscle, agonist-binding curves are also shallow and exhibit a similar response to that of Gpp(NH)p. Basal receptor affinity in myocardium is significantly higher (3.4-fold) than in vascular smooth muscle. Unlike vascular smooth muscle, in which \( \alpha \)-adrenergic receptor affinity is increased by estrogen or reserpine treatment of the animal, the receptor in myocardium is unaffected by these treatments. In vascular smooth muscle, following reserpine-induced increase in \( \alpha \)-adrenergic receptor affinity, the Gpp(NH)p effect is still present. Thus, \( \alpha \)-adrenergic receptors in both myocardium and vascular smooth muscle exist in two affinity states and are subject to regulation by several factors, including guanine nucleotides, mono- and divalent cations, tissue of origin, sex hormones, and the level of sympathetic stimulation. Potentially, alterations in \( \alpha \)-adrenergic receptor affinity, independent of a change in receptor number, may play an important role in the regulation of cardiovascular tissue responsiveness to catecholamines.

been reported recently that the rat myocardial \( \alpha_1 \)-receptor, unlike many receptors for hormones and neurotransmitters, including the hepatic and renal \( \alpha_1 \)-receptors (Goodhardt et al., 1982; Snively and Insel, 1983), is not regulated by guanine nucleotides (Stiles et al., 1983). Guanine nucleotides regulate the affinity of receptors for agonists by modulating the interaction of the receptor with membrane guanine nucleotide regulatory proteins (Rodbell, 1980). Since, as noted, an apparent qualitative difference in the manner in which the vascular and myocardial \( \alpha_1 \)-receptors respond to chemical sympathectomy involves affinity changes, differences in the association of the receptors in the two tissues with guanine nucleotide regulatory proteins may contribute to their unique regulatory responses. Other factors known to regulate \( \alpha_1 \)-adrenergic receptor affinity in nonvascular tissues are mono- and divalent cations which interact with receptor-associated sites (Goodhardt et al., 1982; Snively and Insel, 1983; Glossman and Hornung, 1980).

The objectives of this study were to compare and contrast in a parallel fashion the manner in which myocardial and vascular \( \alpha_1 \)-adrenergic receptors respond to regulatory influences such as denervation and estrogen administration which markedly affect cardiovascular responsiveness (Altura and Altura, 1977; Carrier and Shibata, 1977). To provide further insight into the similarities and dissimilarities of the two receptors, responsiveness to guanine nucleotides and cations was characterized. The data indicate that, in the basal state, the myocardial \( \alpha_1 \)-adrenergic receptor has a higher affinity for agonists than does the vascular receptor, and after estrogen treatment or denervation, only the vascular receptor increases in affinity. Both myocardial and vascular \( \alpha_1 \)-adrenergic receptors, however, have associated guanine nucleotide- and cation-sensitive sites which regulate receptor affinity.

**Methods**

**Reagents**

\( [\text{H}]\)-WB-4101 (specific activity = 25.7 Ci/mmol), \( [\text{H}]\)-prazosin (specific activity = 23 Ci/mmol), and \( [\text{H}]\)-auwalscine (specific activity = 88 Ci/mmol) were obtained from New England Nuclear. An injectible preparation of reserpine-HCl (Serpasil) was obtained from Ciba Pharmaceuticals, and 17-\( \beta \)-estradiol valve rate suitable for subcutaneous administration was obtained from E.R. Squibb and Sons. All other compounds used were purchased from standard chemical suppliers.

**Membrane Preparations**

Male Sprague-Dawley rats weighing 300-350 g (Charles River Breederries) were utilized for all experiments. A membrane-rich particulate fraction of myocardium was prepared by methods similar to those previously described by Karliner et al. (1979). Briefly, rats were killed by a blow to the head and cervical dislocation. The heart was rapidly excised, placed in ice-cold phosphate-buffered saline, and rinsed free of blood. The atria and great vessels were discarded, the ventricular tissue coarsely minced with scissors, and homogenized in 0.25 M sucrose with a Brinkman Polytron (setting no. 8, 10 seconds X 2). The homogenate was centrifuged at 1500 g for 10 minutes at 4°C, and the supernatant centrifuged at 48,000 g for 10 minutes. The particulate fraction from four to eight hearts was suspended in assay buffer to a final protein concentration of 1–2 mg/ml. Protein concentration was determined by the Lowry method with bovine serum albumin as standard (Lowry et al., 1951).

A particulate fraction of dissected mesenteric arteries was prepared by modifications of the methods of Wei et al. (1976), as previously described in detail (Colucci et al., 1980). This preparation differed from that used for myocardial membranes in that there was an additional mechanical step to remove adventitial fat and connective tissue from the vessels, and the final centrifugation was performed at 100,000 g. The particulate fraction from 6 to 12 mesenteric arterial arcades was utilized for each experiment, resulting in a final protein concentration of 1–2 mg/ml.

**Animal Treatment Protocols**

Reserpine (0.25 mg/kg) was administered by intramuscular injection each morning for 5 days as previously described (Colucci et al., 1981). 17-\( \beta \)-Estradiol (0.25 mg/100 g body weight) was injected subcutaneously daily for 3 days as previously described (Colucci et al., 1982).

**Radioligand-Binding Assays**

The assay buffer used for all experiments, unless otherwise stated, was of the following composition: Trisma-HCl, 50 m\( \mu \)M; MgCl\( _2 \), 5 or 10 m\( \mu \)M; pH 7.50 (20°C). The binding of \( [\text{H}]\)-WB-4101 to the mesenteric artery particulate fraction was performed in a total volume of 150 \( \mu \)l, as previously described (Colucci et al., 1981). An identical binding assay was used in the myocardial particulate fraction for those experiments in which the binding to mesenteric arterial and myocardial receptors was compared directly.

For experiments in which the effects of cations and guanine nucleotides were evaluated by computer analysis, binding of \( [\text{H}]\)-WB-4101 and \( [\text{H}]\)prazosin (Karliner et al., 1979) to myocardium was performed in a total volume of 1.0 ml. Incubations were carried out at 30°C for 30 minutes, the assay mixture diluted with 4.5 ml of assay buffer (room temperature), and immediately filtered through a Whatman GF/C glass fiber filter, followed by two additional washes with 4.5-ml portions of assay buffer. In all experiments, filtration was performed on a single 25-\( \mu \)m filter support, as previously described (Colucci et al., 1980).

Specific binding for both radioligands was defined as the portion of total bound counts displaced by 1 mm \( l \)-epinephrine or 100 nm prazosin. At ligand concentrations equivalent to the respective \( K_a \)'s, specific binding was approximately 75% for \( [\text{H}]\)-WB-4101 and greater than 90% for \( [\text{H}]\)prazosin. Data in all figures and tables refer to specific binding.

**Calculations**

Saturation radioligand-binding curves were analyzed by the method of Scatchard (1949) to determine the equilibrium dissociation constant \( (K_a) \) and the amount of radioligand bound at saturation \( (B_{max}) \). Inhibition of radioligand binding by agonists was normalized to maximum inhibition of binding (i.e., inhibition caused by 1 mm \( l \)-epinephrine or 100 nm prazosin) for each experiment, and fitted to the Hill equation (Hill, 1913) from which a straight line was generated by least squares linear regression of the data. From this line, the mean inhibitory...
concentration of agonist (IC50), and the slope of the linear regression line, or slope factor, were determined. The dissociation constants (Kd) for unlabeled agonists and antagonists were determined from the IC50 by the method of Cheng and Prusoff (1973). Where indicated, the inhibition of radioligand binding by agonists was analyzed by Ligand, a nonlinear curve-fitting program (Munson and Rodbard, 1980). All data are expressed as the arithmetic mean ± standard error of the mean, and analyzed by nonpaired two-tailed t-test unless otherwise specified.

Results

Binding of [3H]Prazosin to Rat Myocardial Membranes

Scatchard analysis of [3H]prazosin binding to rat myocardial membranes over a wide concentration range (0.03–15 nM) revealed that binding was of high affinity and to a single class of binding sites (Fig. 1). The mean Kd and Bmax were 0.16 ± 0.04 nM and 111 ± 6 fmol/mg protein (n = 5), respectively. The α-adrenergic antagonists prazosin (Kd = 0.17 ± 0.04 nM; slope factor = 0.99 ± 0.05; n = 4) and yohimbine (Kd = 500 ± 140 nM; slope factor = 1.05 ± 0.04; n = 3) competed for the [3H]prazosin-binding site in the order expected for an α1-adrenergic receptor (Fig. 2). At 30°C, binding was complete by 20 minutes, and dissociation was complete within 20 minutes after the addition of an excess of l-epinephrine (1 μM).

Effect of Guanine Nucleotides on Agonist Binding to α1-Adrenergic Receptors in Rat Myocardium

Addition of the nonhydrolyzable guanine nucleotide analog Gpp(NH)p (guanyl imidodiphosphate) by guest on April 5, 2017 http://circres.ahajournals.org/ Downloaded from
resulted in a concentration-related shift of the $\beta$-epinephrine competition curve to the right, the IC$_{50}$ increasing significantly by 4.6-fold ($P < 0.003$) (Fig. 3) (Table 1). Compared to control experiments, competition curves performed in the presence of Gpp(NH)$_p$ were significantly steeper with a mean slope-factor of 1.05 ± 0.04 ($P < 0.01$). The effect of Gpp(NH)$_p$ was concentration-related, the maximal effect occurring at 1 mM (Fig. 3, inset). ATP (1 mM) resulted in an effect similar to that of Gpp(NH)$_p$, whereas GMP and GDP had little effect at concentrations of 1 mM. The maximum density of [H]$^+$-prazosin-binding sites was not affected by Gpp(NH)$_p$.

Analysis by nonlinear curve-fitting (Ligand program) of the combined data from four experiments performed in the presence of Gpp(NH)$_p$ (1 mM) indicated a significantly better fit of the data ($P < 0.01$) to a one-site model ($K_d = 685$ nM) than to a two-site model (Table 1).

Influence of Cations on Agonist Binding to $\alpha_1$-Adrenergic Receptors

Sodium ion resulted in a concentration-dependent shift of the $\beta$-epinephrine competition curve for the [H]$^+$-prazosin-binding site to the right, the IC$_{50}$ for $\beta$-epinephrine increasing significantly by 4.6-fold ($P < 0.01$) (Fig. 4) (Table 1). The effect was evident at 5 mM, and maximal between 100 and 200 mM (Fig. 4). In contrast, 200 mM sucrose had no effect, indicating that the sodium effect was not due simply to increased osmolarity of the buffer. In the presence of 100 mM NaCl, $\beta$-epinephrine competition curves were steeper, the mean slope factor increasing significantly to 1.01 ± 0.01 ($P < 0.03$) (Table 1). Nonlinear curve-fitting of the combined data demonstrated a significantly better fit of the data to a one-site model ($P < 0.01$) with a $K_d$ of 926 nM (Table 1). Sodium chloride (100 mM) had no effect on maximum $[H]^+$-prazosin binding.

Omission of magnesium from the assay buffer resulted in a rightward shift and steepening of the $\beta$-epinephrine competition curve, the IC$_{50}$ increasing 2.9-fold ($P < 0.001$) and the mean slope factor increasing to 0.88 ± 0.02 ($P = \text{NS vs. control}$) (Fig. 5) (Table 1). Nonlinear curve-fitting of the data analyzed* indicated a significantly better fit of the data to a one-site model ($P < 0.01$) with a $K_d$ of 560 nM (Table 1). Magnesium-free buffer had no effect on maximum $[H]^+$-prazosin binding.

Each experiment used myocardial membranes from 6–10 rats and was performed in duplicate. IC$_{50}$ is concentration of $\beta$-epinephrine which inhibits specific $[H]^+$-prazosin binding by 50% (calculated as per Methods). The slope factor was calculated by the method of Hill, per Methods.

* Analysis by nonlinear curve-fitting (LIGAND program), per Methods. R$_1$ and R$_2$ are presented as percentage of total sites.

† Control buffer consisted of Trisma-HCl, 50 mM; MgCl$_2$, 10 mM; pH 7.50 (20°C).

§ $p < 0.05$ vs. control.

|| $p < 0.01$ vs. control.

\| $p < 0.001$ vs. control.

### Table 1

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>IC$_{50}$ (nM)</th>
<th>Slope factor</th>
<th>$K_1$ (nM)</th>
<th>R$_1$ (%)</th>
<th>$K_d$ (nM)</th>
<th>R$_2$ (%)</th>
</tr>
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<tr>
<td>Control†</td>
<td>9</td>
<td>1,900 ± 300</td>
<td>0.80 ± 0.04</td>
<td>11</td>
<td>15</td>
<td>400</td>
<td>85</td>
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<tr>
<td>Gpp(NH)$_p$ (1 mM)</td>
<td>4</td>
<td>8,400 ± 4,400†</td>
<td>1.05 ± 0.02†</td>
<td>0</td>
<td>685</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>NaCl (100 mM)</td>
<td>3</td>
<td>8,500 ± 2,600§</td>
<td>1.01 ± 0.01§</td>
<td>0</td>
<td>926</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Magnesium-free</td>
<td>4</td>
<td>5,600 ± 800∥</td>
<td>0.88 ± 0.02∥</td>
<td>0</td>
<td>625</td>
<td>100</td>
<td>100</td>
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</tbody>
</table>

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FIGURE 4. Effect of sodium chloride (100 mM) on l-epinephrine competition for the [3H]prazosin-binding site in rat myocardial membranes. Compared to control experiments (○), sodium chloride (□) results in a rightward shift and steepening of the curve, the slope factor increasing to 1.01 ± 0.01 (n = 3). The data presented are the means ± SEM for nine control or three sodium chloride experiments performed in duplicate. Inset: dose-relationship for the sodium chloride effect on l-epinephrine competition for the [3H]prazosin-binding site. The vertical axis depicts the inhibition of [3H]prazosin binding by 1,000 nM l-epinephrine as the percent of control inhibition in the absence of sodium chloride. The data presented represent the means of two experiments.

was most compatible with a one-site model (K_d = 625 nM). The influence of magnesium on l-epinephrine binding was concentration-related over the range of 1–10 mM (Fig. 5, inset). Unless specifically noted, all experiments were performed in the presence of 5 or 10 mM MgCl_2.

Binding of [3H]-WB-4101 to Myocardial Membranes

Binding of [3H]-WB-4101 to myocardial membranes was analyzed over a wide range of concentrations (0.05–25 nM). Scatchard analysis revealed a curvilinear plot, in contrast to the linear Scatchard plot which was evident for [3H]prazosin binding (Fig. 1). Analysis of [3H]-WB-4101 binding by a multiple-fit program revealed that the data were compatible with a two-site model with a high affinity site (K_a = 0.17 ± 0.04 nM) representing approximately 16% of total binding, and a low affinity site (K_a = 3.8 ± 0.5 nM) representing approximately 84% of total binding.

Experiments were performed to determine whether binding to myocardial α₂-adrenergic recep-

FIGURE 5. Effect of magnesium on l-epinephrine competition for the [3H]prazosin-binding site. Compared with control binding experiments (○) performed in the presence of 10 mM MgCl_2, experiments performed in magnesium-free buffer (△) or magnesium-free buffer plus 3 mM EDTA (□) were shifted to the right and steeper. Data presented represent the means of nine (control), two (magnesium-free), or two (magnesium-free + EDTA) experiments. Inset: Concentration-relationship of MgCl_2 for the inhibition of [3H]prazosin binding by l-epinephrine. Data presented represent the percent inhibition of [3H]prazosin binding by 1000 nM l-epinephrine as the percent of control inhibition in the presence of 10 mM magnesium chloride. Data presented represent the means of two experiments performed in duplicate.
The \( \alpha_2 \)-epinephrine competition curve for the \([3H]\)-WB-4101 binding site (IC\(_{50} = 1,500 \pm 200 \text{ nM}; n = 6\)) was shallow, with a slope factor of 0.68 ± 0.01 (\( n = 6 \)) suggesting the presence of multiple binding sites (Fig. 7). Addition of Gpp(NH)p (0.1 mM) to the assay mixture resulted in a steeper and rightward shift of the curve, the IC\(_{50}\) increasing to 7,600 ± 2,200 nM (\( P < 0.03; n = 4 \)) and the slope factor increasing to 0.82 ± 0.03 (\( P < 0.001; n = 4 \)) (Fig. 7). Nonlinear curve-fitting was not performed in these experiments due to the complex binding pattern of the radioligand.

**[3H]-WB-4101 Binding to Vascular Smooth Muscle Membranes**

The binding of [3H]-WB-4101 to vascular smooth muscle membranes from rat mesenteric artery was performed as previously described (Colucci et al., 1980). The \( \alpha_2 \)-epinephrine competition curve (IC\(_{50} = 8,100 \pm 1,700 \text{ nM})\) was shallow with a slope factor of 0.71 ± 0.06 (Fig. 8) (Table 2). Addition of Gpp(NH)p (0.1 mM) to the incubation mixture resulted in a rightward shift and steepening of the curve (Fig. 8) (Table 2).

**Effects of Estrogen and Reserpine Treatment on Agonist Binding to Myocardial and Vascular Smooth Muscle Membranes**

Control \( \alpha_2 \)-epinephrine competition curves in vascular smooth muscle (IC\(_{50} = 8,100 \pm 1,700 \text{ nM}; n = 6\)) were located to the right of competition curves in myocardium (IC\(_{50} = 1,500 \pm 200 \text{ nM}; n = 6\)), the IC\(_{50}\) 's differing significantly (\( P < 0.02 \)) (Fig. 9). Reserpine treatment had no effect on the \( \alpha_2 \)-epinephrine competition curve (IC\(_{50} = 8,100 \pm 1,700 \text{ nM}; n = 6\)) (Table 2).
TABLE 2
Effects of Gpp(NH)p and Prior Reserpine Treatment on the Mean Inhibitory Concentration (IC50) and Slope Factor for the Inhibition of [3H]-WB-4101 Binding by l-Epinephrine in Rat Mesenteric Artery

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>IC50 (nM)</th>
<th>Slope factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control*</td>
<td>6</td>
<td>8,100 ± 1,700</td>
<td>0.71 ± 0.06</td>
</tr>
<tr>
<td>Gpp(NH)p (0.1 mM)</td>
<td>6</td>
<td>12,400 ± 1,100</td>
<td>1.09 ± 0.11§</td>
</tr>
<tr>
<td>Reserpine</td>
<td>6</td>
<td>2,600 ± 200γ</td>
<td>0.69 ± 0.06</td>
</tr>
<tr>
<td>Reserpine + Gpp(NH)p</td>
<td>6</td>
<td>5,200 ± 600γ</td>
<td>0.92 ± 0.06§|</td>
</tr>
</tbody>
</table>

Each experiment used mesenteric arteries from 6 to 10 rats, and was performed in duplicate. IC50 = concentration of l-epinephrine which inhibits [3H]-WB-4101 binding by 50% (calculated as per Methods). The slope factor was calculated by the method of Hill, see Methods. Reserpine was administered as described in Methods.

* Control buffer consisted of: Trizma-HCl, 50 mM; MgCl2, 10 mM; pH, 7.50 (20°C).

‖ p < 0.01 vs. control.
γ p < 0.01 vs. reserpine.
§ p < 0.05 vs. control.
\| p < 0.03 vs. reserpine.

A significant change in the slope factor following treatment with reserpine.

To test the possibility that the reserpine-induced increase in vascular a1-adrenergic receptor affinity might reflect changes in the relationship of the receptor with putative guanine nucleotide regulatory proteins, the effect of Gpp(NH)p on the l-epinephrine competition curve in vascular smooth muscle

competition curve in myocardium (IC50 = 2,500 ± 300 nM; n = 6). In contrast, after reserpine treatment for 5 days, there was a symmetrical 3.1-fold leftward shift of the l-epinephrine competition curve in vascular smooth muscle (IC50 = 2,600 ± 200 nM; n = 6) (Table 2) (Fig. 10), similar to the effect previously reported (Colucci et al., 1981). After reserpine treatment, the IC50's in myocardium and vascular smooth muscle were not significantly different. In neither vascular smooth muscle nor myocardium was there
was examined in rats treated with reserpine. When compared to reserpine-treated animals assayed without Gpp(NH)p, Gpp(NH)p (0.1 mM) resulted in a significant rightward shift and steepening of the l-epinephrine competition curve, the IC$_{50}$ increasing 2-fold to 5,200 ± 600 nM and the slope factor increasing to 0.92 ± 0.06 (n = 6) (Table 2) (Fig. 10).

The responses of the myocardial and vascular α$_1$-receptors to estrogens also were compared. Treatment of rats with 17-β-estradiol (0.25 mg/kg per day intramuscularly) resulted in a 2.5-fold leftward shift of the l-epinephrine competition curve in vascular smooth muscle as previously described (Colucci et al., 1982). In contrast, in myocardium from rats treated with 17-β-estradiol, there was no change in the l-epinephrine competition curve (IC$_{50}$ = 2,000 ± 400 nM; n = 6).

**Discussion**

The objective of this work was to compare, using ligand-binding assays, the characteristics of α-adrenergic receptors in myocardium and vascular smooth muscle. A number of major issues were addressed and include: (1) whether myocardial α$_1$-adrenergic receptor affinity is regulated by guanine nucleotides, and by inference, is associated with a membrane nucleotide regulatory protein; (2) whether conflicting data concerning regulation of α$_1$-adrenergic receptor affinity by guanine nucleotides can be explained on the basis of differences in the ligands which have been used to study the phenomenon or whether tissue-specific differences in α$_1$-adrenergic receptors exist; (3) whether myocardial and vascular α-adrenergic receptors are regulated in similar fashions in response to stimuli which enhance cardiovascular responsiveness to catecholamines, such as chemical sympathoectomy and estrogen administration. The data provide firm evidence for the existence of a high affinity binding state of the receptor which is converted to a lower affinity state by guanine nucleotides. Guanine nucleotide regulation of myocardial α$_1$-adrenergic receptor affinity was observed by using both [³H]prazosin and [³H]-WB-4101. Since [³H]prazosin is considered to be specific for the α$_1$-subtype (Stiles et al., 1983; Karliner et al., 1979), and since [³H]-WB-4101 was shown not to interact with putative α$_2$-subtype receptors in heart, these data constitute strong evidence that the guanine nucleotide-sensitive receptor is, in fact, the α$_1$-receptor. Finally, the data show that the basal affinity of the α$_1$-receptor for agonists appears to be lower in mesenteric arteries than in heart, and that estrogens and denervation enhance vascular but not myocardial α$_1$-adrenergic receptor affinity.

**Agonist Binding to Rat Myocardium**

In rat myocardium, we consistently observed that l-epinephrine competition for the [³H]prazosin-binding site described shallow curves, which, by application of the Hill equation, demonstrated slope factors less than unity. By contrast, and similar to previous studies by Karliner et al. (1979) and others (Stiles et al., 1983), we found that the Scatchard analysis of [³H]prazosin binding was linear, and that the competition curves for antagonists consistently were steep with slope factors of unity. By analogy to other biochemical reactions, a slope factor less than unity is consistent with at least three possibilities: (1) the presence of more than one discrete receptor type, (2) the existence of more than a single affinity state of a homogenous population of receptors, or (3) negative cooperativity of binding such that receptor affinity decreases as the fraction of receptors occupied increases (Limbird et al., 1975).

With rat myocardium, it was possible to generate highly reproducible 15-point competition curves, and, therefore, to analyze the data by Ligand, a computerized nonlinear curve-fitting program described by Munson and Rodbard (1980). The analysis of control l-epinephrine competition curves by Ligand is consistent with two populations of binding sites, of which approximately 15% are of high affinity, and the remainder of low affinity. The fit of the data to a two-site model is significantly better than the fit to a one-site model. Interpretation of the analysis of this data by Ligand must be guarded, since the precise quantification of binding sites is less reliable when the percentage of one site is low. Nevertheless, both the slope factors of less than unity, and the results of analysis by Ligand, strongly suggest that agonist binding to α$_1$-adrenergic receptors in rat myocardium is not consistent with a single-site model.

**Guanine Nucleotide Effects on Agonist Binding to α$_1$-Receptors in Myocardium and Vascular Smooth Muscle**

Although guanine nucleotides are well known to regulate agonist binding affinity to adenylyl cyclase-coupled receptors (Rodbell, 1980), the role of guanine nucleotides in the regulation of α$_1$-adrenergic receptors has been controversial. Initial studies utilizing computer analysis of the binding of the non-subtype selective α-adrenergic ligand [³H]di-hydroergocryptine in liver suggested that there was no effect of guanine nucleotides on agonist binding affinity (Hoffman et al., 1980). However, other investigators using the α$_1$-selective radioligand [³H]prazosin subsequently showed that α$_1$-adrenergic receptors in hepatic membranes are sensitive to both guanine nucleotides and cations (Goodhardt et al., 1982). Rat myocardial α$_1$-receptors identified by the ligand [³H]-WB-4101 initially were reported to have shallow agonist competition curves which steepened and developed lower affinity in the presence of guanine nucleotides (Yamada et al., 1980).
[125I]-HEAT* failed to demonstrate a guanine nucleotide effect on rat myocardial α1-adrenergic receptors (Stiles et al., 1983), and it was suggested that the previously reported results with [3H]-WB-4101 were due to anomalous binding properties of this ligand. The suggestion had been made previously that [3H]-WB-4101 does not discriminate well between α-adrenergic receptors of the α1- and α2-subtypes in uterus (Hoffman and Lefkowitz, 1980).

The data in Figure 1 show that [3H]prazosin and [3H]-WB-4101 differ in their binding characteristics in that the former results in a linear Scatchard plot, whereas the latter results in a curvilinear plot which is resolvable into two sites of differing affinities. In myocardium, the total number of sites identified by the two ligands is similar providing evidence that [3H]-WB-4101 does not identify a separate, large pool of receptors. Moreover, [3H]-WB-4101 does not bind to α2-receptors in the myocardium, since the binding is not affected by the α2-selective antagonist yohimbine (Stiles et al., 1983), nor is specific binding detectable utilizing the α2-selective (Perry and U'Prichard, 1981) ligand [3H]rauwalscine. Although the implications of the differences in the binding properties of [3H]prazosin and [3H]-WB-4101 are not clear, these differences cannot account for discrepancies which have been reported by others (Stiles et al., 1983; Yamada et al., 1980b) with regard to the guanine nucleotide sensitivity of myocardial α1-receptors, since we observed qualitatively similar effects with both ligands.

The mesenteric artery α-adrenergic receptor is similar in that in myocardium in that the interaction of the receptor with agonists, but not antagonists, is modulated by guanine nucleotides. Thus, the epinephrine competition curve is shallow in the absence of guanine nucleotides, and increases in steepness in the presence of guanine nucleotides (Table 2). [3H]-WB-4101 also appears to identify exclusively α1-subtype receptors in the mesenteric artery, since the binding is not affected by high concentrations (100 nM) of yohimbine (Colucci et al., 1981). Although technical constraints of limited tissue availability from mesenteric artery precluded generating the large number of data points necessary for computer analysis, the data are consistent with the notions that vascular α1-adrenergic receptors exist in high and low affinity states, and, in the presence of guanine nucleotides, are converted to a homogeneous population of low affinity sites.

In view of the controversy concerning guanine nucleotide sensitivity of α1-adrenergic receptors, it seems premature to attempt to generalize these results in rat cardiovascular tissues. Very recent reports have demonstrated guanine nucleotide-induced shifts in α1-adrenergic agonist binding curves in kidney (Snavely and Insel, 1983), as well as in the liver (Goodhardt et al., 1982), as noted above. On the other hand, another report demonstrated the absence of effect of guanine nucleotides on α1-receptors in a cultured line of smooth muscle cells (Hughes et al., 1982). Using our standard methodology, we also saw no guanine nucleotide effects on α1-receptors in a related cell line (unpublished observations). Thus, there may be tissue specificity for the property of association of α1-adrenergic receptors with guanine nucleotide-sensitive sites. Differing experimental conditions may account for the differences between our results and those of Stiles et al. (1983) with regard to guanine nucleotide sensitivity of myocardial α1-receptors. Although it is not clear what the fundamental differences are, the estimate of basal l-epinephrine affinity in that study was approximately 10-fold lower than we found (Stiles et al., 1983). If the basal affinity is lower, then interventions that further lower affinity may not produce an appreciable change. Another difference between the two studies is that the Gpp(NH)p concentration used by Stiles et al. (0.1 mM) produced only about 50% of the effect that we observed with a concentration of 1 mM (Fig. 3, inset).

The functional significance of an α1-adrenergic receptor-associated site which is sensitive to guanine nucleotides is not yet clear, but some insights can be gained by analogy with adenylate cyclase-coupled receptors. In the case of adenylate cyclase-coupled receptors such as the β-adrenergic receptor, a distinct guanine nucleotide binding regulatory protein (N1) is involved in the coupling of the receptor to the catalytic unit of the enzyme (Rodbell, 1983). A fundamental property of an agonist is to induce the formation of an interaction between the receptor and the nucleotide regulatory protein which results in the development of a high affinity state for an agonist but not for an antagonist. Binding of guanosine triphosphate to the receptor-regulatory protein complex results in the development of low affinity for the agonist and activation of adenylate cyclase. Receptors inhibiting adenylate cyclase are thought to be coupled through a separate regulatory protein (N0), and receptors neither stimulating nor inhibiting adenylate cyclase have been speculated to interact with a third regulatory protein (N2) (Rodbell, 1980). We have not observed α1-adrenergic inhibition of adenylate cyclase in rat myocardium and heart (Wright et al., 1983), adrenal (Glossman et al., 1974), and vascular smooth muscle (Wright et al., 1982), although not thought to be adenylate cyclase-coupled, are regulated by guanine nucleotides. The role of such a receptor-regulatory protein interaction in the overall process of receptor-

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* 2-[β-(4-hydroxyphenyl)-ethylaminomethyl] tetralone.
effector coupling remains to be elucidated, as does the issue of coupling of $\alpha_1$-receptors to adenylate cyclase. This latter issue must be considered unresolved, since, although $\alpha_1$-receptors are generally considered not to be coupled to adenylate cyclase, there is a report of $\alpha_1$-adrenergic inhibition of cAMP production in heart (Watanabe et al., 1977), and a very recent report of $\alpha_1$-adrenergic stimulation of cAMP in liver (Morgan et al., 1983). At the least, the existence of high affinity receptor binding states may have important implications for the determination of cell sensitivity to $\alpha_1$-adrenergic stimulation.

**Effects of Cations on $\alpha_1$-Adrenergic Receptor Binding in Rat Myocardium**

Sodium and magnesium exert striking effects on the affinity of agonist binding to $\alpha_1$-adrenergic receptors in rat myocardium. Magnesium appears necessary for the expression of high affinity sites, and, hence, for the demonstration of a guanine nucleotide effect. This permissive action of magnesium is concentration-related, becoming maximal between 5 and 10 mM. In the presence of magnesium, sodium causes a rightward shift and steepening of the agonist competition curve, similar to that produced by guanine nucleotides, or the omission of magnesium. The sodium effect is concentration-related, becoming maximal between 100 and 200 mM.

In the case of the $\alpha_1$-adrenergic receptor, the site of action of cations is not known. The effects of magnesium, sodium, and guanine nucleotides on the agonist binding affinity to $\alpha_1$-adrenergic receptors are qualitatively similar, albeit less pronounced, to their effects on agonist binding to $\alpha_2$-adrenergic receptors in several tissues (Rodbell, 1980; Mooney et al., 1982). In the case of the $\alpha_2$-adrenergic receptor, it is believed that magnesium and sodium affect agonist binding affinity as a result of their interaction with the guanine nucleotide regulatory protein (Rodbell, 1980). Although our data are consistent with mediation of the cation effect through an interaction with a guanine nucleotide regulatory protein, it is also possible that the cations exert a direct effect on the $\alpha_1$-receptor, itself, or an indirect effect due to a cation-induced change in the membrane microenvironment of the receptor. The data do suggest that there are discrete monovalent and divalent cation-sensitive sites associated with the myocardial $\alpha_1$-adrenergic receptor. Since our studies were performed in a membrane preparation, it cannot be determined whether the cation effects are mediated through an interaction with components on the inner or outer surface of the plasma membrane. Therefore, although substantial alterations in agonist binding affinity can be induced by changes in cation concentration, it is not known whether physiological changes in cation concentration would result in an important change in receptor-binding characteristics in situ.

**Regulation of Myocardial and Vascular $\alpha_1$-Adrenergic Receptor Affinity**

A comparison of agonist-binding affinity to $\alpha_1$-adrenergic receptors in myocardium and vascular smooth muscle suggests that tissue-specific features of the membrane microenvironment may play a role in determining both basal receptor affinity, and the receptor affinity response to perturbations such as catecholamine depletion and estrogen administration. Thus, basal affinity of epinephrine binding was 5.4-fold higher in membranes from myocardium than in membranes from mesenteric artery, whereas, after catecholamine depletion by reserpine, only mesenteric artery $\alpha_1$-adrenergic receptor affinity increased, becoming similar to that in myocardium. These observations suggest that basal agonist binding affinity is determined, at least in part, by the level of chronic adrenergic stimulation. Consistent with this hypothesis is the fact that the norepinephrine content of rat myocardium is only 10% of that in rat mesenteric artery (Head et al., 1982), presumably reflecting the lower density of adrenergic innervation in myocardium.

The lack of effect of estrogen on $\alpha_1$-adrenergic receptor affinity in myocardium is in contrast to the previously demonstrated effect in mesenteric artery smooth muscle membranes (Colucci et al., 1982), and may relate to the inherently higher basal receptor affinity in myocardium. Alternately, it is possible that myocardium is insensitive to estrogens, or responds in a way that does not influence the plasma membrane $\alpha_1$-receptor.

The effect of guanine nucleotides on agonist binding affinity in rat mesenteric artery particulate fraction from reserpine-treated rats was similar to that demonstrated in control rats: the curve was made steeper by a rightward shift of the lower portion (Fig. 10). It should be noted that the upper portion of the curve was unchanged, and is superimposable on the curve observed in the absence of guanine nucleotide, which is well to the left of the control curve. Since the reserpine-induced affinity change is partially reversed by guanine nucleotide, these data raise the possibility that $\alpha_1$-adrenergic receptor affinity can be mediated by a second mechanism, independent of the guanine nucleotide regulatory protein.

In summary, $\alpha_1$-adrenergic receptors in both the myocardium and mesenteric arteries of the rat show evidence of high and low affinity binding states for agonists. The high affinity state is regulated by guanine nucleotides, providing inferential evidence that the receptors are associated with a membrane guanine nucleotide regulatory protein. Myocardial and vascular $\alpha_1$-adrenergic receptors differ in their basal affinity, the myocardial receptor having a 5.4-fold higher affinity. The vascular receptor appears to be basally down-regulated with respect to affinity since, in contrast with the myocardial receptor, its...
affinity increases after estrogen or chemical sympathectomy. This increase in affinity appears to be independent of guanine nucleotide-dependent mechanisms, since guanine nucleotide modulation of the high affinity site is unchanged after either intervention. The existence of high affinity binding states of cardiovascular α₁-adrenergic receptors may have important implications for determination of the threshold sensitivity to catecholamine stimulation of myocardial and vascular tissues.

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INDEX TERMS: Myocardium • Vascular smooth muscle • α-Adrenergic receptor • Affinity • Guanine nucleotides
Regulation of myocardial and vascular alpha-adrenergic receptor affinity. Effects of guanine nucleotides, cations, estrogen, and catecholamine depletion.

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doi: 10.1161/01.RES.55.1.78

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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