Regulation of the Postsynaptic α-Adrenergic Receptor in Rat Mesenteric Artery

Effects of Chemical Sympathectomy and Epinephrine Treatment

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SUMMARY  Vascular smooth muscle sensitivity to catecholamine-induced contraction is increased after catecholamine depletion by chemical sympathectomy and decreased after exogenous catecholamine administration. To investigate the role of the vascular α-adrenergic receptor in these alterations, we used the α-1 selective radioligand, [(3H)-WB-4101, (phenoxy-3-3H(N)](2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane, to characterize the vascular postsynaptic α-adrenergic receptor in a membrane-rich particulate fraction of the rat mesenteric artery. Binding of [(3H)-WB-4101 was rapid, readily reversible, stereospecific, and saturable. Scatchard analysis described a single class of binding sites with a dissociation constant (Kd) of 0.76 ± 0.15 nm, and a maximal binding capacity (Bmax) of 99 ± 13 fmol/mg protein. Alpha-adrenergic agonists competed for the [(3H)-WB-4101 binding site in the α-adrenergic potency order of (-)-epinephrine (Kd = 3.0 μM) > (-)-norepinephrine (Kd = 8.8 μM) > (-)-isoproterenol (Kd = 57 μM), and the α-adrenergic antagonist phenolamine (Kd = 7.6 nm) was 1000 times more potent than the β-adrenergic antagonist, (-)-propranolol (Kd = 7400 nm). The number and affinity of α-adrenergic binding sites were studied in hyper- and hypo-adrenergic states. In mesenteric arteries from rats treated with exogenous epinephrine for 4 days, Bmax was decreased to 53 ± 10 fmol/mg protein (n = 4, P < 0.01), whereas Kd for [(3H)-WB-4101 was unchanged. Conversely, in mesenteric arteries from rats depleted of catecholamines with reserpine, Bmax was unchanged (96 ± 15 fmol/mg protein), whereas Kd for [(3H)-WB-4101 was decreased to 0.34 ± 0.07 nm (n = 6, P < 0.01), and Kd for (-)-epinephrine was decreased to 0.40 ± 0.19 μM (n = 5, P < 0.01). The effect of reserpine treatment was time dependent, being evident within 1 hour, and reaching a maximum after 3-7 days. The effects of catecholamine depletion with 6-hydroxydopamine were similar to those of reserpine. Thus, experimentally induced hyper- and hypo-adrenergic states can result in regulation of the vascular α-adrenergic receptor through changes in either receptor number or affinity. Circ Res 48: 104–111, 1981

AN ALTERATION in the sensitivity of blood vessels to catecholamines has been observed in a number of physiological and disease states including pregnancy (Altura and Altura, 1977), hypertension (Lais and Brody, 1975) and pheochromocytoma (Kuchel, 1977). Norepinephrine induces a specific desensitization to α-adrenergic agonists in rat aortic strips, in vitro (Carrier et al., 1978). Conversely, catecholamine depletion with reserpine (Burn and Rand, 1959) or 6-hydroxydopamine (Shibata et al., 1972) results in an increase in vascular sensitivity to catecholamines, in vitro. The molecular mechanism by which vascular sensitivity to α-adrenergic agonists is regulated is unknown; however, observations made in a number of nonvascular tissues raise the possibility that an alteration in vascular α-adrenergic receptors may be involved. In both human platelets (Cooper et al., 1978) and dispersed rat parotid acinar cells (Strittmatter et al., 1977), a catecholamine-induced reduction in the number of α-adrenergic binding sites correlates with a decrease in responsiveness to α-adrenergic agonists. Alternatively, reserpine has been shown to increase the number of α-adrenergic receptors in rat salivary gland (Pointon and Banerjee, 1979) and rat brain (U’Prichard and Snyder, 1978). Thus, there is considerable reason to hypothesize that the α-adrenergic receptor may be an important molecular locus for the regulation of vascular reactivity to catecholamines.

The small, highly reactive muscular arteries of the rat mesentery are richly innervated and, thus, are an ideal tissue in which to study the vascular α-adrenergic receptor. We have reported previously on the characterization of α-adrenergic receptors in a crude particulate fraction of the rat mesenteric artery using the radioligand [(3H)]-dihydroergocryptine (Colucci et al., 1980). Two subtypes of α-adrenergic receptors, termed α-1 and α-2, recently have
been characterized (Langer, 1974; Berthelsen and Pettinger, 1977). The \( \alpha \)-adrenergic receptors in rat mesenteric artery appear to be predominantly \( \alpha-1 \) (Colucci et al., 1980). Since the nonselective radioligand \( (^{3}H) \)-dihydroergocryptine would also bind to any presynaptically located \( \alpha-2 \) receptors present in this preparation, the use of a radioligand specific for \( \alpha-1 \) receptors offers the advantage of allowing selective study of the postsynaptic receptor.

In this study we used the radioligand, \( (^{3}H) \)-WB-4101, \( [\text{phenoxy-3-}^{3}H(N)]-(2-(2,6\text{-dimethoxyphenoxymethyl})amino methyl-1,4\text{-benzodioxane}, \) a potent \( \alpha \)-adrenergic antagonist of the benzodioxane series which is selective for the \( \alpha-1 \) (postsynaptic) receptor (Kapur and Mottram, 1978; Raisman et al., 1979) to characterize postsynaptic \( \alpha \)-adrenergic receptors in a particular fraction of muscular arteries from the rat mesentery. This technique then was applied to the study of \( \alpha \)-adrenergic receptors in mesenteric arteries from in vivo rat models of hyper- (epinephrine-treated) and hypo- (reserpine- and 6-hydroxydopamine-treated) adrenergic states to determine if the level of catecholamine stimulation alters the \( \alpha-1 \) receptor.

**Methods**

**Reagents**

\( (^{3}H) \)-WB-4101 (specific activity = 24.4 Ci/mmol; obtained from New England Nuclear) was greater than 98.5% pure on thin layer chromatography in three systems: \( n \)-butanol:acetic acid: water (4:1:1), ethyl acetate:ethanol (5:1), and chloroform:benzene:ethanol (4:2:1). Stock solutions in ethanol: benzene (9:1) were stored at \(-10\degree \)C and periodically checked for purity. After storage for 4 weeks, significant decomposition usually was evident by thin layer chromatography, and correlated with a markedly decreased specific binding. Immediately prior to use, \( (^{3}H) \)-WB-4101 was diluted to the desired concentration in distilled water in a polypropylene test tube (Fisher #14-595-16). Polypropylene test tubes resulted in a smaller loss of activity (presumably through surface adsorption) than did polystyrene or glass test tubes.

Phentolamine-HCl and an injectable preparation of reserpine-HCl (Serpasil) were obtained from Ciba Pharmaceutical Company, (+)-epinephrine and (-)-norepinephrine from Sterling-Winthrop, phenoxybenzamine from Smith, Kline, and French Laboratories, (-)-propranolol from Ayerst Laboratories, clonidine-HCl from Boehringer Ingelheim Laboratories, (±)-propranolol from Ayerst Laboratories, phenoxybenzamine from Smith, Kline, and French Laboratories, (±)-propranolol from Ayerst Laboratories, prazosin from Pfizer Pharmaceuticals, clonidine-HCl from Boehringer Ingelheim, WB-4101 from Ward Blenkinsop and Company, Ltd., and epinephrine, 1:200 aqueous suspension in glycerol (Sus-Phrine) from Cooper Laboratories, Inc. All other compounds used were from standard chemical suppliers.

**Mesenteric Artery Preparation**

A membrane-rich particulate fraction of mesenteric artery (predominantly smooth muscle) was prepared using modifications of the methods described by Wei et al. (1976). Male Sprague-Dawley rats (300–350 g) (Charles River Breeder) were killed by a blow to the head and cervical dislocation. The small intestine was doubly ligated at the proximal duodenum and terminal ileum, and cut between ligatures. The superior mesenteric vascular arcade was severed near its origin at the aorta, and transferred still attached to the small intestine into ice-cold phosphate-buffered saline on the surface of a chilled petri dish. The entire vascular arcade then was freed from its attachments to the bowel, and the mesenteric vein, fat, and lymph nodes removed from the artery by blunt dissection. Arteries from four animals were placed in 10 ml of cold \((4\degree C) 0.25 \text{ m sucrose solution (pH } 6.1) \) in a Potter-Elvejhem homogenizer, and the fatty adventitial tissues removed with 2 gentle strokes of a specially ground, tapered Teflon pestle (clearance, 0.016 in.). Arteries cleaned in this fashion then were combined in 40 ml of cold \((4\degree C) 0.25 \text{ m sucrose solution in a 50-ml beaker and coarsely minced with scissors. The tissue was transferred to a 125-ml Erlenmeyer flask and another 20 ml of sucrose solution added. The mix then was homogenized with a Brinkman Polytron (setting 8, 10 sec \( \times \) 2) and poured into two 40-ml tubes which were centrifuged at 1500 \( g \) for 10 minutes at \( 4\degree C \). The supernatant was centrifuged further at 100,000 \( g \) for 30 minutes at \( 4\degree C \) in a Beckman ultracentrifuge. The resultant pellet was resuspended in assay buffer (5 \( \text{mM MgCl}_2\), 50 \( \text{mM Tris-HCl, pH 7.55} \)). The mesenteric arteries from six to 1200-g rats were combined for each experiment to yield a final protein concentration of \( 1.0-2.0 \text{ mg/ml} \). Protein concentration was determined by the Lowry method with bovine serum albumen as standard (Lowry et al., 1951).

\( (^{3}H) \)-WB-4101 Binding Assay

The binding assay mixture consisted of 25 \( \mu \)l of \( (^{3}H) \)-WB-4101, 100 \( \mu \)l of mesenteric artery particulate fraction (0.10–0.20 mg of protein), and 25 \( \mu \)l of distilled water or various agonists and antagonists dissolved in distilled water. Under standard assay conditions, particulate fraction protein was incubated with 0.5–1.0 \( \text{nm (}^{3}H) \)-WB-4101 for 20 minutes at \( 22\degree C \). After incubation, the assay mixture was diluted with 4.5 ml of assay buffer (22\degree C), immediately filtered through a Whatman GF/C glass fiber filter, and washed with two additional 4.5-ml portions of assay buffer (22\degree C). The temperature and volume of the wash buffer were chosen to yield the highest specific binding. The filtration system consisted of a single 25-mm filter support (Department of Physiology Shop, Duke University, Durham, NC) mounted on a 1.0-liter filtration flask in which the vacuum pressure was regulated by a variable...
flow meter. Specific binding for (3H)-WB-4101 is defined as the percentage of total bound counts displacable by 1 mM (-)-epinephrine. In a standard saturation experiment, from 700 to 11,000 counts/min of (3H)-WB-4101 were added to the reaction mixture. At a ligand concentration of 0.8 nm (approximately the Kd) total counts/min retained by the filter after washing were approximately 475, and 150 in the presence of 10-3 M 1-epinephrine, for a specific binding of approximately 75% (filter blank = 50 counts/min). Data in all figures and tables refer to specific binding.

In Vivo Drug Treatments

To obtain a chronic hyperadrenergic state, a slow release aqueous 1:200 suspension of epinephrine in glycerol (Sus-Phrine) was administered by subcutaneous injection (0.00075 mg/kg) every 12 hours for up to a total of 48 hours. Within one hour after epinephrine administration, the rats became diaphoretic with matting of their fur, and tachypneic with an increase in respiratory rate from 100 to 130 respirations per minute. These effects had largely disappeared by 12 hours. Rats were killed 12 hours after the last injection unless otherwise specified.

Reserpine (0.25 mg/kg) was administered by intramuscular injection each morning for 7 days, and animals were killed 24 hours after the last injection unless otherwise specified. 6-Hydroxydopamine (80 mg/kg) was dissolved in a sterile solution of isotonic saline and 0.1% ascorbic acid immediately prior to intravenous injection via the tail vein. Animals were killed 24 to 48 hours after injection of the drug.

Calculations

Saturation curves for (3H)-WB-4101 were analyzed by the method of Scatchard (1949) to determine the equilibrium dissociation constant (Kd) and the amount of radioligand bound. The Kd values for nonradioactive agonists and antagonists were calculated by the method of Cheng and Prusoff (1973) using the formula Kd = IC50/[1 + (concentration ligand/Kd ligand)]; where IC50 = concentration of drug which inhibits radioligand binding by 50%, and ligand = (3H)-WB-4101. To calculate the IC50 for each agent, a composite dose-response curve was generated from the indicated number of experiments in which each individual point is the mean of duplicate or triplicate determinations. This composite sigmoid curve then was made linear by plotting the data on log-logit paper and the IC50 determined directly. Data were analyzed by comparing the means of the indicated number of experiments by Student’s unpaired t-test. All data are expressed as the mean ± standard deviation.

Results

Saturability and Kinetics of (3H)-WB-4101 Binding

Specific binding of (3H)-WB-4101 to rat mesenteric artery particulate fraction was saturable (Fig. 1). Scatchard analysis of these data (Fig. 1, inset) demonstrated that binding was to a single class of sites with a dissociation constant of 0.76 ± 0.15 nM and a calculated total binding capacity of 99 ± 13 fmol/mg of protein. Binding was rapid, reaching equilibrium in 4 minutes, and remaining stable for 20 minutes (not shown). On addition of 10-6 M (-)-epinephrine, dissociation was also rapid, reaching completion by 5 minutes.

Specificity of Binding

Catecholamines displaced (3H)-WB-4101 with the α-adrenergic potency order of (−)-epinephrine (Kd = 3.0 μM) > (−)-norepinephrine (Kd = 8.8 μM) > (−)-isoproterenol (Kd = 57 μM) (Fig. 2; Table 1); and (−)-stereoisomers of epinephrine and norepinephrine each were several times more potent than their (+) analogs in competing for (3H)-WB-4101 binding sites (Fig. 2). Dopamine (Kd = 60 μM) and serotonin (Kd = 120 μM) were much less potent than (−)-epinephrine in competing for binding. The α-adrenergic antagonist, phentolamine (Kd = 7.6 nM), was 1000 times more potent in displacing (3H)-WB-4101 than was the β-adrenergic antagonist, (+)-propranolol (Kd = 7400 nM). Unlabeled WB-4101 displaced (3H)-WB-4101 with a calculated Kd of 0.45 nM. The α-1 selective antagonist prazosin (Kd = 1.6 nM) was 2800 times more potent in competing for (3H)-WB-4101 binding sites than was the α-2 selective antagonist yohimbine (Kd = 4500 nM) (Fig. 3; Table 2). Catechol and vanillylmandelic acid did not displace (3H)-WB-4101 at concentrations of 1 mM.

![Saturation curve demonstrating specific binding of (3H)-WB-4101, an α-1 selective antagonist, to a particulate fraction of rat mesenteric artery. Data presented are the means ± standard deviation of five experiments, each performed in duplicate. Inset: Scatchard analysis of the saturation curve describes a straight line by the method of least squares (r = 0.97). The equilibrium dissociation constant (Kd), determined as the negative reciprocal of the slope, is 0.76 ± 0.15 nM. The maximal binding capacity, determined by the x-intercept, is 99 ± 13 fmol of (3H)-WB-4101 per mg of particulate fraction protein.](image-url)
FIGURE 2  Alpha-adrenergic agonists compete for the $^{3}$(H)-WB-4101 binding site in the order of the α-adrenergic potency series. Each point represents the mean of duplicate determinations in two experiments.

Influence of Chronic Epinephrine Administration on $(^3$H)-WB-4101 Binding

Saturation binding experiments performed with mesenteric artery preparations from rats treated with epinephrine for 48 hours showed a 46% decrease in total $(^3$H)-WB-4101 binding (control = 99 ± 13, epinephrine treated = 53 ± 10 fmol/mg protein; $P < 0.01$, $n = 4$), whereas the affinity of $(^3$H)-WB-4101 binding was unchanged (control, $K_d$ = 0.76 ± 0.15 nM; epinephrine treated, $K_d$ = 0.57 ± 0.13 nM; $P = NS$, $n = 4$) (Fig. 4). The apparent $K_d$ of $(−)$-epinephrine as determined by competition binding curves with $(^3$H)-WB-4101 (not shown) was unchanged in two experiments, suggesting that the affinity of the binding site for agonists is also unchanged.

TABLE 1  Equilibrium Dissociation Constants ($K_d$)* for Adrenergic Agonists in Rat Mesenteric Artery Particulate Fraction

<table>
<thead>
<tr>
<th>Agonists and partial agonists</th>
<th>$K_d$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$(−)$-Epinephrine</td>
<td>3.0</td>
</tr>
<tr>
<td>$(−)$-Norepinephrine</td>
<td>8.8</td>
</tr>
<tr>
<td>$(+)$.Epinephrine</td>
<td>20</td>
</tr>
<tr>
<td>$(−)$-Isoproterenol</td>
<td>57</td>
</tr>
<tr>
<td>$(+)$.Norepinephrine</td>
<td>180</td>
</tr>
<tr>
<td>Clonidine</td>
<td>0.48</td>
</tr>
<tr>
<td>Dopamine</td>
<td>60</td>
</tr>
</tbody>
</table>

* Dissociation constants were calculated by the method of Cheng and Prusoff (1973). Each value is the mean of duplicate determinations in two or three experiments.

The decrease in $(^3$H)-WB-4101 binding with epinephrine administration was time dependent (Fig. 5). Saturation experiments performed 4 and 8 hours after the administration of $(−)$-epinephrine revealed no change in the total $(^3$H)-WB-4101 binding, whereas binding was decreased to 77 fmol/mg of protein after the administration of epinephrine for 12 hours and was decreased to 53 ± 10 fmol/mg protein after 48 hours of intermittent epinephrine administration (every 12 hours).

Influence of Reserpine Treatment on $(^3$H)-WB-4101 Binding

As shown in Figure 6, pooled data from saturation binding experiments performed on mesenteric artery preparations from rats treated with reserpine revealed no change in total $(^3$H)-WB-4101 binding (control = 99 ± 13, reserpine treated = 99 ± 13 fmol/mg protein; $P = NS$, $n = 4$) (Fig. 6). The apparent $K_d$ of $(−)$-epinephrine as determined by competition binding curves with $(^3$H)-WB-4101 (not shown) was unchanged in two experiments, suggesting that the affinity of the binding site for agonists is also unchanged.

TABLE 2  Equilibrium Dissociation Constants ($K_d$)* for Adrenergic Antagonists and Other Compounds in Rat Mesenteric Artery Particulate Fraction

<table>
<thead>
<tr>
<th>Antagonists</th>
<th>$K_d$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB-4101</td>
<td>0.45</td>
</tr>
<tr>
<td>Prazosin</td>
<td>1.6</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>7.6</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>4,500</td>
</tr>
<tr>
<td>$(±)$-Propranolol</td>
<td>7,400</td>
</tr>
<tr>
<td>Other compounds</td>
<td></td>
</tr>
<tr>
<td>Serotonin</td>
<td>120,000</td>
</tr>
<tr>
<td>Catechol</td>
<td>NH†</td>
</tr>
<tr>
<td>Vanillylmandelic acid</td>
<td>NH†</td>
</tr>
</tbody>
</table>

* Dissociation constants were calculated by the method of Cheng and Prusoff (1973). Each value is the mean of duplicate determinations in two or three experiments.
† Not inhibited at 1 mM.
artery preparations from rats treated with reserpine for 3 to 7 days demonstrated a significant increase in the affinity of \(^{(3)}\)H-WB-4101 binding (control, \(K_d = 0.76 \pm 0.15 \text{ nM}\); reserpine treatment, \(K_d = 0.34 \pm 0.07 \text{ nM}\); \(P < 0.01, n = 6\)). However, there was no change in total \(^{(3)}\)H-WB-4101 binding (control = 99 \pm 13 \text{ fmol/mg of protein}; treatment = 96 \pm 15 \text{ fmol/mg}; \(P = \text{NS}, n = 6\)).

To determine if a change in agonist affinity also occurred with reserpine treatment, \((-\text{-epinephrine}} displacement curves were performed after 1 hour, 24 hours, and 3 to 7 days of reserpine pretreatment (Fig. 7). The calculated affinity of \((-\text{-epinephrine}} binding was increased approximately 2-fold at 1 hour \((K_d = 1.6 \text{ \text{\(\mu\text{M}}\)), 5-fold at 24 hours \((K_d = 0.57 \text{ \text{\(\mu\text{M}}\)), and 8-fold at 3 days \((K_d = 0.40 \text{ \text{\(\mu\text{M}}\)), but showed no further increase after 5 to 7 days of treatment. The change in affinity for \((-\text{-epinephrine}} after 3 to 7 days of reserpine pretreatment was highly significant (control, \(K_d = 3.0 \pm 0.4 \text{ \text{\(\mu\text{M}}\)); reserpine,
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Catecholamines compete for binding stereospecifically, and with the $\alpha$-adrenergic potency series of binding is rapid, readily reversible, and saturable. Postsynaptic $\alpha$-Adrenergic Receptor using Characterization of the Vascular

FIGURE 8 Competition for the ($^3$H)-WB-4101 binding site by (−)-epinephrine in mesenteric artery particulate fraction from control rats () and rats pretreated with 6-hydroxydopamine (●). Results of reserpine treatment are presented for comparison.

$K_d = 0.40 \pm 0.19 \mu M; P < 0.01, n = 6$, and was larger (8-fold) than the increase in affinity for the antagonist ($^3$H)-WB-4101, as determined by the saturation experiments (2-fold).

Influence of 6-Hydroxydopamine Treatment on ($^3$H)-WB-4101 Binding

In mesenteric artery particulate fraction from rats treated for 24 and 48 hours with 6-hydroxydopamine, (−)-epinephrine displacement curves (Fig. 8) demonstrated an increase in (−)-epinephrine affinity which was approximately 88% of the increase produced by reserpinization for 3 to 7 days (control, $K_d = 3.0 \pm 0.40 \mu M$; 6-hydroxydopamine $K_d = 0.88 \mu M; n = 2$).

Discussion

Characterization of the Vascular Postsynaptic $\alpha$-Adrenergic Receptor using ($^3$H)-WB-4101

($^3$H)-WB-4101 is a potent $\alpha$-adrenergic antagonist which previously has been used to identify $\alpha$-adrenergic receptor binding sites in rat brain (U’Prichard and Snyder, 1978), liver (Wood et al., 1979a), and heart (Raisman et al., 1979). Specific binding sites for ($^3$H)-WB-4101 in our particulate fraction of rat mesenteric artery also demonstrate the characteristics of $\alpha$-adrenergic receptors. Thus, binding is rapid, readily reversible, and saturable. Catecholamines compete for binding stereospecifically, and with the $\alpha$-adrenergic potency series of (−)-epinephrine > (−)-norepinephrine > (−)-iso-

prototerol. The $\alpha$-adrenergic antagonists, phentolamine and prazosin, are much more potent than the $\beta$-adrenergic antagonist, (±)-propranolol, in competing for the ($^3$H)-WB-4101 binding site.

There is now considerable physiological (Langer, 1974; Berthelsen and Pettinger, 1977), pharmacological (Wikberg, 1978), and direct radioligand binding (Hoffman et al., 1979) evidence that there are at least two $\alpha$-adrenergic receptor subtypes, termed $\alpha$-1 and $\alpha$-2. Alpha-1 receptors are located postsynaptically, whereas $\alpha$-2 receptors are located on presynaptic neurons where they exert a negative feedback influence on norepinephrine release (Langer, 1974). Alpha-2 receptors also have been identified in rat platelets, uterus, brain, submandibular and parotid glands, thus leading to the suggestion that the $\alpha$-adrenergic receptors in a given tissue may be either $\alpha$-1, $\alpha$-2, or a mixture of the two (Bethelsen and Pettinger, 1977; Hoffman et al., 1979). We previously have used the ligand ($^3$H)-dihydropyroergocryptine to show that the predominant subtype present in the rat mesenteric artery particulate fraction is $\alpha$-1 (Colucci et al., 1980).

The radioligand ($^3$H)-WB-4101 has been suggested as a probe for the selective identification of $\alpha$-1 adrenergic receptors (U’Prichard and Snyder, 1979; Wood et al., 1979b). That ($^3$H)-WB-4101 identifies $\alpha$-1 adrenergic receptors appears to be confirmed by the observation in this study that the $\alpha$-1 selective (Cambridge et al., 1977) antagonist prazosin is 2800 time more potent in competing for ($^3$H)-WB-4101 binding sites than is the $\alpha$-2 selective (Starke et al., 1975) antagonist yohimbine. Since $\alpha$-1 adrenergic receptors appear to be present only postsynaptically, it is likely that the binding site identified by ($^3$H)-WB-4101 is located on the vascular smooth muscle cell, and not on presynaptic neurons which may potentially contaminate this preparation. The observation that the maximum binding of the $\alpha$-2 selective (Wood et al., 1979b) radioligand ($^3$H)-clonidine to rat mesenteric artery particulate fraction is only about 8 fmol/mg of protein (unpublished observation), as compared to 99 fmol/mg protein for ($^3$H)-WB-4101 (Fig. 1), further supports the suggestion that the predominant $\alpha$-adrenergic receptor subtype present in this preparation is $\alpha$-1.

The highly catecholamine-reactive muscular arteries of the rat mesentery were chosen for this study because they are representative of a class of vessels that is important in the regulation of systemic vascular tone. Such vessels are distinguished by specific anatomical and functional traits (Bevan, 1971) and possess smaller synaptic clefts (Bevan, 1979), factors which may influence the concentra-
tion of endogenous norepinephrine to which the postsynaptic α-adrenergic receptor is exposed.

Decrease in Vascular α-Adrenergic Receptor Number after Chronic in Vivo Epinephrine Treatment

In the current study, the treatment of rats with (−)-epinephrine resulted in a significant decrease in total \(^3\)H)-WB-4101 binding to the mesenteric arterial parietal fraction, with no change in affinity. This decrease in maximum binding capacity was time dependent, being first detectable after 12 hours of exposure, and then falling to 54% of control values after 48 hours (Fig. 6).

Studies in nonvascular tissues have demonstrated a correlation between the reduced number of α-adrenergic receptors caused by exposure to a high in vitro concentration of (−)-epinephrine and a desensitization of the effector cell response to α-adrenergic stimulation. In human platelets, in vitro exposure to (−)-epinephrine \((10^{-4} \text{ M})\) resulted in a decrease in the number of α-adrenergic receptors and a densitization of α-adrenergic induced aggregation (Cooper et al., 1978), whereas in dispersed parotid acinar cells the decrease in α-adrenergic receptors was correlated with a decrease in α-adrenergic stimulated potassium secretion (Strittmatter et al., 1977). Thus, the observed decrease in postsynaptic α-adrenergic binding sites following in vivo exposure to exogenous epinephrine may contribute to the desensitization of vascular smooth muscle to α-adrenergic agonists in various physiological, pharmacological, and disease states, such as pheochromocytoma.

Proposed mechanisms by which agonist-induced desensitization may occur include: receptor occupation and inactivation, a change in receptor conformation, internalization of receptors, and a change in the turnover of receptors such that there is a net reduction in number (i.e., increased degradation, decreased synthesis, or both) (Catt et al., 1979). In the current experiments, it is unlikely that the reduced \(^3\)H)-WB-4101 binding observed was due to occupation of receptors by the administered epinephrine, since there was no change in the binding capacity 4 and 8 hours after treatment, whereas a decrease was noted 12 hours after drug administration, at which time the ambient level of epinephrine would have returned toward normal. Although the relatively slow time course observed would tend to favor a change in receptor turnover, clarification of the mechanism(s) responsible for the decrease in α-adrenergic receptors will require further investigation.

Increase in Vascular α-Adrenergic Receptor Affinity following Chemical Sympathectomy

Reserpine administration is a classic method of producing postjunctional supersensitivity (Fleming, 1976). Using rat aorta, Burns and Rand (1959) demonstrated that both depletion of norepinephrine and supersensitivity developed after treatment with 0.5 mg/kg of reserpine for 2 days. Resolution of supersensitivity coincided with the reaccumulation of vascular norepinephrine as evidenced by a return of the tyramine response. Thus, they concluded that the normal tonic release of norepinephrine from nerve endings maintains the effector cell at a low sensitivity, whereas a loss of tonic exposure due to reserpine-induced norepinephrine depletion causes a state of increased sensitivity. Frequently postulated mechanisms of postjunctional supersensitivity to catecholamines have involved alterations in the characteristics of the effector cell α-adrenergic receptor (MacMillan et al., 1962; Carrier and Holland, 1965).

In the current study, the affinities of \(^3\)H)-WB-4101 and the agonist (−)-epinephrine for postsynaptic α-adrenergic receptors were increased 2- and 8-fold, respectively, after treatment with reserpine. The increase in agonist affinity was time dependent; increasing as soon as one hour after reserpine administration, and reaching a plateau after 3 days of treatment (Fig. 7). Hudgins and Fleming (1966) used a similar protocol for reserpine treatment and noted an approximately 12-fold increase in sensitivity to the agonist norepinephrine as assessed by muscle contraction, maximal change occurring between 7 and 14 days of treatment. Thus, the time course and magnitude of change in α-adrenergic receptor affinity for α-adrenergic agonists as demonstrated by \(^3\)H)-WB-4101 binding is in reasonable agreement with that seen with muscle bath techniques, supporting the suggestion that an increase in α-adrenergic receptor affinity may be involved in the phenomenon of postjunctional supersensitivity to catecholamines as observed in vascular smooth muscle.

Similarly, the affinity of (−)-epinephrine for \(^3\)H)-WB-4101 binding sites was increased by 6-hydroxydopamine, an agent which also depletes tissues norepinephrine, but is markedly different from reserpine in terms of structure, mechanism of action, and direct cellular effects. Thus, the similar effect on affinity of 6-hydroxydopamine and reserpine suggests that they are acting, at least partially, through a common mechanism, the depletion of tissue norepinephrine.

Unlike the α-adrenergic specific desensitization produced by norepinephrine, the vascular postjunctional supersensitivity that follows reserpine or 6-hydroxydopamine is not specific for norepinephrine, but is also seen for acetylcholine and potassium, although not for serotonin, histamine, or angiotensin II (Hudgins and Fleming, 1966; Carrier and Shibata, 1977). A number of cellular alterations have been observed in reserpine-treated tissues, and postulated to play a role in postjunctional supersensitivity. These include changes in calcium homeostasis (Carrier and Hester, 1976), intracellular ATP (Westfall et al., 1975), membrane electrical potential (Fleming et al., 1975) and sodium/potassium ATPase (Gerthoffer et al., 1979). Our results suggest...
that alterations in the postsynaptic \( \alpha \)-adrenergic receptors could contribute to \( \alpha \)-adrenergic supersensitivity in vascular smooth muscle. The relationship between the change in \( \alpha \)-adrenergic receptor affinity and the alterations in cellular function observed by others after denervation requires further investigation.

The present studies indicate that the vascular \( \alpha \)-adrenergic receptor is capable of undergoing marked alterations in number and/or affinity in response to the tonic level of agonist stimulation. Normal levels of sympathetic nerve input appear to be sufficient to maintain receptor affinity at its lowest level. That is, with respect to affinity, the receptor is “down-regulated” under normal conditions. With respect to number, however, the receptor appears to be “up-regulated” under these conditions, since removal of tonic input does not result in a further increase in receptor number, whereas an increase in agonist concentration results in a reduction in receptor number. Further studies will be required to delineate the molecular mechanisms of these alterations, and to determine the role of the observed changes in the variable sensitivity of vascular smooth muscle to catecholamines. This system should facilitate study of the role of the vascular \( \alpha \)-adrenergic receptor in the regulation of cardiovascular function.

References


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