Beta1 and Beta2 Adrenergic Binding Sites in Bovine Retina and Retinal Blood Vessels

Gabryleda Ferrari-Dileo

To supplement previous demonstration of angiotensin II and alpha-adrenergic binding sites, beta-adrenergic binding sites for dihydroalprenolol were demonstrated in vessel-free bovine neural retina (Kd = 2.5 nM) and bovine retinal vessels (Kd = 2.8 nM). By means of competition of 3H-dihydroalprenolol with betaxolol and ICI 118,551, approximately equal proportions of Beta1 and Beta2 subtypes were found in both the retinal vessels and the neural retina. Further studies are needed to determine the microanatomic location of the binding sites and the physiologic responses, if any, they mediate.

Adrenergic agents initiate their physiological responses by acting on specific receptor proteins located on the plasma membrane of target cells. Blood vessels from the peripheral and central nervous systems possess specific receptors (alpha-adrenergic and beta-adrenergic), through which both epinephrine and norepinephrine are able to maintain or change the tone of a given vascular bed.

We have been interested in the possible effect of vasoactive substances on the vasculature of the optic nerve head and the retina. We have already determined that vessels from the bovine retina, although lacking adrenergic innervation, have alpha1 and alpha2 adrenergic binding sites,1 which suggests that catecholamines present in the bloodstream may affect the tone of these vessels. We wanted to determine if beta-adrenergic binding sites are also present in the retinal vasculature in order to have a more complete picture of the possible adrenergic mechanisms present in these vessels. To achieve this, we used 3H-dihydroalprenolol (3H-DHA) as a nonselective beta-agent to determine the total population of beta-adrenergic sites, and betaxolol and ICI 118,551 as \( \beta_1 \) and \( \beta_2 \)-selective antagonists, respectively2 to define the proportions of both binding sites in the bovine retina and retinal vessels.

Materials and Methods

Tissue Preparation

Retinal vessels were isolated from bovine retinas as previously described.1 In brief, the whole retinas were homogenized by a hand-driven Teflon-glass homogenizer, and the first separation of vessels from retinas was performed by filtration through a 200 μm nylon sieve. Both the vessels trapped on the filter and the filtrate retina were rehomogenized and sieved again. After an almost pure “vascular fraction” and “retinal fraction” were obtained, further purification was achieved by centrifugation steps at low and high speed. The “vesSEL enriched fraction” was a mesh of tangled vessels with very little retinal debris as confirmed by scanning electron microscopy.1 The “retinal fraction” did not contain vascular tissue fragments as observed by light microscopy (not shown).

For the determination of beta-adrenergic sites, both tissue suspensions obtained after the low speed centrifugation were each diluted with an equal volume of 1 M KCl, homogenized with a Polytron blender for 1 min at medium speed and left on ice for 10 min in order to reduce nonspecific binding.3 Membranes from each fraction were then collected by centrifugation at 15,000 rpm for 10 min, resuspended in 50 mM Tris-HCl buffer at pH 8.0, and centrifuged again to obtain the sedimented membranes used for the binding assays.

Binding of 3H-DHA

Saturation studies: Binding conditions were basically as described before.4 Vascular and retinal fractions were diluted 1:10 in 50 mM Tris-HCl pH 8.0. One hundred microliter aliquots of the vascular and retinal fractions were incubated with 0.03–30.0 nM...
**Beta-adrenergic sites in retinal vessels and retinal neuronal tissue**

**A.**

**Vessels**

**Retina**

---

### **3H-DHA**

(Tables and graphs are shown but not transcribed.)

---

**B.**

**Scatchard plots for retinal vessels (•) and neural retina (○) of the specific binding of 3H-DHA.**

---

**Competition studies:** Analysis of the presence of β₁ (β₁) and β₂ (β₂)-adrenergic sites was determined by competitive inhibition of the binding of 5 nM 3H-DHA (a nonselective beta blocker) and increasing concentrations (10⁻²–10⁻¹⁰ M) of betaxolol, a selective β₁-adrenergic antagonist, or ICI 118,551, a selective β₂-adrenergic antagonist. The conditions for the binding were the same as those described for the saturation studies. Competition experiments were analyzed with a computer program to fit the binding curves to a two-site model, and the IC₅₀ values obtained were transformed into Kᵢ values with the Cheng and Prusoff equation.

### **Results**

Our first approach was to determine the beta-adrenergic binding sites' density in the retinal vascu-
lar and the neural retina preparations. In both tissues, the specific binding increased with the radioligand concentration up to a $^3$H-DHA concentration of 10 nM, but remained constant thereafter (Fig. 1A). At radioligand concentrations higher than 30 nM the nonspecific binding became too high to calculate specific binding accurately. After plotting the data of the specific binding as bound/free vs. bound (Fig. 1B), the dissociation constants (Kd) and maximum capacities of binding (B$_{\text{max}}$) for $^3$H-DHA were obtained for each preparation. Figure 1B shows that both preparations exhibited similar Kds for $^3$H-DHA (2.8 nM for retinal vessels and 2.5 nM in neural retina homogenates). The B$_{\text{max}}$ for retinal vessels was of 1.5 pmol/g of tissue, and 1.0 pmol/g for retinal membranes.

Competitive inhibition binding of $^3$H-DHA and two subtype-selective antagonists were performed in each tissue homogenate, and in both cases the competition curves were biphasic, each possessing a high- and a low-affinity component, reflecting the distribution of beta$_1$ and beta$_2$ adrenergic binding subtypes.

Competition curves using betaxolol, a beta$_1$-selective antagonist, are shown in Figure 2 for retinal vessels and retina. The IC$_{50}$ of betaxolol for the site to which it has high affinity (the beta$_1$ site) was 40 nM and 100 nM for retinal vessels and retina, respectively. The percent of high affinity sites for betaxolol was 42% for retinal vessels and 52% for neural retinal homogenates. The IC$_{50}$ of betaxolol for the sites to which it has a lower affinity (known to be beta$_2$ sites) were in the micromolar range for both tissues.

Competition curves were also performed with ICI 118,551, a beta$_2$ selective blocker. Figure 3 shows again biphasic curves for retinal vessels and neural retina. The IC$_{50}$ for the high affinity component (beta$_2$ sites) was 5 nM in retinal vessels and 2 the retina. The proportions of beta$_2$ sites were 45% and 54% for vessels and for neural retina, respectively. The IC$_{50}$s for the lower affinity component of the ICI 118,551 binding (beta$_1$ sites) were in the low micromolar range for both vessels and neural retina.

The dissociation constant values (Kds), obtained from the IC$_{50}$ values for betaxolol and ICI 118,551 in each tissue (Table 1), agree well with those found in coronary arteries, lung and heart membranes for the same specific beta blockers.$^7$.

**Discussion**

This study demonstrates the presence of $^3$H-DHA-specific binding sites in bovine retinal vessels and in bovine neural retina. The presence of these beta types of adrenergic sites was previously shown in cat and rat retinal membranes.$^9$,$^{10}$ However, these studies did not specify if these sites were located in the neural retina or in the vascular tissue present in the retina, and did not identify the subtypes present. So, this is the first demonstration that the retinal vessels, like peripheral and other central nervous system vascular beds, possess beta-adrenergic sites. In addition, this study shows vessels, as well as the neural retina, have both beta$_1$ and beta$_2$-adrenergic subtypes of the $^3$H-DHA binding sites.

Beta-adrenergic receptors are coupled to adenylate cyclase and their stimulation by catecholamines in-

![Fig. 2. Competition curves for $^3$H-DHA and betaxolol in neural retina (■) and retinal vessels (●). Each point represents duplicate determinations of four separate experiments. These data are consistent with the concept that both tissues have beta$_1$ and beta$_2$ binding types. (-----) model indicating one single class of binding site, in this case, the beta$_1$ binding site (the high affinity site for betaxolol). Numbers close to the curves indicate the proportions of high affinity sites vs. low affinity sites for betaxolol (%H:%L). In retinal vessels: IC$_{50}$ beta$_1$ = 40 nM; IC$_{50}$ beta$_2$ = 40 pM. In neural retina: IC$_{50}$ beta$_1$ = 100 nM; IC$_{50}$ beta$_2$ = 16 pM.]

![Fig. 3. Competition curves for $^3$H-DHA and ICI 118,551 in neural retina (■) and retinal vessels (●). Each point represents duplicate determinations of four separate experiments. The data are consistent with two types of binding sites. (-----) indicates the model for a single class of binding sites, in this case, the beta$_1$ binding site (the high affinity site for ICI 118,551, the beta$_2$ binding site. Numbers close to the curves indicate the relative proportions of the high affinity vs. the low affinity component for ICI 118,551 (%H:%L). In retinal vessels: IC$_{50}$ beta$_1$ = 13 pM; IC$_{50}$ beta$_2$ = 5 nM. In retina: IC$_{50}$ beta$_1$ = 1.6 pM; IC$_{50}$ beta$_2$ = 2 nM.]

Downloaded From: http://iovs.arvojournals.org/pdfaccess.ashx?url=data/journals/iovs/933142/ on 05/14/2018
duces an increase in the activity and an accumulation of cyclic AMP. The subdivision of beta-adrenergoreceptors into beta_1 and beta_2 subtypes was first developed in 1967, on the basis of the diverse potency of several adrenergic agonists on lipolysis, cardiac stimulation, bronchodilation and vasodilation. In addition to other cellular effects these receptors, the beta_1 and beta_2 subtypes, both promote a relaxing response in smooth muscle cells, but their affinities for catecholamines are different. The beta_1 type is equally stimulated by epinephrine or norepinephrine, whereas the beta_2 type shows greater affinity for epinephrine. Vessels from the periphery and the CNS have beta_1 and beta_2 receptors present with different predominances according to the vessels studied. We have found that similar proportions of beta_1 and beta_2 binding sites are present in our preparation of bovine retinal arterioles and microvessels. So, from the present results and our previous findings of alpha_1, alpha_2 and angiotensin II binding sites in these vessels, it seems that the vessels of the retina, and probably those of the optic nerve head, can be influenced by catecholamines and vasoactive peptides.

Our data is limited to the description of the type of binding sites present in retinal vessels. Further studies are required to demonstrate that they are receptors that mediate physiologic responses, and to determine the distribution of these sites in the layers of the vascular walls. Since retinal vessels do not have adrenergic innervation, the only physiologic source of agonist in the circulation. If the receptors are mainly in the smooth muscle cells of the arteries or pericytes in the capillaries, it might therefore be expected that the role of these receptors would be limited to those situations when there is an opening in the tight junctions of the endothelial layers. Thus, the retinal vessels may not respond to circulating agonists. The optic nerve head vessels might respond because of the window in the blood-tissue barrier here, and this may play a role in glaucomatous damage to the optic nerve head. However, it must also be kept in mind that vessels with blood-organ barrier, such as cerebral microvessels, possess beta- and alpha-adrenergic receptors and angiotensin II receptors in the endothelial cells. When in culture, the beta sites present in these cells induce an increase in adenylate cyclase activity, whereas there is an inhibition of such enzyme upon stimulation of alpha_2-adrenergic and angiotensin II sites. If something similar is present in retinal vessels, it can be speculated that vascular tone and perhaps autoregulatory responses might be influenced by circulating vasoactive substances throughout the retinal vascular system.

Finally, when inhibitory or stimulatory hormone-receptors are coupled to the same enzyme (adenylate cyclase), the effects resulting from the activation of one of them can be counteracted or inhibited by the activation of the other one. Such interaction has been shown between beta-adrenergic and vasopressin receptors in cultured smooth muscle cells and might be speculated to occur in retinal vessels where we have already shown a variety of binding sites of the kinds typically coupled to adenylate cyclase to serve as receptors.

In conclusion, the data presented clearly show the presence of beta_1 and beta_2-adrenergic binding sites in the retinal blood vessels and in the neural component of the sensory retina. However, experiments to localize these sites to inner or outer vessel wall and to demonstrate the type of physiologic response will be important in understanding the physiology and pathophysiology of the retinal and optic nerve head circulations.

Key words: retina, retinal blood vessels, beta-adrenergic binding sites, 3H-dihydroalprenolol, ICI 118,551, betaxolol

Acknowledgments

Betaxolol was kindly provided by ALCeON Laboratories, Fort Worth, Texas. ICI 118,551 was a gift from Imperial Chemical Industries PLC, MacClesfield, Cheshire, United Kingdom. I would like to thank Dr. Lincoln T. Potter for his helpful suggestions and for letting me use his computer program, and to Dr. Douglas R. Anderson for his guidance in the direction of the work and the manuscript preparation.

References

5. Dickinson K, Richardson A, and Nahorski SR: Homogeneity of beta_2-adrenoceptors on rat erythrocytes and reticulocytes: A


