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# CHARACTERIZATION OF α- AND β-ADRENERGIC RECEPTORS IN MEMBRANES PREPARED FROM THE RABBIT IRIS BEFORE AND AFTER DEVELOPMENT OF SUPERSENSITIVITY

ELLEN D. PAGE and ARTHUR H. NEUFELD*

Department of Ophthalmology and Visual Science, Yale University School of Medicine, New Haven, CN 06510, U.S.A.

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**Abstract**—α-Adrenergic and β-adrenergic receptors were studied by measuring the binding of [$^3$H]dihydroergocryptine and [$^3$H]dihydroalprenolol, respectively, to membranes prepared from homogenized rabbit irides. The binding of [$^3$H]dihydroergocryptine appears to be specific for α-adrenergic receptors as adrenergic agents displace this radioligand with the following order of potency: phentolamine > epinephrine ⩾ norepinephrine ⩾ isoproterenol = propranolol. The binding of [$^3$H]dihydroalprenolol appears to be specific for β-adrenergic receptors as adrenergic agents displace this radioligand with the following order of potency: propranolol ⩾ isoproterenol ⩾ epinephrine > norepinephrine ⩾ phentolamine. Several weeks after removal of the superior cervical ganglion, when all the adrenergic nerves to the tissue have degenerated, membranes prepared from denervated irides have an increased density of β-adrenergic receptors with no increase in the density of α-adrenergic receptors. A small decrease in the total number of α-adrenergic receptors probably occurs, which is due to the loss of pre-junctional receptors. The affinities of the receptors do not change. These findings suggest that unlike skeletal muscle, the supersensitivity that occurs in smooth muscle is not due to an increase in the population of receptors governing contraction. However, the change in population of β-adrenergic receptors is consistent with the hypothesis that, as in other tissues, the level of cyclic AMP modulates the density of the β-adrenergic receptor.

One of the classical examples of cell–cell interaction is the trophic influence that a nerve has on the muscle that it innervates. Our best understanding of this phenomenon is for skeletal muscle, where denervation leads to a large increase in the number of cholinergic receptor sites on the muscle membrane [1, 2]. Thus, the muscle becomes supersensitive to exogenously administered neurotransmitter and numerous investigations have confirmed this hypothesis [3, 4].

Supersensitivity also develops in smooth muscle upon denervation. For example, denervation of the nictitating membrane of the cat, leads to development of supersensitivity that has both pre-junctional and post-junctional components [5]. At an adrenergic neuromuscular junction, the primary mechanism for terminating the influence of the released norepinephrine is uptake of the neurotransmitter into the nerve terminal [6]. Thus, the pre-junctional component of supersensitivity is due to the actual loss of neuronal uptake and, therefore, an increase in the effective concentration of administered catecholamine in the biophase at the receptor [5]. This type of supersensitivity develops quickly, corresponding with the degeneration of the nerves [7].

In addition, there is a post-junctional component of supersensitivity that develops slowly after denervation and also after decentralization, which interrupts the neural input to the tissue but maintains the presence of the nerve endings [8]. Although a complete explanation of post-junctional supersensitivity at an adrenergic neuromuscular junction is not available, physiological and pharmacological evidence suggests that the post-junctional changes are non-specific and due to changes in excitability of the smooth muscle membrane rather than changes in the adrenergic receptor population [9]. Nevertheless, there have been no biochemical proofs. There is quantitative evidence, however, that increases in sensitivity at non-muscular adrenergic junctions are associated with increases in the density of the population of β-adrenergic receptors [10].

We have, therefore, studied both the α- and β-adrenergic receptor populations in membranes prepared from the same smooth muscle tissue, the heavily adrenergically innervated iris of the rabbit, before and after the development of supersensitivity. We have used *in vitro* methods recently developed by Lefkowitz and co-workers [11, 12] for characterizing the affinity and density of adrenergic receptors prepared from homogenized tissue. These techniques are demonstrated capable of biochemical quantification of both α- and β-adrenergic receptor populations.

## MATERIALS AND METHODS

*Preparation of membranes.* Unilateral extirpation of the superior cervical ganglion was performed on 2–3 kg, male, albino New Zealand rabbits, anesthetized intravenously (i.v.) with sodium pentobar-

* Present address. Eye Research Institute of Retina Foundation, 20 Staniford Street, Boston, MA 02114 U.S.A.

bital. Three to 4 weeks later, when all the adrenergic nerves had degenerated [13], the animals were sacrificed, eyes were enucleated, cut into anterior and posterior poles, and irides dissected free of sclera and vitreous. Irides of different animals, in groups of 5–6, from the ipsilateral side of the surgery were pooled for the denervated preparation and irides from the contralateral side were pooled for the control preparation.

Similar procedures were followed for decentralization of the superior cervical ganglion. Although isolated surgically from the CNS and other parts of the ANS, the neural connections between the ganglion and the eye were left intact. Animals were used 3–4 weeks after surgery.

Following described procedures [11], iridial tissue was homogenized in 20 ml of cold 0.25 M sucrose, 1 mM $MgCl_2$, 5 mM Tris–HCl, pH 7.4 in motor-driven Teflon–glass homogenizers and, after filtration through cheesecloth, centrifuged at 310 *g* for 10 min at 4°. The supernate obtained was centrifuged at 28,000 *g* for 10 min at 4° and washed twice, with re-homogenization in 5 ml of cold 10 mM $MgCl_2$, 50 mM Tris–HCl, pH 7.55. The final pellet was suspended in 2 ml and stored frozen overnight, or for several days, with no apparent loss of activity. Immediately prior to use, this suspension was re-homogenized and a sample was taken for protein determination by the method of Lowry *et al.* [14], with bovine serum albumin as standard. This membrane preparation was assayed for both α- and β-adrenergic receptors. Irides from normal, unoperated rabbits, as well as from frozen eyes obtained from Pel-Freez Biologicals, Inc. were processed in the same manner. No differences were noted.

*Assay for α-adrenergic receptors.* α-Adrenergic receptors were assayed by determining the amount of binding to membranes, prepared as described above, of [$^3$H]dihydroergocryptine, at $5 \times 10^{-5}$ M, that can be displaced by phentolamines at $5 \times 10^{-5}$ M [11]. 50 micrograms of membrane protein were incubated for 15 min at 25° in 150 μl of the following incubation medium: 10 mM $MgCl_2$, 50 mM Tris–HCl, pH 7.55, 50 nM [$^3$H]dihydroergocryptine. When agents were present to displace the [$^3$H]dihydroergocryptine from the binding site, e.g. phentolamine, epinephrine, etc. they were added to the 150-μl volume to give the final molar concentration indicated. Incubation was terminated by the addition of 1.5 ml of cold incubation medium and filtration through Whatman GF/C filters. Filters were washed twice with 10 ml, dried for 1 hr at 65° and prepared for scintillation counting by solubilization in 0.5 ml Protosol$^R$ followed by 10 ml Econofluor$^{TM}$. Nonspecific binding, or that amount of [$^3$H]dihydroergocryptine not displaced by phentolamine, was 10–50 per cent of the total binding, dependent upon the concentration of [$^3$H]dihydroergocryptine used, and had a much lower affinity than specific binding.

*Assay for β-adrenergic receptors.* β-Adrenergic receptors were assayed by determining the amount of binding to membranes, prepared as described above, of [$^3$H]dihydroalprenolol, at $10^{-8}$ M, that can be displaced by propranolol, at $5 \times 10^{-5}$ M [12]. The procedures used for the assay of the β-adrenergic receptor were identical to that for the α-adrenergic receptor except that the 15-min incubation was at 37°. Membranes were collected and washed on Whatman GF/A filters and prepared for scintillation counting as above. Non-specific binding, or that amount of [$^3$H]dihydroalprenolol not displaced by propranolol, was usually 10–15 per cent of the total binding.

*Materials.* [$^3$H]dihydroergocryptine (24.1 Ci/m-mole) and [$^3$H]dihydroalprenolol (32.6 Ci/m-mole) were from New England Nuclear. *l*-Epinephrine, *d*-bitartrate, *l*-artereno, *d*-bitartrate, *l*-isoproterenol, *d*-bitartrate, dopamine, serotonin and *d,l*-propranolol were from Sigma. The following compounds were generously supplied: phentolamine (Ciba–Geigy), phenoxybenzamine (Smith, Kline and French), clorgyline (May and Baker), U-0521 (Upjohn), cocaine (Merck).

## RESULTS

*General observations on binding of radioligands.* The same preparation of membranes can be used to study both the α-adrenergic receptor with [$^3$H]dihydroergocryptine and the β-adrenergic receptor with [$^3$H]dihydroalprenolol. Specific binding of these radioligands was unaltered by $5 \times 10^{-5}$ M of the following compounds: cocaine, an inhibitor of neuronal uptake [6], clorgyline, an inhibitor of monoamine oxidase [15], and U-0521, an inhibitor of catechol-*O*-methyl transferase [16]. The latter two compounds were routinely added to the incubation solution.

*α-Adrenergic receptor.* As determined by Scatchard analysis, the $K_D$ for [$^3$H]dihydroergocryptine

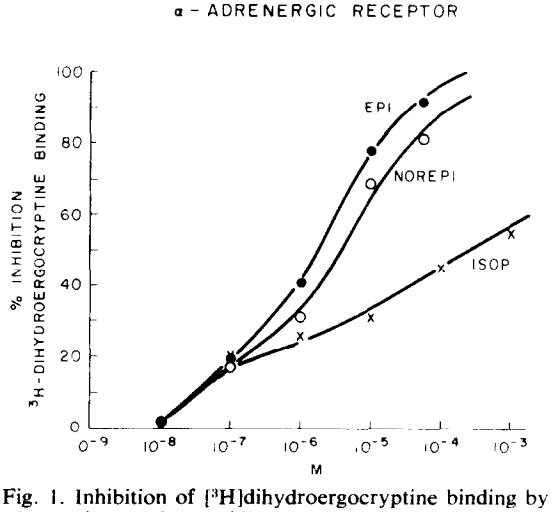


Fig. 1. Inhibition of [$^3$H]dihydroergocryptine binding by adrenergic agonists. Iridial membranes prepared from rabbit eyes obtained from Pel-Freez Biologicals, Inc. were incubated with [$^3$H]dihydroergocryptine in the absence and presence of the indicated agonists. The ability of each agonist to displace the radioligand was compared to 100 per cent inhibition of binding by $5 \times 10^{-5}$ M phentolamine, which corresponds to maximal specific binding of 0.53 pmoles [$^3$H]dihydroergocryptine/mg protein. Each value is the mean of duplicate determinations of at least two separate experiments. EPI: epinephrine; NOREPI: norepinephrine; ISOP: isoproterenol.

Table 1. Inhibition of [$^3$H]-ligand binding to membranes prepared from rabbit irides

| | $K_D$* | |
|---|---|---|
| | α-Receptor μM | β-Receptor μM |
| *l*-Norepinephrine | 1.2 | 4.0 |
| *l*-Epinephrine | 0.6 | 0.7 |
| *l*-Isoproterenol | 100.0 | 0.3 |
| Phentolamine | 0.06 | > 100.0 |
| Phenoxybenzamine | 1.8 | — |
| *d,l*-Propranolol | 100.0 | 0.004 |
| Dopamine | 3.3 | > 100.0 |

* The dissociation constant, $K_D$, was calculated from the following equation:

$$K_D = EC_{50}(1+[^3H]\text{-LG}/[^3H]\text{-LGK}$$

where [$^3$H]-LG is the concentration of the radioligand in the assay, K[$^3$H]-LG is the dissociation constant for the radioligand computed by Scatchard analysis, and $EC_{50}$ is the concentration of the displacing compound which inhibits 50 per cent of the binding of the radioligand [11].

binding to sites on membranes prepared from homogenized rabbit irides is 22 nM. Figure 1 demonstrates the specificity for adrenergic agonists of these sites. Displacement of [$^3$H]dihydroergocryptine by each agonist was compared to maximal displacement, or 100 per inhibition, of the specific binding of [$^3$H]dihydroergocryptine by $5 \times 10^{-5}$ M phentolamine. The order of potency for displacing the radioligand is epinephrine ≥ norepinephrine ≫ isoproterenol. This order of potency is identical to that reported for rabbit uteri and agrees with the known α-adrenergic activity of these compounds [11]. The $K_D$ values are given in Table 1 and are

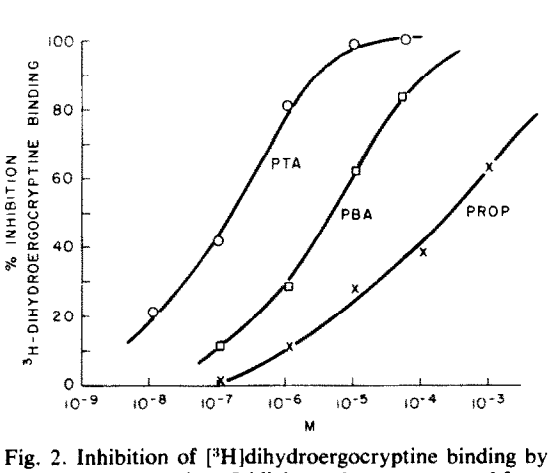


Fig. 2. Inhibition of [$^3$H]dihydroergocryptine binding by adrenergic antagonists. Iridial membranes prepared from rabbit eyes obtained from Pel-Freez Biologicals, Inc. were incubated with [$^3$H]dihydroergocryptine in the absence and presence of the indicated agonists. The ability of each agonist to displace the radioligand was compared to 100 per cent inhibition of binding by $5 \times 10^{-5}$ M phentolamine, which corresponds to maximal specific binding of 0.53 pmoles [$^3$H]dihydroergocryptine/mg protein. Each value is the mean of duplicate determinations of at least two separate experiments. PTA: phentolamine; PBA: phenoxybenzamine; PROP: propranolol.

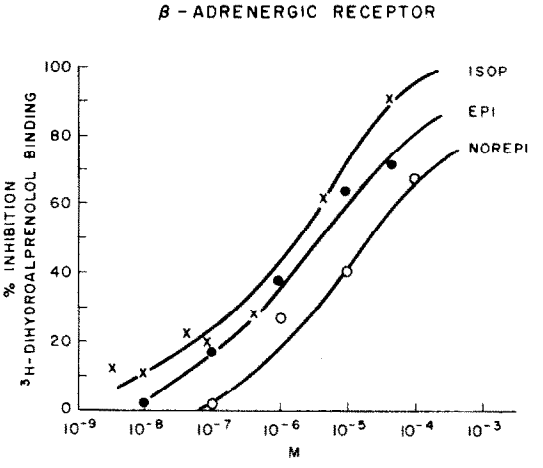


Fig. 3. Inhibition of [$^3$H]dihydroalprenolol binding by adrenergic agonists. Iridial membranes prepared from rabbit eyes obtained from Pel-Freez Biologicals, Inc. were incubated with [$^3$H]dihydroalprenolol in the absence and presence of the indicated agonists. The ability of each agonist to displace the radioligand was compared to 100 per cent inhibition of binding by $5 \times 10^{-5}$ M propranolol, which corresponds to maximal specific binding of 0.33 pmoles [$^3$H]dihydroalprenolol/mg protein. Each value is the mean of duplicate determinations of at least two separate experiments. ISOP: isoproterenol; EPI: epinephrine; NOREPI: norepinephrine.

approximately the same as that reported for membranes prepared from rabbit uteri [11]. Dopamine was active at the α-adrenergic receptor, whereas, serotonin had little activity ($K_D > 10^{-3}$ M).

Figure 2 demonstrates the specificity for adrenergic antagonists of the binding of [$^3$H]dihydroergocryptine to rabbit iridial membranes. The α-adrenergic specificity can clearly be seen when the ability of the competitive α-adrenergic antagonist, phentolamine, to displace [$^3$H]dihydroergocryptine is compared with that of propranolol. Phenoxybenzamine, a non-competitive α-adrenergic antagonist has intermediate activity for displacing [$^3$H]dihydroergocryptine. The $K_D$ values for these compounds, which are given in Table 1, are similar to those reported for membranes prepared from rabbit uteri, in which phentolamine and phenoxybenzamine are equipotent [11].

*β-Adrenergic receptor.* As determined by Scatchard analysis, the $K_D$ for [$^3$H]dihydroalprenolol binding to sites on membranes prepared from homogenized rabbit irides is 2.4 nM. Figure 3 demonstrates the specificity for adrenergic agonists of these sites. Displacement of [$^3$H]dihydroalprenolol by each agonist was compared to maximal displacement, or 100 per cent inhibition, of the specific binding of [$^3$H]dihydroalprenolol by $5 \times 10^{-5}$ M propranolol. The order of potency for displacing the radioligand is isoproterenol > epinephrine > norepinephrine. This order of potency is identical to that reported for frog erythrocytes and rat pineal gland and agrees with the known β-adrenergic activity of these compounds, such as the stimulation of adenylate cyclase [12, 17]. The $K_D$ values are given in Table 1 and are in reasonable agreement with other reported values [12, 17].

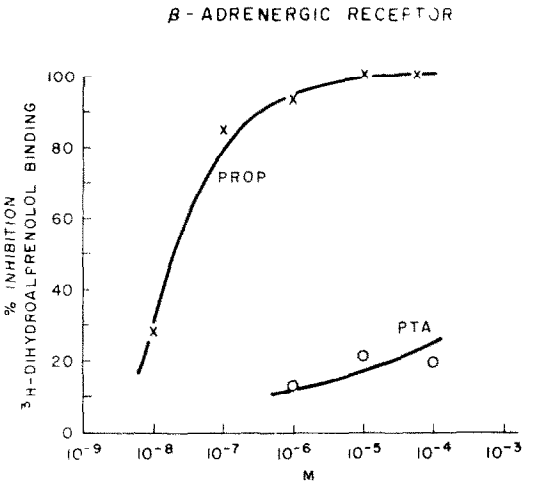


Fig. 4. Inhibition of [$^3$H]dihydroalprenolol binding by adrenergic antagonists. Iridial membranes prepared from rabbit eyes obtained from Pel-Freez Biologicals, Inc. were incubated with [$^3$H]dihydroalprenolol in the absence and presence of the indicated agonists. The ability of each agonist to displace the radioligand was compared to 100 per cent inhibition of binding by $5 \times 10^{-5}$ M propranolol, which corresponds to maximal specific binding of 0.33 pmoles [$^3$H]dihydroalprenolol/mg protein. PROP: propranolol; PTA: phentolamine.

Figure 4 demonstrates the specificity for adrenergic antagonists of the binding of [$^3$H]dihydroalprenolol to rabbit iridial membranes. The β-adrenergic specificity can clearly be seen when the ability of propranolol to displace [$^3$H]dihydroalprenolol is compared to that of phentolamine, which is essentially inactive. The $K_D$ values are given in Table 1 and are in agreement with those presented for the frog erythrocyte [12].

*Adrenergic receptors before and after decentralization and denervation.* Table 2 compares the densities of the α- and β-adrenergic receptors before and 25–30 days after decentralization and after denervation due to removal of the superior cervical ganglion. The phentolamine-sensitive binding of [$^3$H]dihydroergocryptine is not significantly different to membranes prepared from control irides compared to membranes prepared from decentralized irides ($P > 0.30$) but there is at least a suggestion of a decrease in density of α-adrenergic receptors with denervation when compared to the control ($P > 0.10$). Because of the paucity of material, the dissociation constants for dihydroergocryptine and for epinephrine in membranes prepared from denervated or decentralized irides were estimated by determining the binding activity at two concentrations along the dose-response curves for these agents. Thus, specific binding of dihydroergocryptine at 10 and 50 nM was found to be the same as that for membranes prepared from control tissue. Percent inhibition of binding of dihydroergocryptine by epinephrine was found to be the same at $10^{-7}$ M and $10^{-5}$ M in the test and control membranes. Therefore, the affinities for dihydroergocryptine and for epinephrine are not different in membranes prepared from control, decentralized or denervated irides.

Table 2. [$^3$H]-ligand binding to membranes prepared from rabbit irides

| | $B_{max}$* pmoles [$^3$H]-ligand/mg protein |
|---|---|
| α-Adrenergic receptor | |
| Control | 0.53 ± 0.05†(16) |
| Denervated | 0.43 ± 0.04(16) |
| Decentralized | 0.48 ± 0.03(8) |
| β-Adrenergic receptor | |
| Control | 0.33 ± 0.02(12) |
| Denervated | 0.39 ± 0.02(12) |
| Decentralized | 0.41 ± 0.02(8) |

* Maximal, specific binding of the [$^3$H]-ligand.
† Mean ± S.E.M. (number of eyes prepared).

However, when the β-adrenergic receptors were studied, increases were noted. Membranes prepared from adrenergically decentralized or denervated rabbit irides have an increased ability, when compared to the control ($P < 0.05$), to bind [$^3$H]dihydroalprenolol to sites at which this radioligand can be displaced by propranolol. Similar to that described above for the α-adrenergic receptor studies, the dissociation constants for dihydroalprenolol and isoproterenol in membranes prepared from denervated or decentralized irides were estimated by determining the binding activity at two concentrations along the dose-response curves. Thus, specific binding of dihydroalprenolol at 2 and 10 nM was found to be the same as that for membranes prepared from control tissue. Percent inhibition of binding of dihydroalprenolol by isoproterenol was found to be the same at $10^{-7}$ M and $10^{-5}$ M in the test and control membranes. Therefore, there is no difference in the affinities for dihydroalprenolol or isoproterenol of membranes prepared from control, decentralized or denervated irides.

## DISCUSSION

Our findings confirm and extend the work by Lefkowitz and colleagues [11, 12] to a densely adrenergically innervated smooth muscle containing both α- and β-adrenergic receptors. The tissue that we have referred to as rabbit iris, and used for the preparation of membranes in these studies, is actually composed of several different smooth muscles all adrenergically innervated via the superior cervical ganglion. These include ciliary muscle, vascular smooth muscle, iris sphincter and iris dilator. Thus, our findings generalize to adrenergic neuromuscular junctions in several types of smooth muscle.

Our observations with [$^3$H]dihydroergocryptine support the hypothesis that this compound binds to the α-adrenergic receptor [11]. We find at least 3-fold more binding sites for [$^3$H]dihydroergocryptine in membranes prepared from the rabbit iris than that reported previously for the rabbit uterus, a sparsely adrenergically innervated tissue. The phentolamine-sensitive binding of [$^3$H]dihydroergocryptine is displaced by adrenergic agonists with the order of potency characteristic of interaction at an α-adrenergic receptor–epinephrine ⩾ norepine-

phrine > isoproterenol. Dopamine, which has α-adrenergic activity demonstrated by its ability to dilate the pupil when applied topically to the rabbit eye, even in the absence of adrenergic innervation, displaces [$^3$H]dihydroergocryptine with a potency about one-fifth that of epinephrine [18].

Our observations with [$^3$H]dihydroalprenolol support the hypothesis that this compound binds to the β-adrenergic receptor [12]. Although there are no values in the literature to compare the density of β-adrenergic receptors in the iris with other smooth muscles, the value that we have obtained is similar to that reported for frog erythrocytes [12], rat pineal gland [17], and rat cerebral cortex using a different ligand [10]. Propranolol-sensitive binding of [$^3$H]-dihydroalprenolol is displaced by adrenergic agonists with the expected order of potency characteristic of interaction at a β-adrenergic receptor: isoproterenol ≥ epinephrine > norepinephrine. This β-adrenergic receptor is presumably associated with adenylate cyclase, as in other tissues, as stimulation of this tissue *in vitro* with adrenergic agents causes the synthesis of cyclic AMP, which can be inhibited by propranolol [19].

The study of the α- and β-adrenergic receptors in membranes prepared from denervated tissue provides a method for localizing the receptors in the tissue and proving their specificity. At an adrenergic neuromuscular junction, a number of potential recognition sites for catecholamines exist, including pre-junctional α-adrenergic receptors, which exert a negative feedback regulation on the release of norepinephrine [20], pre- and post-junctional sites for the uptake of noreinephrine [6], sites that stimulate the synthesis of protaglandins, which may, or may not, be intimately associated with the post-junctional, α-adrenergic receptors that stimulate the muscle to contract, and post-junctional, β-adrenergic receptors which, through the production of cyclic AMP, cause relaxation of the muscle [22]. After denervation, a small decrease in the total number of α-adrenergic receptors probably occurs. This is undoubtedly due to the loss of the pre-junctional, α-adrenergic receptors with the degeneration of the nerves because this decrease does not occur after decentralization. Of the post-junctional adrenergic sites that could bind norepinephrine, phentolamine, which is extremely potent at displacing [$^3$H]dihydroergocryptine in our experiments, does not inhibit extraneuronal uptake or the synthesis of prostaglandins in the rabbit iris [23]. Thus, in membranes prepared from innervated tissue, perhaps 80–90 per cent of the specific binding of [$^3$H]dihydroergocryptine is to the true post-junctional α-adrenergic receptors associated with the contraction of smooth muscle. Similarly, most, and probably all of the [$^3$H]dihydroalprenolol binds to the post-junctional, β-adrenergic receptors associated with relaxation of smooth muscle.

The supersensitivity that develops after denervation of skeletal muscle is due, primarily, to an increased number of receptor sites for the neurotransmitter, acetylcholine, perhaps as much as a 20-fold increase in the total number of receptor sites, and may represent some developmentally primitive state of the muscle before the presence of neurotransmitter exerts a trophic influence [24]. The supersensitivity that develops after adrenergic denervation of smooth muscle is, qualitatively, quite different from that which occurs in skeletal muscle. The major component in the development of supersensitivity to administered catecholamines in smooth muscle after degeneration of the adrenergic nerves is the loss, from the tissue, of the primary mechanism for inactivating the exogenous neurotransmitter, i.e., uptake into the nerve terminals [5]. Neuronal uptake is inhibited by cocaine, and the pre-junctional component of supersensitivity can be mimicked by administration of this agent [25]. However, the smooth muscle does undergo changes upon loss of its innervation which contribute to the total supersensitive response. This post-junctional supersensitivity can be demonstrated in the nictitating membrane and the vas deferens after complete denervation and also after decentralization, when the nerves remain intact in the tissue but neural input and, therefore, the release of norepinephrine is abolished. Physiological and pharmacological experiments have indicated that post-junctional supersensitivity, which develops slowly, is non-specific; e.g., a muscle such as the vas deferens, made supersensitive, responds to norepinephrine, histamine or potassium [9]. However, because of the lack or specific biochemical ligands, changes in the density of affinity of the receptor populations that might contribute to the phenomenon of supersensitivity have not been investigated.

The work of Lefkowitz and his colleagues [11, 12], and the extension of their work presented in this paper, indicates that the tritium-labeled compounds, dihydroergocryptine and dihydroalprenolol, can be used in an *in vitro* binding assay for characterizing α- and β-adrenergic receptors in membranes prepared from homogenized rabbit irides. We find that in response to chronic and complete loss of the adrenergic nerves and norepinephrine from the tissue, the two adrenergic receptors on the smooth muscle behave quite differently. The post-junctional α-adrenergic receptor does not change in density or affinity for [$^3$H]dihydroergocryptine or catecholamine. The α-adrenergic receptor in smooth muscle is functionally analogous to the cholinergic receptor in skeletal muscle in that stimulation of these sites, by the appropriate neurotransmitter, initiates the events which lead to contraction of the muscle. Thus, the difference in the trophic influences of the neurotransmitters on their post-junctional receptors is striking: at a cholinergic nerve-skeletal muscle junction, acetylcholine chronically released from the nerve influences the density of post-junctional cholinergic receptors, whereas at adrenergic nerve-smooth muscle junctions, norepinephrine chronically released from the nerve has no influence on the density or affinity of post-junctional α-adrenergic receptors.

The difference is even more striking when one considers our findings concerning the β-adrenergic receptor. Unlike the α-adrenergic receptor, the β-adrenergic receptor does increase in density on the muscle membrane upon denervation. This modest increase, about 20 per cent, is approximately the same as the increase in β-adrenergic receptors demon-

strated in the rat cerebral cortex following destruction of the adrenergic nerve terminals with 6-hydroxydopamine [10]. The $\beta$-adrenergic receptors prepared from denervated irides do not exhibit altered affinities for either [$^3$H]dihydroalprenolol or the $\beta$-adrenergic agonist, isoproterenol. Thus, our findings in the peripheral nervous system are consistent with $\beta$-adrenergic supersensitivity as described in other tissues, particularly those in the CNS, where increases $\beta$-adrenergic receptors and catecholamine-sensitive adenylate cyclase and cyclic AMP accumulation have been demonstrated [10, 26]. The observation that changes in the density of $\beta$-adrenergic receptors occur without changes in the $\alpha$-adrenergic receptors suggests that the intracellular level of cyclic AMP modulates the change in density of the $\beta$-adrenergic receptors.

In conclusion, unlike skeletal muscle, in adrenergically innervated smooth muscle, the neurotransmitter itself does not exert a trophic influence on post-junctional receptors. Thus, finding no increase upon denervation in the adrenergic receptor population that stimulates contraction of smooth muscle indicates that supersensitivity at an adrenergic neuromuscular junction can be explained by a pre-junctional component, due to loss of the neural elements, and a post-junctional component, due to a non-specific increase in the excitability of the smooth muscle membrane, as previously suggested [5, 9]. Why there should be changes in one receptor population and not in another, when both receptors influence the same physiological process, but in opposite directions, remains a question for further investigation.

In addition, we have previously demonstrated that catecholamines decrease intraocular pressure, at least in part, through a mechanism mediated by cyclic AMP [27, 28]. In the adrenergically denervated rabbit eye, catecholamines cause a more pronounced decrease of the intraocular pressure and a greater increase in the level of cyclic AMP in the aqueous humor [29]. Clinically, local treatment with 6-hydroxydopamine of patients with primary open angle glaucoma has been used to render these eyes more sensitive to administered epinephrine [30]. Although loss of the adrenergic terminals will allow more epinephrine to be available, perhaps an increased density of $\beta$-adrenergic receptors, providing more cyclic AMP, also contributes to the influence of catecholamines on intraocular pressure in eyes made supersensitive.

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## REFERENCES

1. J. Axelsson and S. Thesleff, *J. Physiol., Lond.* **147**, 178 (1959).
2. R. Miledi, *J. Physiol., Lond.* **151**, 24 (1960).
3. H. C. Hartzell and D. M. Fambrough, *J. gen. Physiol.* **60**, 248 (1972).
4. M. G. McConnell and L. L. Simpson, *J. Pharmac. exp. Ther.* **198**, 507 (1976).
5. U. Trendelenburg, *Pharmac. Rev.* **18**, 629 (1966).
6. L. L. Iverson, *Biochem. Pharmac.* **23**, 1927 (1974).
7. U. Trendelenburg, *J. Pharmac. exp. Ther.* **142**, 335 (1963).
8. U. Trendelenburg, *Pharmac. Rev.* **15**, 225 (1963).
9. W. W. Fleming, J. J. McPhilips and D. P. Westfall, *Ergebn. Physiol.* **68**, 56 (1973).
10. J. R. Sporn, T. K. Harden, B. B. Wolfe and P. B. Molinoff, *Science, N.Y.* **194**, 624 (1976).
11. L. T. Williams, D. Mullikin and R. J. Lefkowitz, *J. biol. Chem.* **251**, 6915 (1976).
12. C. Mukherjee, M. G. Caron, M. Coverstone and R. J. Lefkowitz, *J. biol. Chem.* **250**, 4869 (1975).
13. A. M. Laties and D. Jacobowitz, *Invest. Ophthal.* **3**, 592 (1964).
14. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
15. R. W. Fuller, in *Monoamine Oxidases—New Vistas, Advances in Biochemical Psychopharmacology* (Eds E. Costa and M. Sandler), p. 339. Raven Press, NY (1972).
16. H. C. Guldberg and C. A. Marsden, *Pharmac. Rev.* **27**, 135 (1975).
17. M. Zatz, J. W. Kebabian, J. A. Romero, R. J. Lefkowitz and J. Axelrod, *J. Pharmac. exp. Ther.* **196**, 714 (1976).
18. R. P. Shannon, A. Mead and M. L. Sears, *Invest. Ophthal.* **15**, 371 (1976).
19. A. H. Neufeld and M. L. Sears, *Invest. Ophthal.* **13**, 475 (1974).
20. J. Häggendal, in *New Aspects of Storage and Release Mechanisms of Catecholamines*, Bayer Symposium 2 (Eds H. J. Schüumann and G. Kroneberg), p. 100. Springer, Berlin (1970).
21. B. N. Davies, E. W. Horton and P. G. Withrington, *J. Physiol., Lond.* **188**, 38 (1967).
22. H.-P. Bar, in *Advances in Cyclic Nucleotide Research* (Eds P. Greengard and G. A. Robinson), Vol. 4, p. 195. Raven Press, NY (1974).
23. A. H. Neufeld and E. D. Page, *Expl Eye Res.* **20**, 549 (1975).
24. S. Thesleff, *Physiol. Rev.* **40**, 734 (1960).
25. U. Trendelenburg, *J. Pharmac. exp. Ther.* **125**, 55 (1959).
26. R. K. Dismukes and J. W. Daly, *J. Cyclic Nucleo. Res.* **2**, 321 (1976).
27. A. H. Neufeld, L. M. Jampol and M. L. Sears, *Expl Eye Res.* **14**, 242 (1972).
28. A. H. Neufeld, D. K. Dueker, T. Vegge and M. L. Sears, *Invest. Ophthal.* **14**, 40 (1975).
29. A. H. Neufeld, R. M. Chavis and M. L. Sears, *Expl Eye Res.* **16**, 265 (1973).
30. M. G. Holland and C. Wei, *Ann. Ophthal.* **5**, 633 (1973).