Role of Neuronal and Extraneuronal Uptake in Responses of Rabbit Iris Dilator Muscle to Levarterenol and Phenylephrine

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Abstract □ The roles of neuronal and extraneuronal uptake mechanisms in the response of iris dilator muscles of rabbit to levarterenol (norepinephrine) and phenylephrine were investigated. Chemical denervation with 6-hydroxydopamine was used to eliminate neuronal uptake. Exposure to corticosterone prior to and during exposure to levarterenol or phenylephrine was used to assess the importance of extraneuronal uptake. Dose–response curves and ED_{50} values for levarterenol or phenylephrine in control and 6-hydroxydopamine-denervated tissues, both in the presence and absence of corticosterone, are shown. Curves illustrating the decay of tension on washout of levarterenol or phenylephrine from treated tissues were analyzed. 6-Hydroxydopamine denervation affected the response to levarterenol more than that to phenylephrine. Washout of both agonists was slower after 6-hydroxydopamine pretreatment, and washout of phenylephrine was more rapid when corticosterone was present. These data indicate that extraneuronal uptake in iris dilator muscles was more important in determining the response to phenylephrine than to levarterenol and that neuronal uptake was more important in determining tissue responsiveness to levarterenol than to phenylephrine.

Keyphrases □ Levarterenol—effect on iris dilator muscle response, roles of neuronal and extraneuronal uptake mechanisms, rabbits □ Phenylephrine—effect on iris dilator muscle response, roles of neuronal and extraneuronal uptake mechanisms, rabbits □ Neuronal and extraneuronal uptake mechanisms—roles in iris dilator muscle response, effect of levarterenol and phenylephrine, rabbits □ Iris dilator muscle response—effect of levarterenol and phenylephrine, roles of neuronal and extraneuronal uptake mechanisms, rabbits □ Adrenergic agents—levarterenol and phenylephrine, effect on iris dilator muscle response, roles of neuronal and extraneuronal uptake mechanisms, rabbits

Recent studies (1, 2) demonstrated the importance of extraneuronal uptake in the alteration of the action of adrenergic agonists in vascular smooth muscle. Earlier, the decline of muscle tension during washout of a stimulant drug was found to be a valid index of the decreasing concentration of the drug at its receptor sites (3, 4). Corticosterone blocked the uptake of adrenergic agonists into extraneuronal stores (5, 6), thus altering the time required to wash out the agonists.

6-Hydroxydopamine selectively destroys the adrenergic nerve terminals in many tissues. Since a recent study (7) demonstrated a virtually complete absence of adrenergic innervation of iris muscles of rabbits after treatment with 6-hydroxydopamine, the iris dilator muscle of the rabbit was selected for study. Chemical denervations by 6-hydroxydopamine and blockade of extraneuronal uptake with corticosterone were used to assess the role of various mechanisms in determining muscle responsiveness to α-adrenergic agonists.

EXPERIMENTAL

Materials—Albino rabbits of both sexes, approximately 1.5 kg, were stunned by a blow to the head and exsanguinated and the eyes were enucleated. Two strips of the iris dilator muscle of about 2 mm width were isolated from each eye by the method of Kern (8). The strips were then mounted in 25-ml baths containing a balanced salt solution of the following composition (micromolar): NaCl, 141.58; KCl, 5.40; $NaHCO_3$, 15.00; glucose, 11.00; NaH_2PO_4, 0.40; and $CaCl_2$, 2.10. The solution was aerated with a mixture of 97% oxygen and 3% carbon dioxide. The pH remained constant at 7.4 throughout the experiment after aeration with this gas mixture.

Strain-gauge transducers with microscale accessories were used to measure isometric contractions. Recordings were made of tension changes on an oscillographic recorder. Resting tension was adjusted to approximately 40 mg. Temperature was maintained at 37° using a heater-circulator.

Drugs—Solutions of drugs were prepared fresh in the balanced salt solution throughout the experiments. Levarterenol (norepinephrine), phenylephrine, corticosterone, and 6-hydroxydopamine were employed.

Procedure—Dose–response relationships were determined for levarterenol and phenylephrine using dose levels from 2.4×10^{-7} to 3.5×10^{-5} M and from 2×10^{-7} to 1×10^{-4} M, respectively, added in a cumulative fashion.

One eye of each animal was chemically denervated by administration of 200 μl of 2% (0.12 M) 6-hydroxydopamine subconjunctivally 24 hr prior to sacrifice. 6-Hydroxydopamine was prepared fresh in normal saline containing sodium bisulfite in equimolar concentration. The control eye was injected in the same manner with 200 μl of the sodium bisulfite solvent solution.

Some tissues, both control and denervated, were treated with 8.7×10^{-5} M corticosterone 10 min prior to exposure to the adrenergic agonists. Corticosterone was allowed to remain in the bath only as long as the agonist was present and was removed concurrently with the agonist. Corticosterone was first solubilized in absolute ethanol and then diluted to the final concentration with the balanced salt solution.

Washout curves were determined by exposing control, 6-hydroxydopamine-, and corticosterone-pretreated tissues to the agonists for 10 min. Tissues were initially washed twice and were then washed once every 5 min until they returned to the precontracted baseline level.

Data Analysis—The data were normalized by calculating tension responses as a percent of the maximum value obtained. Semilogarithmic plots represent the mean values of responses obtained for no less than eight tissues for each group. Means ± SE for various groups were calculated; data were compared using the Student t test for paired variables and considered significantly different when $p < 0.05$. The degree of supersensitivity was assessed by the ED_{50} values calculated from tension responses. A programmable calculator was used for all calculations in this study.

RESULTS

Preliminary results revealed no differences in dose–response curves or washout curves for nontreated iris dilator muscles as compared to those injected with the solvent solution for 6-hydroxydopamine. Therefore, muscles treated in this manner are referred to as controls.

Figure 1 illustrates dose–response relationships of iris dilator strips to levarterenol of both control and denervated tissues, obtained in the presence and absence of corticosterone. The dose–response curves for denervated tissues exhibited supersensitivity, as shown by the shift to the left. Also, a slight increase in sensitivity was seen in both control and denervated tissues when corticosterone was present during exposure to the agonist.

Dose–response relationships for phenylephrine in control and denervated tissues both in the presence and absence of corticosterone are illustrated in Fig. 2. The responses to phenylephrine suggested a slight supersensitivity of denervated tissues, but statistical analysis did not support this finding. Both control and denervated tissues appeared slightly more sensitive to phenylephrine when corticosterone was present

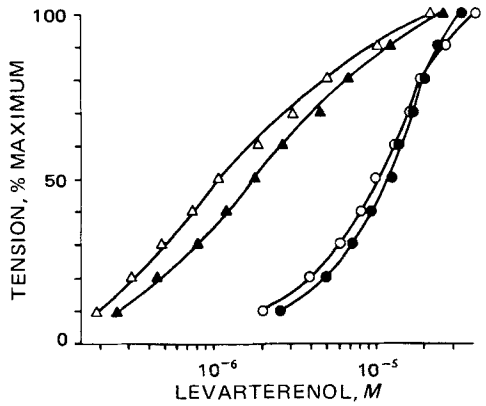

Figure 1—*Effect of 6-hydroxydopamine denervation and corticosterone blockade of extraneuronal uptake on the response of the rabbit iris dilator muscle to levarterenol. Key: ●, control; ○, control + corticosterone; ▲, denervated; and △, denervated + corticosterone. See text for* n *values and procedures of drug administration.*

before and during exposure to this agonist. Again, this finding was not supported by statistical analysis.

The ED_{50} values for levarterenol and phenylephrine on control and denervated iris dilator muscles, both in the presence and absence of corticosterone, are shown in Table I. Supersensitivity of denervated strips to levarterenol, but not to phenylephrine, was demonstrated by a significant decrease in the ED_{50} value for levarterenol as compared to the control value. Conversely, exposure of tissues to corticosterone prior to and during exposure to the agonists significantly decreased ED_{50} values for phenylephrine but not those for levarterenol.

Figure 3 shows tracings of typical response to, and washout of, levarterenol in control and denervated tissues. The marked prolongation of drug washout after chemical denervation is illustrated.

Figure 4 illustrates the washout of levarterenol (3.5×10^{-5} *M*) from control and denervated tissues in the presence and absence of corticosterone. Tension returned to the resting level significantly faster in control tissues ($t_{50\%}$ = 33 sec; time to 50% tension decay) than in denervated tissue ($t_{50\%}$ = 225 sec). The presence of corticosterone before and during exposure to levarterenol slightly increased the time required for washout in control ($t_{50\%}$ with corticosterone = 60 sec) and denervated ($t_{50\%}$ with corticosterone = 255 sec) strips.

The washout of phenylephrine (1×10^{-4} *M*) from control and denervated iris dilator muscle strips in the presence and absence of corticosterone is illustrated in Fig. 5. The washout of phenylephrine from denervated tissues ($t_{50\%}$ = 432 sec) was slower than from control tissues ($t_{50\%}$ = 177 sec), but this alteration was not as dramatic as the effect seen on levarterenol washout. Corticosterone had an effect on phenylephrine washout opposite to its effect on levarterenol washout. The washout times for phenylephrine decreased in both control ($t_{50\%}$ corticosterone = 139 sec) and denervated ($t_{50\%}$ corticosterone = 246 sec) tissues.

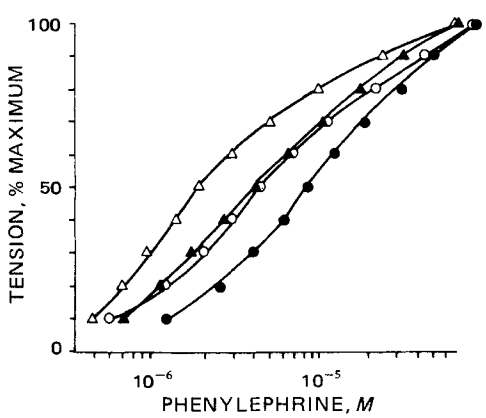

Figure 2—*Effect of 6-hydroxydopamine denervation and corticosterone blockade of extraneuronal uptake on the response of the rabbit iris dilator muscle to phenylephrine. Key: ●, control; ○, control + corticosterone; ▲, denervated; and △, denervated + corticosterone. See text for* n *values and procedures of drug administration.*

Table I—Effect of 6-Hydroxydopamine Denervation and Corticosterone Blockade of Extraneuronal Uptake on ED_{50} Values for Levarterenol and Phenylephrine on Rabbit Iris Dilator Muscle[a]

	Drug Concentration, × 10^6 *M*	
	Levarterenol	Phenylephrine
Control	21.0 ± 6.8	8.6 ± 1.9
Corticosterone	11.4 ± 0.9	3.9 ± 0.9[b]
Denervated	1.7 ± 0.7[b]	4.5 ± 1.3
Corticosterone	1.2 ± 0.4[b]	2.0 ± 0.5[b]

[a] See text for *n* values and methods of calculation. [b] Significantly different from control, $p < 0.05$.

DISCUSSION

Previous studies demonstrated that several factors are involved in the response of smooth muscle to adrenergic agents and that these factors also play a role in the decay of the response during washout of the agonists. The major factors involved are uptake by adrenergic neurons, enzymatic destruction by monoamine oxidase or catechol *O*-methyltransferase, and extraneuronal uptake.

The dose–response curves and ED_{50} values reported here for levarterenol for control and denervated tissues demonstrate the eminent importance of neuronal uptake and enzymatic (monoamine oxidase) mechanisms governing the response to this compound. The dose–response curves and ED_{50} values for phenylephrine were not altered to the same extent. This finding indicates that the neuronal uptake system of iris dilator muscle shows greater affinity for levarterenol than for phenylephrine. Support can be found for this conclusion in studies of the neuronal uptake process in other tissues (9, 10).

Corticosterone at the concentration used has been shown to block extraneuronal uptake of α-receptor agonists in other tissues (5, 6, 11). In this study, the dose–response curves and ED_{50} values for muscles treated with corticosterone suggest that extraneuronal uptake plays a more important role in determining the response to phenylephrine than to levarterenol.

The curves representing washout of agonists are sigmoid in nature and are believed to represent a valid measure of the amount of agonist present at the receptor site at any given time during washout (2). The agonist that is active at the receptor site may represent the agonist that originally interacted with the receptor or a fraction of agonist leaving some storage site such as an extraneuronal store. It is reasonable to suggest that the decline of tension upon washout of an adrenergic agonist is related to the sensitivity of that tissue to the agonist (*i.e.*, denervation-supersensitive tissues *versus* control tissues). Destruction of the adrenergic nerve terminals by 6-hydroxydopamine can potentially alter responses to agonists by two mechanisms. First, the neuronal uptake mechanism is totally absent, and second, the level of monoamine oxidase is decreased. Since corticosterone blocks extraneuronal uptake, the washout of compounds taken up into this store should be more rapid if it is blocked.

The washout of levarterenol is significantly prolonged in denervated tissues. This finding indicates that neuronal uptake and/or monoamine oxidase activity are important in the termination of its action. Levar-

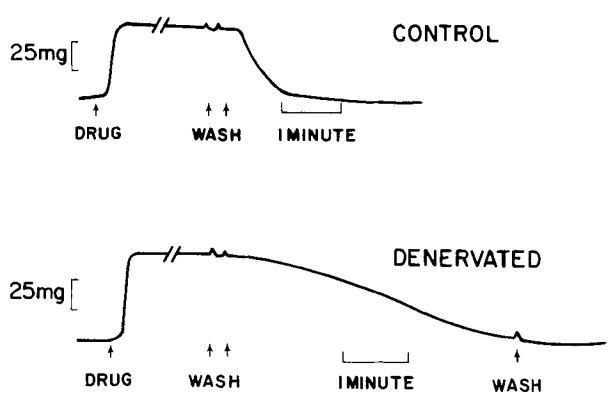

Figure 3—*Typical recording showing the effect of 6-hydroxydopamine denervation on the washout of levarterenol from the rabbit iris dilator muscle. Tissues were washed twice initially and then once every 5 min until baseline levels were reached.*

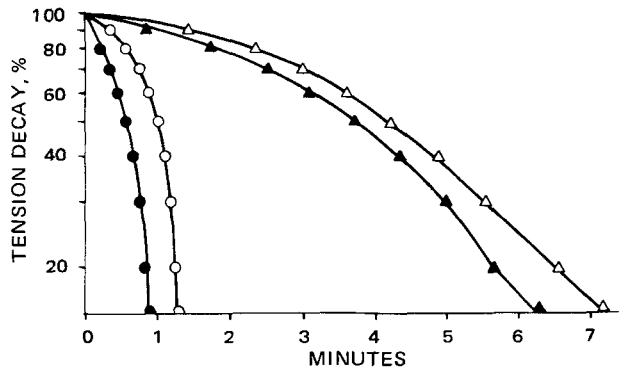

Figure 4—*Effect of 6-hydroxydopamine denervation and corticosterone blockade of extraneuronal uptake on washout of levarterenol from the rabbit iris dilator muscle. Key: ●, control; ○, control + corticosterone; ▲, denervated; and △, denervated + corticosterone. See text for* n *values and drug administration and removal protocol.*

terenol washout in tissues treated with corticosterone is slightly delayed. Thus, if extraneuronal stores are important in the termination of action of this compound, they are corticosterone insensitive in this tissue. Corticosterone-insensitive extraneuronal stores have been demonstrated (10). However, the slight but consistent delay in washout of levarterenol may be due merely to the slight shift in the ED_{50} of levarterenol under these conditions. Another possibility is that the destruction of levarterenol by catechol *O*-methyltransferase obscures the role played by extraneuronal uptake. Some support can be seen for this explanation in the data shown for washout of phenylephrine, which is not destroyed by

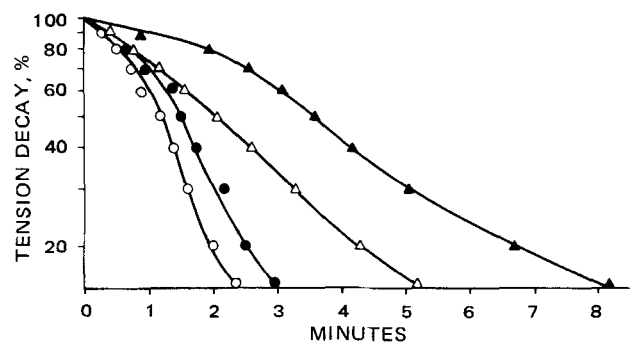

Figure 5—*Effect of 6-hydroxydopamine denervation and corticosterone blockade of extraneuronal uptake on washout of phenylephrine from the rabbit iris dilator muscle. Key: ●, control; ○, control + corticosterone; ▲, denervated; and △, denervated + corticosterone. See text for* n *values and drug administration and removal protocol.*

catechol *O*-methyltransferase.

The washout of phenylephrine from iris dilator muscle strips previously denervated with 6-hydroxydopamine was delayed as compared to control values, but not nearly so dramatically as was that of levarterenol. This finding again indicates that the affinity of the neuronal uptake mechanism is lower for phenylephrine than for levarterenol. The rate of washout of phenylephrine from corticosterone-treated tissues (both control and denervated) was more rapid than that of tissues not subjected to this treatment, indicating that extraneuronal uptake does play a role in the termination of the action of phenylephrine.

The data presented here support the view that extraneuronal uptake in iris dilator muscles from rabbits was more important in the determination of the response to phenylephrine than to levarterenol. Also, neuronal uptake was more important in determining the responsiveness to levarterenol than to phenylephrine. To clarify further the role of catechol *O*-methyltransferase and extraneuronal uptake in the termination of action of adrenergic agents in this tissue, experiments involving catechol *O*-methyltransferase inhibition and uptake of radioactively labeled compounds will be undertaken.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 28, 1975, from the *Department of Pharmacology, Medical College of Georgia, Augusta, GA 30902.*

Accepted for publication March 24, 1976.

Supported in part by U.S. Public Health Service Grants RR-05365-13 and AM-18424-01 and by a grant from Fight for Sight, Inc.

The technical assistance of Ms. J. Taylor is gratefully acknowledged.

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