Evidence for the Existence of Vascular Alpha₂-Adrenergic Receptors in Humans

MICHAEL R. GOLDBERG AND DAVID ROBERTSON

SUMMARY Many studies have suggested that α-adrenergic receptors on vascular smooth muscle are heterogeneous and that both α₁- and α₂-adrenergic receptors can cause vasoconstriction when stimulated. We explored this hypothesis in normal humans by comparing the capacity of yohimbine, an α₂-adrenergic receptor antagonist, and prazosin, an α₁-adrenergic receptor antagonist, with differentially blocked pressor responses to phenylephrine, an α₁-adrenergic receptor agonist, and epinephrine, a nonselective α-agonist. We studied these responses in normal male volunteers who had been pretreated with propranolol (80 mg orally every 8 hours for 5 days) to obviate stimulation of β-receptors by either agonist. We found differential effects of the antagonists on responses to the two agonists. Yohimbine induced a 3.1-fold (± 0.5) shift in the dose of epinephrine, which raised blood pressure 25 mm Hg, and only a 1.9-fold (± 0.2) shift in the response to phenylephrine (p < 0.01). Prazosin induced a 2.4-fold (± 0.5) shift in the responses to epinephrine and a 4.5-fold (± 1.2) shift in the response to phenylephrine (p < 0.05). These data are consistent with the notion that α-adrenergic receptors in the human vasculature are not homogeneous, but rather may be subdivided into at least two subtypes, one resembling α₁-adrenergic receptors and the other resembling α₂-adrenergic receptors. (Hypertension 6: 551–556, 1984)

KEY WORDS • vascular alpha₂-adrenergic receptors • yohimbine • prazosin • epinephrine • phenylephrine

The differentiation, widespread distribution, and multiple functions of α₂-adrenergic receptors have been intensively studied and reviewed.¹⁻⁴ Recent studies have suggested that responses of vascular smooth muscle to α-agonists are mediated by both α₁- and α₂-adrenergic receptors (for review, see references 3 and 4). Several investigators have suggested that vascular α₂-adrenergic receptors can be differentiated in humans by agonists⁵⁻⁶ or antagonists⁷⁻⁸ of appropriate selectivity. A concern in these studies is that blockade of agonist responses by α₂-selective antagonists was not demonstrated⁹⁻¹⁰ and may have been confounded by β-effects of the agonists.⁸ Accordingly, to study these responses in normal humans we used an approach similar to that used in animal models to demonstrate vascular α₂-adrenergic receptors.³⁴ Thus, we compared the ability of the α₁- and α₂-selective antagonists prazosin and yohimbine to inhibit pharmacologically mediated responses to phenylephrine (α₂-selective) and epinephrine (nonselective). As suggested by the study of Reid et al.,⁸ we combined α₁ and α₂-selective blockade with β-adrenergic receptor blockade to avoid the confounding cardiovascular effects of β-adrenergic receptor stimulation or its withdrawal.

The antagonists we used were selected on the basis of data showing that, in humans, yohimbine raises the blood pressure and enhances pressor reflexes. These findings have been compatible with blockade of central or peripheral α₂-receptors that inhibit sympathetic outflow.¹⁰ In contrast, others have shown that prazosin, an α₁-selective antagonist,² lowers blood pressure and has no effect or slightly inhibits pressor reflexes.¹¹⁻¹³ We compared the ability of these antagonists to attenuate responses to two agonists of differing alpha selectivity.¹,² Phenylephrine is a directly acting sympathomimetic amine that selectively stimulates α₁-receptors. Epinephrine is nonselective and shows similar potency at α₁- and α₂-receptors. However, several studies suggest that responses to injected catecholamines may be due to preferential stimulation of vascular α₂-receptors.¹⁴ Based upon these and other studies,⁴ we hypothesized that yohimbine would preferentially block responses to epinephrine and that prazosin would preferentially block responses to phenylephrine.
Methods

Eleven normal male paid volunteers were used in these studies. Their ages ranged from 23 to 40 years. All procedures and consent forms were approved by the Vanderbilt University Committee for the Protection of Human Subjects. These studies were performed at the Elliot V. Newman Clinical Research Center (CRC).

After they had been recruited and screened by physical examinations, laboratory profiles, and electrocardiograms, individuals were supplied with propranolol to be taken at a dose of 80 mg every 8 hours for 5 days prior to and throughout the time of the study. This regimen has previously been shown to provide steadystate levels of propranolol of about 130 ng/ml and shift dose-response curves for isoproterenol about 25-fold (J. Nadeau, personal communication). On the fourth night of propranolol administration, subjects were admitted to the CRC and were kept in bed until the time of the study. In the morning, the last 80 mg dose of propranolol was given at 0800 hours, an intravenous line was inserted for drug administration, and a Teflon catheter was inserted into the radial artery on the nondominant side for measurement of intraarterial blood pressure. Blood pressure, electrocardiogram, and heart rate triggered from the electrocardiogram (ECG) were continuously monitored on a Hewlett-Packard four-channel recorder. After a 20-minute accommodation period, the study began.

In this study, we used mean intraarterial blood pressure as the measure of the response to each agonist. This methodology does not separate agonist effects on cardiac function and venous tone from those on resistance vessels. However, it has proven to be useful in demonstrating putative vascular $\alpha_2$-responses previously, and results using this technique should later be applicable to more precise delineation of the response of each vascular segment. Cardiac effects were minimized by pretreatment of volunteers with propranolol.

Dose-response curves for phenylephrine and epinephrine were determined in random sequence. To assure safety, agonist injections were stopped when a mean arterial pressure increment of 25 mm Hg was achieved. Minimum doses of agonist used were 0.5 and 50 $\mu$g for epinephrine and phenylephrine, respectively. The dose of agonist was doubled until the 25 mm Hg goal response was achieved exactly or bracketed. This approach produced a maximum pressor response of 32 $\pm$ 7 mm Hg. After each dose of agonist, blood pressure was allowed to return to baseline for a minimum of 5 minutes before administration of the next dose. After the first response to each agonist had been determined, a second dose-response curve was obtained for each dose. After this second control response, an antagonist was administered. Yohimbine HCl (Sigma Chemical Company, St. Louis, Missouri) was dissolved in bacteriostatic saline (0.5 mg/ml) and administered in a fashion previously shown to produce sustained, but modest, elevations in blood pressure and plasma norepinephrine (125 $\mu$g/kg bolus, then 1 $\mu$g/kg/min infusion). Sequential determinations of responses to each agonist were evaluated during the yohimbine infusion beginning 20 minutes after the yohimbine bolus with the same 25 mm Hg goal response. Prazosin was administered as a single oral dose of 1.0 mg, and agonist responses were repeated 1 hour after ingestion, an interval sufficient for plasma levels to reach a transient plateau. Placebo-treated (single-blind) subjects ($n=2$) were given bacteriostatic saline in a fashion similar to yohimbine (0.25 ml/kg bolus, then 0.002 ml/kg/min), and responses to agonists were again evaluated following 20 minutes of infusion. Two subjects received only yohimbine and two received only prazosin. Five subjects were studied twice, once with yohimbine and once with prazosin, on separate occasions 1–12 months apart.

The individuals given placebo infusions had reproducible responses to phenylephrine and epinephrine, a dose-response curve to each being determined three times over a 6-hour period. Therefore, the first two responses to the agonists were averaged and compared to a single dose-response curve for each that was determined after administration of antagonist. Least squares linear regression analysis of dose-response curves was performed on the responses before and after the antagonist. The dose of agonist that raised mean arterial pressure 25 mm Hg (D-25, either EPI-25 or PHE-25) was calculated from this analysis. From these values, the fold-shift in the response was determined by dividing the D-25 following antagonist by the control D-25. If our hypothesis were correct, we would expect differential shifts of responses to each agonist following each antagonist. Therefore, as an index of the selectivity of each antagonist and our ability to distinguish $\alpha_2$-adrenergic receptor subtypes, we defined the selectivity ratio as the fold-shift for epinephrine divided by the fold-shift for phenylephrine in each individual following administration of each antagonist (yohimbine or prazosin).

Statistical comparisons were made using paired and unpaired t tests on an Apple II computer or with Clinfo.

Results

As in our previous study, but with propranolol present, yohimbine induced a 15 to 20 mm Hg increment in mean arterial pressure and did not affect heart rate. Prazosin elicited no changes in blood pressure or heart rate while subjects were supine. Following the study, one of the seven individuals given prazosin was mildly orthostatic.

The increase in D-25 for each agonist in each subject is shown in Table 1 and the average fold-shift for each agonist following each antagonist is compared in Figure 1. A study in one individual is depicted in Figure 2. Differential blockade of pressor responses to epinephrine and phenylephrine was noted following yohimbine. The $\alpha_2$-selective antagonist induced a 3.1-fold shift (± 0.5) in the response to epinephrine and a 1.9-fold (± 0.2) shift in the response to phenylephrine.
Table 1. Changes in Sensitivity to Epinephrine and Phenylephrine Induced by Saline, Yohimbine, and Prazosin

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<th>Prazosin</th>
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TABLE 1. Changes in Sensitivity to Epinephrine and Phenylephrine Induced by Saline, Yohimbine, and Prazosin

Dose ratios for epinephrine and phenylephrine were calculated by dividing the dose that raised mean arterial pressure 25 mm Hg after antagonist by the dose that raised mean arterial pressure 25 mm Hg before the antagonist. The responses to agonists were compared before and after administration of each agonist. The dose that raised mean arterial pressure 25 mm Hg after antagonist was divided by the dose that raised mean arterial pressure 25 mm Hg before the antagonist to determine the dose ratio in each subject for each agonist.

The differential blockade of these responses was statistically significant (p < 0.01).

Prazosin also produced a differential blockade of responses to each agonist (Table 1 and Figure 1). Epinephrine was shifted 2.4-fold (± 0.5), and phenylephrine was shifted 4.5-fold (± 1.2) following prazosin. The differential blockade of these responses was statistically significant (p < 0.05).

The selectivity ratio for each antagonist is represented in Figure 3. On the average, the fold-shift for epinephrine was 60% greater than the fold-shift for phenylephrine following yohimbine. In contrast, following prazosin, the fold-shift for phenylephrine was 100% greater than that for epinephrine, a highly significant difference between the effects of the two antagonists.

At baseline and during administration of antagonists, the pressor response to each agonist was accompanied by a reflex bradycardia. The relationship between each fall in heart rate and the pressor response was similar for epinephrine and phenylephrine and was not altered by prazosin or the elevation in baseline blood pressure induced by yohimbine (data not shown).

Discussion

The goal of these studies was to further investigate observations suggesting that α-adrenergic receptor-mediated pressor responses in humans are mediated by at least two definable populations of α-receptors.5-9 We used four pharmacologic probes to investigate this hypothesis. Epinephrine and phenylephrine were chosen as agonists that are either nonselective or α₁-selective. Prazosin and yohimbine were chosen as antagonists with selectivity for α₁- and α₂-adrenergic receptors, respectively. Subjects were pretreated with propranolol to obviate β-receptor stimulation by either of our agonists, particularly epinephrine. If our hypothesis were correct, pressor responses to epinephrine would be mediated by stimulation of both α₁- and α₂-receptors, and responses to phenylephrine would be
mediated largely by α₁-receptors. If a single population of α-receptors were mediating the pressor response to both agonists, then receptor theory would predict that, regardless of antagonist selectivity, responses to each agonist should be shifted to similar degrees following administration of a given antagonist.

Alternatively, if different populations of receptors were mediating the pressor response to each agonist, then preferential inhibition of responses by each antagonist should be demonstrable according to the relative affinity of each antagonist for each receptor. Our data suggest this latter possibility to be the case. As indicated by the selectivity ratios summarized in Figure 3, yohimbine preferentially inhibited responses to epinephrine, and prazosin preferentially inhibited responses to phenylephrine. These findings confirm that the vasculature of humans is endowed with both α₁- and α₂-adrenergic receptors.

Each antagonist inhibited to some degree the response to each agonist. In view of the known high order of selectivity of both prazosin and phenylephrine for α₁-receptors, we interpret these findings as demonstrating the capacity of epinephrine to stimulate α₁-receptors in addition to α₂-receptors. In addition, these
data suggest that, at the doses used, yohimbine also blocks α₂-receptors, although to a lesser degree than α₁-receptors.

In our earlier study, yohimbine enhanced reflexly mediated pressor responses. In contrast, in our present study, yohimbine antagonized responses to the agonists injected intravenously at doses that produced increments in blood pressure similar to that evoked by these reflexes. Although there were some differences in the methodology in these two studies (bolus vs infusion of yohimbine, and propranolol pretreatment in the present study), comparisons would appear to be justifiable. Differential effects of yohimbine on these equivalent pressor responses suggest that reflex and pharmacologic adrenergic pressor responses could be mediated by different receptor subtypes. Alternatively, amplification of sympathetic outflow by yohimbine at central or peripheral sites could obscure inhibition of the reflex vascular response by the alkaloid.

Differential blockade of neurogenic and pharmacologic pressor responses was noted in animals in 1935 and 1941, but, although noted, has not been emphasized in humans. Later animal studies suggested that responses to electrical stimulation of sympathetic nerves were mediated by α₁-receptors while responses to injected catecholamines were mediated by α₂-receptors. In studies of normal humans, however, neurogenic pressor responses can only be elicited by multicomponent reflexes. These reflexes are not well blocked by α-antagonists that affect α₁-receptors (phenolamine, prazosin, and phenoxybenzamine), and are enhanced by α₂-receptors. Alternatively, amplification of sympathetic outflow by yohimbine at central or peripheral sites could obscure inhibition of the reflex vascular response by the alkaloid.

A hypothesis advanced by Hirst and Niel21 may explain differences between reflex and pharmacologic pressor responses that are not explained by drug effects on sympathetic outflow per se. Hirst and Nield showed two types of vasoconstrictor responses to norepinephrine in guinea pig mesenteric artery. A depolarization-coupled response was not inhibited by phentolamine at concentrations sufficient to block α₁- and α₂-receptors, while a localized, nonpropagated constrictor response was blocked by phentolamine. Thus, the vasculature may possess a third catecholamine receptor that is unrelated to α₁- and α₂-receptors and that may mediate a portion of the neurogenic pressor response.

The identification of distinct subtypes of vascular α-adrenergic receptors has implications for a variety of pathological, physiological, and pharmacological situations. For example, in conditions with elevated circulating catecholamines, such as pheochromocytoma and clonidine-withdrawal, our data suggest that the resultant hypertension could be mediated by both α₁- and α₂-receptors. Effective treatment should then be directed at both receptor subtypes. Thus, phentolamine and not prazosin would be the preferred therapy for acute lowering of blood pressure. Studies in animals have suggested that vascular α₂-receptors are not evenly distributed among arteries and veins. At least in the dog, veins are more responsive to drugs that stimulate late α₂-receptors. In rats, mesenteric arteries do not have α₂-receptors identifiable by [3H] clonidine binding. In dogs, femoral artery and aorta have a greater proportion of α₁-relative to α₂-receptors, and renal and mesenteric arteries have equivalent numbers of each as identified by [3H] prazosin and [3H] yohimbine binding. If, in humans, subsequent studies show that α₂-receptors are preferentially distributed on the venous side of the circulation, then selective α₂-agonists might provide more specific therapy in conditions associated with inadequate venous return (such as idiopathic orthostatic hypotension). Similarly, selective, peripherally active α₂-agonists might be useful in reducing preload in heart failure. Finally, recent studies have shown that vascular responses to α₂-agonists are more sensitive to blockade by slow channel calcium antagonists than are the responses to α₁-agonists. Our findings may, therefore, be relevant to the use of calcium antagonists in the treatment of hypertension.

References

Evidence for the existence of vascular alpha 2-adrenergic receptors in humans.
M R Goldberg and D Robertson

Hypertension. 1984;6:551-556
doi: 10.1161/01.HYP.6.4.551

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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