Preferential Noradrenergic Innervation of
Alpha-Adrenergic Receptors in Vascular Smooth Muscle

SALOMON Z. LANGER, M.D., NICHOLAS B. SHEPPERSON, PH.D., AND ROY MASSINGHAM, PH.D.

SUMMARY In the isolated perfused hindlimb preparation of the dog, pressor responses to norepinephrine (NE) are mediated by postsynaptic α1- and α2-adrenergic receptors. Results obtained using preferential α1- and α2-adrenergic receptor antagonists suggest that the α1-subtype is predominantly innervated while both α1- and α2-adrenergic receptor subtypes in vascular smooth muscle are accessible to circulating agonists and antagonists. Preliminary studies in the isolated perfused cat spleen support these in vivo findings in the dog. In contrast to vascular smooth muscle, the cat nictitating membrane appears to contain only α1-adrenergic receptors postsynaptically. (Hypertension 3 (supp I): 1-112-1-118, 1981)

KEY WORDS • postsynaptic α1- and α2-adrenergic receptors • prazosin • rauwolscine • hind limb perfusion pressure • presynaptic α2-adrenergic receptors

Much evidence now supports the existence of two subtypes of alpha-adrenergic receptors. Originally this subdivision was made when α-adrenergic receptors were discovered to be located presynaptically on noradrenergic nerves regulating transmitter release, as well as postsynaptically mediating the responses of most adrenergically innervated tissues. Studies of these two alpha-receptors showed that they possessed different affinities for a given series of agonists and antagonists and therefore were probably of a slightly different structure. Thus, these receptors were differentiated by denoting them as pre- and post-synaptic α-adrenergic receptors — an anatomical designation.

Since this original classification was made, it has been found that receptors with the characteristics of postsynaptic receptors are located on non-neuronal tissues, for example on platelets, frog skin melanocytes, and rat pancreatic islets. For this reason, it has been proposed that those α-adrenergic receptors with affinities for drugs corresponding to the original postsynaptic receptor should be called α1-receptors, and those corresponding to the presynaptic receptor, α2. Thus, the original anatomical designation of these receptors has been replaced by a pharmacological differentiation.

Recent evidence has shown that in dogs, rats, and cats, the pressor response to norepinephrine (NE) is resistant to blockade by the α1-adrenergic receptor antagonist, prazosin. In the rabbit and the rat, it has been shown that α2-adrenergic receptor agonists produce pressor responses that are blocked by selective α2-adrenergic receptor antagonists. These findings have led to the conclusion that in these species the pressor response to exogenous agonists may be mediated via both postsynaptic α1- and α2-adrenergic receptors. Following our earlier work in the anesthetized dog, we investigated the pressor responses to several exogenous α-adrenergic receptor agonists and sympathetic nerve stimulation to evaluate the presence and role of postsynaptic α-adrenergic receptors in this species.

Methods

Mongrel dogs (10 to 25 kg body weight) were anesthetized with pentobarbitone (35 mg/kg + 6 mg/kg/hr i.v.). The animals were respired artificially and blood pressure monitored from the left carotid artery. The right jugular vein and left brachial vein were cannulated and used for drug infusions or injections respectively. After heparinizing the animals, blood was taken, via a roller pump, from the right femoral artery and used to perfuse the left hindlimb via the left femoral artery. Resting hindlimb perfusion pressure was measured using a Statham P23D pressure transducer and was set to mean arterial
pressure (MAP) at the start of the experiment by adjusting the perfusion rate of the pump. In some animals, the left lumbar sympathetic chain was stimulated (supramaximal voltage, 1 ms for 1 min) every 3 minutes at various frequencies (0.1 to 2 Hz), and between each series of stimulations dose-response curves were constructed to NE (0.3 to 3 μg/kg i.v.). After stable control responses to nerve stimulation and injected NE were obtained, prazosin (10 μg/kg i.v.) a selective α-adrenergic receptor antagonist or rauwolscine, a preferential α₂-adrenergic receptor antagonist, was injected and the series of nerve stimulations and injections of NE repeated. Finally, the effect of the combination of the two antagonists was assessed on endogenously released and exogenously injected NE.

In a second group of animals, ganglion blocked with chlorisondamine (1 mg/kg i.v.) and atropine (1 mg/kg i.v.) and β-adrenergic receptor blocked with propranolol (0.5 mg/kg i.v. + 0.25 mg/kg/hr i.v.), dose response curves on hindlimb perfusion pressure were constructed to NE (0.1 to 1 μg/kg i.v.), guanabenz (1-10 μg/kg i.v.), and to phenylephrine (PE) (1 to 10 μg/kg i.v.). Animals were then given prazosin (10 μg/kg i.v.) or rauwolscine (10 μg/kg i.v.), and responses to NE, PE, and guanabenz were re-examined in the presence of each antagonist given separately, and finally in the presence of both antagonists in combination.

Results are expressed as changes in hindlimb perfusion pressure since administration of the blocking agents did not markedly affect basal pressure levels in most experiments. Statistical significance was assessed by analyzing differences in the area under the dose-response curve; a value of \( p < 0.05 \) was taken as significant.

The nictitating membrane experiments were performed in cats prepared as described by Langer and Pinto. Contractions of the nictitating membrane to nerve stimulation (0.1 to 20 Hz, supramaximal voltage, 2 ms pulse width) and exogenous NE were recorded. The blood pressure (BP) was recorded from a cannula passed into the abdominal aorta via the femoral artery.

Results

Prazosin (10 μg/kg) significantly inhibited (\( p < 0.05 \)) the hindlimb pressor response to lumbar sympathetic nerve stimulation at all the frequencies of stimulation employed (0.1 to 2.0 Hz). This stimulation produced increases in hindlimb perfusion pressure of 28 ± 3 to 95 ± 7 mm Hg (fig. 1). Similar responses were obtained by i.v. injections of NE at 0.3 to 3 μg/kg (28 ± 3 to 86 ± mm Hg), but these responses were not significantly affected by this dose of prazosin (\( p > 0.05 \)). Nerve mediated dilator mechanisms did not account for the different sensitivity of these responses to prazosin, since neither atropine nor propranolol modified the blockade of the nerve-mediated responses (fig. 2). Rauwolscine had no significant effect on the hindlimb pressor response to lumbar sympathetic stimulation in doses up to 100 μg/kg. At a dose of 300 μg/kg, small decreases in the responses to all frequencies of nerve stimulation occurred, but these were only significant for the response to 0.1 Hz (\( p < 0.05 \); fig. 3 left). However, rauwolscine produced a dose-dependent (10 to 300 μg/kg) decrease in the hindlimb pressor response to exogenous NE (1 μg/kg, fig. 3 right).

In ganglion and β-blocked dogs, i.v. injections of PE, NE, and guanabenz produced dose-dependent increases in hindlimb perfusion pressure (figs. 4, 5, 6). The response to PE was inhibited to a greater extent by prazosin (10 μg/kg) than by rauwolscine (10 μg/kg)
The effect of atropine and propranolol on the inhibition by prazosin of the pressor response to lumbar sympathetic stimulation in the perfused hindlimb of the dog. Black circles are the results of control experiments; black squares are the effect of prazosin 10 μg/kg; open circles represent the effect of prazosin in presence of atropine (1 mg/kg) and propranolol (0.5 mg/kg); black triangles show the effect of prazosin in the presence of atropine (1 mg/kg) and propranolol (0.5 mg/kg). Each point is the mean and standard error of four experiments.

In the experiments carried out with the cat nictitating membrane, prazosin (10 μg/kg) shifted the dose-response curve for exogenous NE and nerve stimulation to the right to approximately the same extent. On the other hand, the pressor response to NE was not significantly reduced by this dose of prazosin (fig. 8).

Discussion

In the isolated hindlimb preparation of the dog, we obtained pressor responses to guanabenz, a preferential α₁-adrenergic receptor agonist; PE, a preferential α₁-adrenergic receptor agonist; and NE, a non-selective agonist. The pressor response to guanabenz was preferentially blocked by rauwolscine, an α₂-adrenergic receptor antagonist, whereas responses to PE were preferentially blocked by prazosin, an α₁-adrenergic receptor antagonist. These results demonstrate the presence of both α₁- and α₂-adrenergic recep-

Combination of both rauwolscine and prazosin produced a greater inhibition of the response to NE than that produced by either drug alone (fig. 5). The response to guanabenz was not affected by prazosin (10 μg/kg) but was reduced by rauwolscine (10 μg/kg) and further reduced by 30 μg/kg rauwolscine (fig. 6).

Inspection of the polygraph recordings of the hindlimb pressor response to exogenous NE showed that, although the peak response was not significantly affected by prazosin, the duration of the response was shortened. Typical tracings are shown in fig. 7.

Figure 2. The effect of atropine and propranolol on the inhibition by prazosin of the pressor response to lumbar sympathetic stimulation in the perfused hindlimb of the dog. Black circles are the results of control experiments; black squares are the effect of prazosin 10 μg/kg; open circles represent the effect of prazosin in presence of atropine (1 mg/kg) and propranolol (0.5 mg/kg); black triangles show the effect of prazosin in the presence of atropine (1 mg/kg) and propranolol (0.5 mg/kg). Each point is the mean and standard error of four experiments.

Figure 3. Effect of rauwolscine on the hindlimb perfusion pressor responses to lumbar sympathetic stimulation, exogenous NE, and phenylephrine (PE). Each point is the mean and standard error of four experiments. Left graph: Black circles represent the response in the absence of antagonist; black squares, in the presence of 100 μg/kg; and black triangles, 300 μg/kg rauwolscine. Right graph: Black circles represent the inhibition of the response to NE; black triangles, the inhibition of the response to PE.
ALPHA-ADRENERGIC RECEPTORS IN VASCULAR SMOOTH MUSCLE/Langer et al.

**Figure 4.** Effect of prazosin (P, 10 ng/kg i.v.) and rauwolscine (R, 10 ng/kg i.v.) on the hindlimb perfusion pressor response to phenylephrine (PE) in ganglion and β-blocked dogs (chlorisondamine 1 mg/kg, atropine 1 mg/kg plus propranolol 0.5 mg/kg and 0.25 mg/kg/hr). Open circles represent control responses in the absence of antagonist; black circles, in the presence of prazosin (10 ng/kg); black triangles, in the presence of rauwolscine (10 ng/kg). Each point is the mean and standard error of at least five experiments.

Tors postsynaptically in the hindlimb vasculature of the dog. The response to exogenous NE was reduced by either antagonist, but required the presence of a combination of both α-adrenergic receptor blocking agents to produce a substantial blockade of the response. This observation would be consistent with the presence of two types of alpha-receptor in vascular smooth muscle, both of which are freely accessible to circulating NE. The fact that the duration of the NE response is shortened by prazosin but not the peak effect, suggests that the α1- and α2-adrenergic receptors in vascular smooth muscle may have different time courses of effect. The profile of the pressor response to NE, and the effect of prazosin upon its time course, has been studied in detail by Cavero and Lefèvre-Borg. It should be noted that the pressor response to nerve stimulation is markedly reduced by a dose of prazosin that does not significantly affect the

**Figure 5.** Effect of prazosin (P, 10 μg/kg), rauwolscine (R, 10 μg/kg), or a combination of both drugs (P + R) on the hindlimb perfusion pressor response to exogenous norepinephrine (NE), in ganglion and β-blocked dogs (chlorisondamine 1 mg/kg, atropine 1 mg/kg plus propranolol 0.5 mg/kg and 0.25 mg/kg/hr). Open circles are the responses in the absence of antagonists; black circles, in the presence of prazosin (10 μg/kg); black triangles, in the presence of rauwolscine (10 μg/kg); and black squares in the presence of both drugs. Each point is the mean and standard error of at least five experiments.
peak response to exogenously administered NE. Furthermore, the response to sympathetic nerve stimulation is resistant to blockade by rauwolscine, and the combination of both \( \alpha \)-adrenergic receptor antagonists only reduced this response to the same degree as prazosin alone. Since the neurotransmitter mediating the pressor response to sympathetic nerve stimulation is NE, which is an agonist capable of stimulating both \( \alpha_1 \)- and \( \alpha_2 \)-adrenergic receptors, it would appear that this catecholamine is liberated from nerves in such a way as to stimulate mainly \( \alpha_1 \)-adrenergic receptors.

We propose therefore that the vascular smooth muscle of the dog hindlimb contains both \( \alpha_1 \)- and \( \alpha_2 \)-adrenergic receptors. Both subtypes are accessible to blood-borne agents; however, the sympathetic vascular nerve supply innervates preferentially the \( \alpha_1 \)-subtype. The results we have presented contradict the general view that alpha-adrenergic receptor antagonists are more effective in blocking vasoconstrictor responses to exogenous NE than to nerve stimulation.\(^{24-28}\) However, our results are in complete agreement with, and explain the findings of, Levin and Beck\(^7\) and Miranda et al.\(^{28}\) that nerve-mediated pressor responses are preferentially blocked by phenoxymenzamine. These authors were not able to provide a reasonable explanation for their observations. However, our experiments using selective \( \alpha_1 \)- and \( \alpha_2 \)-adrenergic receptor agonists and antagonists clearly demonstrate that two different populations of postsynaptic \( \alpha \)-adrenergic receptors may mediate pressor responses. Noradrenergic innervation of only the \( \alpha_1 \)-type of receptor in vascular smooth muscle would explain the preferential blocking effect of prazosin, and of phenoxymenzamine too, since the latter drug has also been shown to be a preferential antagonist at \( \alpha_1 \)-adrenergic receptors.\(^6\)
Our interpretation could be complicated by two factors. First, the presence of vasodilator fibers within the sympathetic nerve supply to the hindlimb. While our results rule out any influence of cholinergic, muscarinic, and β-adrenergic receptor-mediated vasodilator effects, the possibility of other nerve-mediated dilator mechanisms exists (e.g., histamine). The second factor to be considered is the presence of presynaptic, release-modulating α₂-adrenergic receptors on the sympathetic nerve supply to the hindlimb. One might suppose that rauwolscine, by blocking these receptors, would prevent the negative feedback mediated by inhibitory presynaptic α₂-adrenergic receptors, thus increasing NE release upon nerve stimulation. The increased concentration of NE in the synaptic cleft may then compete with rauwolscine for any α₂-adrenergic receptors located postsynaptically within the synaptic cleft. However, were this the case, released NE would also compete with prazosin for α₁-receptors and this would result in a decrease of the prazosin blockade in the presence of rauwolscine; this does not occur. Thus, we might conclude that there are few postsynaptic α₂-adrenergic receptors located intrasynaptically in dog hindlimb vascular smooth muscle.

In a recent study carried out in pithed rats, Yamaguchi and Kopin²⁹ reached similar conclusions. Based on the different effectiveness of phenoxybenzamine and piperoxan on the pressor response and the change in plasma NE levels upon spinal cord stimulation, these authors concluded that in the rat the α₂-adrenergic receptor subtype predominates in the region of the vascular neuroeffector junction while the pressor effect of exogenous NE is mediated predominantly by α₁-adrenergic receptors.³⁰

To further study and characterize postsynaptic α₂-adrenergic receptors, we have used in vitro preparations of dog arteries and veins. We have confirmed with selective α₂-agonists the results of De Mey and Vanhoutte³⁰ that dog saphenous veins contain α₁- and α₂-adrenergic receptors, while dog femoral arteries contain almost exclusively α₁-adrenergic receptors. However, since the pressor response to α₂-agonists in this study is mediated via the arterial side of the circulation, one must conclude that α₂-adrenergic receptors are present postsynaptically in arteries, also. This apparent paradox may be explained by the fact that small arteries contain different receptor populations than the large conduit vessels which we have examined in vitro. To investigate this possibility, we have conducted further in vitro studies using the entire vascular bed of the isolated perfused cat spleen. In this preparation prazosin antagonizes preferentially the pressor response to nerve stimulation when compared to responses elicited by exogenous NE.³¹ Thus, these in vitro experiments support the conclusions from our in vivo studies and suggest that the α₂-receptor subtype predominates in the smaller resistance vessels.

In contrast to the results obtained with vascular smooth muscle, we have found that the responses of the smooth muscle of the cat nictitating membrane to both exogenous and endogenous NE are equally in-
hibited by prazosin (10 \( \mu g/\)kg). Thus, in this preparation both blood-borne NE and neurally released NE have access to \( \alpha_2 \)-adrenergic receptors, there being no differential blockade between responses to these two stimuli. It is of interest to note that under these experimental conditions the pressor response to exogenous NE was not affected by this dose of prazosin, confirming the results obtained in the dog and suggesting that \( \alpha_2 \)-adrenergic receptors are probably present in the vascular smooth muscle of the cat.

In conclusion, we have presented evidence that the vasculature of the dog hindlimb contains both \( \alpha_1 \)- and \( \alpha_2 \)-adrenergic receptors located postsynaptically. Furthermore, the noradrenergic innervation of this vasculature supplies the \( \alpha_1 \)-subtype predominantly if not exclusively. Blood-borne NE acts on both subtypes of \( \alpha \)-adrenergic receptors and is thus relatively resistant to prazosin blockade. Evidence from the cat nictitating membrane shows that in this nonvascular smooth muscle the response to exogenous NE is not resistant to prazosin blockade, suggesting that \( \alpha_1 \)-adrenergic receptors are not located postsynaptically in this tissue.

If our findings in the perfused dog hindlimb can be extrapolated to other vascular beds and to man, the susceptibility of nerve-mediated vasoconstrictor responses to prazosin blockade may explain the efficacy of this drug and its favorable hemodynamic profile in the treatment of hypertension.

References

15. Constantine JW, Gunnell D, Weeks RA: \( \alpha_1 \) and \( \alpha_2 \)-vascular adrenergic receptors in the dog. Eur J Pharmacol 66: 281, 1980
21. Langer SZ, Pinto JEB: Possible involvement of a transmitter different from noradrenaline in the residual responses to nerve stimulation of the cat after pressor responses with reserpine. J Pharmacol Exp Ther 196: 697, 1976
Preferential noradrenergic innervation of alpha-adrenergic receptors in vascular smooth muscle.
S Z Langer, N B Shepperson and R Massingham

Hypertension. 1981;3:I112
doi: 10.1161/01.HYP.3.3_Pt_2.I112

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1981 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/3/3_Pt_2/I112

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at: http://hyper.ahajournals.org//subscriptions/