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

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SUMMARY In the isolated perfused hindlimb preparation of the dog, pressor responses to norepinephrine (NE) are mediated by postsynaptic \( \alpha_1 \)- and \( \alpha_2 \)-adrenergic receptors. Results obtained using preferential \( \alpha_1 \)- and \( \alpha_2 \)-adrenergic receptor antagonists suggest that the \( \alpha_1 \)-subtype is predominantly innervated while both \( \alpha_1 \)- and \( \alpha_2 \)-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 \( \alpha_1 \)-adrenergic receptors postsynaptically. (Hypertension 3 (suppl I): I-112-I-118, 1981)

KEY WORDS • postsynaptic \( \alpha_1 \)- and \( \alpha_2 \)-adrenergic receptors • prazosin • rauwolscine • hind limb perfusion pressure • presynaptic \( \alpha_2 \)-adrenergic receptors

Much evidence now supports the existence of two subtypes of alpha-adrenergic receptors. Originally this subdivision was made when \( \alpha \)-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 \( \alpha \)-adrenergic receptors — an anatomical designation.

Since this original classification was made, it has been found that receptors with the characteristics of presynaptic 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 \( \alpha \)-adrenergic receptors with affinities for drugs corresponding to the original postsynaptic receptor should be called \( \alpha_1 \)-receptors, and those corresponding to the presynaptic receptor, \( \alpha_2 \). Thus, the original anatomical designation of these receptors has been replaced by a pharmacological differentiation.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 \( \alpha_1 \)-adrenergic receptor antagonist, prazosin. In the rabbit and the rat, it has been shown that \( \alpha_2 \)-adrenergic receptor agonists produce pressor responses that are blocked by selective \( \alpha_2 \)-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 \( \alpha_1 \)- and \( \alpha_2 \)-adrenergic receptors. Following our earlier work in the anesthetized dog, we investigated the pressor responses to several exogenous \( \alpha \)-adrenergic receptor agonists and sympathetic nerve stimulation to evaluate the presence and role of postsynaptic \( \alpha_2 \)-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 α₁-adrenergic receptor antagonist, was injected and the series of nerve simulations 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. Contraction 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.

Recordings from all the above experiments were made on Grass model 7D polygraphs.

**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 \( (p < 0.05, \text{fig. 3 left}) \). 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).
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 \( \mu \)g/kg; open circles represent the effect of prazosin in presence of atropine (1 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 \( \mu \)g/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 \( \alpha_1 \)-adrenergic receptor agonist; PE, a preferential \( \alpha_2 \)-adrenergic receptor agonist; and NE, a non-selective agonist. The pressor response to guanabenz was preferentially blocked by rauwolscine, an \( \alpha_2 \)-adrenergic receptor antagonist, whereas responses to PE were preferentially blocked by prazosin, an \( \alpha_1 \)-adrenergic receptor antagonist. These results demonstrate the presence of both \( \alpha_1 \)- and \( \alpha_2 \)-adrenergic recep-
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PRAZOSIN • RAUWOLSCINE

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.

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 α-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 α₁- and α₂-adrenergic receptors, it would appear that this catecholamine is liberated from nerves in such a way as to stimulate mainly α₁-adrenergic receptors.

We propose therefore that the vascular smooth muscle of the dog hindlimb contains both α₁- and α₂-adrenergic receptors. Both subtypes are accessible to blood-borne agents; however, the sympathetic vascular nerve supply innervates preferentially the α₂-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. However, our results are in complete agreement with, and explain the findings of, Levin and Beck and Miranda et al. that nerve-mediated pressor responses are preferentially blocked by phenoxybenzamine. These authors were not able to provide a reasonable explanation for their observations. However, our experiments using selective α₁- and α₂-adrenergic receptor agonists and antagonists clearly demonstrate that two different populations of postsynaptic α-adrenergic receptors may mediate pressor responses. Noradrenergic innervation of only the α₁-type of receptor in vascular smooth muscle would explain the preferential blocking effect of prazosin, and of phenoxybenzamine too, since the latter drug has also been shown to be a preferential antagonist at α₁-adrenergic receptors.
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 \( \beta \)-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 \( \alpha_2 \)-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 \( \alpha_2 \)-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 \( \alpha_2 \)-adrenergic receptors located postsynaptically within the synaptic cleft. However, were this the case, released NE would also compete with prazosin for \( \alpha_1 \) 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 \( \alpha_2 \)-adrenergic receptors located intrasynaptically in dog hindlimb vascular smooth muscle.

In a recent study carried out in pithed rats, Yamaguchi and Kopin\(^{29}\) 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 \( \alpha_2 \)-adrenergic receptor subtype predominates in the region of the vascular neuroeffector junction while the pressor effect of exogenous NE is mediated predominantly by \( \alpha_1 \)-adrenergic receptors.\(^{29}\)

To further study and characterize postsynaptic \( \alpha_2 \)-adrenergic receptors, we have used in vitro preparations of dog arteries and veins. We have confirmed with selective \( \alpha_2 \)-agonists the results of De Mey and Vanhoutte\(^{30}\) that dog saphenous veins contain \( \alpha_2 \)- and \( \alpha_1 \)-adrenergic receptors, while dog femoral arteries contain almost exclusively \( \alpha_1 \)-adrenergic receptors. However, since the pressor response to \( \alpha_2 \)-agonists in this study is mediated via the arterial side of the circulation, one must conclude that \( \alpha_2 \)-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 \( \alpha_2 \)-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

![Graph](http://hyper.ahajournals.org/)

**Figure 8.** Effect of prazosin (0.1 \( \mu g/kg \)) on the diastolic pressor response to NE and on the response of the cat nictitating membrane to nerve stimulation and exogenous NE. Black circles represent control responses in the absence of antagonists; black triangles represent responses in the presence of prazosin. Each point is the mean and standard error of four experiments. Note that prazosin blocks responses to both nerve stimulation and exogenous NE in the nictitating membrane while it fails to modify the diastolic pressor responses to exogenous NE.
hibited by prazosin (10 μg/kg). Thus, in this preparation both blood-borne NE and neurally released NE have access to α₁-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 exogenously infused NE was not affected by this dose of prazosin, confirming the results obtained in the dog and suggesting that α₁-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 α₁- and α₂-adrenergic receptors located postsynaptically. Furthermore, the noradrenergic innervation of this vasculature supplies the α₁-subtype predominantly if not exclusively. Blood-borne NE acts on both subtypes of α₁-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 α₂-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.

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