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Alpha-Adrenoreceptor Subtypes in Blood Vessels: Physiology and Pharmacology

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Summary: A significant advance in the field of neurotransmission was made with the discovery of presynaptic release-modulating alpha-adrenoreceptors on noradrenergic nerve terminals. The concept of presynaptic modulation of noradrenaline release developed in parallel with the pharmacological evidence for two subtypes of alpha-adrenoreceptors as defined by a different profile of affinity and relative order of potencies for agonists and for antagonists. The $alpha_1$-adrenoreceptor is stimulated preferentially by methoxamine and cirazoline and blocked selectively by prazosin or corynanthine. The $alpha_2$-adrenoreceptor is stimulated preferentially by agonists such as clonidine, TL-99, GHT-933, and UK-14,304, and the responses mediated by these agonists are selectively blocked by the $alpha_2$-adrenoreceptor antagonist idazoxan. In blood vessels, both the $alpha_1$- and the $alpha_2$-adrenoreceptor subtypes are present postsynaptically, where they mediate vasoconstriction, although the $alpha_1$-adrenoreceptor is the predominant receptor in vascular smooth muscle. Presynaptically on noradrenergic nerve terminals, the stimulation of inhibitory $alpha_2$-adrenoreceptors reduces the depolarization-evoked release of the transmitter. In most vascular beds, the $alpha_1$-adrenoreceptor is also the preferentially innervated subtype. In spontaneously hypertensive rats, smooth-muscle $alpha_2$-adrenoreceptors mediate vasoconstrictor responses to exogenous noradrenaline and to sympathetic nerve stimulation to a greater extent than in the corresponding normotensive Wystar-Kyoto rats, which may point to a pathophysiological role of these $alpha_2$-adrenoreceptors in hypertension. **Key Words:** $Alpha_1$-and $alpha_2$ - adrenoreceptors—Noradrenaline—Noradrenergic neurotransmission—Presynaptic receptors—Dopamine—Hypertension.

Until approximately 10 years ago, it was generally accepted that alpha-adrenoreceptors represented a homogeneous population. The discovery of presynaptic alpha-adrenoreceptors and their role in the modulation of noradrenergic neurotransmission (1–3) provided the stimulus for the subclassification of alpha-adrenoreceptors into $alpha_1$- and $alpha_2$-subtypes. This subclassification developed as a result of the pharmacological differences between presynaptic ($alpha_2$) and postsynaptic ($alpha_1$) adrenoreceptors (2).

The original proposal for the subclassification of alpha-adrenoreceptors was based on pharmacological differences observed using alpha-adrenoreceptor blocking agents (4,5) on peripheral presynaptic and postsynaptic alpha-adrenoreceptors in the perfused cat spleen (2,4,5). Subsequent work in this field, using a number of selective agonist and antagonist drugs, has confirmed the existence of $alpha_1$- and $alpha_2$-adrenoreceptor subtypes and allowed a characterization of their location and function (3,6).

In blood vessels, the presynaptic receptor, which inhibits the release of noradrenaline, corresponds to the $alpha_2$-subtype, while postsynaptically, in smooth muscle, $alpha_1$- as well as $alpha_2$-adrenoreceptors are present and both are involved with the contractile process.

The present article deals with the physiological and pharmacological relevance of the alpha-adrenoreceptor subtypes in blood vessels under normal conditions and in the hypertensive state.

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TABLE 1. *Pharmacological basis for the subclassification of alpha-adrenoreceptors*

Order of selectivity for agonists	
α_1	Methoxamine
	Amidephrine
	Cirazoline
	Phenylephrine
	Noradrenaline
	Adrenaline
	Alpha-CH_3-noradrenaline
	Dopamine
	6-F-noradrenaline
	Clonidine
	Para-aminoclonidine
	M-7
	UK-14304
	TL-99
	BHT-920
α_2	BHT-933

Alpha-adrenoreceptor agonists in their order of selectivity for the $alpha_1$- and $alpha_2$-adrenoreceptor subtypes.

M7 = [2,*N*,*N*-dimethylamino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene].

TL99 = [2,*N*,*N*-dimethylamino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene].

BHT920 = [2-amino-6-allyl-5,6,7,8-tetrahydro-4*H*-thiazolo-(4,5-d)azepine].

BHT933 = [2-amino-6-allyl-4,5,7,8-tetrahydro-6*H*-oxazolo-(5,4-d)azepine].

UK14304 = [5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline].

AGONISTIC AND ANTAGONISTIC DRUGS ACTING ON ALPHA-ADRENORECEPTOR SUBTYPES: PHARMACOLOGICAL BASIS FOR RECEPTOR SUBCLASSIFICATION

The relative order of selectivities of alpha-adrenoreceptor agonists acting on $alpha_1$- and $alpha_2$-adrenoreceptor subtypes is shown in Table 1. Methoxamine, amidephrine, and cirazoline, followed by phenylephrine, are among the most selective $alpha_1$-adrenoreceptor agonists. Whereas noradrenaline and adrenaline stimulate both subtypes of alpha-adrenoreceptors, the alpha-methyl and the 6-fluoro analogs of noradrenaline are preferential $alpha_2$-adrenoreceptor agonists (Table 1).

Among the preferential $alpha_2$-adrenoreceptor agonists which are not phenylethylamines, clonidine, para-aminoclonidine, BHT-920, BHT-933, UK-14304, and the aminotetralines M-7 and TL-99 are the most widely used drugs (Table 1). It is of interest that in these series of compounds, M-7, TL-99, and BHT-920 are also agonists at the D_2-receptor (7). Finally, although dopamine itself is a weak alpha-adrenoreceptor agonist, it is also of interest that in several experimental models, dopamine preferentially stimulates the $alpha_2$- rather than the $alpha_1$-adrenoreceptor subtype (8,9). Consequently, when the relative order of potency for dopamine, compared with noradrenaline, on the alpha-adrenoreceptor is determined, it is important to differentiate

TABLE 2. *Pharmacological basis for the subclassification of alpha-adrenoreceptors*

Order of selectivity for antagonists	
α_1	Prazosin
	Corynanthine
	WB4101
	Phentolamine
	Mianserin
	Tolazoline
	Piperoxan
	Yohimbine
	Rauwolscine
α_2	Idazoxan

Alpha-adrenoreceptor antagonists in their order of selectivity for the $alpha_1$- and $alpha_2$-adrenoreceptor subtypes.

WB4101 = (*N*-[2-(2,6-dimethoxyphenoxy)-ethyl]1,4-benzodioxane-2-methylamine).

the corresponding values for the $alpha_1$- and the $alpha_2$-adrenoreceptor subtypes.

In addition to the relative order of selectivity of the agonists, the alpha-adrenoreceptor antagonists have provided the most solid piece of evidence in support of the subclassification of the alpha-adrenoreceptor. Prazosin is a highly selective, competitive $alpha_1$-adrenoreceptor antagonist (Table 2), and corynanthine (stereoisomer of yohimbine), although less potent than prazosin, is also rather selective as a blocking agent at $alpha_1$-adrenoreceptors. This is not the case for WB4101, which is a preferential $alpha_1$-adrenoreceptor antagonist, but is less selective than the former two antagonists. Phentolamine blocks both the $alpha_1$- and the $alpha_2$-adrenoreceptor subtypes, and there are three other antagonists which belong to the group of nonselective alpha-adrenoreceptor blocking agents: mianserin, tolazoline, and piperoxan (Table 2) (although in some species, e.g., dog, piperoxan may be more selective for the $alpha_2$-adrenoreceptor). Both yohimbine and rauwolscine are preferential $alpha_2$-adrenoreceptor antagonists, while idazoxan is at present the most selective $alpha_2$-adrenoreceptor antagonist available, showing a 100-fold selectivity ratio between the $alpha_2$- and $alpha_1$-adrenoreceptors under *in vitro* conditions. Some analogs of idazoxan have recently been reported to possess an even greater selectivity ratio for the blockade of the $alpha_2$- over the $alpha_1$-adrenoreceptor subtype (10).

LOCATION AND FUNCTION OF ALPHA-ADRENORECEPTOR SUBTYPES IN BLOOD VESSELS: DIFFERENCES BETWEEN VASCULAR BEDS

The neuroeffector junction at the adventitial medial border in vascular smooth muscle is shown schematically in Fig. 1. The alpha-adrenoreceptor subtype located presynaptically on noradrenergic nerve terminals and mediating an inhibition of nor-

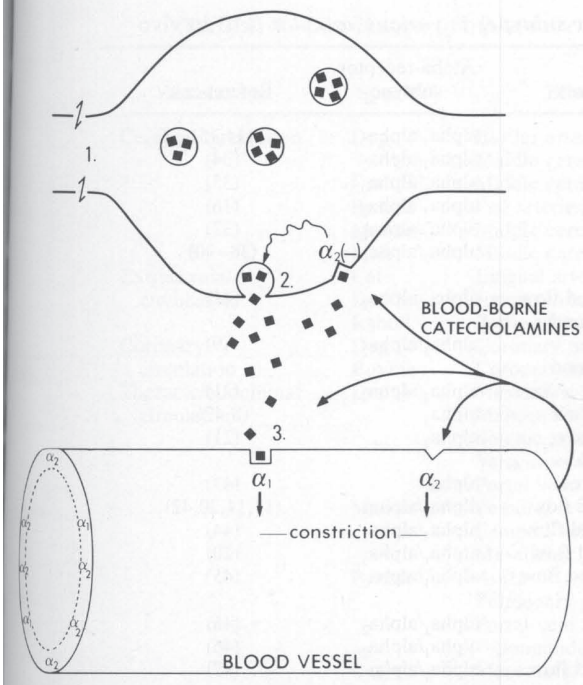

FIG. 1. Alpha-adrenoreceptor subtypes in blood vessels. Schematic representation of a noradrenergic varicosity of the post-ganglionic sympathetic neuron innervating vascular smooth muscle. Electrical stimulation (1) leads to the exocytotic release (2) of noradrenaline (squares) that crosses the junctional cleft to occupy (3) vascular alpha$_1$-adrenoreceptors (squares indent) mediating vasoconstriction. Neuronally released noradrenaline may act at prejunctional alpha$_2$-adrenoreceptors to inhibit the quantity of transmitter subsequently released per impulse. Postjunctional alpha$_2$-adrenoreceptors mediate vasoconstriction and appear to be predominantly extrasynaptic. When postjunctional alpha$_2$-adrenoreceptors are stimulated by exogenously administered agonists or by circulating catecholamines, a vasoconstrictor response is elicited.

adrenaline release corresponds to the alpha$_2$-subtype of adrenoreceptor. It is generally accepted that the presynaptic alpha$_2$-adrenoreceptor is the target for exogenously administered agonists which reduce transmitter release (e.g., clonidine), but the physiological role of the presynaptic alpha$_2$-adrenoreceptor in modulating noradrenaline release is still controversial (11). Depending on the frequency of nerve impulses and on the duration of depolarization of noradrenergic nerves, alpha$_2$-adrenoreceptor antagonists enhance the overflow of noradrenaline during nerve stimulation. This indicates that there is an operational negative feedback mechanism through which the released transmitter noradrenaline can autoregulate its own release, once a threshold concentration of noradrenaline is achieved in the synaptic cleft (12).

Postsynaptically, in the vascular smooth muscle, the alpha$_1$-adrenoreceptor is the predominant receptor mediating vasoconstriction, but there are also postsynaptic alpha$_2$-adrenoreceptors which mediate vasoconstriction in arterial as well as in venous vascular smooth muscle (Fig. 1, Tables 3,4).

With the exception of the cerebral vascular bed, where the postsynaptic alpha$_2$-adrenoreceptor appears to be the predominant receptor in some of the species examined (Table 4), in most other peripheral vascular beds it is either the alpha$_1$-adrenoreceptor that predominates or both postsynaptic alpha$_1$- and alpha$_2$-adrenoreceptors that are present (Tables 3,4).

It is of interest that, in the renal vascular bed, there are almost exclusively alpha$_1$-adrenoreceptors, which subserve vasoconstriction, whereas in the femoral and mesenteric vascular beds, both alpha$_1$- and alpha$_2$-adrenoreceptors appear to mediate vasoconstriction (Tables 3,4). Not only do the relative proportions of postsynaptic alpha$_1$- and alpha$_2$-adrenoreceptors vary as a function of the vascular bed, but within a given vascular bed it is also possible that the proportions of alpha$_1$- and alpha$_2$-adrenoreceptors vary with the diameter of the blood vessel. Additional studies are required to establish the presence as well as the density of postsynaptic alpha$_1$- and alpha$_2$-adrenoreceptors in the smooth muscle of arteries and veins as a property of the vascular bed and also in relation to the caliber of the blood vessels. Finally, as shown in Tables 3 and 4, it appears that there may be considerable species variations in the relative contributions of alpha$_1$- and alpha$_2$-adrenoreceptor subtypes to vasoconstriction in the different vascular beds. Therefore, one should be cautious in extrapolating results from one species to another.

PREFERENTIAL NORADRENERGIC INNERVATION OF THE ALPHA-ADRENORECEPTOR SUBTYPE IN BLOOD VESSELS

A number of studies have been carried out under *in vivo* as well as *in vitro* experimental conditions, in which the effectiveness of alpha-adrenoreceptor antagonists was compared with respect to blocking the responses to exogenous noradrenaline and to the endogenously released transmitter (13–23).

In all these studies, the alpha$_1$-selective adrenoreceptor antagonist prazosin was more effective in blocking the responses to noradrenaline released by nerve stimulation than in antagonizing the same end-organ responses induced by exogenous noradrenaline (13–23). Similar results were obtained with the use of other alpha$_1$-selective antagonists (18). These results were interpreted as follows: exogenous noradrenaline can activate both alpha$_1$- and alpha$_2$-adrenoreceptors mediating vasoconstriction (Table 1, Fig. 1), whereas the transmitter released by nerve stimulation elicits vasoconstric-

TABLE 3. *Distribution of alpha-adrenoreceptor subtypes in various vascular beds* in vivo

Vascular bed	Species	Experiments	Alpha-receptor subtype	References
Whole animal Blood pressure	Dog	Anesthetized	$alpha_1/alpha_2$	(14,15)
		Pithed	$alpha_1/alpha_2$	(34)
	Cat	Anesthetized	$alpha_1/alpha_2$	(35)
	Rabbit	Pithed	$alpha_1/alpha_2$	(36)
		Conscious	$alpha_1/alpha_2$	(37)
	Rat	Pithed	$alpha_1/alpha_2$	(38–40)
Coronary circulation	Dog	Circumflex Coronary blood flow	$alpha_1/alpha_2$	(41)
Thoracic/abdominal circulation	Dog	Mesenteric blood flow	$alpha_1/alpha_2$	(9)
	Cat	Mesenteric blood flow	$alpha_1/alpha_2$	(21)
Renal vasculature	Dog	Renal blood flow	$alpha_1$	(8,42)
	Cat	Renal blood flow	$alpha_1$	(21)
	Rat	Renal blood flow (microspheres)	$alpha_1$	(43)
Femoral/hindlimb circulation	Dog	Femoral blood flow	$alpha_2/alpha_1$	(13,14,20,42)
	Dog	Forelimb blood flow	$alpha_1/alpha_2$	(44)
	Cat	Femoral blood flow	$alpha_1/alpha_2$	(20)
	Rabbit	Hindlimb blood flow	$alpha_1/alpha_2$	(45)
	Rat	Hindquarter perfusion	$alpha_1/alpha_2$	(46)
		Hindlimb	$alpha_1/alpha_2$	(46)
	Man	Forearm blood flow	$alpha_1/alpha_2$	(47)

The references quoted refer to the positive identification of postsynaptic $alpha_1$- or $alpha_2$-adrenoreceptor subtypes in these vascular beds *in vivo*.

tion through the activation of $alpha_1$-adrenoreceptors (14,15). This point is exemplified *in vitro* in Sprague-Dawley rat-tail arteries (Fig. 2), since both prazosin and the $alpha_1$-selective diastereoisomer of yohimbine, corynanthine (19,24), are particularly potent and effective antagonists of the response elicited by electrical field stimulation (for prazosin a concentration of only 1 n*M* virtually abolished the vasoconstrictor response to sympathetic nerve stimulation (Fig. 2). In the vascular beds examined thus far, it appears that the $alpha_1$-adrenoreceptor is the preferentially innervated receptor, and it has been demonstrated that the neuronally mediated vasoconstriction is exquisitely sensitive to blockade by prazosin (14,15,18,20,21).

It is therefore possible that the $alpha_1$-adrenoreceptor predominates in the adventitial-medial border, where most of the noradrenergic nerve terminals are present, whereas the postsynaptic $alpha_2$-adrenoreceptors are located mainly near the intima (Fig. 1) and therefore may be the target of circulating catecholamines (16). However, one should be cautious in generalizing about this arrangement in blood vessels, because the preferential noradrenergic innervation of the $alpha_1$-adrenoreceptor may also vary among vascular beds and with the diameter of the blood vessels. In addition, there may be differences between arteries and veins with respect to the alpha-adrenoreceptor subtype which is preferentially innervated; studies concerning this question are presently underway in several laboratories.

In recent years, a number of electrophysiological studies have been carried out on arteriolar preparations, in which the excitatory junctional potentials (EJPs) have been recorded in response to low-frequency stimulation of the sympathetic nerves to the blood vessel (25,26). Although the EJPs are thought to be mediated by release of noradrenaline in these vessels, the electrophysiological responses remain resistant to blockade with all alpha-adrenoreceptor antagonists (25–27). It is clearly premature to ascribe these phenomena to a new type of receptor [e.g., gamma-receptor (25)] in the absence of an antagonist which blocks the EJPs (27). Nevertheless, the phenomenon should not be dismissed, since these determinations represent a physiological measurement of the transmitter-receptor interaction, although it does not seem to involve alpha-adrenoreceptors.

CONTRIBUTION OF POSTSYNAPTIC $ALPHA_1$- AND $ALPHA_2$-ADRENORECEPTORS TO VASOCONSTRICTOR RESPONSES IN SPONTANEOUSLY HYPERTENSIVE RATS

Postsynaptic $alpha_1$- and $alpha_2$-adrenoreceptors mediate vasoconstriction in both hypertensive rats [spontaneously hypertensive (SHR) and DOCA-salt] and in normotensive controls (WKY) (28). Under *in vivo* conditions, in pithed SHR or WKY, the intravenous administration of $alpha_1$-selective agonists, such as phenylephrine, or the $alpha_2$-selective agonists (TL-99) (29) (Fig. 3), and BHT-993 (30) causes similar increases in blood pressure, with

TABLE 4. *Distribution of alpha-adrenoreceptor subtypes in isolated blood vessels*

Vascular bed	Species	Blood vessels	Alpha-receptor subtype	References
Cerebral circulation	Dog	Basilar artery	$Alpha_2/alpha_1$	(48)
		Middle cerebral	$Alpha_2$	(49)
	Cat	Middle cerebral	$Alpha_2$	(50,51)
	Bovine	Pial arteries	$Alpha_2 >> alpha_1$[a]	(52)
	Monkey	Middle cerebral	$Alpha_1$	(49)
	Man	Middle cerebral	$Alpha_1$	(49)
Extracranial circulation	Cat	Lingual artery	$Alpha_1 > alpha_2$	(51)
	Dog	Jugular vein	$Alpha_1 > alpha_2$	(53)
	Rabbit	Perfused ear artery	$Alpha_1/alpha_2$	(54)
Coronary circulation	Dog	Coronary artery	$Alpha_1$	(55–57)
	Bovine	Coronary artery	$Alpha_1 >> alpha_2$[a]	(58)
Thoracic/abdominal circulation	Dog	Mesenteric artery	$Alpha_1/alpha_2$	(57)
		Mesenteric vein	$Alpha_1/alpha_2$	(53)
		Splenic artery	$Alpha_1$	(59)
		Vena cava	$alpha_1$	(53)
		Portal vein	$Alpha_1$	(53)
	Man	Femoral artery	$Alpha_1$	(61)
		Femoral vein	$Alpha_2/alpha_1$	(61)
		Mesenteric/jejunal arteries	$Alpha_1$	(60)
	Rabbit	Aorta	$Alpha_1$	(62)
		Pulmonary artery	$Alpha_1$	(63)
		Portal vein (longitudinal muscle)	$Alpha_1$	(62)
	Cat	Mesenteric artery	$Alpha_1$	(51)
		Perfused spleen	$Alpha_1/alpha_2$	(64)
	Rat	Mesenteric bed	$Alpha_1 >> alpha_2$	(65)
		Thoracic aorta	$Alpha_1$	(66)
			$Alpha_1 >> alpha_2$	(67)
		Portal vein (longitudinal muscle)	$Alpha_1$	(66,68)
		Portal vein (phasic activity)	$Alpha_1/alpha_2$	(68)
Renal vasculature	Dog	Renal vein	$Alpha_1$	(53)
	Rat	Perfused kidney	$Alpha_1$	[c]
		Renal artery	$Alpha_1$	[c]
Femoral/hindlimb circulation	Dog	Femoral artery	$Alpha_1$	(59)
		Femoral vein	$Alpha_1/alpha_2$	(59)
		Saphenous vein	$Alpha_1/alpha_2$	(69,70)
	Rabbit	Saphenous vein	$Alpha_1/alpha_2$	(71)
		Saphenous artery	$Alpha_1$	(72)
	Rat	Tail artery (SHRSD)	$Alpha_1/alpha_2$	(22,23,75)
		Tail artery (WKY)	$Alpha_1$	(22,23)
	Man	Digital arteries	$Alpha_2$[b]	(60,73)
		Metacarpal veins	$Alpha_1/alpha_2$[b]	(73)
		Umbilical arteries	No evidence found	(74)

[a] Experiments on receptor binding.
[b] Alpha-receptor subtype may not conform with classical $alpha_1$- or $alpha_2$-adrenoreceptor subtype.
[c] Hicks, unpublished observations.
The references quoted refer to the positive identification of postsynaptic $alpha_1$- or $alpha_2$-adrenoreceptor subtypes in these isolated blood vessels.

no apparent difference in sensitivity between the normotensive and the hypertensive rats (Fig. 3). In this study, both phenylephrine and noradrenaline caused greater maximal vasoconstrictor responses in SHR, which is probably related to the adaptive structural changes (increased wall/lumen ratio) known to occur in hypertension (31), since similar effects were observed with angiotensin II (28). Furthermore, in pithed rats, there was no apparent change in the potency of yohimbine to block $alpha_2$-adrenoreceptor-mediated vasoconstriction (28) in SHR as compared with WKY.

Despite the overwhelming evidence for the presence *in vivo* of post-synaptic $alpha_2$-adrenoreceptors which mediate vasoconstriction, it has been particularly difficult to demonstrate their presence in arterial smooth muscle *in vitro*. It is most likely that only arteriolar constriction, and not an influence on the venous circulation, accounts for the changes in blood pressure, blood flow, and vascular resistance (32) which are observed *in vivo* in response to $alpha_2$-adrenoreceptor agonists.

In vitro, the perfused/superfused tail artery of SHR appears to contain, in addition to the well-established $alpha_1$-adrenoreceptor, a population of postsynaptic $alpha_2$-adrenoreceptors which me-

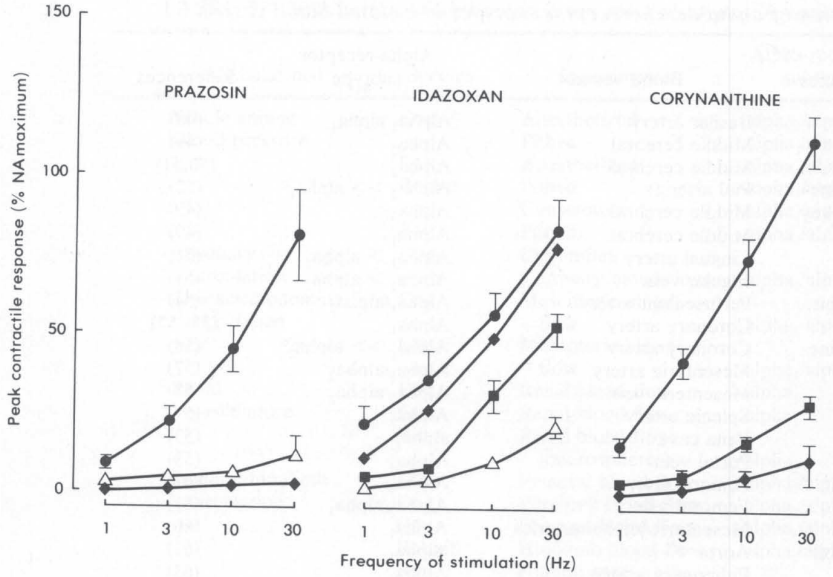

FIG. 2. Antagonism produced by alpha-adrenoreceptor antagonists of the vasoconstrictor responses induced by electrical field stimulation in the isolated perfused rat-tail artery (Sprague-Dawley). Ordinate, peak contractile responses to electrical stimulation expressed as percentage of the maximal response to noradrenaline (NA). Abscissae, frequency of stimulation (Hz), monophasic square-wave pulses of 0.3 ms duration. Supramaximal voltage. **Left panel,** (●) controls; prazosin (△)1 n*M*, (◆)100 n*M*. **Middle panel,** (●) controls; idazoxan (◆)100 nM, (■)1 μ*M*, (△)10 μ*M*. **Right panel,** (●) controls; corynanthine (■) 1 μ*M*, (◆)10 μ*M*. Incubation time with each antagonist was 20 min. Cocaine (4 μ*M*) and propranolol (1 μ*M*) were present in the Krebs' perfusion medium. Vertical bars indicate mean ± SEM for at least four experiments. Data taken from reference 75.

diate vasoconstriction in this vessel (22,23). Responses induced by noradrenaline and the alpha$_2$-adrenoreceptor agonist TL-99 are antagonized by the selective alpha$_2$-adrenoreceptor antagonist idazoxan (22,23) at concentrations which do not antagonize responses induced by the alpha$_1$-adrenoreceptor agonist methoxamine (Fig. 4). This antagonistic effect of low concentrations of idazoxan has not been observed in tail arteries from WKY (Fig. 4.)

Similarly to the vasoconstrictor responses provoked by exogenous noradrenaline, those mediated by electrical field stimulation in SHR tail arteries were significantly greater than those obtained in WKY arteries (22,23) (Fig. 5). The alpha$_1$-adrenoreceptor is the predominant subtype in both SHR and WKY tail arteries. Although prazosin was a very potent antagonist of the responses to sympathetic nerve stimulation, a significant antagonism of the neuronally-mediated vasoconstriction could also be demonstrated in tail arteries of SHR with idazoxan (Fig. 5) at concentrations which do not block alpha$_1$-adrenoreceptor-mediated constriction. This profile of antagonism, with low concentrations

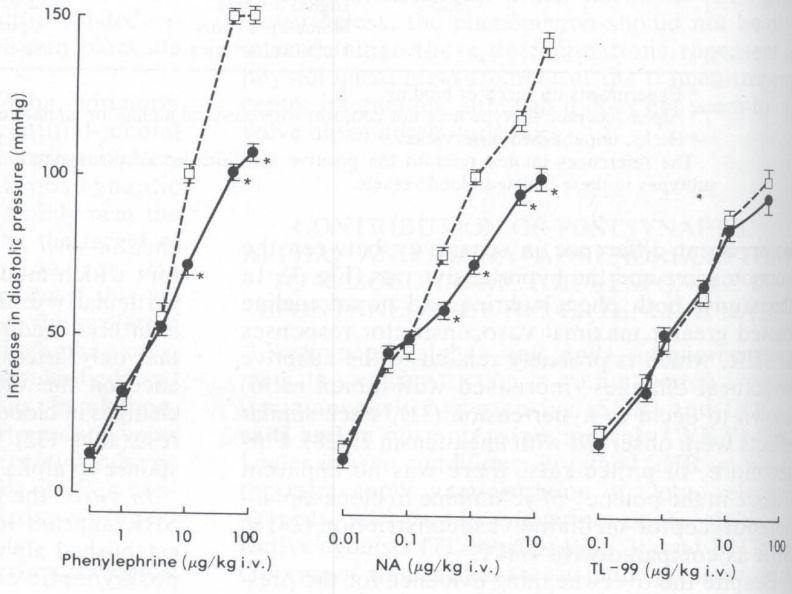

FIG. 3. Vasoconstrictor responses induced by alpha-adrenoreceptor agonists in pithed (SHR) or normotensive WKY. SHR and WKY were selected on the basis of systolic blood-pressure measurements (tail-cuff method in conscious rats). SHR were used if the systolic pressure exceeded 185 mm Hg; WKY were only used if systolic blood pressure was less than 140 mm Hg. Basal diastolic blood pressures of these rats 30 min after pithing were not significantly different (SHR = 39 ± 4 mm Hg, n = 27; WKY = 42 ± 3 mm Hg, n = 28). Ordinate, increase in diastolic blood pressure (mm Hg). Abscissae, log-dose agonists (μg/kg i.v.). **Left panel,** phenylephrine. **Middle panel,** noradrenaline (NA). **Right panel,** TL-99. ●—●, response curves obtained in WKY; □---□, response curves obtained in SHR. * $p < 0.05$, significantly different from SHR data. Vertical bars indicate mean ± SEM. Data taken from reference 28.

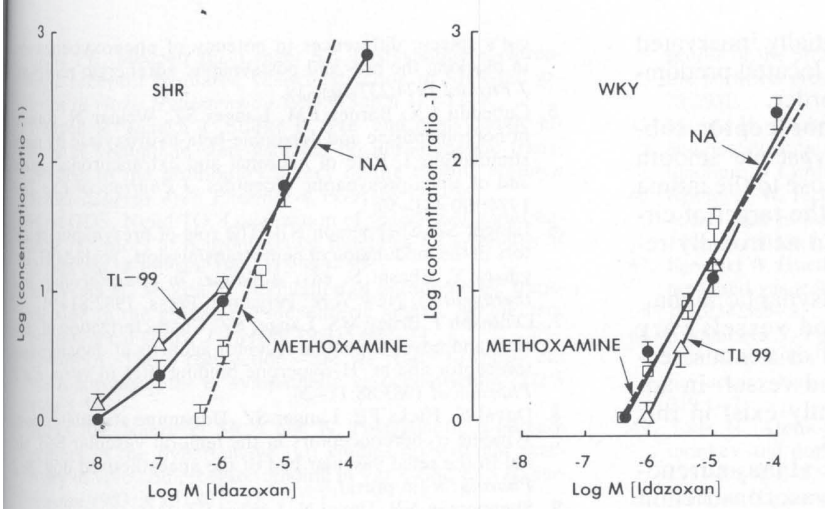

FIG. 4. Antagonistic effects of idazoxan against vasoconstrictor responses induced by alpha-adrenoreceptor agonists in perfused tail arteries from SHR or WKY normotensive controls. Schild plots for idazoxan against TL-99 (△—△), noradrenaline (NA) (●—●), or methoxamine (□—□) in SHR tail arteries **(left panel)** or WKY tail arteries **(right panel).** Ordinate, log (concentration ratio^{-1}). Abscissae, log molar concentration of idazoxan. Vertical bars indicate mean ± SEM. n = at least four experiments for each point. Note that idazoxan was significantly more potent as an antagonist of responses evoked by TL-99 or noradrenaline than against methoxamine in SHR tail arteries, but was equipotent against these three agonists in WKY tail arteries.

of idazoxan, was not demonstrated for the vasoconstriction elicited by nerve stimulation in WKY tail arteries (Fig. 5).

Idazoxan has been shown to act as a partial agonist at alpha$_2$-adrenoreceptors in perfused rabbit-ear arteries (33); however, we have now demonstrated that low concentrations of idazoxan, which inhibit end-organ responses to field stimulation, do not reduce the electrically evoked overflow of ^{3}H-noradrenaline from SHR tail arteries (Langer and Hicks, unpublished observations). We therefore conclude that, in the SHR-tail artery preparation, a significant population of postsynaptic alpha$_2$-adrenoreceptors can be demonstrated. Furthermore, in hypertensive animals, these alpha$_2$-adrenoreceptors may also be activated by endogenously released noradrenaline to elicit vasoconstriction. The difficulties in demonstrating changes in post-synaptic alpha$_2$-adrenoreceptor-mediated vasoconstriction *in vivo* (involving the total circulation) emphasize the necessity to evaluate these mechanisms in localized vascular beds, where the postsynaptic alpha$_1$- and alpha$_2$-adrenoreceptor subtypes are likely to play an important role in the modulation of regional blood flow. Such studies in regional vascular beds in hypertensive models may provide valuable information on the role of vascular alpha$_2$-adrenoreceptors in the development or maintenance of hypertension.

CONCLUSIONS

There is now a wealth of experimental evidence in support of the subclassification of alpha-adrenoreceptors into alpha$_1$- and alpha$_2$-subtypes.

The alpha$_1$-adrenoreceptor subtype, linked to vasoconstriction, is the predominant postsynaptic receptor in vascular smooth muscle and in most blood

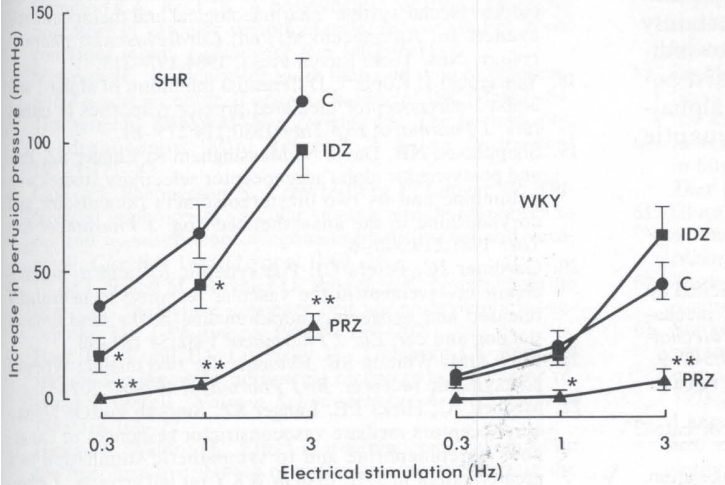

FIG. 5. Blockade by the alpha-adrenoreceptor antagonists idazoxan or prazosin of the responses induced by electrical field stimulation of the isolated perfused tail artery preparation from SHR, or normotensive controls WKY. Ordinate, increase in perfusion pressure (mm Hg). Abscissae, frequency of stimulation (Hz), monophasic square-wave pulses of 0.3 ms duration, supramaximal voltage. Control (C) frequency-response curves (●—●). Antagonistic effects of idazoxan (IDZ) (■—■; 10 n*M;* 20 min preincubation, n = 6), or prazosin (PRZ) (▲—▲; 10 n*M;* 20 min preincubation, n = 6) in SHR **(left panel)** and WKY **(right panel).** Vertical bars indicate mean ± SEM. *$p < 0.05$; **$p < 0.01$, significantly different from control data. Note that idazoxan (10 n*M*) significantly reduced the responses to field stimulation in SHR at 0.3 and 1 Hz, whereas it did not affect the corresponding responses in WKY normotensive tail arteries. Data taken from reference 23.

vessels, appears to be the preferentially innervated receptor. This subtype seems to be located predominantly in the medial adventitial border.

The postsynaptic alpha$_2$-adrenoreceptor subtype also mediates contraction of vascular smooth muscle and appears to be located close to the intima of blood vessels, where it may be the target of circulating catecholamines rather than neuronally released noradrenaline.

The relative proportions of postsynaptic alpha$_1$- and alpha$_2$-adrenoceptors in blood vessels vary with the vascular bed, the arterial or venous sections, and the diameter of the blood vessel. In addition, species differences apparently exist in this proportion in certain vascular beds.

In SHR there is a postsynaptic alpha$_2$-adrenoreceptor-mediated component of vasoconstriction to both exogenous and endogenous noradrenaline. On the other hand, alpha$_2$-adrenoreceptors in vascular smooth muscle may contribute significantly less to vasoconstriction in normotensive WKY rats. These results suggest that postsynaptic vascular alpha$_2$-adrenoreceptors mediating vasoconstriction may play an important role in the pathophysiology of hypertension and contribute to the increased vascular reactivity to noradrenaline observed in hypertensive states.

At a presynaptic level, inhibitory alpha$_2$-adrenoreceptors are present on noradrenergic nerve terminals, and their activation leads to a reduction in the output of noradrenaline during nerve stimulation. The physiological role of presynaptic inhibitory alpha$_2$-adrenoreceptors in the modulation of noradrenergic neurotransmission depends on the frequency and duration of depolarization and on the presence of a certain threshold concentration of noradrenaline in the synaptic cleft. From the pharmacological point of view, the presynaptic inhibitory alpha$_2$-adrenoreceptors are the target of action of alpha$_2$-adrenoreceptor agonists like clonidine, which inhibit noradrenergic neurotransmission.

The pharmacological profiles of presynaptic and postsynaptic alpha$_2$-adrenoreceptors are certainly similar and, as yet, there is little evidence to indicate that pharmacological differences may exist between the presynaptic, release-modulating alpha$_2$-adrenoreceptors and those on the postsynaptic vascular smooth muscle.

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Discussion

Kreye: I should like to comment on your idea of a differential distribution of alpha-adrenoreceptors in the vessel wall, i.e., $alpha_1$ being located predominantly in the outer media and $alpha_2$ predominantly in the inner media. Ten years ago, Dr. Keatinge reported that the inner media is more sensitive to norepinephrine than the outer media is. He suggested that the higher sensitivity of the inner media to the agonist might compensate for the lack of innervation of that zone. His finding is in contrast with your assumption of a preferential location of $alpha_1$-adrenoreceptors in the outer media.

Langer: The view of a preferential innervation of $alpha_1$-adrenoreceptors by noradrenergic nerves is only valid if, postsynaptically, there are both receptor subtypes. In vascular beds with $alpha_1$-adrenoreceptors only, such as the renal bed, the only receptor that can be innervated is the one that is present. On the other hand, when there are $alpha_1$- and $alpha_2$-adrenoreceptors, it depends on which vascular bed you are dealing with. Talking about preferential innervation of the $alpha_1$-adrenoreceptors implies that a vascular bed is studied where both $alpha_1$- and $alpha_2$-adrenoreceptors are present postsynaptically and elicit contractile responses. At the time you mentioned, the idea of $alpha_1$- and $alpha_2$-subtypes had not yet been developed and documented. I think that, provided we are dealing with a tissue where the smooth muscle has both $alpha_1$- and $alpha_2$-adrenoreceptors, the blockade by selective antagonists would be the answer to the question: Are both responses to norepinephrine equally blocked by prazosin, or are they selectively and preferentially blocked by prazosin or idazoxan?

Bühler: If one transfers this concept to the clinical setting, there are some questions. One: Does the plasma concentration of norepinephrine or epinephrine suffice to activate post-junctional $alpha_2$-adrenoreceptors in the intima? Two: Does norepinephrine get to the $alpha_2$-adrenoreceptor? Dr. Vanhoutte has some indication that norepinephrine is being metabolized in the endothelial cell.

Langer: First of all, the plasma concentrations of both catecholamines are exceedingly low, they are in the pg/ml level. They may increase to the ng/ml level, e.g., under stress conditions. Thus, at best, one may have a situation in which contractions are elicited, if these $alpha_2$-adrenoreceptors are close to the intima and are sufficiently sensitive to circulating catecholamines. If, of the two catecholamines that are present in the circulation, one (norepinephrine) is metabolized by the cells of the intima, it may not reach the smooth muscle. If this is not the case for epinephrine, then this compound would have the physiological role of acting on these $alpha_2$-adrenoreceptors that are not innervated and probably located more closely to the intima.

Daniel: In your experiments on the coronary artery, there was an apparent predominance of $alpha_2$-adrenoreceptors *in vivo* and a predominance of $alpha_1$-adrenoreceptors *in vitro*. I should like to know what model you used to explain these differences.

Langer: When you are looking at changes in perfusion flow under *in vivo* conditions, you are dealing with the whole of the vascular bed and particularly with the small vessels, which contribute the major part to the responses. The small decrease in flow, elicited with norepinephrine and epinephrine under these conditions, in which you block all other receptors, is mediated by $alpha_2$-adrenoreceptors. On the other hand, when the large circumflex coronary artery is set up *in vitro* and contractions are elicited by the exogenous agonist, then you are dealing only with the large vessel, which responds with a contraction mediated by an overwhelming majority of $alpha_1$-adrenoreceptors. Hence, we presume that, as you go from the large to the small and to the precapillary vessels, there is a change in the relative proportion of $alpha_1$- and $alpha_2$-adrenoreceptors in this particular vascular bed. This does not mean that every single vascular bed would have the same sequence of changes in density of alpha-adrenoreceptor subtypes as a function of diameter. Yet, it appears to be the case for the coronary vessels, but certainly not for the renal vascular bed, because, by intraarterial injections of agonists *in vivo*, we could not elicit vasoconstriction that would be sensitive to $alpha_2$-antagonistic drugs.

Kobinger: I am afraid we get into methodological difficulties if we compare the alpha$_1$- and alpha$_2$-adrenoreceptor subtypes under *in vitro* and *in vivo* conditions, respectively. Under reserpine pretreatment, we were not able to show alpha$_2$-agonistic effects in large arteries, but we succeeded in small arteries. Thus, I think it is not correct to compare *in vitro* results with big arteries to *in vivo* results with small arteries.

Langer: I fully agree that it is exceedingly difficult to demonstrate, *in vitro,* alpha$_2$-adrenoreceptors mediating constriction. Yet, it has been achieved with cerebral vascular vessels. If you take a vascular bed in which you can elicit responses *in vivo* that are partly sensitive to alpha$_2$-blockade and you do not see this when you take the large arteries *in vitro,* then I think one of the possible interpretations might be that there is a difference in the density of the subtypes of alpha-adrenoreceptors, related to the diameter of the blood vessel. Furthermore, under *in vitro* conditions, there are tissues—and I am now talking about the rat-tail artery—where the pA$_2$ values are unequivocally in favor of a subpopulation of alpha$_2$-adrenoreceptors mediating constriction. We are still at the beginning of the characterization of the alpha-adrenoreceptor subtypes in different vascular beds, in arterial versus venous vascular beds, and of comparing the conditions *in vivo* and *in vitro*. There are still many open questions but, in some cases, you can get evidence for the presence of vascular alpha$_2$-adrenoreceptors, *in vitro* one of them being the cerebral vascular bed.

Hoefke: It is difficult to differentiate between alpha$_1$- and alpha$_2$-adrenoreceptors in isolated vessels under *in vitro* conditions. Schumann and Lues (*Naunyn-Schmiedebergs Arch Pharmacol* 1983; 323:328–34) could differentiate only between alpha$_1$- and alpha$_2$-adrenoreceptors in isolated rabbit saphenous veins after addition of angiotensin II. They concluded that post-junctional alpha$_2$-adrenoreceptors require the blood-borne angiotensin for being demonstrated.

Langer: There are several tricks, such as reserpine pretreatment or the addition of angiotensin II, to sensitize alpha$_2$-adrenoreceptors. But if one is really trying to see what is happening under the classical experimental conditions, then one must absolutely respect the rules of the game and look at the alpha$_1$- under the same conditions as one looks at the alpha$_2$-adrenoreceptors. I believe that, unless you have clear pA$_2$ determinations, you are not entitled to say that there is an alpha$_2$-mediated contraction, either because you look only at single agonist responses or because you examine a single concentration of the antagonist. You have to deal at least with several concentrations of antagonists, over a range of no less than 100-fold, and naturally with full dose-response curves to the agonist. I insist that pA$_2$ values are essential, and one should not be misled by results from single concentrations of antagonists.

Berkowitz: You showed a frequency-response curve of the tail artery, and a greater response in the SHR than in the WKY. You interpreted this reponse as being receptor-mediated, because you could also show a shift with exogenous norepinephrine, with a greater response in the SHR than in the WKY. Is that correct?

Langer: Not quite. I said that the response was receptor-mediated to the extent that it was exquisitely sensitive to prazosin, as exogenous norepinephrine is, the difference being that, in WKY and SHR, prazosin is equally effective, but idazoxan is not. In other words, idazoxan does not inhibit the responses to nerve stimulation in the WKY tail arteries; it only potentiates these responses, probably because of its presynaptic alpha$_2$-blockade that enhances release. On the other hand, the same concentration of idazoxan reduces significantly the responses to nerve stimulation in the SHR, in spite of its presynaptic effect on release, and that could only be due to blockade of the post-synaptic alpha$_2$-adrenoreceptor component. Hence, there is an alpha$_2$-component in the vasoconstriction elicited by electrical stimulation in the SHR, but not in the WKY rat-tail artery.

Berkowitz: The increased concentration of norepinephrine in the vascular nerves of the SHR compared with that of the WKY should give you an enhanced response to electrical stimulation. This increased vascular concentration of norepinephrine in the SHR is one of the factors causing a difference, not only the receptor activity. When you put in cocaine, you see a shift to the left after electrical stimulation, which is exactly what you would expect if norepinephrine release were enhanced in the SHR.

Langer: Yes, but the experiments with the antagonist were all carried out in the presence of cocaine.

Berkowitz: But you said you got a shift to the left after cocaine. Is there any direct evidence for increased alpha$_2$-adrenoreceptor density by binding studies?

Langer: Yes. If you look at ^{3}H-clonidine binding, there is an increase in the B_{max} of the recognition sites for ^{3}H-clonidine in SHR as compared with WKY tissues. This has been done in the brain and in the smooth muscle of rat-tail arteries by Webb. These results would be consistent with our hypothesis. Thus, it turns out that the available evidence, which was published recently by Webb et al. (*J Pharmacol Exp Ther* 1983;225:599–605) would fully support this view. In fact, they find also an increased density of ^{3}H-clonidine binding sites in platelets of SHR. Furthermore, there are the results of Pettinger, obtained with alpha$_2$-adrenoreceptor binding in the kidney. He has shown that, in the hypertensive state, you have an increased number of binding sites for ^{3}H-clonidine in the kidney of

SHR as compared with that of WKY, and the same is the case in the DOCA-salt model. All findings follow the same direction: increased alpha$_2$-adrenoreceptor involvement in hypertension.

Marshall: Idazoxan has become the standard antagonist for classifying alpha$_2$-adrenoreceptors, but it is also a partial agonist at some alpha$_1$-adrenoreceptors (Paciorek PM, Shepperson NB. *Br J Pharmacol* 1983;79:12–4) and alpha$_2$-adrenoreceptors (Limberger N, Starke K. *Naunyn-Schmiedebergs Arch Pharmacol* 1983;324:75–8). Do these properties complicate the interpretation of your results with idazoxan? Second, blockade of presynaptic alpha$_2$-adrenoreceptors by idazoxan will increase the release of norepinephrine, which will offset the blockade of post-junctional alpha$_2$-adrenoreceptors mediating vasoconstriction. The potential difference between pre- and post-junctional alpha$_2$-adrenoreceptors [Alabaster VA, Peters CJ. *Br J Pharmacol* 1984;81(suppl):(in press).] may help develop more selective compounds.

Langer: It is clearly established that idazoxan has partial agonist properties, because the compound produces vasoconstriction *in vivo*. If you have very low frequencies of stimulation, you can demonstrate partial agonist effects on the presynaptic alpha$_2$-adrenoreceptors at least in the rabbit-ear artery preparation, as shown by Starke. However, in the rat-tail artery, we were unable to demonstrate any agonist action at the concentrations of idazoxan used, either on alpha$_1$- or on alpha$_2$-adrenoreceptors. Whether alpha$_2$-adrenoreceptors presynaptically modulating norepinephrine release and those post-synaptically mediating contraction of vascular smooth muscle are identical pharmacologically or whether there are any differences, I do not know. Yet, I should like to see them show pharmacological differences, because that would be interesting from the point of view of drug discovery. We should, however, not ignore the fact that, for a large number of agonists and for a number of antagonists, the profile is very similar, if not identical. Hence, there is no convincing evidence to indicate that there are pharmacological differences between the presynaptic and the postsynaptic alpha$_2$-adrenoreceptors.

Daniel: I should like to return to the question of binding. We have just completed a study of binding in the SHR and WKY using plasma-membrane-enriched preparations of mesenteric arteries. We have found that you can differentiate quite clearly the binding of prazosin from that of yohimbine. ^{3}H-prazosin binding is not displaced by yohimbine, except at very high concentrations, and ^{3}H-yohimbine binding is not displaced by prazosin. We cannot so far distinguish the ability of any agonist to displace yohimbine or prazosin binding; we have tried phenylephrine, clonidine, naphazoline, etc. There was no significant change in prazosin or yohimbine binding (K_D or B_{max}) between SHR and WKY. We are quite critical of a recently published study which showed apparent increases in ^{3}H-clonidine binding in crude membranes from rat caudal artery of SHR as compared with WKY. We suspect that the authors are dealing with binding of clonidine to presynaptic alpha$_2$-adrenoreceptors because, in their binding study, they failed to denervate the artery or to carefully remove the adventitia.

Langer: I agree with you and, as I mentioned, binding data showing changes in numbers of recognition sites for receptor subtypes are not relevant to the possible functional implication, because one is only measuring densities or affinities of recognition sites. Supposing you have the same number of recognition sites but an amplification mechanism that is changed in such a way that the activation of an alpha$_2$-adrenoreceptor by an agonist could produce a larger response than it does normally, then the fact that you have an equal or a different number of receptors is not a conclusive piece of evidence.

Bühler: Some of Dr. Bolli's recent clinical and pharmacological work in our laboratory supports Dr. Langer's concept. Infusion of yohimbine into the forearm circulation antagonizes post-junctional alpha$_2$-adrenoreceptor-mediated vasoconstriction, and this effect is enhanced in patients with essential hypertension. These post-junctional alpha$_2$-adrenoreceptors are highly sensitive to epinephrine. Following alpha$_1$-adrenoreceptor blockade with prazosin and beta-adrenoreceptor blockade with propranolol, superinfusion of minute doses of epinephrine into the forearm circulation produced a vasoconstrictor effect, which could be prevented by pretreatment with yohimbine. This suggests an epinephrine-sensitive post-junctional alpha$_2$-adrenoreceptor vasoconstrictor mechanism in man, and this is enhanced in essential hypertension.

Vanhoutte: Are the innervated alpha-adrenoreceptors in the saphenous vein of the dog of the alpha$_1$- or the alpha$_2$-subtype?

Langer: We never looked at that, but we have shown in the dog saphenous vein that, as far as responses to the exogenous agonist are concerned, alpha$_1$- and alpha$_2$-adrenoreceptor subtypes are located post-synaptically. But we have not looked at responses mediated by nerve stimulation or at their sensitivity to blockade by prazosin or idazoxan.

Vanhoutte: We believe that this preparation is an example of preferential alpha$_2$-innervated adrenoreceptors.

Langer: This is not excluded in any way. I am not saying that the preferential innervation of alpha$_1$-adrenoreceptors is a universal statement for all vascular beds, arterial or venous and irrespective of the diameter. I am restricting our observations to the tissues in which they were described. The density of alpha$_1$- and alpha$_2$-adrenoreceptors postsynaptically may vary in each vascular bed and with the size of the blood vessels. Consequently, this question should be examined separately.