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Characterization of Postjunctional *Alpha*-1 and *Alpha*-2 Adrenoceptors Activated by Exogenous or Nerve-Released Norepinephrine in the Canine Saphenous Vein

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ABSTRACT

Experiments were designed to characterize *alpha*-1 and *alpha*-2 adrenoceptor-mediated effects in the canine saphenous vein. Rings of saphenous vein were mounted for isometric tension recording in physiological saline solution. Contractile responses evoked by *alpha*-1 adrenoceptor agonists, cirazoline or St 587 were inhibited by *alpha*-1 antagonists, prazosin (pA_2 = 7.9) or phenoxybenzamine, but were relatively resistant to the *alpha*-2 adrenoceptor antagonist rauwolscine. The responses to *alpha*-2 adrenoceptor agonists, xylazine or B-HT 920, were relatively resistant to prazosin or phenoxybenzamine but were antagonized by rauwolscine (pA_2 = 8.7). After phenoxybenzamine, the *alpha*-2 agonists, M-7, guanfacine, UK 14304, B-HT 920 and xylazine evoked similar maximal increases in tension which were considerably smaller (approximately 50%) than that attained by *alpha*-1 adrenoceptor stimulation. The different concentration-effect characteristics of these responses were also revealed using norepinephrine. Prazosin produced a biphasic effect on the concentration-response curve of norepinephrine, being more potent against responses above 50% of the maximum (pA_2 = 7.9) compared to lower increases in tension (pA_2 = 6.2). After *alpha*-1 adrenoceptor blockade with prazosin, rauwolscine was more effective against responses below 50% of the maximum, compared to higher increases in tension. The results suggest that the *alpha*-1 and *alpha*-2 adrenoceptor-mediated concentration-effect curves to norepinephrine are almost coincident and that *alpha*-2 adrenergic stimulation produces only partial activation of the vascular smooth muscle. Contractile responses produced by sympathetic nerve stimulation or by tyramine were antagonized more effectively by the combination of prazosin plus rauwolscine than by either blocker given alone, suggesting that *alpha*-1 and *alpha*-2 adrenoceptors are both innervated by sympathetic nerves in the canine saphenous vein.

Vascular, postjunctional *alpha* adrenoceptors have been subclassified using agonists and antagonists which are selective for *alpha*-1 or *alpha*-2 adrenoceptors (McGrath, 1982; Timmermans and van Zwieten, 1982). In some blood vessels, however, the postjunctional excitatory adrenoceptors activated by the physiological agonist, norepinephrine, cannot be classified simply as *alpha*-1 or *alpha*-2. In canine isolated saphenous veins (De Mey and Vanhoutte, 1980, 1981), human isolated digital arteries (Stevens *et al.*, 1981; Stevens and Moulds, 1981, 1982) and human isolated femoral veins (Glusa and Markwardt, 1983), selective *alpha*-1 or *alpha*-2 adrenoceptor blockers cause noncompetitive antagonism of the contractile responses of norepinephrine. Stevens and Moulds (1981, 1982) suggested that this was due to the presence of atypical adrenoceptors. However, this explanation seems unlikely for the canine saphenous vein that contains a mixed population of postjunctional *alpha*-1 and *alpha*-2 adrenoceptors (De Mey and Vanhoutte, 1980, 1982; Shepperson and Langer, 1981; Constantine *et al.*, 1982). The aim of the present study was to characterize *alpha*-1 and *alpha*-2 adrenoceptor-mediated effects *in vitro* in the canine saphenous vein (using selective agonists and antagonists) and to determine if these receptor subtypes were activated by norepinephrine, whether added exogenously or librated by sympathetic nerves.

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Materials and Methods

Preparation of blood vessels. Experiments were performed using lateral sephenous veins taken from mongrel dogs of either sex (15–30 kg) anesthetized with pentobarbital sodium (30 mg/kg i.v.). Immediately after excision, the tissue was placed into cold physiological salt solution of the following composition (millimolar): NaCl, 118.3; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; edetate calcium disodium, 0.026; and glucose, 11.1. The blood vessels were cleaned of adherent connective tissue and cut into rings (5 mm in length) without disturbing the intima. Rings were suspended in organ chambers filled with 25 ml of physiological salt solution (37°C) aerated with 95% O_2-5% CO_2 (pH = 7.4). The rings were connected to force transducers (Statham Universal UC2 or Grass FT 03C) and changes in isometric force were recorded (Hewlett Packard 7418A or Gould Brush 260 recorder). For electrical stimulation of the adrenergic nerve endings, two platinum electrodes were placed parallel to the rings. Electrical impulses (9V, 2 msec, 0.2–16 Hz) were provided by a stimulator (Grass model SM6) and a d.c. amplifier (Mayo Clinic, Section of Engineering). Before the actual experiment the rings were stretched to the optimal point of their length-tension relationship using a standard electrical

stimulation (16 Hz for 10 sec) (Vanhoutte and Leusen, 1969). After this procedure, the rings were allowed to equilibrate for 30 min.

Drugs. The following drugs were used: B-HT 920 (2 HCl) (Boehringer Ingelheim, Ltd., Ridgefield, CT); calcium disodium versenate (edetate calcium disodium) (Riker Laboratories, Inc., Northridge, CA); cirazoline HCl (L.E.R.S. Synthélabo, Paris, France); cocaine HCl (generic); dimethyl sulfoxide (Sigma Chemical Company, St. Louis, MO); guanfacine HCl (Sandoz, Ltd., Basel, Switzerland); hydrocortisone 21-hemisuccinate (sodium salt) (Sigma); methoxamine HCl (Burroughs Wellcome Co., Research Triangle Park, NC); M-7 (HBr) (Glaxo Group Research Limited, Ware, Hertfordshire, UK); *l*-norepinephrine bitartrate (Sigma); pentobarbital sodium (Fort Dodge Laboratories, Inc., Fort Dodge, IA); phenoxybenzamine HCl (Smith Kline and French Laboratories, Philadelphia, PA); *l*-phenylephrine HCl (Sigma); prazosin HCl (Pfizer, Inc., Brooklyn, NY); *dl*-propranolol HCl (Sigma); rauwolscine HCl (Carl Roth, Karlsruhe, Federal Republic of Germany); St 587 (nitrate) (Boehringer Ingelheim, Ltd.); tyramine HCl (Sigma); UK 14,304 (tartrate) (Pfizer Central Research, Sandwich, Kent, UK); and xylazine HCl (Bayvet, Shawnee, KS).

Drugs were prepared and diluted using distilled water, kept on ice and added to the organ chambers in volumes of 500 μl or less. Stock solutions of prazosin were prepared using dimethyl sulfoxide (highest bath concentration: 5×10^{-4} M) with further dilution with distilled water; dimethyl sulfoxide had no significant effect on contractile responses evoked by adrenoceptor agonists or sympathetic nerve stimulation. Stock solutions of phenoxybenzamine were prepared by dissolving 50 mg in 0.5 ml of absolute alcohol (highest bath concentration: 3×10^{-5} M) and 4.5 ml of distilled water with one drop of 1 M HCl added to remove turbidity and further dilution with distilled water. Norepinephrine, phenylephrine and M-7 stock solutions (10^{-4} M) contained 0.01% L-ascorbic acid as an antioxidant. Concentrations of drugs are expressed as final organ chamber concentrations (molar).

Experimental protocol, calculations and statistical analysis. The rings were pretreated for 30 min with cocaine (5×10^{-6} M), hydrocortisone (3×10^{-5} M) and propranolol (5×10^{-6} M) before exposure of the tissues to adrenoceptor agonists or sympathetic nerve stimulation in order to inhibit neuronal uptake, extraneuronal uptake and *beta* adrenoceptors, respectively. The rings remained in contact with these drugs throughout the experiment. Rings that had spontaneous contractile activity or developed spontaneous activity after drug addition [*e.g.*, cocaine (Verbeuren and Vanhoutte, 1982)] were excluded. At the start of each experiment the maximal adrenergic response of the venous rings (De Mey and Vanhoutte, 1980, 1981; Rimele and Vanhoutte, 1983) was determined using a maximal concentration of norepinephrine (10^{-4} M) or electrical stimulation (16 Hz). The subsequent responses of the tissues to adrenoceptor agonists or sympathetic nerve stimulation were expressed as a percentage of these responses.

When the indirect sympathomimetic action of tyramine was studied, cocaine was omitted from the bath solution. In these experiments, the contractile responses to tyramine, and to sympathetic nerve stimulation, are expressed as a percentage of the response to 16 Hz nerve stimulation obtained before blockade of extraneuronal uptake and *beta* adrenoceptors.

Concentration-effect (10^{-9} to 10^{-4} M) curves to adrenoceptor agonists were constructed by increasing organ chamber concentrations cumulatively by half-log increments. Frequency-effect curves to sympathetic nerve stimulation were constructed in a similar fashion by a stepwise increase in stimulus frequency (0.2–16 Hz). Concentrations (agonists) or frequencies (nerve stimulation) were increased only when the contraction to the preceding stimuli had stabilized. Only one concentration-effect curve or frequency-effect curve was performed in each ring.

For irreversible blockade of *alpha* adrenoceptors, some rings were incubated for 30 min with phenoxybenzamine (5×10^{-9} to 5×10^{-7} M) and then were rinsed repeatedly (Constantine *et al.*, 1982). When the competitive antagonists, prazosin and rauwolscine, were used, the preparations were incubated for 45 min with the drugs, which remained in the bath solution during exposure of the tissue to the agonist or to nerve stimulation. When several concentrations of antagonists were studied, this was done on different rings of the same vein. In each experiment, one untreated ring served as a control.

The pA_2 values for the antagonists were determined from concentration-effect curves by calculating the ratio of agonist-concentrations producing equal responses in the presence and absence of different concentrations of the antagonist. Because only one concentration-effect curve was performed in each preparation, a series of concentration-ratios was calculated from each animal by comparing the control and test rings. Except where otherwise stated, this was done by using concentrations of the agonist that caused increases in tension equal to 50% of the maximal response (ED_{50}) to the agonist. In the case of competitive antagonism, a plot of the logarithm of (concentration ratio − 1) against the negative logarithm of the molar concentration of the antagonist should yield a straight line with a slope of unity and an intercept along the abscissa giving the pA_2 ($-\log K_B$) (Arunlakshana and Schild, 1959).

Results are expressed as means ± S.E.M. Except where otherwise stated, statistical evaluation of the data was by Student's *t* test for paired or unpaired observations. When P was smaller than .05, means were considered to be significantly different. In all experiments, *n* equals the number of dogs.

Results

***Alpha*-1 adrenoceptor agonists.** Phenylephrine, methoxamine and cirazoline produced maximal contractions of the saphenous vein that were not significantly different from that evoked by norepinephrine (table 1). However, the maximal response to St 587 was less than that evoked by norepinephrine (table 1).

Phenoxybenzamine (5×10^{-9} to 5×10^{-7} M) produced a dose-dependent attenuation of the concentration-effect curves evoked by phenylephrine (fig. 1A), cirazoline (fig. 1B) and methoxamine (data not shown). Phenoxybenzamine (5×10^{-8} M) virtually abolished the response to St 587 (fig. 1C).

Rauwolscine (10^{-7} M), did not significantly affect the concentration-effect curves to cirazoline or St 587 (data not shown). In the presence or absence of rauwolscine (10^{-7} M), prazosin (5×10^{-9} to 5×10^{-7} M) produced dose-dependent, parallel shifts in the concentration-effect curves to cirazoline (fig. 2) or St 587 (data not shown) without affecting the maximal responses to these agents. The inhibition by prazosin of the responses to cirazoline or St 587 fulfilled the criteria for competitive antagonism and the pA_2 values obtained for each agonist were the same (table 2). The responses to cirazoline

TABLE 1

Responses of canine saphenous veins to *alpha* adrenergic agonists (n = 4–6)

	Agonist	pD_2[a]	Maximal Response[b]
Selective *alpha*-1 agonists	Cirazoline	7.10 ± 0.19	104.3 ± 2.7
	Phenylephrine	5.91 ± 0.07	104.3 ± 5.6
	St 587	5.83 ± 0.08	48.9 ± 6.4[c]
	Methoxamine	5.42 ± 0.92	99.5 ± 5.3
Selective *alpha*-2 agonists	M-7	7.34 ± 0.31	87.6 ± 7.6
	UK,14304	7.14 ± 0.21	71.4 ± 5.5[c]
	B-HT 920	7.02 ± 0.09	50.1 ± 12.7[c]
	Guanfacine	6.96 ± 0.23	101.0 ± 5.4
	Xylazine	6.46 ± 0.26	68.1 ± 9.5[c]
alpha-1 and *alpha*-2 agonist	Norepinephrine	6.51 ± 0.12	103.7 ± 3.2

[a] $pD_2 = -\log (ED_{50})$.

[b] Maximal responses are expressed as a percent of the initial contraction to norepinephrine (10^{-4} M).

[c] Denotes significant difference from the maximal response of norepinephrine.

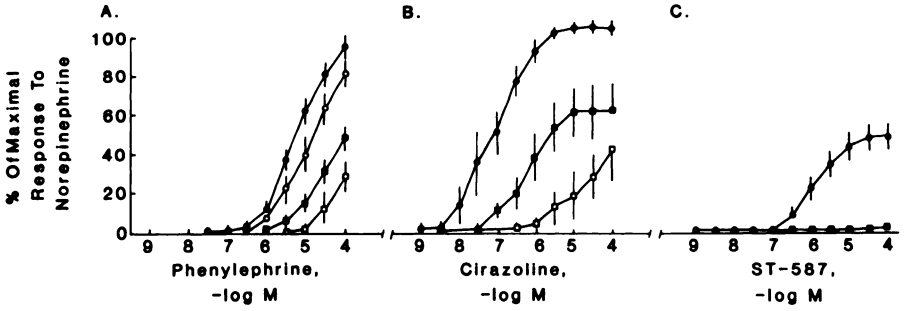

Fig. 1. Effects of phenoxybenzamine (○, 5×10^{-9} M; ■, 5×10^{-8} M; □, 5×10^{-7} M) on the contractile responses (●, control) to A) phenylephrine, B) cirazoline, or C) St 587. Data are expressed as percentage of the maximal response to norepinephrine and are shown as mean ± S.E.M. ($n = 6$).

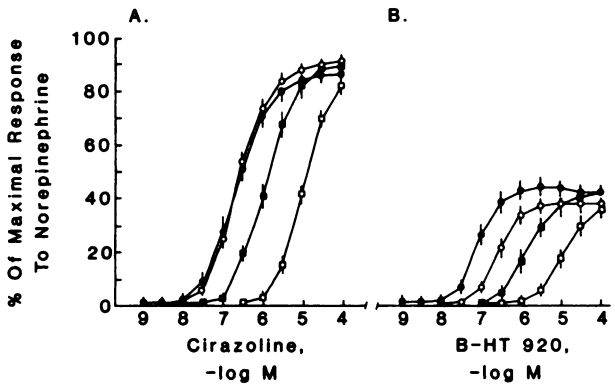

Fig. 2. Competitive antagonism of *alpha*-1 and *alpha*-2 adrenoceptor-mediated effects in the saphenous vein. A, effects of prazosin on the concentration-response curve to cirazoline. Experiments were performed in the presence of rauwolscine (10^{-7} M) to block *alpha*-2 adrenoceptors ($n = 6$): B, effects of rauwolscine on the concentration-response curve to B-HT 920. Experiments were performed after pretreatment with phenoxybenzamine (5×10^{-8} M) to block *alpha*-1 adrenoceptors ($n = 7$). Symbols: ●, control. Antagonist concentrations: ○, 5×10^{-9} M; ■, 5×10^{-8} M; □, 5×10^{-7} M. Data are expressed as percentage of the maximal response to norepinephrine and are shown as mean ± S.E.M.

TABLE 2

Comparison of adrenoceptor blocking actions of rauwolscine and prazosin in the isolated canine saphenous vein

Values were obtained from lines fitted by least-square linear regression. The correlation coefficients for the lines were not significantly different from unity. Values were obtained from data at ED_{50}. Numbers in parentheses, 95% confidence limits for the means.

Antagonist	Agonist	*n*	Slope of Arunlakshana and Schild Plot	pA_2
Rauwolscine[a]	B-HT 920	7	0.95 (1.21–0.69)	8.7 (9.3–8.0)
Rauwolscine[a]	Xylazine	9	0.97 (1.25–0.69)	8.7 (9.2–8.2)
Prazosin[b]	Cirazoline	6	1.06 (1.27–0.85)	7.9 (8.4–7.4)
Prazosin[b]	St 587	5	1.12 (1.38–0.86)	7.9 (8.6–7.2)

[a] The preparations were pretreated with phenoxybenzamine (5×10^{-8} M).
[b] Experiments performed in the presence of rauwolscine (1×10^{-7} M).

remaining after phenoxybenzamine (5×10^{-8} M) were inhibited more effectively by prazosin (5×10^{-7} M) than by rauwolscine (5×10^{-7} M [concentration ratios of 25.3 ± 11.4 and 3 ± 1.2, respectively, determined at 25% of the maximal response of norepinephrine ($n = 4$)].

***Alpha*-2 adrenoceptor agonists.** The maximal effects attained by the *alpha*-2 adrenoceptor agonists tested varied from 50 to 100% of the maximal response to norepinephrine (table 1; fig. 3A). After phenoxybenzamine (5×10^{-8} M), the maximal responses evoked by M-7, UK14,304, guanfacine, B-HT 920 and xylazine were not significantly different from each other ($P > .05$ for overall f test, one-way analysis of variance), but were less than that produced by norepinephrine (fig. 3B). Phenoxybenzamine did not significantly affect the concentration-effect curves to B-HT 920, UK14,304 or xylazine but reduced the responses to guanfacine and M-7 significantly (figs. 3, A and B). The concentration-effect curve to B-HT 920 was not significantly affected by a higher dose of phenoxybenzamine (5×10^{-7} M) or by prazosin (5×10^{-7} M) (data not shown).

In the presence or absence of phenoxybenzamine (5×10^{-8} M), rauwolscine (5×10^{-9} to 5×10^{-7} M) produced dose-dependent parallel shifts of the concentration-effect curves to B-HT 920 (fig. 2) or xylazine (data not shown), without affecting their maximal responses. The inhibition by rauwolscine fulfilled the criteria for competitive antagonism and the pA_2 values obtained from each agonist were the same (table 2).

Norepinephrine. Prazosin (5×10^{-7} M) had a greater inhibitory effect on the contractile responses evoked by high concentrations of norepinephrine than on those evoked by lower doses (fig. 4) [concentration ratios for the ED_{75} and ED_{25} responses were 10.6 ± 3.2 and 2.7 ± 0.7, respectively ($n = 6$; $P < .01$)]. Rauwolscine (10^{-7} M) was equieffective against high and low concentrations of norepinephrine producing a parallel shift in the concentration-effect curve (fig. 4). However, after prazosin (5×10^{-7} M), rauwolscine (10^{-7} M) was more effective against the contractile responses evoked by low concentrations of norepinephrine [concentration-ratios for the ED_{75} and ED_{25} responses were 9.1 ± 6 and 22.2 ± 6.9, respectively ($n = 6$, $P < .01$)]. In the presence of rauwolscine (10^{-7} M), prazosin (5×10^{-7} M) produced a parallel shift in the concentration-effect curve of norepinephrine (fig. 4). The inhibitory effect of

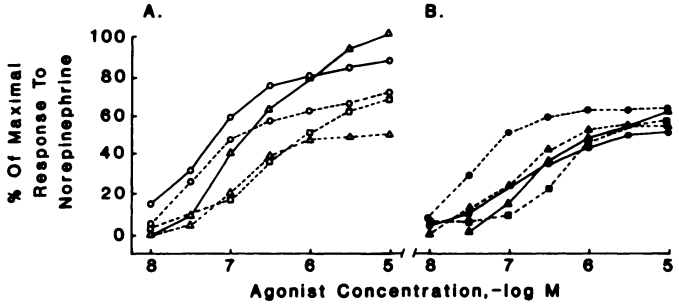

Fig. 3. Effects of phenoxybenzamine on the contractile responses evoked by selective *alpha*-2 adrenoceptor agonists. A, control responses; B, responses after phenoxybenzamine (5×10^{-8} M). Agonists studied: M7 (○——○, ●——●); UK 14,304 (○- - -○, ●- - -●); guanfacine (△——△, ▲——▲); B-HT 920 (△- - -△, ▲- - -▲); xylazine (□- - -□, ■- - -■). Data expressed as percentage of the maximal response to norepinephrine. For clarity S.E. bars are not shown ($n = 4$ or 5).

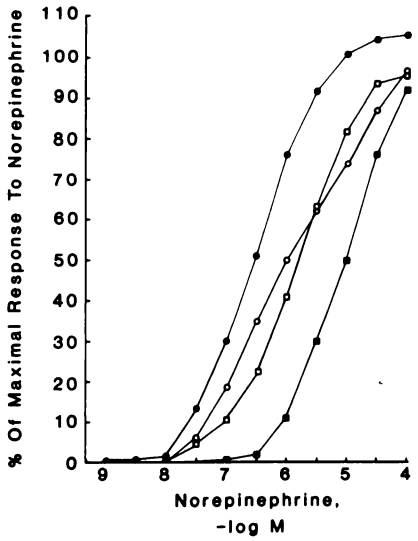

Fig. 4. Effects of selective *alpha*-1 and *alpha*-2 adrenoceptor antagonists on the concentration-effect curve to norepinephrine. Control responses (●) and those after prazosin (5×10^{-7} M, ○), rauwolscine (10^{-7} M, □) or rauwolscine (10^{-7} M) plus prazosin (5×10^{-7} M, ■) are shown. Data are expressed as percentage of the initial maximum response to norepinephrine. For clarity, S.E. bars have been omitted ($n = 6$).

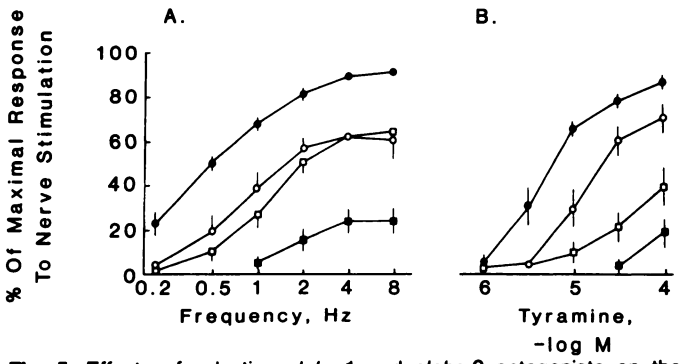

Fig. 5. Effects of selective *alpha*-1 and *alpha*-2 antagonists on the contractile responses evoked by A) sympathetic nerve stimulation or B) tyramine. Control responses (●) and those after prazosin (5×10^{-7} M, ○), rauwolscine (10^{-7} M, □) or prazosin (5×10^{-7} M) plus rauwolscine (10^{-7} M, ■) are shown. Data are expressed as percentage of the initial maximal response to nerve stimulation (16 Hz), in the absence of uptake or *beta* receptor blockade. S.E. bars are indicated ($n = 6$).

phenoxybenzamine (5×10^{-8} M) was similar to that produced by prazosin (5×10^{-7} M) (data not shown).

Nerve stimulation. Electrical stimulation (0.2–16 Hz) of the adrenergic nerve endings produced sustained, frequency-dependent increases in tension. Blockade of neuronal uptake (cocaine, 5×10^{-6} M), extra-neuronal uptake (hydrocortisone, 3×10^{-5} M) and *beta* adrenoceptors (propranolol, 5×10^{-6} M) significantly increased the contractile responses to nerve stimulation at low frequencies (0.2–2 Hz) but depressed those evoked by higher frequencies (8 and 16 Hz) (data not shown).

Rauwolscine (10^{-7} M) or prazosin (5×10^{-7} M) significantly reduced the responses evoked by each frequency ($P < .05$; fig. 5). After rauwolscine (10^{-7} M) plus prazosin (5×10^{-7} M), the contractile responses evoked by each frequency were significantly less than those obtained in the presence of either antagonist given alone ($P < .05$; fig. 5).

Tyramine. Tyramine produced dose-dependent increases in tension that were significantly reduced by either prazosin or rauwolscine ($P < .05$; fig. 5). The inhibitory effect of rauwolscine (10^{-7} M) was greater than that of prazosin (5×10^{-7} M) at high concentrations of tyramine ($P < .05$; fig. 5). The combination of prazosin (5×10^{-7} M) and rauwolscine (10^{-7} M) caused greater attenuation of the responses to tyramine than either antagonist alone ($P < .05$; fig. 5).

Discussion

Heterogeneous populations of postjunctional *alpha* adrenoceptors can be demonstrated in the canine saphenous vein by using selective *alpha*-1 and *alpha*-2 adrenoceptor agonists (De Mey and Vanhoutte, 1980, 1981; Shepperson and Langer, 1981). In the present study, the contractile effects produced by selective *alpha*-1 adrenergic agonists (cirazoline or St 587) were relatively resistant to rauwolscine, a selective *alpha*-2 adrenergic antagonist, but were inhibited in a competitive manner by prazosin, a selective *alpha*-1 adrenergic antagonist. The pA_2 values for prazosin are low compared to other *alpha*-1 adrenoceptor systems (*e.g.*, rabbit pulmonary artery: Starke, 1981; Shepperson and Langer, 1981; canine coronary artery: Rimele *et al.*, 1983) but are consistent with *alpha*-1 adrenoceptor blockade observed by others in the canine saphenous vein (Sullivan and Drew, 1980; Shepperson and Langer, 1981). *Alpha*-2 adrenoceptor-mediated contractile responses were relatively resistant to prazosin but sensitive to rauwolscine. The pA_2 values of the antagonism by rauwolscine of the response to B-HT 920 or xylazine are consistent with *alpha*-2 adrenoceptor blockade (Perry and U'Prichard, 1981; Bylund and U'Prichard, 1983).

Another way to dissociate *alpha*-1 and *alpha*-2 adrenoceptor-mediated effects is by using phenoxybenzamine to block selectively and irreversibly *alpha*-1 adrenoceptors (Constantine *et al.*, 1982; Starke and Docherty, 1982). In concentrations up to 5×10^{-7} M, phenoxybenzamine caused a dose-dependent attenuation of the concentration-effect curves produced by selective *alpha*-1 adrenoceptor agonists (cirazoline, phenylephrine, methoxamine and St 587), but did not affect *alpha*-2 receptor-mediated responses evoked by the selective *alpha*-2 adrenoceptor agonist B-HT 920. Because phenoxybenzamine is an irreversible antagonist, its potency will depend on the receptor subtype (*alpha*-1 or *alpha*-2) activated by an agonist and also on the efficacy of the agonist (Kenakin, 1984). The greater potency of phenoxybenzamine against responses to St 587, compared to those of other agonists, probably results from a lower efficacy of St 587 at the *alpha*-1 adrenoceptor (Beckeringh *et al.*, 1984). The antagonism by prazosin, but not by rauwolscine, of the response to cirazoline remaining after phenoxybenzamine (5×10^{-8} M) indicates that the difference in the potency of phenoxybenzamine does not result from differences in agonist selectivity. As with selective *alpha*-1 adrenoceptor agonists, the inhibitory effect of phenoxybenzamine against selective *alpha*-2 agonists was not uniform. The contractile responses evoked by B-HT 920, UK 14,304 and xylazine were not affected by the irreversible antagonist whereas those produced by guanfacine or M-7 were reduced. This probably does not result from differences in agonist efficacy because guanfacine and M-7 produced greater maximal responses than the other agonists but suggests that guanfacine and M-7 can activate *alpha*-1 adrenoceptors in the saphenous vein (Shepperson and Langer, 1981).

After *alpha*-1 adrenoceptor blockade with phenoxybenzamine, the selective *alpha*-2 adrenoceptor agonists each caused

a similar maximal increase in tension. Because these maximal contractions approximate only 50% of those attained after *alpha*-1 receptor activation, it is likely that *alpha*-2 adrenoceptor stimulation can produce only partial activation of the vascular smooth muscle. The physiological agonist, norepinephrine, also evoked a low-maximum, *alpha*-2 adrenergic response, suggesting that the reduced maximal effects do not result from low efficacy of the agonists at the *alpha*-2 adrenoceptor. It is likely that this inequality of *alpha*-1 and *alpha*-2 adrenoceptor-mediated effects, that also occurs *in vivo* (Timmermans and van Zwieten, 1980), results from a decreased efficiency of *alpha*-2 adrenoceptor/response coupling.

The present study suggests that the different concentration-effect relationships of *alpha*-1 and *alpha*-2 adrenoceptor stimulation are partly responsible for the inconclusive results obtained previously with norepinephrine in the canine saphenous vein and in other vascular preparations that probably contain a mixed population of postjunctional *alpha*-1 and *alpha*-2 adrenoceptors (Drew and Whiting, 1979; De Mey and Vanhoutte, 1980, 1981; Sullivan and Drew, 1980; Stevens and Moulds, 1981, 1982; Glusa and Markwardt, 1983; Flavahan and McGrath, 1984). Because norepinephrine is a nonselective agonist of these receptors, its *alpha*-1 and *alpha*-2 concentration-effect curves will be almost coincident. It is, therefore, difficult to analyze the effects of selective *alpha*-1 or *alpha*-2 adrenoceptor antagonists on the response to the catecholamine. For example, when prazosin would tend to cause dose-dependent, parallel shifts of the concentration-effect curve to the *alpha*-1 adrenergic component of the response to norepinephrine, this is partially obscured by the persistence of the effect of norepinephrine at *alpha*-2 adrenoceptors. At low concentrations of norepinephrine producing responses less than 50% of the maximum, prazosin is virtually ineffective because of the persistence of the low-maximum, *alpha*-2 adrenoceptor-mediated contraction. However, at concentrations of norepinephrine producing responses greater than 50% of the maximum (and out of the range of the *alpha*-2 adrenergic component), prazosin can cause significant attenuation of the contractile response to the agonist. Thus, prazosin produces antagonism that resembles "noncompetitive" blockade (Drew and Whiting, 1979; De Mey and Vanhoutte, 1981; Stevens and Moulds, 1981, 1982; Timmermans and van Zwieten, 1982; Glusa and Markwardt, 1983) but which results from selective competitive antagonism of *alpha*-1 adrenoceptors (Flavahan and McGrath, 1984). This conclusion is supported by the observation that after inhibition of the *alpha*-2 adrenoceptors by rauwolscine, prazosin produced a parallel shift in the concentration-effect curve to norepinephrine.

De Mey and Vanhoutte (1981) attempted to characterize the contractile response to the ED_{50} of norepinephrine in the canine saphenous vein by constructing Arunlakshana and Schild plots (1959) using selective (prazosin), preferential (yohimbine) and nonselective (phentolamine) *alpha* adrenergic antagonists. However, such plots were designed for single receptor populations (Arunlakshana and Schild, 1959; Furchgott, 1972). When used in the saphenous vein, they indicated that the antagonism produced by prazosin or yohimbine was not competitive (De Mey and Vanhoutte, 1981). Because the results of the present study suggest that the contractile responses to norepinephrine above 50% of the maximal response are mediated by *alpha*-1 adrenoceptor stimulation, and below this level by combined *alpha*-1 and *alpha*-2 adrenergic activation, we have reanalyzed the data obtained by De Mey and Vanhoutte (1981), using ED_{25} and ED_{75} concentrations to construct Arunlakshana and Schild plots (fig. 6). As expected, the nonselective *alpha* adrenergic antagonist, phentolamine, had similar pA_2 values at the ED_{25} or ED_{75} levels. The antagonism by prazosin at the ED_{75} level fulfilled the criteria for competitive antagonism (slope = 1.03) and produced a pA_2 value of 7.9, which is the same as that obtained against St 587 or cirazoline. This then implies that the response to norepinephrine at the ED_{75} level is determined predominantly by the activation of *alpha*-1 adrenoceptors. The Arunlakshana and Schild plot constructed for the antagonism of the response to the ED_{25} of norepinephrine yielded a biphasic plot with a slope of less than unity (see also De Mey and Vanhoutte, 1981). However, if the biphasic pattern is analyzed, it can be divided into two Arunlakshana and Schild plots, each with slopes close to unity and with different intersections (real or projected) with the abscissa (fig. 6). At low concentrations of prazosin, the antagonism appears to be similar to that produced with the ED_{75} of norepinephrine and therefore probably reflects inhibition of the *alpha*-1 adrenergic effect of norepinephrine. However, this inhibitory effect of prazosin is not observed at higher antagonist concentrations; prazosin (10^{-7} and 3×10^{-7} M) produce similar antagonism of the response to norepinephrine. When the concentration of prazosin is increased further, a second inhibitory effect occurs, with a pA_2 value (6.94) that is close to that observed for *alpha*-2 adrenoceptor antagonism produced by prazosin ($pA_2 = 6.2$; calculated from fig. 8 in Davey, 1980), and thus probably reflects inhibition of the action of norepinephrine at *alpha*-2 adrenoceptors. The Arunlakshana and Schild plots (fig. 6) indicate that the *alpha*-1 and *alpha*-2 adrenoceptor-mediated concentration-effect curves to norepinephrine are not coincident but that the *alpha*-1 curve lies to the left of the *alpha*-2 curve. This displacement of the two receptor-mediated components probably explains the overestimation in the *alpha*-2 adrenoceptor potency of prazosin. Prazosin, at low concentrations, will cause selective, competitive antagonism of the *alpha*-1 adrenergic response

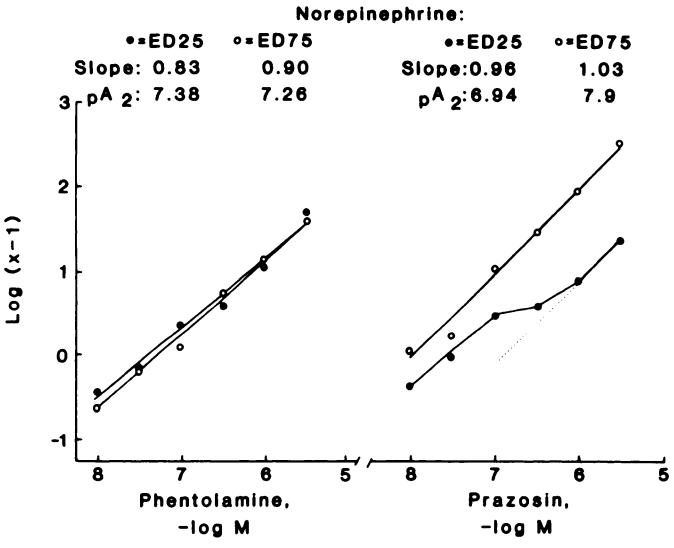

Fig. 6. Reanalysis of the results obtained by De Mey and Vanhoutte (1981). Schild plots are shown for the antagonism produced by A) phentolamine or B) prazosin of the contractile response of norepinephrine at ED_{25} (●) and ED_{75} (○) levels. Slopes and pA_2 values are indicated. For prazosin's antagonism of the ED_{25} response of norepinephrine, the slope and pA_2 value refer to the projected line.

producing a rightward shift in the concentration-effect curve to norepinephrine. However, because of the presence of the *alpha*-2 adrenergic component, the Arunlakshana and Schild plot for *alpha*-1 adrenoceptor blockade by prazosin is partially concealed. Because this occurs at prazosin (10^{-7} M), it suggests that the ED_{25} response to norepinephrine remaining after this concentration of prazosin represents the *alpha*-2 adrenoceptor-mediated, concentration-effect curve. By using this response to represent the *alpha*-2 adrenergic effect to norepinephrine, an Arunlakshana and Schild plot was constructed for the antagonism, produced by prazosin, of the *alpha*-2 adrenergic component. This plot produced a pA_2 value of 6.2 (slope = 1.1) which is the same as that obtained for the inhibitory effect of prazosin against the selective *alpha*-2 adrenoceptor agonist, UK14,304, (Davey, 1980).

The present study, taken in conjunction with earlier work (De Mey and Vanhoutte, 1981), suggests that in the canine saphenous vein, the responses to low concentrations of norepinephrine (*i.e.*, small increases in tension) represent predominantly the *alpha*-2 adrenergic component, whereas those to higher concentrations (*i.e.*, larger increases in tension) the *alpha*-1 adrenergic component. However, because the *alpha*-1 adrenergic concentration-effect curve to norepinephrine lies to the left of the *alpha*-2 adrenergic effect curve, *alpha*-2 adrenergic antagonists, under control conditions, do not cause selective inhibition of the responses to low concentrations of norepinephrine. Indeed, the preferential *alpha*-2 adrenoceptor blocker, yohimbine, produces equal antagonism of the ED_{25} and ED_{75} responses (slopes of 0.76 and 0.77, respectively; pA_2 values of 7.52 and 7.28, respectively) (Arunlakshana and Schild plots not shown, data obtained from De Mey and Vanhoutte, 1981). These pA_2 values are consistent with *alpha*-1 adrenoceptor antagonism produced by yohimbine in the canine saphenous vein (pA_2 = 7.5, Sullivan and Drew, 1980; Shepperson and Langer, 1981), but not for *alpha*-2 adrenoceptor blockade (pA_2 = 8.2–8.6, Davey, 1980; Shepperson and Langer, 1981; Constantine *et al.*, 1982; Cavero *et al.*, 1983). Although yohimbine, at low concentrations, should cause selective inhibition of the *alpha*-2 adrenergic component (low-maximum) of the response to norepinephrine, this apparently is obscured by the persistence of the *alpha*-1 adrenoceptor-mediated effect (high-maximum). When the concentration of yohimbine is increased, it causes inhibition of the *alpha*-1 adrenergic component, which, because the *alpha*-2 adrenergic receptors are already inhibited, results in parallel, rightward displacements of the concentration-effect curve of norepinephrine (mimicking the shift produced by prazosin in the presence of rauwolscine). The inhibitory effect of *alpha*-2 adrenoceptor blockers on the low-maximum, *alpha*-2 adrenergic component of the response to norepinephrine can be observed only if the *alpha*-1 adrenergic response is already antagonized. For example, after prazosin, rauwolscine produced greater antagonism of the ED_{25} compared to the ED_{75} response (fig. 4).

In the absence of prazosin, rauwolscine (10^{-7} M) produced a parallel displacement in the concentration-effect curve of norepinephrine. Because this concentration of rauwolscine appears to be selective for *alpha*-2 adrenoceptors, the inhibitory effect of rauwolscine, in contrast to that produced by yohimbine, cannot be explained by *alpha*-1 adrenoceptor blockade. This would be explained best if activation of *alpha*-2 adrenoceptors by norepinephrine augmented the *alpha*-1 adrenoceptor-mediated component of the response to the adrenergic transmitter. If this were the case, antagonism of *alpha*-2 adrenoceptors by rauwolscine may produce attenuation in the *alpha*-1 adrenoceptor-mediated response. The influence of this interaction on the potency of yohimbine is probably less pronounced due to its decreased selectivity for *alpha*-2 adrenoceptors.

The results of the present study indicate that norepinephrine, released from sympathetic nerves, can also activate postjunctional *alpha*-1 and *alpha*-2 adrenoceptors in the canine saphenous vein. This contrasts with recent studies which have suggested that postganglionic sympathetic nerves innervate preferentially vascular *alpha*-1 or *gamma* adrenoceptors (Docherty and McGrath, 1980; Yamaguchi and Kopin, 1980; Langer *et al.*, 1980; Wilffert *et al.*, 1982a; Langer and Shepperson, 1982; Nield and Zelcer, 1982). In the present study, the contractile responses evoked by sympathetic nerve stimulation in the saphenous vein were reduced only partly after prazosin or rauwolscine, but were virtually abolished after combined *alpha*-1 and *alpha*-2 adrenoceptor blockade. Because of prejunctional *alpha*-2 adrenoceptor-mediated autoinhibition of adrenergic neurotransmission (Gillespie, 1980; Langer, 1980; Starke, 1981), the relative contribution of each receptor subtype to the neuronal response is difficult to determine. *Alpha*-2 adrenoceptor blockade will increase the release of norepinephrine from the sympathetic nerves during nerve stimulation (Sullivan and Drew, 1980) which may reduce the postjunctional antagonism produced by rauwolscine. To bypass prejunctional facilitation due to *alpha*-2 adrenoceptor antagonism, we examined the contractile effects of the indirect sympathomimetic, tyramine. Prazosin and rauwolscine do not antagonize the prejunctional action of tyramine (Wilffert *et al.*, 1982b). The contractile responses evoked by tyramine were inhibited to a greater extent by rauwolscine than by prazosin, suggesting that norepinephrine released from adrenergic nerves activates preferentially postjunctional *alpha*-2 adrenoceptors in the canine saphenous vein (see also Cooke *et al.*, 1984). The responses evoked by low-frequency nerve stimulation or by low doses of tyramine (fig. 5) were virtually abolished by either prazosin or rauwolscine, at concentrations which display selectivity at *alpha*-1 and *alpha*-2 adrenoceptors, respectively. This suggests further that activation of one receptor subtype (*e.g.*, *alpha*-2) may facilitate the response mediated by the other receptor (*e.g.*, *alpha*-1). Because this summation appears to be greater with nerve-released compared to exogenous norepinephrine, it might indicate differences between "junctional" and "extrajunctional" adrenoceptors.

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