

## SUBTYPES OF $\alpha_1$ -ADRENOCEPTORS IN RABBIT SAPHENOUS VEIN

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### ABSTRACT

We investigated the  $\alpha_1$ -adrenoceptor subtypes of rabbit saphenous vein which has a mixed functional population of  $\alpha_1$  and  $\alpha_2$ -adrenoceptors. Lateral saphenous veins were obtained from male rabbits weighing 3.20-4 kg, which were killed by overdose with pentobarbitone sodium (i.v. injection). They were easily dissected out and placed in cold, oxygenated modified Krebs-Henselite solution (Krebs). Each preparation was cut transversely into 3-4 mm rings and suspended between thick wire supports. The vein rings were mounted in 10 mL isolated organ bath, bathed in Krebs maintained at 37°C and gassed with 95% O<sub>2</sub> plus 5% CO<sub>2</sub>. Cumulative concentration-response curves (CCRC) were constructed by increasing the concentration of the agonists in half-log increments. The preparations were left for a further period of 45-60 min before re-exposure to the agonist. Competitive antagonists like prazosin and rauwolscine were incubated in preparations at least for 45 minutes prior to the onset of a second CCRC. The strategy was based on using the  $\alpha_1$ -adrenoceptor selective agonist, phenylephrine (PE). Prazosin, an  $\alpha_1$ -adrenoceptor selective antagonist, competitively inhibited contractile responses to phenylephrine with a pA<sub>2</sub> value of 8, WB-4101 had a pA<sub>2</sub> of 8.6 but a low Schild plot slope, while low potency was found with 5MU (pA<sub>2</sub> 7.2) and HV-723 (pA<sub>2</sub> 7.97). This data is not consistent with a definitive for  $\alpha_{1A}$  or  $\alpha_{1N}$  and taken alone the evidence from prazosin is in favour of  $\alpha_{1L}$ . However the selective  $\alpha_2$ -adrenoceptor antagonist delequamine inhibited phenylephrine-induced contractions. Overall the data is consistent with phenylephrine-induced contractions being mediated by  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors. The best estimate of the subtype of  $\alpha_1$ -adrenoceptor mediating contraction is  $\alpha_{1L}$  due to the relatively low absolute pA<sub>2</sub> values for prazosin.

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**Keywords:**  $\alpha_1$ -adrenoceptor subtypes, phenylephrine, prazosin, delequamine, WB-4101, 5MU, HV-723.

### INTRODUCTION

The sympathetic nervous system plays an important role in regulating the tone of the peripheral blood circulation. Catecholamines bind and activate  $\alpha$ -adrenoceptors. It has now been clearly shown that postsynaptic  $\alpha$ -adrenoceptors in the peripheral blood circulation are composed of  $\alpha_1$  and  $\alpha_2$ -adrenoceptors, with both receptors mediating vasocon-

striction.<sup>1</sup> Functional studies and experiments have demonstrated the existence of two major  $\alpha_1$ -adrenoceptor subtypes:  $\alpha_{1L}$  and  $\alpha_{1H}$ .  $\alpha_{1L}$  displays low affinity for prazosin and  $\alpha_{1H}$  displays high affinity for prazosin.<sup>2,3</sup> The  $\alpha_{1L}$ -adrenoceptor seems to mediate vasoconstriction of some vessels in human and experimental animals.<sup>4,5</sup>

The rabbit lateral saphenous vein has a mixed functional population of  $\alpha_1$  and  $\alpha_2$ -adrenoceptors.<sup>6</sup> It represents a tis-

sue which has a mixed population of postjunctional  $\alpha$ -adrenoceptors where  $\alpha_2$ -adrenoceptors dominate to a greater extent.<sup>7</sup> We chose the saphenous vein to characterise the  $\alpha_1$ -adrenoceptor subtypes which co-exist with  $\alpha_2$ -adrenoceptors in this tissue. It is possible that different selective  $\alpha_1$ -adrenoceptor agonists and antagonists interact with  $\alpha_2$ -adrenoceptors. The aim of this study was to examine and interpret the pharmacological subclassification of  $\alpha_1$ -adrenoceptors in this atypical preparation that has both postjunctional  $\alpha_1$  and  $\alpha_2$ -adrenoceptors.

## MATERIAL AND METHODS

Lateral saphenous veins were obtained from male rabbits weighing 3.2-4 kg, which were killed by overdose with pentobarbitone sodium (i.v. injection). They were easily dissected out and were placed in cold, oxygenated modified Krebs-Henselite solution (Krebs). The veins were cleared of any extraneous connective tissue using fine scissors. Each preparation was cut transversely in to 3-4mm rings and suspended between thick wire supports. Each was suspended horizontally by means of two stainless-steel L-shaped hooks carefully passed through the lumen. The upper support was connected by cotton to an isometric transducer while the lower support was connected to a glass tissue holder. The vein rings were mounted in a 10 mL isolated organ bath, bathed in Krebs, maintained at 37°C and gassed with 95% O<sub>2</sub> plus 5% CO<sub>2</sub>.<sup>8</sup>

The rings were then placed under resting tension at 2g for each group of vein rings. Isometric contractions were measured by a Grass FT03 transducer connected to a Linseis (TYP 7208) pen recorder. In all experiments, tissues were left to equilibrate for a 60 min period, during which time the tension was re-adjusted to a set value which was maintained constant throughout the rest of the experimental day. Each preparation was then exposed to noradrenaline (1 $\mu$ M) and allowed to contract for 5-10 min. This first contraction to an agonist minimises changes in the sensitivity of preparations to further addition of agonists. Following complete wash-out, an additional one hour equilibration period was allowed before commencement of any other experimental procedure. Cumulative concentration-response curves (CCRC) were constructed by increasing the concentration of the agonists in half-log increments. When responses to agonists were not maintained, addition of the next concentration was made as close to the peak as possible. An initial control CCRC, to any given agonist, was obtained in each preparation. Following attainment of the maximal control contraction, preparations were washed until complete relaxation was effected. The preparations were then left for a further period of 45-60 min before re-exposure to the agonist. When the competitive antagonists prazosin and rauwolscine were used, the preparations were incubated at least for 45 minutes with the drugs prior to the onset of a second CCRC. Results were

expressed as mean  $\pm$  standard error of mean (s.e.mean). Comparisons between two groups were performed using the paired or unpaired Student's t-test with values. Comparisons among several groups were performed using one-way analysis of variance. A value of  $p < 0.05$  was taken as statistically significant.

## Solutions and drugs

The composition of the modified Krebs-Henselite solution was as follows (in mM): NaCl 118.4, NaHCO<sub>3</sub> 25, KCl 4.7, KH<sub>2</sub>PO<sub>4</sub> 1.6, MgSO<sub>4</sub> 0.6; CaCl<sub>2</sub> 2.5 and glucose 11. Na<sub>2</sub>EDTA (23 $\mu$ M) was also included in the Krebs in all experiments to prevent degradative oxidation of NA, and propranolol (1 $\mu$ M) and cocaine hydrochloride (10 $\mu$ M) were also included to inhibit  $\beta$ -adrenoceptors and neuronal uptake of NA respectively. The following compounds were used: prazosin HCl (Pfizer); (-)-phenylephrine HCl (Sigma); (-)-noradrenaline bitrate (Sigma); propranolol HCl (Sigma); cocaine HCl (MacCarthys); HV-723 (Gift from Dr. Muramatsu, Japan); 5-methylurapidil (Research Biochemicals International); WB-4101 (Research Biochemicals International); chloroethylclonidine (Research Biochemicals International); Delequamine (RS-15385-197, Syntex, Gift from Dr. Whiting).

All drugs were dissolved in distilled water. All concentrations of the drugs used are expressed as final concentration in the organ bath.

## RESULTS

### Potency of phenylephrine

Phenylephrine produced concentration-dependent contractions in the isolated lateral saphenous vein. Phenylephrine produced isometric contraction with a pD<sub>2</sub> value of 5.84 and a maximum contraction of 3.36 $\pm$ 0.15g.

### Effects of $\alpha$ -adrenoceptor antagonists

Prazosin produced a concentration dependent rightward displacement of the phenylephrine CCRC. The pA<sub>2</sub> value for prazosin was 8 and the slope of Schild plot was close to unity (0.98), indicating competitive antagonism (Table I, Figure 1a).

HV-723 produced parallel shifts of the concentration-response curve to phenylephrine. Schild regression analysis yielded line with a pA<sub>2</sub> value of 7.97 and slope of 0.61, different from unity, indicating non-competitive antagonism (Table I).

5MU produced concentration-dependent shifts in the potency of phenylephrine without reducing the maximum response. The pA<sub>2</sub> value for 5MU was 7.2 and the slope of the Schild plot was 0.81, different from unity, indicating non-competitive antagonism (Table I).

Concerning the effects of various concentrations of the selective  $\alpha_1$ -adrenoceptor antagonist WB-4101 on responses

**Table I.** List of  $pA_2$  values with the slopes of the Schild plots (with 95% confidence limits) for  $\alpha$ -adrenoceptor antagonists against responses to phenylephrine in the rabbit isolated lateral saphenous vein.

Antagonist	$pA_2$	Slope
Prazosin	8 (7.6-8.95)	0.98 (0.93-1.1)
5MU	7.2 (6.77-8.85)	0.81 (0.74-0.95)
WB-4101	8.6 (7.93-9.37)	0.74 (0.63-0.91)
HV-723	7.97 (7.75-8.19)	0.61 (0.502-0.706)
Delequamine	8.31 (7.94-8.69)	0.71 (0.56-0.83)

$pA_2$  values were determined from a regression analysis of the logarithm of dose ratio-1 against the negative logarithm of the molar concentration of the antagonist.  $pA_2$  values were obtained for the  $\alpha$ -adrenoceptor antagonists using Schild analysis by plotting  $\log(DR-1)$  on the y-axis against  $\log M$  [antagonist] on the x-axis and fitting using linear regression.

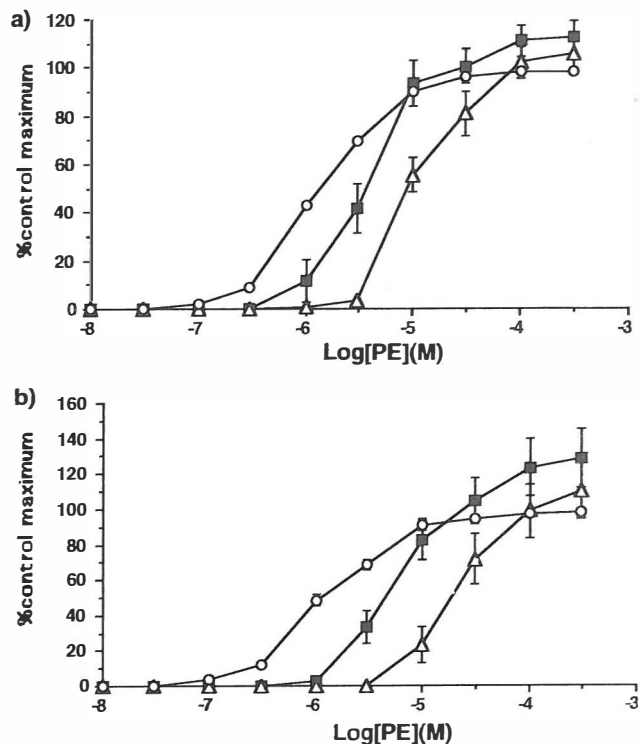
to PE, the  $pA_2$  value for WB-4101 was 8.6 and slope of the Schild plot was different from unity (0.74), indicating non-competitive antagonism (Table I, Figure 1b).

The irreversible antagonist chloroethylclonidine (CEC) (100  $\mu$ M) that preferentially alkylates  $\alpha_{1B}$ -adrenoceptors, failed to significantly reduce the maximum response to phenylephrine but produced small shifts in the potency of phenylephrine. The tissues were treated with CEC (100  $\mu$ M) for 30 minutes followed by washout of the irreversible antagonist for an additional 30-45 minutes (Figure 2a). The shifts in potency of phenylephrine produced by CEC (100  $\mu$ M) was 12.8 times. The  $\alpha_2$ -adrenoceptor antagonist delequamine produced a shift of the concentration-response curve to phenylephrine. The  $pA_2$  value was 8.3 and slope of the Schild plot was different from unity (0.7), indicating non-competitive antagonism (Table I, Figure 2b).

The rank order of potencies for these  $\alpha$ -adrenoceptor antagonists were as follows: WB-4101 > delequamine > prazosin > HV-723 > 5MU (Table I).

## DISCUSSION

Although phenylephrine is considered to be a selective  $\alpha_1$ -adrenoceptor agonist<sup>9</sup> and has been used to activate the postjunctional  $\alpha_1$ -adrenoceptors, the potency of phenylephrine in the rabbit saphenous vein is low (with  $pD_2$  value of 5.84) and it is possible that phenylephrine mediates contraction by activation of  $\alpha_2$ -adrenoceptors. A difficulty is that postjunctional  $\alpha_1$  and  $\alpha_2$ -adrenoceptors do not co-exist

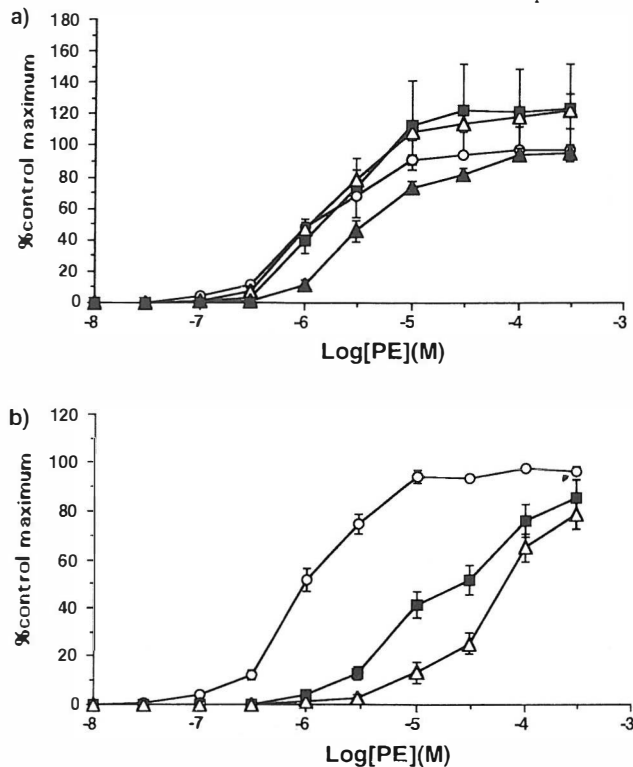


**Fig. 1.** Effects of  $\alpha_1$ -adrenoceptor antagonists a) prazosin 0.01  $\mu$ M ( $\blacksquare$ ) or 0.1  $\mu$ M ( $\triangle$ ) b) WB-4101 0.01  $\mu$ M ( $\blacksquare$ ) or 0.1  $\mu$ M ( $\triangle$ ) on responses to phenylephrine [PE] (O) in the rabbit isolated lateral saphenous vein. Results are expressed as % of the maximum response of the control CCR in the absence of antagonist. Each point represents mean  $\pm$  S.E. mean (n = 6).

in a simple manner and they interact at the level of a common post-receptor site in the events leading to contraction. Schumann and Lues<sup>6</sup> reported that phenylephrine responses were insensitive to prazosin but sensitive to rauwolscine in the rabbit saphenous vein. In another report rauwolscine competitively antagonised the contractile responses to phenylephrine in the rabbit saphenous vein with a  $pA_2$  of 7.16.<sup>10</sup> In the current study prazosin competitively inhibited contractile responses to phenylephrine with a  $pA_2$  value of 8.

The selective  $\alpha_2$ -adrenoceptor antagonist delequamine<sup>11</sup> inhibited phenylephrine-induced contractions. The  $pA_2$  and slope were 8.3 and 0.7 respectively. If it is accepted that delequamine is highly selective for  $\alpha_2$ -adrenoceptors then this would be consistent with phenylephrine contraction being mediated by  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors and corollary is that its responses can be attenuated by antagonists of either receptor type. In dog saphenous vein, concentration-dependent contractile response curves obtained to selective  $\alpha_2$ -adrenoceptor agonist BHT-920 were progressively displaced to the right of controls by delequamine. Schild analysis of these data gave a  $pA_2$  of 10 with a slope of 0.85. Pretreatment of the tissues with phenoxybenzamine at a concentration (10 nM) which irreversibly inactivates the  $\alpha_1$ -

## $\alpha_1$ -Adrenoceptor Subtypes



**Fig. 2.** Effects of **a)** treatment with the CEC [1nM (■), 10 nM ( $\Delta$ ) or 100 $\mu$ M ( $\blacktriangle$ )]; tissues were incubated with 1nM or 10nM CEC for 45 min prior to the onset of CCRC to phenylephrine (PE). **b)** selective  $\alpha_2$ -adrenoceptor antagonist delequamine 0.1  $\mu$ M (■) or 1 $\mu$ M ( $\Delta$ ) on response to phenylephrine [PE] (O) in the rabbit isolated lateral saphenous vein. Results are expressed as % of the maximum response of the first control CCRC to PE

adrenoceptors did not modify the antagonist effects of delequamine. In rabbit aorta delequamine was a weak antagonist of phenylephrine-induced contractions.  $pA_2$  was 6.05 with slope of 0.9.<sup>12</sup> Also in the present study in precontracted preparations of rat carotid artery induced by noradrenaline, delequamine produced weak relaxation of noradrenaline-induced contractions compared with rauwolscine. Thus delequamine does appear to be highly selective for  $\alpha_2$ .

### Subtype of $\alpha_1$ -adrenoceptor mediating contraction is consistent with $\alpha_{1L}$

In this preparation there is evidence for phenylephrine-induced contraction being mediated by  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors and a synergistic effect of  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors. The relatively low absolute  $pA_2$  values for prazosin in rabbit saphenous vein is consistent with blockade of the  $\alpha_{1L}$  type as defined by Muramatsu and co-workers.<sup>2</sup> An alternative explanation is that all antagonists appear to have low potency due to the synergism between  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors but the  $\alpha_{1L}$  hypothesis will be discussed further. The  $\alpha_{1L}$ -adrenoceptors are also less sensitive to WB-

4101 and 5-MU ( $pK_B$ : approximately 8) and are relatively resistant to CEC. HV-723 can subdivide the  $\alpha_{1L}$ -adrenoceptor into two subtypes:  $\alpha_{1L}$  (HV723-low, <lnM) and  $\alpha_{1N}$  (neither  $\alpha_{1H}$  or  $\alpha_{1L}$ ) (HV723-high).<sup>2</sup> In this study the  $pA_2$  of HV-723 was 7.97, that is less than 9. Therefore the subtype of  $\alpha_1$ -adrenoceptor mediating contraction in this preparation can be interpreted as the  $\alpha_{1L}$  type. Lack of inhibition of phenylephrine-induced contraction by chloroethylclonidine (CEC) supports that the response is mediated by a subtype other than  $\alpha_{1B}$  or  $\alpha_{1D}$  subtypes. The same pattern was seen when  $\alpha_1$ -adrenoceptor-mediated contraction of the guinea-pig ileum by phenylephrine was examined. Phenylephrine-mediated contraction was not affected by treatment with CEC. In addition, both WB-4101 and 5-MU antagonised the  $\alpha_1$ -adrenoceptor mediated contraction with low affinity.<sup>12</sup> In the present investigation, prazosin, WB-4101, 5-MU and HV-723 antagonised the phenylephrine-mediated contraction with lower affinity than we expected for  $\alpha_1$ -adrenoceptors. In the classical  $\alpha_1$ -adrenoceptor preparation, rat aorta, WB-4101 and 5-MU competitively antagonised contractions to noradrenaline. The  $pA_2$  values were 9.21 and 8.12 respectively.<sup>13</sup> HV-723 competitively inhibited the contractile responses induced by phenylephrine in rabbit thoracic aorta and rat aorta. The  $pA_2$  values were 8.71 and 9.21 respectively.<sup>2</sup> Although they are low for  $\alpha_2$ , the affinities of these antagonists are much higher than we would expect for  $\alpha_2$ -adrenoceptors. This indicates that in this preparation the relative resistance of responses to phenylephrine shown by the selective  $\alpha_1$ -adrenoceptor antagonists may be because phenylephrine interacts not only with the  $\alpha_1$ -adrenoceptors. However the data can support the presence of the prazosin-low affinity sites ( $\alpha_{1L}$ -adrenoceptors) which have also been detected in many other tissues: human, dog and rabbit prostates, human coronary vein, rat vas deferens, rat anococcygeus muscle, rat portal vein, and dog femoral artery and vein.<sup>14</sup> The  $\alpha_{1L}$ -adrenoceptor is found in vascular smooth muscle (the thoracic aorta) of the guinea-pig and is insensitive to inactivation by CEC. In addition to having a low affinity for prazosin (>1nM), they also have a relatively low affinity (1-10nM) for WB-4101.<sup>15</sup> The present data may fit with the  $\alpha_{1L}$  subtype within the  $\alpha_{1H}$ ,  $\alpha_{1L}$  and  $\alpha_{1N}$  subclassification proposed by Muramatsu and co-workers<sup>2</sup> although this subtype has not yet been identified by molecular cloning techniques. In conclusion, our study demonstrates that in the rabbit isolated lateral saphenous vein, the subtype of  $\alpha_1$ -adrenoceptor mediating contraction is  $\alpha_{1L}$ , and contraction induced by the selective  $\alpha_1$ -adrenoceptor agonist phenylephrine is mediated via  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors.

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