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Interaction of 5-methyl-urapidil with α_1-adrenoceptors in canine blood vessels: impact of pacing-induced heart failure

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Abstract

This study examines whether the α_1-adrenoceptors in canine endothelium-denuded dorsal pedal artery and endothelium-denuded saphenous vein can be differentiated by 5-methyl-urapidil before (non-paced) and at end-stage heart failure induced by rapid ventricular pacing. Noradrenaline and phenylephrine produced concentration-dependent contractions of the dorsal pedal artery and the saphenous vein which were enhanced at end-stage heart failure. In non-paced animals, 5-methyl-urapidil was shown to be insurmountable against noradrenaline with the artery being more sensitive compared to the vein. At end-stage heart failure, 5-methyl-urapidil was a competitive antagonist against noradrenaline in both the artery and the vein with pA_2 values of 8.1 (7.9–8.4) and 8.6 (8.2–9.1), respectively. A different antagonist profile was seen against phenylephrine. Similar to noradrenaline, insurmountable antagonism was observed in the artery and the vein before the development of heart failure. In contrast to noradrenaline, at end-stage heart failure, no antagonism was seen with the concentrations of 5-methyl-urapidil tested against phenylephrine. These results suggest that the mechanisms mediating contractions in the dorsal pedal artery and saphenous vein to noradrenaline and phenylephrine are heterogeneous and dependent on the heart failure state.

Keywords: Ventricular pacing, rapid; Heart failure; Dorsal pedal artery; Saphenous vein; α_1-Adrenoceptor; 5-Methyl-urapidil

1. Introduction

Congestive heart failure is characterised by a decreased cardiac output, for which several compensatory mechanisms evolve. Activation of the sympathetic nervous system results in elevated levels of circulating catecholamines, which mediate vasoconstriction and increased peripheral vascular resistance via stimulation of vascular α_1-adrenoceptors, ensuring blood flow to vital vascular beds (Cody and Laragh, 1988; Goldsmith and Kubo, 1988). In congestive heart failure, induced by rapid right ventricular pacing in the dog, haemodynamic and neurohumoural changes occur which are similar to those seen in the clinical setting of heart failure in man (Armstrong et al., 1986; Parmley, 1985).

This laboratory has been concerned with defining α_1-adrenoceptor characteristics in blood vessels following development of pacing-induced heart failure. It was found, in mild heart failure (1 week pacing) and at end-stage heart failure (approximately 4 weeks of pacing), that contractile responsiveness of the dorsal pedal artery and saphenous vein to α-adrenoceptor agonists was enhanced (Forster et al., 1989a,1992a). This occurred with selective α_1-adrenoceptor agonists and mixed α-adrenoceptor agonists, but not with α_2-adrenoceptor agonists (Forster and Armstrong, 1990; Forster et al., 1989a,b,1992a).

The existence of α_1-adrenoceptor subtypes has been the subject of debate for several years. Morrow and Creese (1986) found high and low affinity binding sites for WB 4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride) in rat brain. Moreover, functional studies showed that chloroethylclonidine alkylated the α_1-adrenoceptors in certain tissues to a much greater extent than in others (Han et al., 1987a,b). The receptors with high affinity for WB 4101 were termed α_{1A}-adrenoceptors and those being sensitive to alkylation by chloroethylclonidine were termed α_{1B}-adrenoceptors (Morrow and Creese, 1986). This classification has been supported by a number of antagonists showing approximately 100-fold greater sensitivity for the α_{1A}-adrenoceptor (Gross et al.,

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1988). pA_2 values for 5-methyl-urapidil in rat tissues with putative α_{1A}-adrenoceptors (submaxillary gland, kidney and in other reported literature) are approximately 9.0 (Clarke et al., 1995), those pA_2 values reported for 5-methyl-urapidil at the α_{1B}- and α_{1D}-adrenoceptors being 7.5 and 8.0, respectively.

With the advent of molecular biology, cloning studies have identified three α_1-adrenoceptor subtypes (α_{1b}-, α_{1c}- and α_{1d}-; recombinant receptors are indicated by a lowercase subscript, whereas functional, pharmacologically defined receptors are indicated by an uppercase subscript: Hieble et al., 1995; Bylund et al., 1994; Cotecchia et al., 1988; Lomasney et al., 1991; Schwinn et al., 1990). The α_{1b}-adrenoceptor clone when expressed in cell lines transfected with the cDNA for this subtype and the tissue α_{1B}-adrenoceptor have similar pharmacology (Lomasney et al., 1991). A clone was identified which had distinctly different pharmacology from the α_{1A}-adrenoceptor and the α_{1B}-adrenoceptor, this was subsequently termed the α_{1d}-adrenoceptor clone (Perez et al., 1991). There is evidence for a functional α_{1D}-adrenoceptor in the vasculature (Kenny et al., 1995; Saussy et al., 1994) and the availability of BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5]decane-7,9-dione dihydrochloride; a relatively selective α_{1D}-adrenoceptor antagonist) will allow functional tissue correlates to be identified (Graham et al., 1995). Much controversy surrounds the notion that the cloned α_{1c}-adrenoceptor and the functional α_{1A}-adrenoceptor are identical counterparts (Clarke et al., 1995; Ford et al., 1994; Michel and Insel, 1994; Laz et al., 1993). This notion has now been settled (Watson and Girdlestone, 1996). Nevertheless, there is support for the existence of other α_1-adrenoceptor subtypes, particularly in vascular smooth muscle (Flavahan and Vanhoutte, 1986; Muramatsu et al., 1991, 1995), which hinders the classification into purely α_{1A}- and α_{1B}- (Oriowa and Ruffolo, 1992).

The present study examines the effects of the selective α_{1A}-adrenoceptor antagonist, 5-methyl-urapidil (Gross et al., 1988) against noradrenaline and phenylephrine in the dorsal pedal artery and saphenous vein from non-paced (before heart failure development) and paced (250 bpm) dogs with the hypothesis that the enhanced contractile response in these blood vessels is mediated, in part, by α_{1A}-adrenoceptors.

2. Materials and methods

2.1. Canine model of congestive heart failure

Adult male mongrel dogs (18–25 kg) were preconditioned to the study environment 2–3 weeks prior to the onset of the study. Two groups of animals were used. (i) Nine dogs which were not paced, acted as controls and were killed acutely after conscious haemodynamics had been evaluated (previous data from this laboratory have indicated that there are absolutely no changes in a group of non-paced animals which were sham-operated and studied 4 and 8 weeks after pacemaker implantation and a group of animals which were acutely killed (Forster et al., 1989b)). (ii) Nine dogs paced to end-stage heart failure as defined previously (Armstrong et al., 1986) or a cut-off period of 4 weeks of pacing (Larosa and Forster, 1996).

Pacemaker implantation was performed according to methods previously described (Armstrong et al., 1986). Briefly, under sodium thiopental (25 $mg \cdot kg^{-1}$; Abbott Laboratories, Montreal, Quebec, Canada) anaesthesia, a pacemaker generator (Medtronics, Mississauga, Ontario, Canada) was inserted into a subcutaneous cervical pocket and a unipolar pacemaker lead was positioned, under fluoroscopy, into the apex of the right ventricle. Dogs were allowed a 1 week recovery period before pulse generators were programmed to deliver 250 bpm. Approval for these studies was obtained from the Animal Care Committee of St Michael's Hospital in accordance with the Animals of Research Act and the guidelines of the Canadian Council on Animal Care.

2.2. Organ bath experiments

Once the desired pacing time had elapsed, respective dogs from either the non-paced or the end-stage heart failure group were killed with an overdose of sodium thiopental and small segments of dorsal pedal artery and saphenous vein (< 4 cm) were removed from the region of the lateral hindpaw. Six ring sections (5 mm) from each vessel had their endothelium removed by inserting the tip of fine forceps through the lumen and rolling the preparation back and forth on Krebs-Henseleit moistened filter paper. Each vessel segment was then mounted in 10 ml organ baths containing Krebs-Henseleit solution (gassed with 95% O_2/5% CO_2) of the following mM composition: NaCl 120, $CaCl_2$ 2.5, KCl 5.6, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, NaH_2PO_4 1.2 and D-glucose 10.0. In addition, the following inhibitors were added to the Krebs-Henseleit solution: propranolol, 10^{-6} M; indomethacin, 2.8×10^{-6} M; desipramine, 10^{-6} M and yohimbine, 10^{-7} M to antagonise β-adrenoceptors, inhibit endogenous prostanoid production, block neuronal uptake and antagonise α_2-adrenoceptors, respectively. These concentrations have been shown to be optimum in affecting these other systems (Forster, 1995). Each ring was attached to a force displacement transducer (Model FT03C, Grass Instrument Co., Quincy, MA, USA) and changes in isometric tension were displayed on a polygraph (Model 7D, Grass Instrument, Quincy, MA, USA). The initial equilibration period was at least 1 h (during which time frequent washing occurred and optima resting tensions of 4 g and 2.5 g (as previously determined in this laboratory (Forster et al., 1991)) were maintained for the dorsal pedal artery and the saphenous vein, respectively.

2.3. Experimental design

After equilibration, cumulative concentration curves were constructed on both preparations to noradrenaline and phenylephrine. Briefly, rings were exposed to the lowest concentration of agonist, the resulting contraction was allowed to develop until it reached a plateau, after which another higher concentration of the same agonist was added. This procedure continued until either no further increase in tension was observed or limited by the highest concentration of agonist (10^{-4} M). A total of four preparations were used from each vessel, one pair of rings received noradrenaline and the other pair received phenylephrine. Once the initial concentration-effect curve was completed, rings were washed frequently until the resting tension returned. 5-Methyl-urapidil (10^{-8} M) was administered to one ring of each pair so that antagonism could be assessed against each of the agonists. The other ring of each pair acted as a time control and did not receive 5-methyl-urapidil. The antagonist was in the bath 30 min before re-constructing the concentration-effect curves. The entire procedure was repeated on at least two more occassions using two higher concentrations of 5-methyl-urapidil. At the end of each experiment, vessels were preconstricted with KCl (20 mM) and when the contraction had developed and plateaued, without washing out, increasing concentrations of acetylcholine (10^{-8} M–10^{-5} M) were added to ensure successful endothelium denudation.

2.4. Data analysis

Contractions were expressed as arithmetic means ± S.E. for n (number of) preparations (one or two from each dog). To eliminate differences due to variability in muscle mass, all contractile data were normalised for cross-sectional area ($g \cdot mm^{-2}$; determined by dividing the blotted mass of the tissue by its length and specific gravity (Herlihy and Berardo, 1986). EC_{50} values were expressed as geometric means with 95% confidence intervals. All concentration-effect curve data were fitted to the logistic function:

$$Y = \left\{ (a - d) / \left(1 + [X/c]^b\right)\right\} + d$$

which derived the maximum response and the EC_{50} from the observed individual data points (Parker and Waud, 1971). For the antagonist data, either Arunlakshana and Schild (1959) analysis was performed for competitive antagonism or IC_{50} (geometric means with 95% confidence intervals) values were calculated for insurmountable antag-

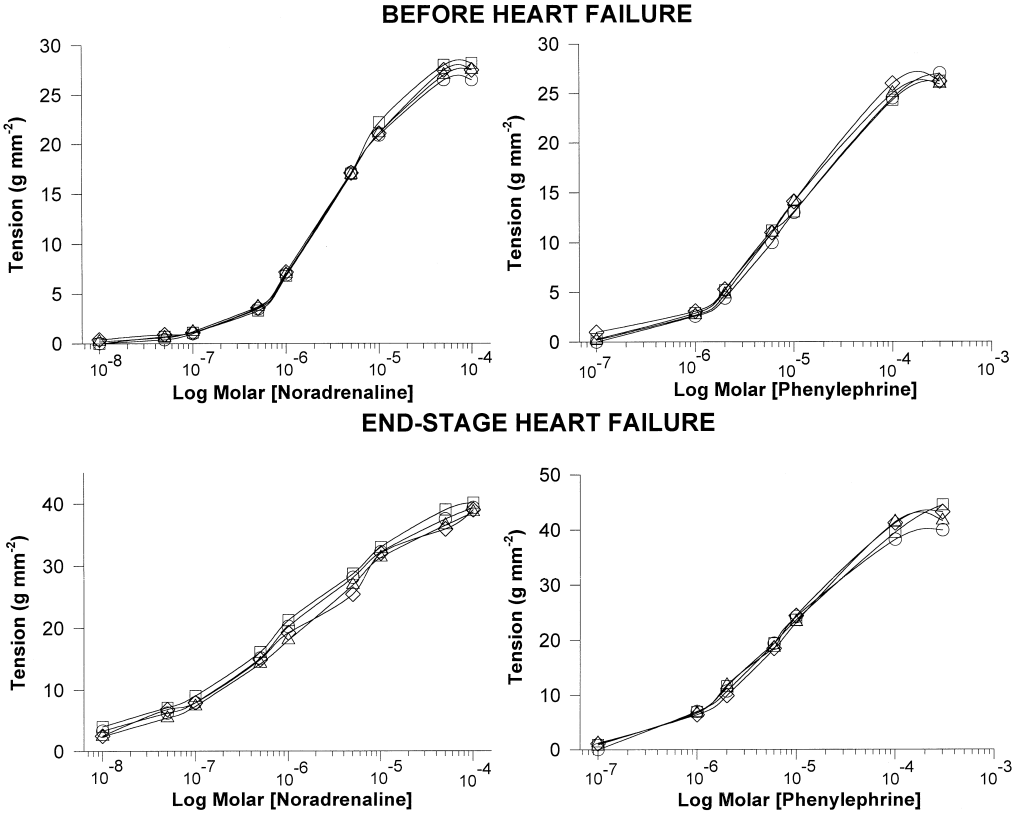

Fig. 1. Time-matched control, concentration-effect curves generated for noradrenaline and phenylephrine before heart failure (upper panel) and at end-stage heart failure (lower panel). The open circles represent the first curve, the open triangles represent the second curve, the open squares represent the third curve and the open diamonds represent the fourth curve. Each curve was separated by a period of approximately 90 min and each point is the mean ± S.E.M. from at least 4 dogs. Data shown are for the dorsal pedal artery, similar time-matched control curves were seen on the saphenous vein.

onism. IC_{50} is defined as that concentration of antagonist which causes a reduction in the maximum response to the agonist by 50% (Jenkinson et al., 1995). Statistical analysis for all data except the contractile data was performed by the non-parametric test, Mann-Whitney *U*-test. Student's *t*-test was performed for end-stage heart failure versus control and if more than one comparison was used, then a Bonferroni correction was applied. In all cases a P value of < 0.05 was considered significant.

2.5. Drugs and solutions

The following drugs were used: acetylcholine iodide, *l*-noradrenaline bitartrate, phenylephrine hydrochloride, desipramine hydrochloride, indomethacin (all from Sigma, St. Louis, MO, USA), propranolol hydrochloride (Ayerst Laboratories, New York, NY, USA) and 5-methyl-urapidil (Research Biochemicals International, Natick, MA, USA).

Stock solutions and dilutions were made in deionised water, with the exception of noradrenaline and indomethacin. Noradrenaline was made up in 0.2% ascorbic acid with subsequent dilutions in deionised water. Indomethacin was dissolved in the bicarbonate mixture before being added to the Krebs-Henseleit solution.

3. Results

3.1. General

The haemodynamic variables of the two groups of dogs are shown in Table 1. Typically, heart failure was associated by a rise in heart rate, left ventricular filling pressure, mean pulmonary artery pressure and mean pulmonary capillary wedge pressure, as well as an increase in systemic vascular resistance. Cardiac output was always significantly decreased in the heart failure group.

Table 1
Haemodynamic profile for the two groups of dogs

	Before heart failure	End-stage heart failure
HR (beats·min^{-1})	105 ± 3	160 ± 7 [b]
MAP (mmHg)	111 ± 4	103 ± 4
LVEDP (mmHg)	10 ± 1	35 ± 2 [b]
PA (mmHg)	14 ± 1	40 ± 2 [b]
PCWP (mmHg)	6 ± 1	30 ± 1 [b]
RA (mmHg)	8 ± 1	16 ± 2 [b]
CO (litres·min^{-1})	5 ± 1	3 ± 0.3 [b]
SVR (Units)	78 ± 5	110 ± 12 [b]

Each value is mean ± S.E.M. for 9 dogs in each group for heart rate (HR), mean arterial pressure (MAP), left ventricular end-diastolic presure (LVEDP), pulmonary artery pressure (PA), pulmonary capillary wedge pressure (PCWP), right atrial pressure (RA), cardiac output (CO) and systemic vascular resistance (SVR) which was calculated from MAP − RA/cardiac index. Conscious haemodynamic variables were measured with the pacemaker turned off (i.e., under normal sinus rhythm) by insertion of a Swan-Ganz catheter and a Millar catheter. [b] Significance at the $P < 0.05$ level for end-stage heart failure versus before heart failure.

Table 2
Maximal response (g tension·mm^{-2}) for 5-methyl-urapidil (5-MU; 10^{-7} M) against noradrenaline and phenylephrine in the dorsal pedal artery and saphenous vein

		Dorsal pedal artery maximum response	Saphenous vein maximum response
Noradrenaline			
Before	(no 5-MU)	28.3 ± 2.4	33.6 ± 2.0
	(with 5-MU)	13.8 ± 0.6 [a]	23.8 ± 1.4 [a,c]
End-stage	(no 5-MU)	41.4 ± 3.4	46.1 ± 2.6 [b]
	(with 5-Mu)	37.7 ± 2.3	37.0 ± 2.4
Phenylephrine			
Before	(no 5-MU)	30.7 ± 1.1	35.6 ± 2.2
	(with 5-MU)	14.2 ± 1.6 [a]	19.2 ± 1.5 [a]
End-stage	(no 5-MU)	42.4 ± 3.8 [b]	60.4 ± 3.6 [b,c]
	(with 5-MU)	36.5 ± 3.4	55.3 ± 2.5 [c]

Maximum response data are expressed as mean ± S.E.M. for at least 4 dogs. [a] Significance at the $P < 0.05$ level for before heart failure with 5-MU versus no 5-MU. [b] Significance at the $P < 0.05$ level for end-stage versus before heart failure in the absence of 5-MU. [c] Significance at the $P < 0.05$ level for saphenous vein versus dorsal pedal artery.

None of the time-control preparations differed in responsiveness throughout the course of the study as can be seen in Fig. 1. Typically, end-stage heart failure was associated with an increased maximum response to both noradrenaline and phenylephrine in both vessels (Table 2). EC_{50} values for noradrenaline on the dorsal pedal artery were 3.8 (2.9–5.0) μM and 2.1 (0.2–3.3) μM for non-paced and end-stage heart failure, respectively. The vein exhibited significantly lower EC_{50} values, compared to the artery, which were 0.8 (0.4–1.5) μM and 0.9 (0.3–3.7) μM for non-paced and end-stage heart failure, respectively. In both preparations, the EC_{50} values for phenylephrine were not significantly different regardless of the heart failure state (before heart failure: 6.7 (5.1–8.8) μM and 5.7 (3.6–8.9) μM for the artery and the vein; and at end-stage heart failure: 9.2 (4.7–11.8) μM and 6.7 (4.5–9.9) μM for the artery and vein, respectively).

None of the vascular preparations reacted with a relaxation in response to increasing concentrations of acetylcholine indicating that the endothelium removal process had been successful.

3.2. The effect of 5-methyl-urapidil on responsiveness of the dorsal pedal artery to noradrenaline

Fig. 2 shows concentration-effect curves constructed to noradrenaline in the absence and presence of increasing concentrations of 5-methyl-urapidil in the canine dorsal pedal artery before and at end-stage heart failure. In arterial rings from non-paced animals, increasing concentrations of 5-methyl-urapidil caused a concentration-depen-

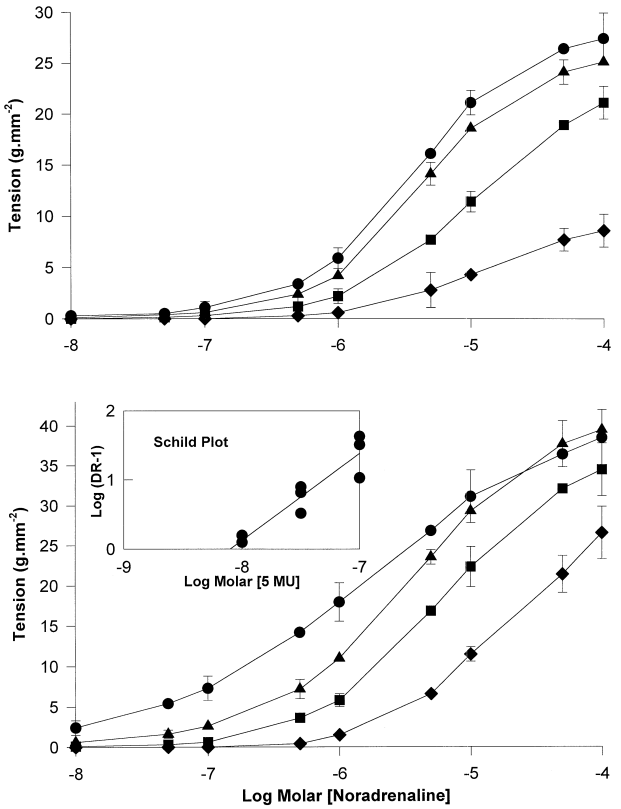

Fig. 2. Concentration-effect curves were constructed for noradrenaline in the absence and presence of increasing concentrations of 5-methyl-urapidil in the dorsal pedal artery before heart failure (upper panel) and at end-stage heart failure (lower panel). Control curves in both data sets are represented by the closed circles. Curves constructed in the presence of 10^{-8} M 5-methyl-urapidil (closed triangles); in the presence of 3×10^{-8} M 5-methyl-urapidil (closed squares); and in the presence of 10^{-7} M 5-methyl-urapidil (closed diamonds). Each point is the mean ± S.E.M. of at least 4 determinations from at least 4 dogs. The inset in the lower panel represents the Schild plot; no Schild plot could be derived for the non-paced group since 5-methyl-urapidil caused a concentration-dependent decrease in the maximum response. For statistical comparisons, see Table 2.

dent decrease in the height of the maximum response by noradrenaline. Further analysis of these data yielded an IC_{50} of 0.1 (0.07–0.2) μM. At end-stage heart failure, the dorsal pedal artery was seen to respond to noradrenaline (in the absence of 5-methyl-urapidil) in an enhanced manner resulting in an increase in the maximum response (Table 2). It is apparent that the gradient of the slope is shallower than that seen before heart failure. 5-Methyl-urapidil caused a concentration-dependent dextral displacement of the concentration-effect curve which was associated with no significant change in the height of the maximum response as determined by the curve-fit analysis (see Section 2.4). The resulting Schild plot (inset Fig. 2) rendered a pA_2 value of 8.1 (7.9–8.4) and a slope of −1.2 (−0.9 to −1.4).

3.3. The effect of 5-methyl-urapidil on the responsiveness of the saphenous vein to noradrenaline

The effect of 5-methyl-urapidil on the responsiveness of the saphenous vein to noradrenaline from non-paced and end-stage heart failure animals is shown in Fig. 3 and Table 2. Before heart failure, 5-methyl-urapidil produced a concentration-dependent decrease in the maximum response generated by noradrenaline. The calculated IC_{50} for these data was 0.5 (0.2–0.8) μM which was significantly greater than the IC_{50} for the dorsal pedal artery (0.1 (0.07–0.2) μM). Furthermore, 5-methyl-urapidil affects the upper portion of the concentration-effect curve to a much greater extent than the lower portion. No significant

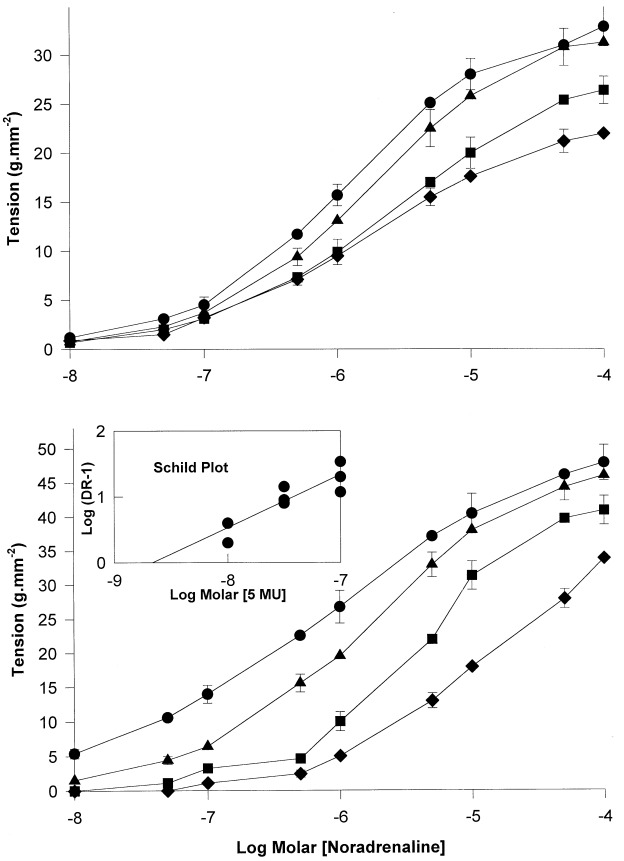

Fig. 3. Concentration-effect curves were constructed for noradrenaline in the absence and presence of increasing concentrations of 5-methyl-urapidil in the saphenous vein before heart failure (upper panel) and at end-stage heart failure (lower panel). Control curves in both data sets are represented by the closed circles. Curves constructed in the presence of 10^{-8} M 5-methyl-urapidil (closed triangles); in the presence of 3×10^{-8} M 5-methyl-urapidil (closed squares); and in the presence of 10^{-7} M 5-methyl-urapidil (closed diamonds). Each point is the mean ± S.E.M. from at least 4 determinations from at least 4 dogs. The inset in the lower panel represents the Schild plot; no Schild plot could be derived for the non-paced group since 5-methyl-urapidil caused a concentration-dependent decrease in the height of the maximum response. For statistical comparisons, see Table 2.

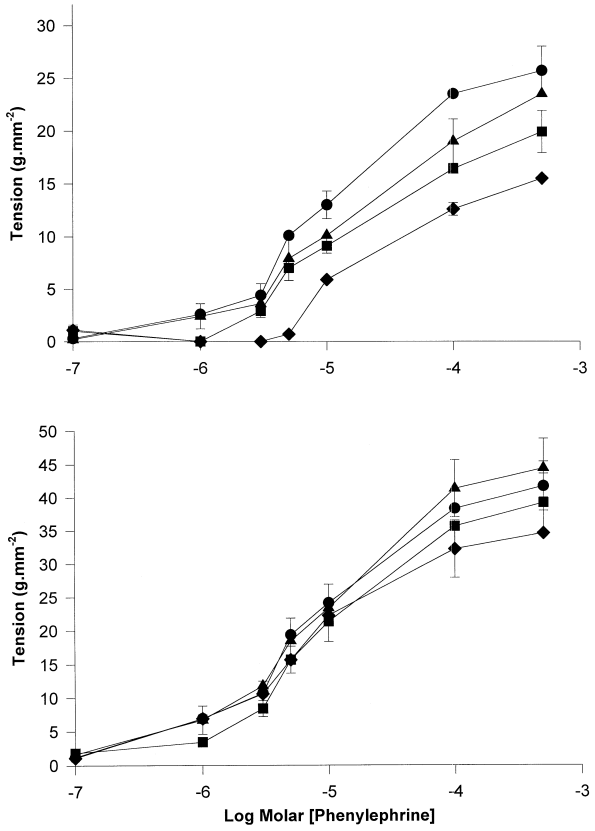

Fig. 4. Concentration-effect curves were constructed for phenylephrine in the absence and presence of increasing concentrations of 5-methyl-urapidil in the dorsal pedal artery before heart failure (upper panel) and at end-stage heart failure (lower panel). Control curves in both data sets are represented by the closed circles. Curves constructed in the presence of 10^{-8} M 5-methyl-urapidil (closed triangles); in the presence of 3×10^{-8} M 5-methyl-urapidil (closed squares); and in the presence of 10^{-7} M 5-methyl-urapidil (closed diamonds). Each point is the mean ± S.E.M. from at least 4 determinations from at least 4 dogs. For statistical comparison, see Table 2.

reduction in the magnitude of the contraction was seen until a noradrenaline concentration of 10^{-6} M was administered.

The effect of 5-methyl-urapidil on the responsiveness of the saphenous vein to noradrenaline from dogs at end-stage heart failure was seen to be distinctly different as shown in the lower panel of Fig. 3. First of all there were no significant differences in the derived maximum responses (according to the logistic function; see Section Section 2.4) by noradrenaline in the presence of any of the concentrations of 5-methyl-urapidil tested. Secondly, there was a concentration-dependent displacement of the concentration-effect curve to the right. Arunlakshana Schild analysis of these data generated a pA_2 value of 8.6 (8.2–9.1) with a slope of −0.8 (−0.6 to −1.1) as shown in the inset of Fig. 3. In addition, and similar to the artery, the gradient of the control curve was less than that seen before heart failure.

3.4. The effect of 5-methyl-urapidil on the responsiveness of the dorsal pedal artery to phenylephrine

In Fig. 4, concentration-effect curves generated by phenylephrine in the absence and presence of 5-methyl-urapidil on the dorsal pedal artery are depicted. It can be seen that before heart failure, 5-methyl-urapidil shifted the concentration-effect curve downwards in a concentration-dependent manner. The maximum response was significantly reduced as derived by the logistic function equation (Table 2) and the IC_{50} was 0.1 (0.06–0.2) μM. In contrast, at end-stage heart failure, 5-methyl-urapidil (in the concentrations used) had no effect on the contractions developed with phenylephrine.

3.5. The effect of 5-methyl-urapidil on the responsiveness of the saphenous vein to phenylephrine

Concentration-effect curves to phenylephrine in the saphenous vein are shown in Fig. 5. Before heart failure,

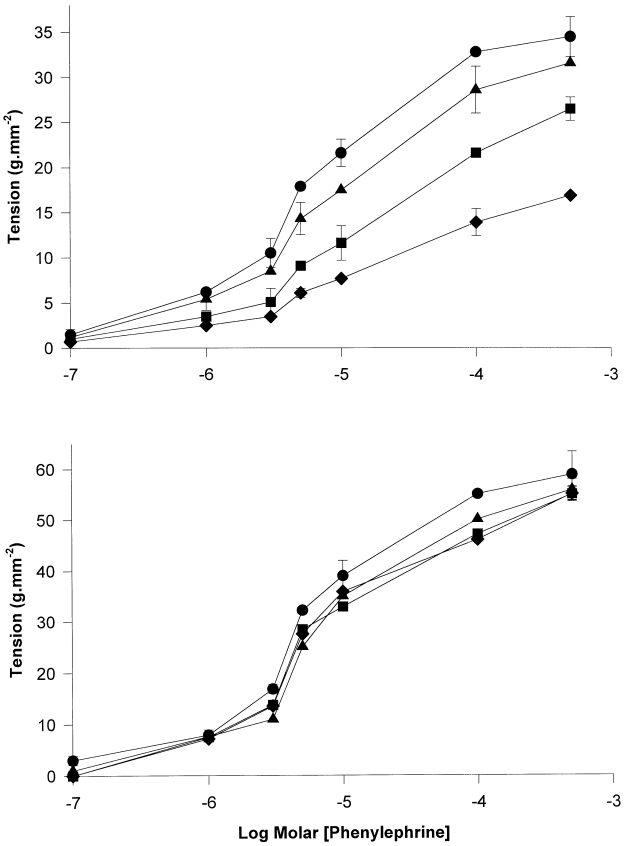

Fig. 5. Concentration-effect curves were constructed for phenylephrine in the absence and presence of increasing concentrations of 5-methyl-urapidil for the saphenous vein before heart failure (upper panel) and end-stage heart failure (lower panel). Control curves for both data sets are represented by the closed circles. Curves constructed in the presence of 10^{-8} M 5-methyl-urapidil (closed triangles); in the presence of 3×10^{-8} M 5-methyl-urapidil (closed squares); and in the presence of 10^{-7} M 5-methyl-urapidil (closed diamonds). Each point is the mean ± S.E.M. of at least 4 determinations from at least 4 dogs. For statistical comparisons, see Table 2.

5-methyl-urapidil significantly shifted the concentration-effect curve downwards by suppression of the maximum response in a concentration-dependent fashion with an IC_{50} of 0.08 (0.06–0.1) μM. At end-stage heart failure, similar to that seen in the dorsal pedal artery, 5-methyl-urapidil failed to antagonise the contractions developed with phenylephrine.

4. Discussion

The key findings of this study were 2-fold. First, prior to heart failure development, 5-methyl-urapidil was an insurmountable antagonist against both noradrenaline and phenylephrine in both the dorsal pedal artery and saphenous vein. Second, at end-stage heart failure, 5-methyl-urapidil became a competitive antagonist against noradrenaline, *but* failed to antagonise phenylephrine. These data support the heterogeneous nature of the α_1-adrenoceptors in blood vessels and suggest that the relative populations of α_1-adrenoceptor subtypes are capable of changing in disease settings, such as heart failure.

Before heart failure, both noradrenaline and phenylephrine produced concentration-dependent contractions in both the artery and the vein with similar maxima suggesting that the contractions to these agents are mediated by α_1-adrenoceptors. On the basis of the EC_{50} values, noradrenaline was more potent, with phenylephrine having half the potency in the artery and being some 8-fold less potent in the saphenous vein. In addition, the vein was more sensitive to noradrenaline, whereas both vessels exhibited similar sensitivity to phenylephrine. Previously, a distinction between noradrenaline- and phenylephrine-induced contractions in the dorsal pedal artery and the saphenous vein was made on the susceptibility to calcium channel inhibition with nifedipine (Forster and Campbell, 1993) and protein kinase C inhibition (Forster, 1995). At end-stage heart failure, both blood vessels exhibited an exaggerated response with noradrenaline and phenylephrine, but no significant changes in EC_{50} were observed. This latter observation differs from that seen previously (Forster et al., 1989a,b) and may be related to different pacing regimens (5.2 weeks versus 4 weeks in the current study). Clearly, these observations are very dependent on the stage of heart failure development. Nevertheless, this exaggerated response appears to be relatively selective for the α_1-adrenoceptor agonists and mixed α-adrenoceptor agonists, as no such enhancement was encountered with angiotensin I, II or III, nor is an increase seen in response to the depolarising agent, potassium chloride (Forster et al., 1989b,1992b).

In order to examine these phenomena further, the effect of 5-methyl-urapidil (a selective α_{1A}-adrenoceptor antagonist; Gross et al., 1988; Aboud et al., 1993) was used. 5-Methyl-urapidil inhibited the contractions by noradrenaline and phenylephrine in an insurmountable manner. This was unusual, since if the response to noradrenaline and phenylephrine involved an α_{1A}-adrenoceptor, then competitive antagonism would have been expected. Therefore, before heart failure, both vessels displayed a heterogenous population of α_1-adrenoceptors confirming other reports (Hicks et al., 1991; Low et al., 1994). In addition, 5-methyl-urapidil affected the upper portion of the concentration-effect curves to a greater extent which further indicates receptor heterogeneity. Moreover, Clarke et al. (1995) demonstrated that 5-methyl-urapidil defined two sites in the noradrenaline-perfused kidney of the rat, and similar to the data reported herein with 5-methyl-urapidil, *S*(+)-niguldipine (selective α_{1A}-adrenoceptor antagonist) acted as an insurmountable antagonist (Boer et al., 1989).

At end-stage heart failure, 5-methyl-urapidil antagonised noradrenaline in a competitive manner in both the dorsal pedal artery and the saphenous vein. The pA_2 value for 5-methyl-urapidil in the saphenous vein is consistent with the α_{1A}-adrenoceptor in various tissues (Burt et al., 1995; Garcia-Sainz and Romera-Avila, 1993; Marshall et al., 1995; Testa et al., 1993). A lower pA_2 estimate (8.1) was found in the dorsal pedal artery. This pA_2 value is very close to that described for the α_{1D}-adrenoceptor (8.0; Clarke et al., 1995). Whether the adrenoceptors in the dorsal pedal artery at heart failure represent further classes of functional α_1-adrenoceptors awaits subsequent studies with selective α_{1B}-antagonists, namely chloroethylclonidine (Han et al., 1987b) and spiperone (Eltze and Boer, 1992; Taddei et al., 1993), as well as the selective α_{1D}-adrenoceptor antagonist, BMY 7378 (Goetz et al., 1995). Preliminary data with chloroethylclonidine have been presented. These results indicated that, in the dorsal pedal artery, chloroethylclonidine (at a concentration of 10^{-4} M) inhibited the noradrenaline response by 66% compared to the saphenous vein before heart failure. However, at end-stage heart failure, the effect of the same concentration of chloroethylclonidine in both the artery and the vein was negligible (Forster and Le Tran, 1996).

5-Methyl-urapidil failed to antagonise contractions developed in response to phenylephrine on both blood vessels at end-stage heart failure. This would indicate that phenylephrine is not producing its contraction via α_{1A}-adrenoceptors. This is in conflict with reports suggesting that phenylephrine is an α_{1A}-adrenoceptor agonist (Piascik et al., 1991) and as described for the human vas deferens (Furukawa et al., 1995). In this latter study, 5-methyl-urapidil was a competitive antagonist against phenylephrine yielding a pA_2 value of 8.8. On the other hand, a recent report indicates that phenylephrine-activated receptors are not recognised by 5-methyl-urapidil in the dog saphenous vein (Daniel et al., 1996). The lack of effect by 5-methyl-urapidil against phenylephrine may indicate that another α_1-adrenoceptor exists in the dorsal pedal artery and the saphenous vein at end-stage heart failure. Therefore, the possibility remains that other α_1-adrenoceptors (atypical) await classification.

In conclusion, it appears that more than one functional α_1-adrenoceptors exist in the dorsal pedal artery and the saphenous vein and that this heterogeneity is altered in the setting of pacing-induced heart failure and is dependent on the stage of heart failure. The lack of effect by 5-methyl-urapidil against phenylephrine may imply that an unusual α-adrenoceptor subtype may exist in these blood vessels in the setting of heart failure. Additionally, the mechanism whereby phenylephrine induces its contractile response may differ from noradrenaline. Notwithstanding, the function of the adrenoceptors in the regulation of vascular tone in different vascular beds cannot be fully appreciated without the use of a full range of selective agonists and antagonists.

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References

Aboud, R., M. Shafi and J.R. Docherty, 1993, Investigations of the subtypes of α_1-adrenoceptor mediating contractions of the rat aorta, vas deferens and spleen, Br. J. Pharmacol. 109, 80.

Armstrong, P.W., T.P. Stopps, S.E. Ford and A.J. De Bold, 1986, Rapid ventricular pacing in the dog: pathophysiologic studies of heart failure, Circulation 74, 1075.

Arunlakshana, O. and H.O. Schild, 1959, Some quantitative uses of drug antagonists, Br. J. Pharmacol. 14, 48.

Boer, R., A. Grassegger, C. Schudt and H. Glossmann, 1989, (+)-Niguldipine binds with high affinity to Ca^{2+} channels and to a subtype of α_1-adrenoceptors, Eur. J. Pharmacol. 172, 131.

Burt, R.P., C.R. Chapple and I. Marshall, 1995, Evidence for a functional α_{1A}-(α_{1C}-)adrenoceptor mediating contraction of the rat epididymal vas deferens and an α_{1B}-adrenoceptor mediating contraction of the rat spleen, Br. J. Pharmacol. 115, 467.

Bylund, D.B., D.C. Eikenberg, J.P. Hieble, S.Z. Langer, R.J. Lefkowitz, K.P. Minneman, P.B. Molinoff, R.R. Ruffolo Jr. and U. Trendelenberg, 1994, International Union of Pharmacology. IV. Nomenclature of adrenoceptors, Pharmacol. Rev. 46, 121.

Clarke, D.E., A.P.D.W. Ford, T.J. Williams, D. Bonhaus, R. Vimont and D.R. Blue Jr., 1995, Pharmacological evidence for the equivalence of the α_{1A}-adrenoceptor of rat and the cloned α_{1C}-adrenoceptor: a revised α_1-adrenoceptor classification scheme, Pharmacol. Commun. 6, 9.

Cody, R.J. and J.H. Laragh, 1988, The renin-angiotensin-aldosterone system in chronic heart failure: pathophysiology and implications for treatment, in: Drug Treatment of Heart Failure, ed. J.N. Cohn (Advanced Therapeutic Communications International, Secaucus, NJ) p. 79.

Cotecchia, S., D.A. Schwinn, R.R. Randall, R.J. Lekkowitz, M.G. Caron and B.K. Kobilka, 1988, Molecular cloning and expression of the cDNA for the hamster α_1-adrenergic receptor, Proc. Natl. Acad. Sci. USA 85, 7159.

Daniel, E.E., A.M. Low, V. Gaspar, H, Lu-Chao, J. Green, J. Akrong, S. Duerksen, C. Soyka, C.K. Chen, J. Boyd and C.Y. Kwan, 1996, Unusual α-adrenoceptor subtype in canine saphenous vein: comparison to mesenteric vein, Br. J. Pharmacol. 117, 1535.

Eltze, M. and R. Boer, 1992, The adrenoceptor agonist, SDZ NVI 085, discriminates between α_{1A}- and α_{1B}-adrenoceptor subtypes in vas deferens, kidney and aorta of the rat, Eur. J. Pharmacol. 224, 125.

Flavahan, N.A. and P.M. Vanhoutte, 1986, α-Adrenoceptor subclassification in vascular smooth muscle, Trends Pharmacol. Sci. 7, 347.

Ford, A.P.D.W., T.J. Williams, D.R. Blue and D.E. Clarke, 1994, α_1-Adrenoceptor classification: sharpening Occam's razor, Trends Pharmacol. Sci. 15, 167.

Forster, C., 1995, Inhibition of vascular contractions to α-adrenoceptor agonists by polymyxin B: impact of heart failure state, Eur. J. Pharmacol. 283, 241.

Forster, C. and P.W. Armstrong, 1990, Pacing-induced heart failure in the dog: evaluation of peripheral vascular α-adrenoceptor subtypes, J. Cardiovasc. Pharmacol. 16, 708.

Forster, C. and P.M. Campbell, 1993, Nifedipine inhibits responses to α-adrenoceptor stimulation in canine blood vessels: impact of heart failure, Eur. J. Pharmacol. 242, 119.

Forster, C. and Y. Le Tran, 1996, Do α_{1B}-adrenoceptors play a role in the exaggerated vascular response to α-agonists in experimental heart failure?, Br. J. Pharmacol. 119, 261.

Forster, C., S.L. Carter and P.W. Armstrong, 1989a, α_1-Adrenoceptor activity in arterial smooth muscle following congestive heart failure, Can. J. Physiol. Pharmacol. 67, 110.

Forster, C., S. Carter and P. Armstrong, 1989b, Vascular smooth muscle responsiveness to noradrenaline and phenylephrine following experimental heart failure in dogs, Cardiovasc. Res. 23, 489.

Forster, C., D. Wanes and P.W. Armstrong, 1991, Novel vascular effects of isoprenaline following pacing-induced heart failure in the dog, Eur. J. Pharmacol. 200, 251.

Forster, C., P.M. Campbell and P.W. Armstrong, 1992a, Temporal alterations in peripheral vascular responsiveness during both the development and recovery from pacing- induced heart failure, J. Cardiovasc. Pharmacol. 20, 206.

Forster, C., G. Larosa and P.W. Armstrong, 1992b, Impact of enalapril therapy on in vitro coronary artery responsiveness in pacing-induced heart failure, Can. J. Pysiol. Pharmacol. 70, 1417.

Furukawa, K., D.J. Rosario, D.J. Smith, C.R. Chapple, T. Uchiyama and R. Chess-Williams, 1995, α_{1A}-Adrenoceptor-mediated contractile responses of the human vas deferens, Br. J. Pharmacol. 116, 1605.

Garcia-Sainz, J.A. and M.I. Romera-Avila, 1993, Characterization of the α_{1A}-adrenoceptors of guinea pig liver membranes using 5-^{3}H-methyl-urapidil, Mol. Pharmacol. 44, 589.

Goetz, A.S., H.K. King, S.D.C. Ward, T.A. True, T.J. Rimele and D.L. Saussy Jr., 1995, BMY 7378 is a selective antagonist of the D subtype of α_1-adrenoceptors, Eur. J. Pharmacol. 272, R5.

Goldsmith, S.R. and S.H. Kubo, 1988, Pathophysiology of heart failure: peripheral vascular factors and neurohormonal mechanisms, in: Drug Treatment of Heart Failure, ed. J.H. Cohn (Advanced Therapeutic Communications International, Secaucus, NJ) p. 49.

Graham, R.M., D.M. Perez, M.T. Piascik, R.P. Riek and J. Hwa, 1995, Characterization of α_1- adrenergic receptor subtypes, Pharmacol. Commun. 6, 15.

Gross, G., G. Hanfi and C. Rugevics, 1988, 5-Methyl-urapidil discriminates between subtypes of the α_1-adrenoceptor, Eur. J. Pharmacol. 151, 333.

Han, C., P.W. Abel and K.P. Minneman, 1987a, α_1-Adrenoceptor subtypes linked to different mechanisms for increasing intracellular Ca^{++} in smooth muscle, Nature 329, 333.

Han, C., P.W. Abel and K.P. Minneman, 1987b, Heterogeneity of α_1-adrenergic receptors revealed by chloroethylclonidine, Mol. Pharmacol. 32, 505.

Herlihy, J.T. and P.V. Berardo, 1986, Effect of preload on rat aortic smooth muscle sensitivity to vasoactive agents, Pharmacology 33, 39.

Hicks, P.E., M. Barras, G. Herman, P. Mauduit, J.M. Armstrong and B.

Rossignol, 1991, α-Adrenoceptor subtypes in dog saphenous vein that mediate contraction and inositol phosphate production, Br. J. Pharmacol. 102, 151.

Hieble, J.P., D.B. Bylund, D.E. Clarke, D.C. Eikenburg, S.Z. Langer, R.J. Lefkowitz, K.P. Minneman and R.R. Ruffolo Jr., 1995, International Union of Pharmacology. X. Recommendation for nomenclature of alpha 1-adrenoceptors; concensus update, Pharmacol. Rev. 47, 267.

Jenkinson, D.H., E.A. Barnard, D. Hoyer, P.P.A. Humphrey, P. Leff and N.P. Shankly, 1995, International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. IX. Recommendations on terms and symbols in quantitative pharmacology, Pharmacol. Rev. 47, 255.

Kenny, B.A., D.H. Chalmers and A.M. Naylor, 1995, Characterization of a functional α_{1D} adrenoceptor on rat aorta, Br. J. Pharmacol. 114, 25P.

Larosa, G. and C. Forster, 1996, Coronary β-adrenoceptor function is modified by the endothelium in heart failure, J. Vasc. Res. 33, 62.

Laz, T.M., C. Forray, K.E. Smith, P. Vaysse, P. Hartig, C. Gluchowski, T.A. Branchek and R.L Weinshank, 1993, Cloned rat homolog of the bovine α_{1C}-adrenergic receptor exhibits an α_{1A}-like receptor pharmacology, Neurosci. Abstr. 733, 2.

Lomasney, J.W., S. Cotecchia, R.J. Lefkowitz and M.G. Caron, 1991, Molecular biology of α-adrenergic receptors: implications for receptor classification and for structure-function relationships, Biochim. Biophys. Acta 1095, 127.

Low, A.M., D.M. Bowdish, T.R. Prashad and V. Gaspar, 1994, Interactions of chloroethylclonidine with rauwolscine- and prazosin-sensitive adrenoceptors in dog saphenous vein, Br. J. Pharmacol. 113, 1263.

Marshall, I., R.P. Burt and C.R. Chapple, 1995, Noradrenaline contractions of human prostate by α_{1A}-(α_{1c}-)adrenoceptor subtype, Br. J. Pharmacol. 115, 781.

Michel, M.C. and P.A. Insel, 1994, Comparison of the cloned and pharmacologically defined rat tissue alpha 1-adrenoceptor subtypes, Naunyn-Schmiedeberg's Arch. Pharmacol. 350, 136.

Morrow, A.L. and I. Creese, 1986, Characterisation of α_1-adrenergic receptor subtypes in rat brain: a reevaluation of [^{3}H]WB 4101 and [^{3}H]prazosin binding, Mol. Pharmacol. 29, 49.

Muramatsu, I., S. Kigoshi and T. Ohmura, 1991, Subtypes of α_1-adrenoceptors involved in noradrenaline-induced contractions of rat thoracic aorta and dog carotid artery, Jpn. J. Pharmacol. 57, 535.

Muramatsu, I., T. Ohmura, S. Hashimoto and M. Oshita, 1995, Functional subclassification of vascular α_1-adrenoceptors, Pharmacol. Commun. 6, 23.

Oriowa, M.A. and R.R. Ruffolo Jr., 1992, Activation of a single alpha-1-adrenoceptor in rat aorta mobilizes intracellular and extracellular pools of calcium, Pharmacology 44, 139.

Parker, R.B. and D.R. Waud, 1971, Pharmacological estimation of drug receptor dissociation constants. Statistical evaluation. (i) Agonists, J. Pharmacol. Exp. Ther. 177, 1.

Parmley, W.W., 1985, Pathophysiology of congestive heart failure, Am. J. Cardiol. 55, 9A.

Perez, D.M., M.T. Piascik and R.M. Graham, 1991, Solution-phase library screening for the identification of rare clones: isolation of an α_{1D}-adrenergic receptor cDNA, Mol. Pharmacol. 40, 876.

Piascik, M.T., B.T. Butler, T.A. Pruitt and J.W. Kusiak, 1991, Agonist interaction with alkylation sensitive and resistant alpha$_1$ adrenoceptor subtypes, J. Pharmacol. Exp. Ther. 254, 982.

Saussy, D.L., A.S. Goetz, H.K. King and T. True, 1994, BMY 7378 is a selective antagonist of α_{1d}-adrenoceptors (AR): further evidence that vascular α_{1d}-AR are of the α_{1d} subtype, Can. J. Physiol. Pharmacol. 72 (Suppl. 1), P31.1.008.

Schwinn, D.A., J.W. Lomasney, W. Lorenz, P.J. Szklut, R.T. Fremeau Jr., T.L. Yang-Feng, M.G. Caron, R.J. Lefkowitz and S. Cotecchia, 1990, Molecular cloning and expression of the cDNA for a novel alpha$_1$-adrenergic receptor subtype, J. Biol. Chem. 265, 8183.

Taddei, C., E. Poggesi, A. Leonardi and R. Testa, 1993, Affinity of different α_1-agonists and antagonists for the α_1-adrenoceptors of rabbit and rat liver membranes, Life Sci. 53, 177.

Testa, R., L. Guarneri, M. Ibba, G. Strada, E. Poggesi, C. Taddei, I. Simonazzi and A. Leonardi, 1993, Characterization of the α_1-adrenoceptor subtypes in prostate and prostatic urethra of rat, rabbit, dog and man, Eur. J. Pharmacol. 249, 307.

Watson, S. and D. Girdlestone, 1996, Receptor and ion channel nomenclature, Trends Pharmacol. Sci. Suppl. 1, 10.