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Chloroethylclonidine and α-adrenoceptor agonist interaction in blood vessels following heart failure

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Abstract

This study examined the interaction of chloroethylclonidine with α-adrenoceptor agonists in canine endothelium-denuded dorsal pedal artery and saphenous vein before (non-paced) and at end-stage heart failure which was induced by rapid ventricular pacing (250 bpm for no more than four weeks). The interaction was heterogeneous in both non-paced and heart failure blood vessels. In the dorsal pedal artery, only chloroethylclonidine (10^{-4} M) reduced the maximum response to noradrenaline. At 10^{-6} and 10^{-5} M, chloroethylclonidine potentiated the response to noradrenaline. In the saphenous vein, chloroethylclonidine was not surmountable against noradrenaline before heart failure, but produced competitive antagonism of noradrenaline in the heart failure group ($pA_2 = 5.7$). In the dorsal pedal artery, chloroethylclonidine potentiated the response to low concentrations of methoxamine, but inhibited the response of higher concentrations. In the saphenous vein, chloroethylclonidine (10^{-6} and 10^{-5} M) potentiated the response to methoxamine from non-paced dogs but did not significantly effect the response at heart failure. In the dorsal pedal artery, chloroethylclonidine (10^{-4} M) potentiated low concentrations and inhibited higher concentrations of phenylephrine from non-paced animals but had no significant effect at heart failure. In contrast, in the saphenous vein, chloroethylclonidine (at all concentrations tested) inhibited the response to phenylephrine in non-paced dogs, whereas the inhibitory effect was not as marked in heart failure. In conclusion, these results indicate that differences in α_1-adrenoceptor populations and distribution are blood vessel dependent and dependent on the pathological state. © 1997 Elsevier Science B.V.

Keywords: Ventricular pacing, rapid; Heart failure; Blood vessel; α_1-Adrenoceptor subtype; Chloroethylclonidine

1. Introduction

Congestive heart failure is characterised by decreased cardiac output, for which several compensatory mechanisms evolve. One of which is activation of the sympathetic nervous system which results in elevated levels of circulating catecholamines mediating vasoconstriction and increasing peripheral vascular resistance via stimulation of vascular α_1-adrenoceptors, ensuring adequate 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 (Parmley, 1985; Armstrong et al., 1986). Indeed, tachycardia-induced heart failure has been described as a specific clinical syndrome in humans (Packer et al., 1986).

This laboratory has been primarily concerned with defining α_1-adrenoceptor characteristics in blood vessels following development of pacing-induced heart failure in the dog. It was found that at end-stage heart failure (approximately 4 weeks of pacing at 250 bpm), the contractile responsiveness of the dorsal pedal artery and saphenous vein to α-adrenoceptor agonists was enhanced (Forster et al., 1989a, 1992). This occurred with α_1-adrenoceptor agonists and mixed α-adrenoceptor agonists, but not with α_2-adrenoceptor agonists (Forster et al., 1989a,b, 1992; Forster and Armstrong, 1990).

Alpha$_1$-adrenoceptors were originally subdivided into α_{1A}- and α_{1B}-adrenoceptor subtypes based on affinities of a series of ligands for binding sites in the cerebral cortex (Morrow and Creese, 1986), vas deferens and spleen (Docherty, 1989) and on the ability of chloroethylclonidine to alkylate α_{1B}-adrenoceptors, but not α_{1A}-adrenoceptor subtypes (Han et al., 1987a,b). Subsequently, although controversial, three recombinant α_1-adrenoceptor proteins have been identified (Hieble et al., 1995) which are desig-

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nated with a lower case subscript. 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 (Cotecchia et al., 1988). During the next few years, a number of α_1-adrenoceptor clones were produced which could not be identified pharmacologically (Schwinn et al., 1990; Lomasney et al., 1991; Perez et al., 1991). A clone was also identified (α_{1d}-) which had distinct pharmacology from either the native α_{1A}- or the α_{1B}-adrenoceptor (Perez et al., 1991). The clone, originally termed, α_{1c}-adrenoceptor, has identical pharmacology to the native α_{1A}-adrenoceptor and therefore, the cloned α_{1c}-adrenoceptor is considered a redundant term and is now called the α_{1a}-adrenoceptor clone (Hieble et al., 1995; Ford et al., 1994; Clarke et al., 1995). This is the current nomenclature (Alexander and Peters, 1997).

The effect of chloroethylclonidine on vascular α_1-adrenoceptor characteristics has been contentious. For example, in rat aortic rings, chloroethylclonidine caused an irreversible blockade of noradrenaline-induced contractions (Vargas et al., 1993). Other reports have indicated that chloroethylclonidine did not affect noradrenaline-induced contractions of blood vessels (Sayet et al., 1993). Still, other studies have suggested that chloroethylclonidine failed to reduce the maximum response generated by noradrenaline, but caused a parallel shift (Oriowo and Bevan, 1990; Oriowo and Ruffolo, 1992; Aboud et al., 1993) or a non-parallel shift (Piascik et al., 1991) of the concentration–effect curves constructed to α_1-adrenoceptor agonists. Moreover, chloroethylclonidine also produced contractions of vascular smooth muscle (Schwietert et al., 1991; Nunes and Guimaraes, 1993) and in the canine saphenous vein this contraction was mediated by an α_2-adrenoceptor (Nunes and Guimaraes, 1993; Low et al., 1994; Daniel et al., 1996).

It has previously been shown that the pattern of the interaction of 5-methyl-urapidil with α_1-adrenoceptors in the canine dorsal pedal artery and saphenous vein is heterogeneous and was dependent on the heart failure state (Forster, 1996). We have, therefore, predicted that the exaggerated response developed to α_1-adrenoceptor stimulation in these blood vessels is due to activation of distinct α1-adrenoceptor subtypes. To further test this, we now report the effects of chloroethylclonidine on the responsiveness of the dorsal pedal artery and saphenous vein to α-adrenoceptor stimulation before and at end-stage heart failure.

2. Materials and methods

2.1. Study group

Adult male mongrel dogs (approximately 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) seven dogs which were not paced, acted as controls and were killed acutely (previous data from this laboratory has 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 compared with a group of animals which were acutely killed (Forster et al., 1989b)) and (ii) six dogs paced to end-stage heart failure as defined previously (Armstrong et al., 1986; Larosa and Forster, 1996). Pacemaker implantation was performed according to methods previously described (Armstrong et al., 1986). Briefly, under sodium thiopental (25 mg/kg; Abbott Laboratories, Montreal, Québec, 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 and the University of Toronto, in accordance with the Animals of Research Act and the Guidelines of the Canadian Council on Animal Care.

2.2. Organ bath experiments

Dogs from either the non-paced or the end-stage heart failure group were killed with an overdose of sodium thiopental and 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 a fine forcep 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% oxygen/5% carbon dioxide) with the following mM composition: NaCl, 120.0; KCl, 5.6; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; NaH_2PO_4, 1.2 and D-glucose, 10.0. In addition, the following antagonists were present throughout the study: 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. Each ring was attached to a force displacement transducer (Model FT03C, Grass Instrument, Quincy, MA, USA) and changes in isometric tension were displayed on a polygraph (Model 7D, Grass Instrument). An equilibration period of at least 1 h was used, during which time frequent washing occurred and optima resting tensions of 4 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.

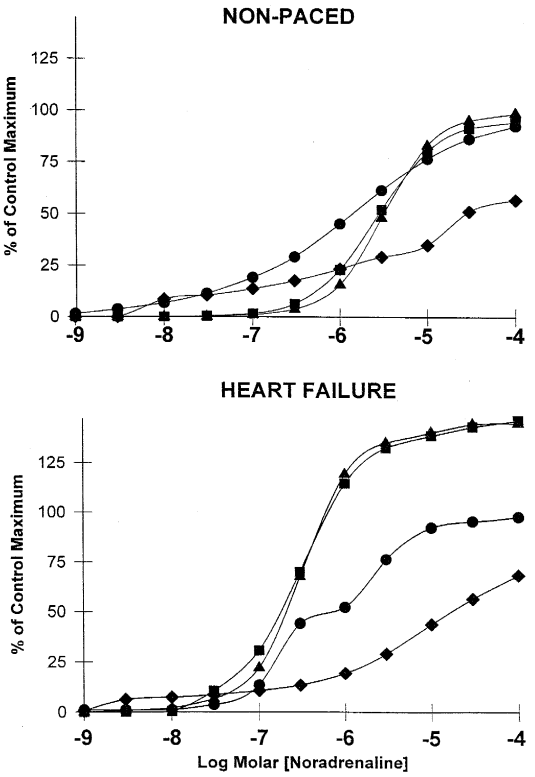

Fig. 1. Concentration–effect curves to noradrenaline in the absence and presence of increasing concentrations of chloroethylclonidine in the canine dorsal pedal artery. The upper panel show data from dogs which were not-paced and the lower panel those data from heart failure dogs. Closed circles are the mean concentration–effect data for noradrenaline in the absence of chloroethylclonidine whereas the closed triangles, squares and diamonds represent the corresponding mean data in the presence of 10^{-6}, 10^{-5} and 10^{-4} M chloroethylclonidine, respectively. Each point is the mean from at least 5 animals and standard errors are omitted for clarity.

2.3. Experimental design

After equilibration, cumulative concentration–effect curves were constructed to noradrenaline, methoxamine and phenylephrine. Briefly, rings were exposed to the lowest concentration of agonist (10^{-9} M), the resulting contraction was allowed to develop and once complete, 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 six preparations were used from each vessel segment, one pair of rings received noradrenaline, the second pair of rings received methoxamine and the remaining pair received phenylephrine. Once the concentration–effect curve was completed, rings were washed until the resting tension returned. Chloroethylclonidine (10^{-6} M) was administered to one ring of each pair, the other ring acted as a time control and did not receive chloroethylclonidine. The antagonist was in the bath 30 min before re-constructing the concentration–effect curves to each of the agonists. The entire procedure was repeated on at least two more occasions using two higher concentrations of chloroethylclonidine on identical preparations. 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.M. for *n* (number) 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/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 a logistic function:

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

which determined 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 antagonism. Statistical analysis for all data except the contractile data was performed by the non-parametric test, Mann

Table 1
Maximum response data in dorsal pedal artery

Agonist	Non-paced				Heart failure			
	control	10^{-6} M	10^{-5} M	10^{-4} M	control	10^{-6} M	10^{-5} M	10^{-4} M
Noradrenaline	27.5 ± 2.2	28.1 ± 2.2	28.4 ± 1.9	16.5 ± 0.5 [a]	38.5 ± 3.0	55.8 ± 8.0 [b]	56.6 ± 9.0 [b]	25.0 ± 13.0
Methoxamine	21.0 ± 2.2	18.4 ± 1.7	19.3 ± 1.0	8.2 ± 0.5 [a]	27.3 ± 1.6	29.8 ± 1.0	38.2 ± 2.8 [b]	13.4 ± 1.8 [a]
Phenylephrine	28.9 ± 0.9	26.4 ± 0.9	33.6 ± 1.4	16.3 ± 0.4 [a]	37.0 ± 2.2	48.8 ± 1.3 [b]	48.5 ± 1.6 [b]	42.6 ± 2.6

Data are for maximum response (mean ± S.E.M.) for the 3 agonists in the absence (control) and presence of (10^{-6}, 10^{-5} and 10^{-4} M) chloroethylclonidine from at least 5 animals.

[a] Indicates significant inhibition versus control ($P < 0.05$).

[b] indicates significant potentiation versus control ($P < 0.05$). For clarity differences between non-paced and heart failure are not shown (see text).

Table 2
EC_{50} data for α-agonists in the absence and presence of chloroethylclonidine in the dorsal pedal artery

Agonist	Non-paced				Heart failure			
	control	10^{-6} M	10^{-5} M	10^{-4} M	control	10^{-6} M	10^{-5} M	10^{-4} M
Noradrenaline	1.2 (0.8–2.6)	3.0 (1.1–5.2)	2.8 (0.9–6.2)	2.3 (1.1–3.9)	0.5 (0.3–0.7)	0.3 (0.1–0.5)	0.3 (0.1–0.5)	10.0 [a] (7.1–18.0)
Methoxamine	5.0 (3.3–7.1)	14.0 [a] (9.2–22.0)	1.2 (0.8–3.6)	0.3 [b] (0.1–0.6)	11.0 (8.3–22.0)	9.0 (5.6–22.2)	5.0 (1.3–9.1)	0.1 [b] (0.09–0.2)
Phenylephrine	3.0 (1.1–5.3)	3.3 (0.9–6.6)	6.0 (1.2–9.7)	0.09 [b] (0.05–0.2)	0.4 (0.1–0.7)	0.3 (0.09–0.6)	0.3 (0.1–0.5)	0.8 (0.3–1.7)

Data are for geometric mean EC_{50} (10^{-6} M) with 95% confidence limits in parentheses for each of the three agonists in the absence (control) and presence of 10^{-6}, 10^{-5} and 10^{-4} M chloroethylclonidine from at least 5 animals.
[a] Indicates a significant increase in EC_{50}.
[b] Indicates a significantly lower EC_{50}. For clarity differences between non-paced and heart failure are not shown.

Whitney *U*-test. Student's *t*-test with a Bonferroni correction was performed for end-stage heart failure versus control. In all cases a P value of < 0.05 was considered significant.

2.5. Drugs and solutions

The following drugs were used: acetylcholine iodide, 1-noradrenaline bitartrate, phenylephrine hydrochloride, desipramine hydrochloride, indomethacin and yohimbine (all from Sigma, St. Louis, MO, USA), propranolol hydrochloride (Ayerst Laboratories, New York, NY, USA) methoxamine (Glaxo Wellcome, Kirkland, Québec, Canada) and chloroethylclonidine (Research Biochemicals International, Natick, MA, USA). Stock solutions and dilutions were made in deionised water, with the exception of noradrenaline and indomethacin. Noradrenaline was

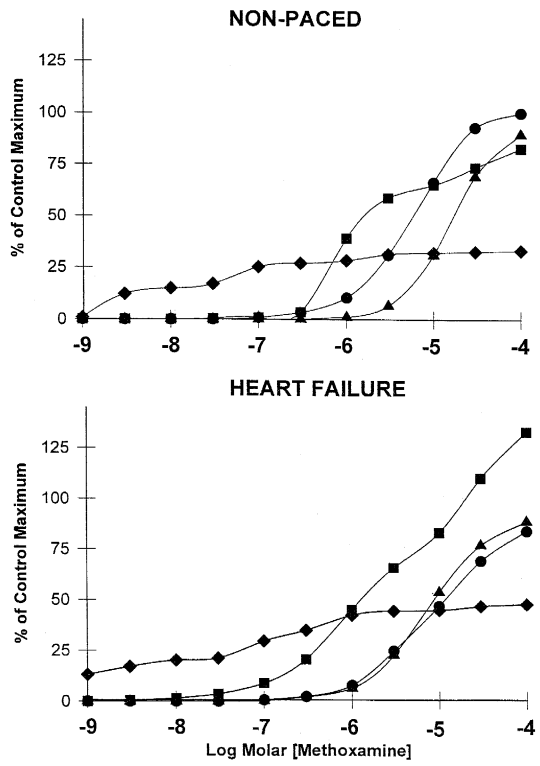

Fig. 2. Concentration–effect curves to methoxamine in the absence and presence of increasing concentrations of chloroethylclonidine in the canine dorsal pedal artery. The upper panel show data from dogs which were not paced and the lower panel those data from heart failure dogs. Closed circles are the mean concentration–effect data for methoxamine in the absence of chloroethylclonidine whereas the closed triangles, squares and diamonds represent the corresponding mean data in the presence of 10^{-6}, 10^{-5} and 10^{-4} M chloroethylclonidine, respectively. Each point is the mean from at least 5 animals and standard errors are omitted for clarity.

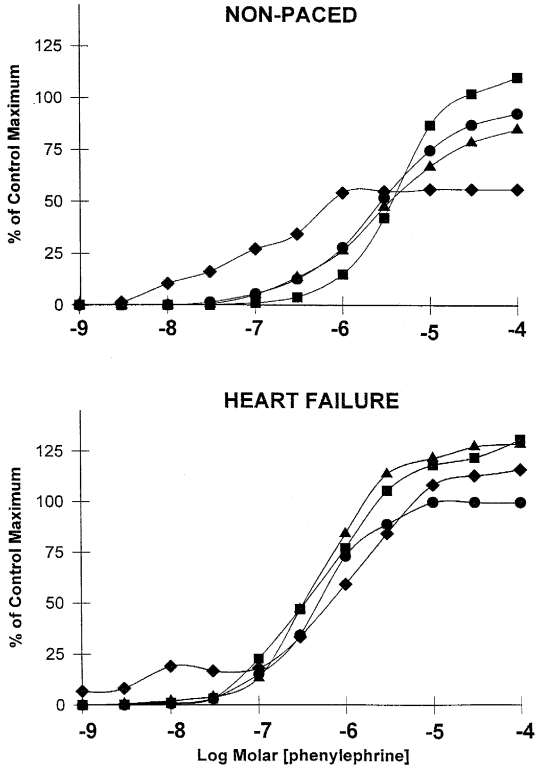

Fig. 3. Concentration–effect curves to phenylephrine in the absence and presence of increasing concentrations of chloroethylclonidine in the canine dorsal pedal artery. The upper panel show data from dogs which were not paced and the lower panel those data from heart failure dogs. Closed circles are the mean concentration–effect data for phenylephrine in the absence of chloroethylclondiine whereas the closed triangles, squares and diamonds represent the corresponding mean data in the presence of 10^{-6}, 10^{-5} and 10^{-4} M chloroethylclonidine, respectively. Each point is the mean from at least 5 animals and standard errors are omitted for clarity.

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

Typically, end-stage heart failure was associated with a rise in heart rate, left ventricular filling pressure, mean pulmonary artery pressure and mean pulmonary wedge pressure, as well as an increase in systemic vascular resistance. Cardiac output was significantly lower in the heart failure group compared to the non-paced group. None of the preparations responded with a relaxation by acetylcholine indicating successful removal of the endothelium and there were no differences in the magnitude of the contraction by KCl as shown previously (Forster et al., 1989a). None of the time-control preparations differed in responsiveness throughout the course of the study and confirms previous data (Forster, 1996). In addition, chloroethylclonidine did not produce any contractions per se.

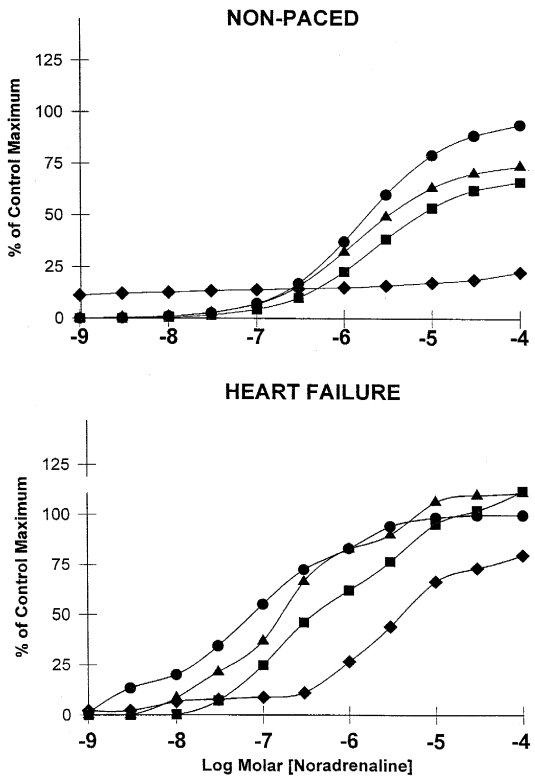

Fig. 4. Concentration–effect curves to noradrenaline in the absence and presence of increasing concentrations of chloroethylclonidine in the canine saphenous vein. The upper panel show data from dogs which were not paced and the lower panel those data from heart failure dogs. Closed circles are the mean concentration–effect data for noradrenaline in the absence of chloroethylclonidine whereas the closed triangles, squares and diamonds represent the corresponding mean data in the presence of 10^{-6}, 10^{-5} and 10^{-4} M chloroethylclonidine, respectively. Each point is the mean from at least 5 animals and standard errors are omitted for clarity.

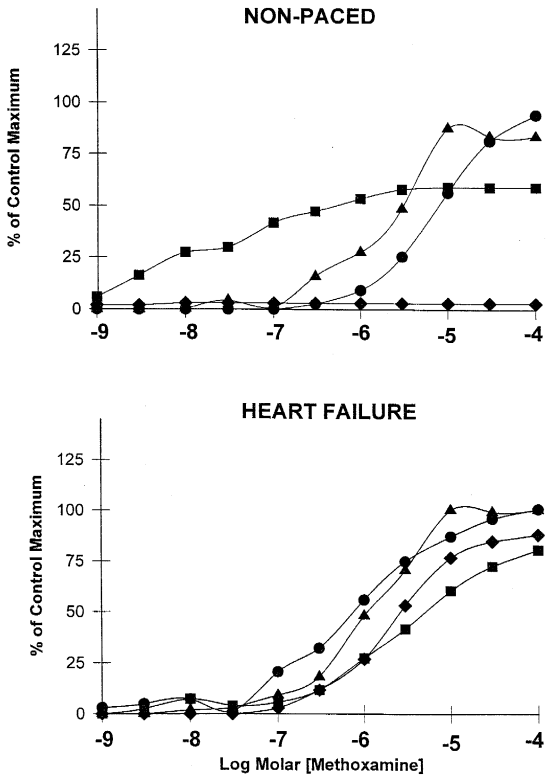

Fig. 5. Concentration–effect curves to methoxamine in the absence and presence of increasing concentrations of chloroethylclonidine in the canine saphenous vein. The upper panel show data from dogs which were not paced and the lower panel those data from heart failure dogs. Closed circles are the mean concentration–effect data for methoxamine in the absence of chloroethylclonidine whereas the closed triangles, squares and diamonds represent the corresponding mean data in the presence of 10^{-6}, 10^{-5} and 10^{-4} M chloroethylclonidine, respectively. Each point is the mean from at least 5 animals and standard errors are omitted for clarity.

3.1. Dorsal pedal artery

Fig. 1 shows concentration–effect curves to noradrenaline in the absence and presence of increasing concentrations of chloroethylclonidine from non-paced and heart failure dogs. In the vessels from non-paced animals, chloroethylclonidine inhibited the responses to noradrenaline and 10^{-4} M chloroethylclonidine significantly reduced the maximum response ($P < 0.05$; Table 1) from 27.5 ± 2.2 to 16.5 ± 0.5 g/mm^2. Chloroethylclonidine (10^{-6} and 10^{-5} M) displayed a non-parallel shift of the concentration–effect curve to the right (Fig. 1) and as a result the concentration–effect curve became steeper. There was, however, no significant difference in EC_{50} values (Table 2). A different profile was seen in heart failure. The maximum response generated to noradrenaline in the absence of chloroethylclonidine was significantly higher than that observed in non-paced arteries (Table 1). In contrast to the non-paced dorsal pedal artery, 10^{-6} and 10^{-5} M chloroethylclonidine significantly potentiated the maximal attainable response to noradrenaline ($P < 0.05$; Table 1), this was not associated with any change in EC_{50} (Table 2).

Table 3
Maximum response data in saphenous vein

Agonist	Non-paced				Heart failure			
	control	10^{-6} M	10^{-5} M	10^{-4} M	control	10^{-6} M	10^{-5} M	10^{-4} M
Noradrenaline	39.2 ± 4.0	28.8 ± 5.0	25.5 ± 3.0	9.0 ± 1.0a	45.2 ± 5.1	51.3 ± 3.5	57.1 ± 4.4	34.6 ± 4.4
Methoxamine	33.7 ± 3.6	28.2 ± 6.0	17.3 ± 4.7 [a]	2.2 ± 0.5 [a]	39.4 ± 3.2	39.7 ± 2.2	38.3 ± 4.2	33.4 ± 1.8
Phenylephrine	31.4 ± 2.4	32.3 ± 4.3	26.6 ± 2.6	8.7 ± 1.6 [a]	55.3 ± 5.3	56.6 ± 3.7	57.1 ± 5.6	21.4 ± 4.7 [a]

Data are for maximum response (mean ± S.E.M.) for the 3 agonists in the absence (control) and presence of (10^{-6}, 10^{-5} and 10^{-4} M) chloroethylclonidine from at least 5 animals.
[a] Indicates significant inhibition versus control ($P < 0.05$). For clarity differences between non-paced and heart failure are not shown (see text).

Although 10^{-4} M chloroethylclonidine produced a significant increase in EC_{50} (Table 2) there was no significant difference in the maximum response (Table 1).

Concentration–effect curves to methoxamine are shown in Fig. 2. In dorsal pedal arteries from non-paced animals, chloroethylclonidine (10^{-6} M) displaced the control curve to the right. The EC_{50} was significantly increased (Table 2) and there was no significant difference in the height of the maximum response (Table 1). In contrast, 10^{-5} M chloroethylclonidine shifted the curve to the left (the EC_{50} was reduced; Table 2). With 10^{-4} M chloroethylclonidine the maximum response was significantly decreased ($P < 0.05$, Table 1), and the response to low concentrations of methoxamine were potentiated. At heart failure, 10^{-6} M chloroethylclonidine had no effect on contractions generated by methoxamine (Fig. 2), whereas 10^{-5} M chloroethylclonidine shifted the curve leftward and increased the maximum response (Tables 1 and 2). A similar pattern, to the non-paced data, was seen with 10^{-4} M chloroethylclonidine (Figs. 2 and 3, Tables 1 and 2).

Chloroethylclonidine did not significantly alter the concentration–effect curves generated to phenylephrine in non-paced arteries with the exception of treatment with 10^{-4} M chloroethylclonidine. In this situation, it potentiated low concentrations and inhibited high concentrations of phenylephrine. At heart failure, chloroethylclonidine (10^{-6} and 10^{-5} M) caused a small, but significant increase in the height of the maximum response (Table 1).

3.2. Saphenous vein

Fig. 4 shows the effect of chloroethylclonidine on responses of the saphenous vein to noradrenaline. In non-paced vessels, chloroethylclonidine produced an insurmountable antagonism with a concentration-dependent decrease in the height of the maximum response (Table 3). The mean IC_{50} value was calculated as 1.7 (1.0–2.4) × 10^{-5} M. In contrast, chloroethylclonidine did not significantly alter the maximum response generated to noradrenaline in veins from dogs with heart failure. In this case, increasing concentrations of chloroethylclonidine produced a concentration-dependent displacement of the concentration–effect curves to the right indicative of competitive antagonism. The mean pA_2 value and slope was calculated to be 5.7 (5.5–6.0) and -0.93 ± 0.1, respectively.

The features of the interaction of chloroethylclonidine and methoxamine in the saphenous vein from non-paced and heart failure dogs are illustrated in Fig. 5. In the non-paced group, chloroethylclonidine produced a concentration-dependent decrease in the height of the maximum response (Table 3) with a mean IC_{50} value of 8.3 (7.1–9.6) × 10^{-6} M. However, 10^{-6} and 10^{-5} M chloroethylclonidine potentiated the contractions produced by low concentrations of methoxamine. Chloroethylclonidine

Table 4
EC_{50} data for α-agonists in the absence and presence of chloroethylclonidine in the saphenous vein

Agonist	Non-paced				Heart failure			
	control	10^{-6} M	10^{-5} M	10^{-4} M	control	10^{-6} M	10^{-5} M	10^{-4} M
Noradrenaline	1.5 (0.7–2.5)	1.8 (0.8–2.8)	2.0 (0.9–3.4)	nd	0.09 (0.07–0.2)	0.13 (0.08–0.35)	0.4 (0.1–0.7)	4.0 [a] (0.9–7.8)
Methoxamine	8.3 (2.3–16.1)	3.1 (0.9–6.7)	0.05 [b] (0.01–0.09)	nd	0.73 (0.31–1.7)	1.1 (0.91–3.0)	2.2 (0.97–5.6)	4.5 (1.1–7.9)
Phenylephrine	0.09 (0.05–0.2)	2.1 [a] (0.9–5.6)	1.9 [a] (0.9–4.7)	nd	0.8 (0.3–3.3)	0.8 (0.4–2.3)	2.3 (1.0–5.5)	10.0 [a] (7.0–19.0)

Data are for geometric mean EC_{50} (10^{-6} M) with 95% confidence limits in parentheses for each of the three agonists in the absence. (control) and presence of 10^{-6}, 10^{-5} and 10^{-4} M chloroethylclonidine from at least 5 animals.
[a] Indicates a significant increase in EC_{50}.
[b] Indicates a significant lower EC_{50} and indicates that EC_{50} could not be determined. For clarity differences between non-paced and heart failure are not shown.

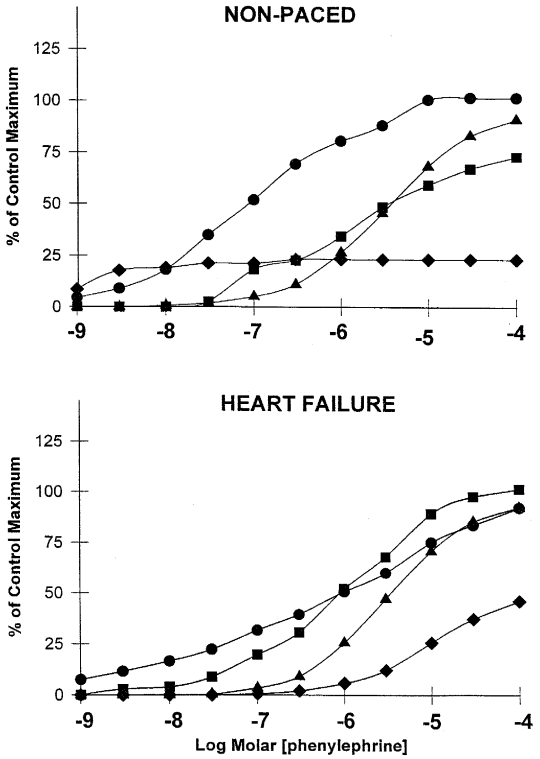

Fig. 6. Concentration–effect curves to phenylephrine in the absence and presence of increasing concentrations of chloroethylclonidine in the canine saphenous vein. The upper panel show data from dogs which were not paced and the lower panel those data from heart failure dogs. Closed circles are the mean concentration–effect data for phenylephrine in the absence of chloroethylclonidine whereas the closed triangles, squares and diamonds represent the corresponding mean data in the presence of 10^{-6}, 10^{-5} and 10^{-4} M chloroethylclonidine, respectively. Each point is the mean from at least 5 animals and standard errors are omitted for clarity.

(10^{-4} M) almost completely abolished the contractions seen by methoxamine. At heart failure, chloroethylclonidine (at the concentrations tested) appeared to shift the concentration–effect curve to the right in a concentration-dependent manner although statistical analysis proved this to be insignificant. Where applicable, the EC_{50} values for these data are summarised in Table 4.

Fig. 6 shows concentration–effect curves constructed to phenylephrine in saphenous vein from non-paced and heart failure dogs. Chloroethylclonidine (10^{-6} M and 10^{-5} M) did not significantly alter the height of the maximum response to phenylephrine (Table 3), but did displace the concentration–effect curves to the right which was not concentration-dependent. With chloroethylclonidine (10^{-4} M) significantly inhibited the contractile response to phenylephrine. Chloroethylclonidine (10^{-6} and 10^{-5} M) did not significantly affect the concentration–effect curves generated by phenylephrine in heart failure saphenous veins, but 10^{-4} M caused a significant decrease in the height of the maximum response (Fig. 6, Table 3).

4. Discussion

This study confirms previous indications that the interaction of chloroethylclonidine (in the setting of α_2-adrenoceptor blockade) with α_1-adrenoceptors is variable (Low et al., 1994). Nevertheless, the key findings indicate that this interaction is not only agonist-dependent and pathophysiological state-dependent, but more importantly blood vessel-dependent. The differences in agonist efficacy and potency occurring with heart failure have already been addressed and therefore will not be discussed further (Forster et al., 1989a, 1992, Forster, 1996).

Chloroethylclonidine had a complex interaction with the α-agonists tested producing unusual patterns of antagonism which was not only dependent on the heart failure state but, also, clearly blood vessel dependent. Since chloroethylclonidine binds to all α_1-adrenoceptors, it cannot be presumed that these interactions are due solely to the α_{1B}-adrenoceptor per se (O'Rourke et al., 1995). Hence, this may lead to the complexities seen in the present study and suggests that analysing these data in terms of only maximum response and EC_{50} may be an oversimplification. However, due to the limited number of data points (half log units) for each concentration–effect curve generated, we cannot perform a more stringent test.

In non-paced dorsal pedal artery, chloroethylclonidine (10^{-4} M) reduced the maximum response by noradrenaline consistent with its insurmountable activity (O'Rourke et al., 1995), but lower concentrations of chloroethylclonidine did not affect the maximum response (this was not consistent with competitive antagonism). In contrast, the antagonism was competitive in the saphenous vein from heart failure dogs. These complex interactions may be due to the fact that noradrenaline is not a selective agonist at α_1-adrenoceptors. Although the α_2-adrenoceptor and β-adrenoceptors were blocked throughout the study there may be an interaction of chloroethylclonidine with these antagonists which may add to the complex picture. In addition, high concentrations of noradrenaline likely overcome the blockade. It has been reported that chloroethylclonidine can inactivate approximately 50% of rauwolscine binding sites in canine saphenous vein yielding a more sensitive residual site to α-adrenoceptor agonists (Daniel et al., 1996). It therefore is likely that the α_2-adrenoceptor may become unmasked to varying degrees by chloroethylclonidine. Secondly, although no initial contractile responses were seen with chloroethylclonidine, it is possible that a slowly developing contraction may occur which is not seen during the initial incubation period. This may be additive to the contraction seen with noradrenaline and may explain some of the potentiating effects. In support of this, it has been reported that the contraction to chloroethylclonidine in the canine saphenous vein is persistent for several hours but occurs within 15 min of the onset of incubation (Daniel et al., 1996). We failed to observe a contraction with an incubation period of 30 min. These

observations, being not the concern of the present study, suggest that subsequent studies be performed to fully evaluate the time-course of chloroethylclonidine's activity.

These mixed interactions of chloroethylclonidine and noradrenaline led to a more stringent examination with selective α_1-adrenoceptor agonists. There was a differential response of the dorsal pedal artery and saphenous vein to the interaction of chloroethylclonidine and methoxamine. This consisted of a distinct combination of potentiation, antagonism and abolishment of the agonist response. The potentiation of the response has been described elsewhere, especially in the canine saphenous vein where it was suggested to occur as a result of a direct contraction to chloroethylclonidine (Daniel et al., 1996). However, as mentioned above, no contractions were observed in these preparations. Like noradrenaline, methoxamine has also been shown to interact with a rauwolscine-sensitive site and therefore a possible interaction with the yohimbine (which was present throughout the study) may still offer some explanation to these disparate results. The interaction between chloroethylclonidine and rauwolscine has been defined to occur as a result of binding to an unusual α-adrenoceptor. This adrenoceptor was not an α_2-adrenoceptor but may represent an atypical α_{1B}-adrenoceptor (Hicks et al., 1991). Notwithstanding, chloroethylclonidine has been reported to recognise a specific α_2-adrenoceptor (Michel et al., 1993).

The nature of the interaction was similar to that seen before heart failure in the dorsal pedal artery from paced animals. However, no rightward shift of the methoxamine concentration–effect curve was seen and the potentiation of the methoxamine response with 10^{-5} M chloroethylclonidine was more exaggerated. In contrast, in the saphenous vein, chloroethylclonidine did not significantly affect the methoxamine curve although there was an apparent concentration-dependent displacement to the right. This may imply an interaction with another α-adrenoceptor subtype, e.g. the α_{1D}-adrenoceptor which is present in the vasculature. The α_{1B}-adrenoceptor subtype could be ruled out since typically chloroethylclonidine would be expected to inactivate this α-adrenoceptor subtype.

To address these discrepancies further, we proceeded to examine chloroethylclonidine's interaction with phenylephrine, an α_1-adrenoceptor agonist which is recognised to a lesser extent by 5-methyl-urapidil (an α_{1A}-adrenoceptor antagonist [Gross et al., 1988]). Similar to that seen with methoxamine, chloroethylclonidine produced some degree of potentiation (albeit not as great as that seen with methoxamine) of the response to phenylephrine in the dorsal pedal artery from non-paced animals. In heart failure, chloroethylclonidine did not significantly affect the concentration–effect curve to phenylephrine. In the saphenous vein, chloroethylclonidine inactivated the response to phenylephrine in a non-concentration-dependent fashion in both the non-paced and the heart failure preparations which indicates the involvement of the α_{1B}-adrenoceptor. Based on these observations, it seems likely that phenylephrine is more selective for the α_{1B}-adrenoceptor which has been implied in previous studies (Daniel et al., 1996; Forster, 1996). That chloroethylclonidine did not affect the phenylephrine contractions from the dorsal pedal artery at heart failure further suggesting that the α_{1B}-adrenoceptor plays a minimal role in this response.

Based on these differential findings, these blood vessels contain a mixed population of α_1-adrenoceptors, comprising the α_{1A}-adrenoceptor, the classical α_{1B}-adrenoceptor, an atypical α_{1B} and, possibly, the α_{1D}-adrenoceptor. The relative proportions of each differ amongst blood vessels and pathophysiological state. Furthermore, the responses to chloroethylclonidine indicate that α-adrenoceptor-mediated contractions are, not only blood vessel-dependent, but also, agonist-dependent. Clearly the differential interaction of chloroethylclonidine with methoxamine and phenylephrine suggest that these agonists activate distinct α_1-adrenoceptor subtypes. Future endeavours to explore this novel finding must be carried out with a much wider range of agonists and antagonists, as well as molecular studies using the recombinant clones for these known subtypes.

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