ELSEVIER

European Journal of Pharmacology 283 (1995) 241–250

ejp

Inhibition of vascular contractions to α-adrenoceptor agonists by polymyxin B: impact of heart failure state

Christine Forster *

Division of Cardiology, Department of Medicine, University of Toronto, Toronto, Ontario, Canada

Received 22 March 1995; revised 1 June 1995; accepted 13 June 1995

Abstract

In this study, the effects of polymyxin B (a protein kinase C inhibitor) on α-adrenoceptor stimulation of the canine dorsal pedal artery and saphenous vein were examined. In addition, the question was asked, whether these effects could be altered by the impact of a heart failure state? Blood vessels were obtained at three time points during the development of pacing-induced heart failure in the dog; control (non-paced), 1 week paced and end-stage heart failure. Concentration-effect curves were constructed to the α-adrenoceptor agonists, namely, noradrenaline and phenylephrine in the absence and presence of polymyxin B (2×10^{-5} and 10^{-4} M). Responses to noradrenaline and phenylephrine were enhanced in the saphenous vein, but not in the dorsal pedal artery, following the onset of heart failure. In the dorsal pedal artery, polymyxin B was found to inhibit the contractions developed to noradrenaline and phenylephrine to a significant degree ($P < 0.05$) at control and end-stage heart failure. In contrast, in the saphenous vein, polymyxin B inhibited responses developed to noradrenaline and phenylephrine at all time points studied. This inhibition was always more marked against noradrenaline compared to phenylephrine and, similar to the dorsal pedal artery, became more pronounced at end-stage heart failure. Furthermore, the vein was always more sensitive compared to the artery. Interestingly, as heart failure developed, a non-classical broad concentration-effect curve was evident. The high affinity component was more sensitive to inhibition by polymyxin B. This component was absent at end-stage heart failure in response to phenylephrine. In this latter case, no inhibition was seen with polymyxin indicating that it is the high affinity component which renders polymyxin B's sensitivity. Whether these effects relate to specific receptor populations is discussed. It can be concluded that some constituent of the α_1-adrenoceptor-mediated contraction involves protein kinase C activation. Therefore, protein kinase C may play a vital role in regulating vascular smooth muscle reactivity which may be an important mechanism in the pathogenesis of disease, i.e., heart failure and hypertension.

Keywords: Pacing-induced heart failure; Protein kinase C; Polymyxin B; α-Adrenoceptor; Smooth muscle, vascular

1. Introduction

It is well known that activation of the sympathetic nervous system represents one of the initial compensatory mechanisms in congestive heart failure (Thomas and Marks, 1978). In this laboratory, the canine model of rapid ventricular pacing has been adopted in order to investigate the pathophysiology of congestive heart failure (Armstrong et al., 1986). This model reliably produces clinical, haemodynamic and neurohumoural perturbations simulating clinical heart failure in man (Coleman et al., 1971; Francis, 1985; Parmley, 1978). Furthermore, it has been suggested that some of these features may be due, in part, to altered peripheral (Forster et al., 1989a, 1992a) and coronary (Main et al., 1991) vascular reactivity. In both the dorsal pedal artery and the saphenous vein, the contractile response to noradrenaline and selective α_1-adrenoceptor agonists (but not to selective α_2-adrenoceptor agonists) is enhanced during developing heart failure (Forster et al., 1992a). This increased responsiveness did not appear to result from a generalised increase in contractile function of these tissues, since a similar increase was not seen with potassium chloride (Forster et al., 1989b). That this enhanced responsiveness is related to the heart failure state, is further supported by the observation that no change in responsiveness was seen in groups of animals which had been sham-operated or

* Corresponding address: Suite 4308, Department of Pharmacology, Medical Sciences Building, University of Toronto, Toronto, Ontario M5S 1A8, Canada. Tel. (416) 978 2049, fax (416) 978 6395.

SSDI 0014-2999(95)00364-9

paced at 180 bpm for 4–8 weeks (Forster et al., 1989a; Forster – unpublished observation). It also has been shown that the increased responsiveness of the peripheral vessels could not be attributed to a diminished effect of endothelium-derived relaxing factors (Forster et al., 1989b). Therefore, it was concluded that the enhanced response was a consequence of α_1-adrenoceptor stimulation (Forster et al., 1992a). It was further speculated that this increase in responsiveness to α_1-adrenoceptor agonists may, in fact, be related to post-receptor events rather than changes at the receptor level per se (Forster et al., 1989a).

Vascular contractions in response to α_1-adrenoceptor stimulation are associated with hydrolysis of phosphatidylinositol 4,5-biphosphate by phospholipase C resulting in the generation of inositol 1.4.5-trisphosphate and 1,2-diacylglycerol (Aub and Putney, 1985; Berridge, 1983) which activates protein kinase C (Nishizuka, 1984). When a contraction is produced in response to noradrenaline, a biphasic pattern of contraction development is often observed (Deth and Van Breemen, 1974; Khalil and Van Breemen, 1988) an initial phasic component, followed by a sustained tonic element. It has been proposed that these two elements of contraction are mediated by different Ca^{2+} mobilising pathways, and possibly involve inositol 1.4.5-trisphosphate and diacylglycerol respectively (Kishimoto et al., 1980). The tumour promoting phorbol esters have been useful pharmacological probes for investigating the protein kinase C activation pathway (Danthaluri and Deth, 1984; Rasmussen et al., 1984). Another possible explanation for the mechanism of these two components is the possibility of a subdivision of the α_1-adrenoceptors. It has been reported that the canine saphenous vein possessed multiple α-adrenoceptor subtypes and it was concluded that there were at least three subtypes, i.e., α_{1A}, α_{2A} and an atypical α_{1B} (Hicks et al., 1991). Furthermore, the α_{1A}-adrenoceptor has been referred to as the imidazoline-preferring receptor and utilises extracellular Ca^{2+} (Han et al., 1987). On the other hand, the α_{1B}-adrenoceptor is phenylalkylamine-sensitive and utilises intracellular Ca^{2+} (Tian et al., 1990). In addition, up to four α_1-adrenoceptor subtypes have been cloned, although their functional significance awaits verification (Ford et al., 1994).

In view of the above observations, an examination was conducted as to whether α_1-adrenoceptor mediated contractions of vascular smooth muscle (namely the dorsal pedal artery and the saphenous vein from the dog) involved protein kinase C activation. Notwithstanding, it is hypothesised that the protein kinase C involvement in α-adrenoceptor-mediated contractions would be more pronounced during developing pacing-induced heart failure which would support previous observations (Forster et al., 1989a, 1992a).

2. Materials and methods

2.1. General

The study group consisted of nine male mongrel dogs (weight range 20–25 kg). All animals were allowed free access to food and water and were preconditioned to the study environment for 2–3 weeks prior to the onset of the study. All animals were anaesthetised with sodium thiopental and approximately 3 cm sections of dorsal pedal artery and lateral saphenous vein in the region of either the right or left hindpaw in alternate dogs (Forster et al., 1992a) were carefully dissected and placed in 4°C Krebs-Henseleit solution (composition in mM: NaCl 120, KCl 5.6, $CaCl_2$ 2.5, $MgSO_4$ 1.2, NaH_2PO_4 1.17, $NaHCO_3$ 25, and D-glucose 10.0; pH 7.4).

Pacemaker implantation was performed according to established methods (Armstrong et al., 1986). Briefly, a pacemaker generator (Medtronics, Mississauga, Ontario, Canada) was inserted into a cervical pocket and a unipolar pacemaker lead was positioned transvenously into the apex of the right ventricle. Animals were treated topically with Ancef (cefazolin sodium; Smith Kline Beecham, Oakville, Ontario, Canada) before the wounds were sutured. No further antibiotic or analgesia management was required. After a 1 week recovery period, the pacemaker was programmed to deliver 250 bpm asynchronously. One group of dogs ($n = 5$) was paced for only 1 week and a second group of animals ($n = 4$) was paced to end-stage heart failure. This latter group was examined biweekly for signs of dyspnoea, pulmonary oedema and the presence of ascites until the criteria for end-stage heart failure was fulfilled (a 25% increase in heart size accompanied by pulmonary oedema on chest X-ray and/or a 10% increase in body weight associated with the development of ascites and/or a left ventricular ejection fraction of $< 25\%$ on echocardiography, all of which were evident at 3 weeks of rapid ventricular pacing; Grima et al., 1994). All animals were re-anaesthetised and the contralateral dorsal pedal artery and saphenous vein were removed and processed as above. All procedures were approved by the Animal Care Committee of St Michael's Hospital in accordance with the Animals for Research Act and the Guidelines of the Canadian Council on Animal Care.

2.2. Tissue bath preparation

Dorsal pedal artery and saphenous vein segments were each cut into four ring sections of 5 mm length. Each ring was mounted in 10 ml organ baths as described previously (Forster et al., 1989b). Indomethacin (2.8 μM), propranolol (3 μM), desipramine (1 μM) and yohimbine (0.1 μM) were incorporated into the

Krebs solution throughout the study to inhibit endogenous prostanoid production, antagonise β-adrenoceptors, inhibit neuronal uptake and antagonise α_2-adrenoceptors respectively. In this study, all vascular segments had their endothelium removed by inserting the tip of a fine forcep through the lumen and carefully rolling the segment back and forth on Krebs-moistened filter paper. Vascular responses to the various agents were measured by means of an isometric force transducer (Grass Model FT03C, Quincy, MA, USA) and displayed on a Grass Polygraph (Model 7D).

2.3. Experimental design

Determination of agonist potency

Following an equilibration period of at least 1 h, during which time frequent washing and resting tensions of 4.0 and 2.5 g were maintained for the dorsal pedal artery and the saphenous vein respectively. These resting tensions had proved to be optimum whilst conducting individual length-tension analyses. All preparations were first tested for successful removal of the endothelium. This was done by inducing a contraction to potassium chloride (20 mM), allowing the contraction to plateau and without washing out, adding increasing concentrations of acetylcholine (10^{-7}–10^{-4} M). Denuded vessels should render no relaxation in response to acetylcholine. Following washout and re-establishment of baseline tension, α-adrenoceptor agonist concentration-effect curves were constructed in a cumulative manner. Of the four vascular preparations from each of the blood vessels at each of the time points (i.e., control, 1 week paced and end-stage heart failure), pairs of rings were treated with the α-adrenoceptor agonists, noradrenaline and phenylephrine. In all cases, a maximum volume of 100 μl at each concentration increment was administered. After completion of the concentration-effect curve, the preparations were washed and allowed to return to baseline resting tension.

Determination of antagonist potency

Once resting baseline tension had been re-established, one vascular preparation of each pair (which had previously been treated with the same agonist) served as a control and no antagonist was added. The remaining ring of the pair acted as the test preparation and received the protein kinase C inhibitor, polymyxin B (2×10^{-5} M; a membrane active polypeptide antibiotic which has been shown to be a relatively selective protein kinase C inhibitor (Kuo et al., 1983; Yuan and Sen, 1986)) which was left in contact for 30 min before concentration-effect curves were reconstructed to the agonists. Once completed, the procedure was repeated using 10^{-4} M polymyxin B.

2.4. Calculations and statistical considerations

At the end of the experiment, tissues were dried and their weights determined in order to calculate the cross-sectional area. Contractile responses of each preparation to the α-adrenoceptor agonists were calculated as the increase in tension $g \cdot mm^{-2}$ in response to each concentration of agonist. Individual concentration-effect curves were fitted to a sigmoidal curve using a curve fit programme using the logistic function (Parker and Waud, 1971) which determined the maximal response and the EC_{50} from individual data sets by applying the formula:

$$Y = \frac{(a-d)}{(1+X/c)^b} + d$$

where Y is the response; X is the arithmetic concentration; a is the response when $X = 0$; d is the response for infinite concentration; c is the EC_{50}; and b is the slope factor. The mean curve was then calculated and expressed with the S.E.M. for the mean response and in some circumstances the maximum response was calculated to occur with a higher concentration (as determined by the formula above) than actually tested, which naturally leads to the data in the figures and the tables being somewhat different. EC_{25} and EC_{75} were generated from individual concentration-effect curves. The EC_{25}, EC_{50} and EC_{75} data were calculated as the geometric mean with 95% confidence limits. Statistical analyses were performed by ANOVA and the Mann-Whitney test for non-parametric data and a P value of < 0.05 was considered statistically significant.

2.5. Drugs and solutions

Indomethacin was added to the $NaHCO_3$ solution prior to being added to the Krebs solution. Noradrenaline (10^{-2} M) was made up in 0.2% ascorbic acid, and subsequent dilutions in deionised water.

Indomethacin, desipramine hydrochloride, noradrenaline bitartrate, phenylephrine hydrochloride, polymyxin B sulphate and yohimbine were all obtained from Sigma (St. Louis, MO, USA). L-Propranolol hydrochloride was generously supplied from Ayerst Laboratories (New Jersey, USA).

3. Results

Table 1 shows the haemodynamic profile of the animals at control, 1 week paced and end-stage heart failure.

All preparations failed to elicit a relaxation response to acetylcholine indicative that the endothelial denudation procedure was successful.

Table 1
Haemodynamic profile of control, 1 week paced and end-stage heart failure dogs

	Control	1 week paced	End-stage heart failure
HR (beats·min^{-1})	87±7	119±10	147±21
MAP (mm Hg)	114±7	116± 3	110± 6
LVEDP (mm Hg)	7±1	23± 2	35± 3
PA (mm Hg)	14±1	32± 1	43± 3
PCWP (mm Hg)	10±1	13± 2	27± 3
RA (mm Hg)	7±1	8± 1	11± 1
CO (litres·min^{-1})	5±1	3± 1	2± 1

Heart rate (HR), mean arterial pressure (MAP), left ventricular end-diastolic pressure (LVEDP), pulmonary arterial pressure (PA), pulmonary capillary wedge pressure (PCWP), right atrial pressure (RA) and cardiac output (CO) are shown for control, 1 week-paced and end-stage heart failure. Conscious haemodynamic evaluations were performed under normal sinus rhythm in all study groups. Values are mean±S.E.M.

Fig. 1 shows concentration-effect curves constructed to noradrenaline on the dorsal pedal artery at control (A), 1 week paced (B) and end-stage heart failure (C). In the absence of polymyxin B, the contractile responsiveness did not change with developing heart failure (Table 2). Polymyxin B did not inhibit the response to noradrenaline at control, nor was any inhibition seen in the 1 week paced. At end-stage heart failure, polymyxin B antagonised the contractile response to noradrenaline in the dorsal pedal artery; however, the two concentrations of polymyxin B produced an identical degree of inhibition. This inhibition was significant at the $P < 0.05$ level against control.

In the saphenous vein, differences in maximal response and EC_{50} values were seen to noradrenaline at 1 week paced and end-stage heart failure. As time progressed, the vein became much more sensitive to noradrenaline, the maximal response significantly increased and the EC_{50} significantly declined (Table 2 and Table 3). In addition, the saphenous vein was always more sensitive to noradrenaline compared to the dorsal pedal artery. Furthermore, the concentration-effect curves became much broader with the development of heart failure (Fig. 2). Following polymyxin B treatment, significant inhibition occurred at control, 1 week paced and end-stage heart failure (Fig. 2A, 2B and 2C respectively). Considering the control data (Fig. 2A), polymyxin B produced a concentration-dependent inhibition of the noradrenaline maximal response with 10^{-4} M producing a 50% decline in the maximum. The EC_{50}, but not the EC_{25} and EC_{75}, values were reduced to a similar degree with both concentrations of polymyxin B. After 1 week of pacing, polymyxin B produced a marked rightward shift, and altered the shape, of the concentration-effect curve. The EC_{25} was increased some 65 and 75 times, whereas the EC_{50} and EC_{75} were only increased 10 times compared with control. Polymyxin B, 2×10^{-5} M and 10^{-4} M, produced the same degree of inhibition. At end-stage heart failure, polymyxin B inhibited the contractile response to noradrenaline in the saphenous vein and on this occassion a concentration-dependent effect was observed. The inhibition was more marked than control and 1 week-paced. Similar to the 1 week-paced data, the degree of inhibition by polymyxin B was more pronounced in the high affinity component.

No significant changes were seen in the response of the dorsal pedal artery to phenylephrine at control, 1

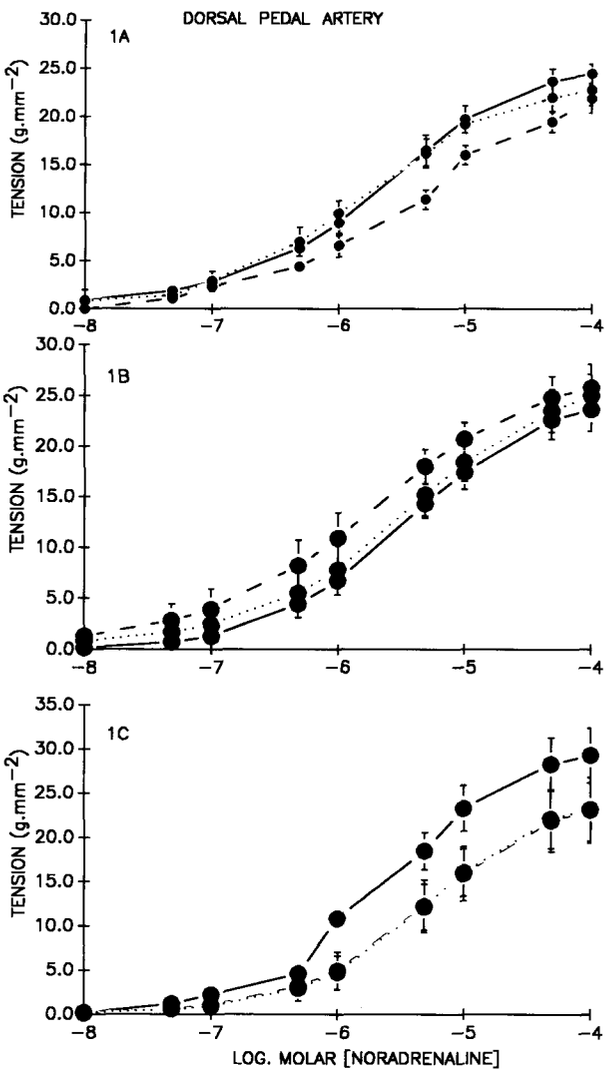

Fig. 1. Concentration-effect curves generated to noradrenaline in the absence and presence of polymyxin B on dorsal pedal arterial rings. Panel A represents data derived at control, before heart failure, panel B, data derived after 1 week of pacing, and panel C, data derived from peak heart failure. Solid lines represent concentration-effect curves in the absence of polymyxin B. Dashed lines represent concentration-effect curves to noradrenaline in the presence of 2×10^{-5} M polymyxin B and dotted lines in the presence of 10^{-4} M polymyxin B. Each point is the mean±S.E.M. from at least four dogs.

Table 2
Efficacy for α-adrenoceptor agonists in the absence and presence of 2×10^{-5} M and 10^{-4} M polymyxin B

	Dorsal pedal artery			Saphenous vein		
	No polymyxin	2×10^{-5} M	10^{-4} M	No polymyxin	2×10^{-5} M	10^{-4} M
(A) Noradrenaline						
Control	26.8 ± 1.0	26.0 ± 3.1	24.2 ± 3.7	19.3 ± 1.3	15.2 ± 1.0	10.0 ± 0.9 [a]
1 week paced	25.4 ± 2.7	28.0 ± 3.2	28.7 ± 4.0	40.0 ± 3.9 [b]	40.0 ± 3.3 [b]	36.2 ± 1.1 [b]
End-stage	30.0 ± 3.0	23.1 ± 3.7	23.2 ± 3.6	40.9 ± 1.5 [b]	29.6 ± 2.4 [b]	23.1 ± 2.6 [a,b]
(B) Phenylephrine						
Control	29.4 ± 2.3	24.2 ± 2.4	22.1 ± 1.4 [a]	21.8 ± 1.8	24.2 ± 3.2	17.6 ± 1.4
1 week paced	25.6 ± 2.1	25.3 ± 2.3	26.3 ± 2.2	53.3 ± 4.6 [b]	44.0 ± 3.5 [b]	42.5 ± 1.8 [b]
End-stage	37.7 ± 3.0	25.9 ± 5.0	22.4 ± 4.6 [a]	32.4 ± 1.5 [b]	29.3 ± 2.8	27.9 ± 2.3 [b]

Each value is the mean ± S.E.M. for at least four dogs. [a] Significantly different from no polymyxin group and [b] significantly different from control, both at the $P < 0.05$ level.

week paced and end-stage heart failure as depicted in Table 2. At control, polymyxin B showed a tendency to inhibit contractile responses to phenylephrine in a concentration-dependent manner. A significant decrease in maximal response was seen with 10^{-4} M polymyxin B (Fig. 3A). This inhibition was lost in vessels studied from dogs paced for 1 week and, like the responses generated to noradrenaline, polymyxin B caused a slight potentiation in the responsiveness (Fig. 3B). For example, significant increases in tension were observed with phenylephrine concentrations of 10^{-6} M and 2×10^{-6} M in the 2×10^{-5} M polymyxin treated rings. In addition, the EC_{50} values were significantly lower in the polymyxin B-treated groups. Similar to noradrenaline, at end-stage heart failure, polymyxin B clearly antagonised the response to phenylephrine, the maximal response being some 50% reduced with 10^{-4} M polymyxin B (Fig. 3C).

Phenylephrine-induced contractions of the saphenous vein were significantly enhanced at 1 week of pacing and end-stage heart failure with both increased maximal response and enhanced potency. The saphenous vein was also more sensitive to phenylephrine, compared to the dorsal pedal artery, at the three time points. Polymyxin B inhibited responsiveness of phenylephrine at all time points as depicted in Fig. 4. After 1 week of pacing, there was no concentration-dependent inhibition by polymyxin B. The inhibition was found to be greater against the EC_{25} and EC_{50} compared to that against the EC_{75} (Table 4). At end-stage heart

Table 3
EC_{25}, EC_{50} and EC_{75} (μM) values for noradrenaline in the absence and presence of polymyxin B

	Dorsal pedal artery			Saphenous vein		
	No polymyxin	2×10^{-5} M	10^{-4} M	No polymyxin	2×10^{-5} M	10^{-4} M
Control						
EC_{25}	0.5	1.0	0.4	0.2	0.2	0.2
	(0.2–0.8)	(0.8–1.2)	(0.2–0.6)	(0.05–0.4)	(0.1–0.3)	(0.1–0.25)
EC_{50}	2.0	3.1	1.1	3.2	1.0 [a]	0.8 [a]
	(1.1–3.2)	(2.4–6.1)	(0.9–2.8)	(2.2–4.5)	(0.9–1.5)	(0.6–1.1)
EC_{75}	12.0	33.0	20.0	5.0 [b]	8.0	5.0
	(9.0–20.0)	(20.0–113.0)	(11.0–43.0)	(2.3–6.3)	(6.8–9.0)	(2.3–8.0)
1 week paced						
EC_{25}	1.0	0.3	0.8	0.02 [b]	1.3 [a]	1.5 [a]
	(0.7–1.4)	(0.1–0.9)	(0.4–1.4)	(0.01–0.08)	(1.0–2.0)	(1.1–2.0)
EC_{50}	3.2	1.3	3.0	0.4 [b]	5.0 [a]	5.0 [a]
	(2.3–4.6)	(0.9–3.0)	(2.2–6.0)	(0.1–1.1)	(3.0–8.6)	(3.2–7.6)
EC_{75}	28.0	9.0	24.0	3.1 [b]	29.0 [a]	33.3 [a]
	(11.0–40.0)	(7.0–12.0)	(14.0–50.0)	(1.3–10.0)	(15.0–50.0)	(13.0–50.0)
End-stage						
EC_{25}	0.7	1.1	1.2	0.05b	0.5a	2.3a
	(0.3–1.0)	(0.7–3.1)	(0.8–2.9)	(0.04–0.09)	(0.3–0.8)	(1.1–5.0)
EC_{50}	4.0	4.2	4.3	0.1b	1.1a	7.6a
	(2.6–6.3)	(2.8–8.0)	(3.0–6.0)	(0.07–0.3)	(0.8–2.1)	(3.1–11.1)
EC_{75}	9.0	30.0	29.0a	1.0b	14.5a	23.3a
	(5.3–18.0)	(13.0–83.0)	(20.0–85.0)	(0.5–2.3)	(6.0–56.0)	(13.0–73.0)

Each value is the geometric mean with 95% confidence limits in parentheses for at least four dogs. [a] Significance versus no polymyxin and [b] significance for saphenous vein versus dorsal pedal artery. $P < 0.05$.

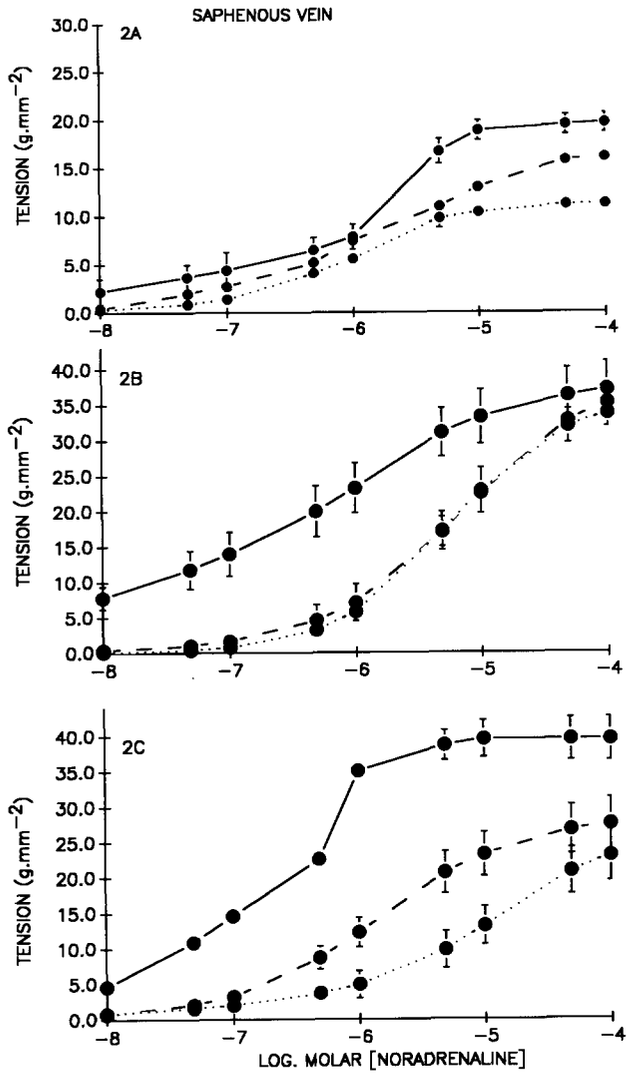

Fig. 2. Concentration-effect curves generated to noradrenaline in the absence and presence of polymyxin B on rings of saphenous vein. Panel A represents data derived at control, before heart failure, panel B, data derived after 1 week of pacing, and panel C, data derived from peak heart failure. Solid lines represent concentration-effect curves in the absence of polymyxin B. Dashed lines, concentration-effect curves to noradrenaline in the presence of 2×10^{-5} M polymxin B and dotted lines in the presence of 10^{-4} M polymyxin B. Each point is the mean ± S.E.M. from at least four dogs.

failure, the concentration-effect curve became much narrower compared to the 1 week-paced data. In this case, polymyxin B only antagonised the higher concentrations of phenylephrine.

4. Discussion

This study demonstrates that in the α_1-adrenoceptor mediated contraction of canine vascular smooth muscle there is a polymyxin B inhibitable component. Secondly, the nature and type of inhibition of α_1-adrenoceptor-mediated contractions by polymyxin B appeared to be different with respect to artery and vein. In addition, the shape of the concentration-effect curves, generated for the vein, varied with developing heart failure and polymyxin B tended to inhibit the high affinity component. Finally, this inhibitory component by polymyxin B of the α_1-adrenoceptor mediated contraction of the saphenous vein (and to a lesser extent the dorsal pedal artery) was dependent on the heart failure state.

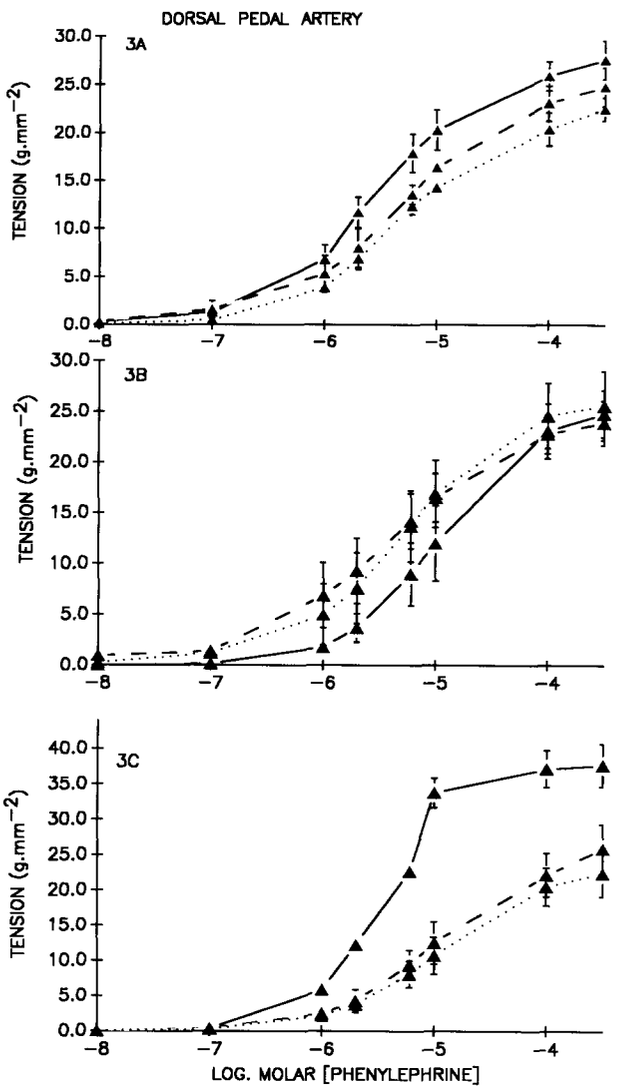

Fig. 3. Concentration-effect curves generated to phenylephrine in the absence and presence of polymyxin B on dorsal pedal arterial rings. Panel A represents data derived at control, before heart failure, panel B, data derived after 1 week of pacing, and panel C, data derived from peak heart failure. Solid lines represent concentration-effect curves in the absence of polymyxin B. Dashed lines represent concentration-effect curves to phenylephrine in the presence of 2×10^{-5} M polymyxin B and dotted lines in the presence of 10^{-4} M polymyxin B. Each point is the mean ± S.E.M. from at least four dogs.

This current investigation was designed to continue the examination into the amplification of peripheral vascular smooth muscle contractions to α_1-adrenoceptor agonists of the dorsal pedal artery and the saphenous vein at heart failure (Forster et al., 1989a,b). In the presence of extracellular Ca^{2+}, α_1-adrenoceptor agonists produce a transient phase contraction followed by a maintained tonic phase. The initial phase being due to Ca^{2+} release from intracellular stores (Deth and Van Breemen, 1974) and the sustained contraction, possibly due to increased Ca^{2+} influx (Bolton, 1979; Van Breemen et al., 1979). It has been suggested that protein kinase C activation is involved in calcium sensitisation mediated by various receptor agonists since, for example, noradrenaline produces a greater contraction than that produced by high potassium at a given intracellular calcium concentration (Hori et al., 1992). In carotid artery smooth muscle, it has been demonstrated that diacylglycerol levels remain elevated after agonist stimulation, implying a role for protein kinase C in the maintenance of tone (Takuwa et al., 1988). Given this information and previous findings (Forster et al., 1989a; Forster and Campbell, 1993), it seemed reasonable that the augmented response to α_1-adrenoceptor stimulation in peripheral blood vessels from dogs with pacing-induced heart failure involved some increase in Ca^{2+} sensitivity due to protein kinase C activation.

Before discussing the participation of protein kinase C and the effects of its inhibition in developing heart failure, on α-adrenoceptor mediated contractions of the dorsal pedal artery and saphenous vein, the contractile responses to the agonists per se should be commented upon. In the dorsal pedal artery, noradrenaline caused concentration-dependent increases in tension. However, unlike previous studies (Forster et al., 1989a, 1992a) there were no differences in the concentration-effect curves at the different time points in heart failure development. The reason for this discrepancy is unclear but may be related to the mongrel canine population. Secondly, in this study, end-stage heart failure was defined after a cut-off period of 3 week pacing; in other studies, pacing continued to a definite biological end point (Forster et al., 1992b; Moe et al., 1993). In contrast to the dorsal pedal artery, the maximal response to noradrenaline in the saphenous vein was potentiated after 1 week of pacing and end-stage heart failure which was associated with the characteristic decrease in EC_{50} values. The fact that polymyxin B had only slight effects on the arterial preparation means that the α-adrenoceptor is less dependent on protein kinase C compared to the saphenous vein. Therefore, protein kinase C is involved in the venous response to noradrenaline in heart failure, indicating that potentially different populations of α_1-adrenoceptors are activated.

Although several agents have been shown to be inhibitory to protein kinase C (Kuo et al., 1984), most, including polymyxin B do not interact with the catalytically active centre of protein kinase C. It appears that

Table 4
EC_{25}, EC_{50} and EC_{75} (μM) values for phenylephrine in the absence and presence of polymyxin B

	Dorsal pedal artery			Saphenous vein		
	No polymyxin	2×10^{-5} M	10^{-4} M	No polymyxin	2×10^{-5} M	10^{-4} M
Control						
EC_{25}	1.1	1.3	1.8	0.016 [b]	1.0 [a]	1.2 [a]
	(0.9–1.3)	(1.0–1.9)	(1.1–2.3)	(0.011–0.03)	(0.7–1.2)	(0.9–1.6)
EC_{50}	4.0	4.2	4.2	1.4	2.2	4.0
	(2.9–5.4)	(3.0–7.6)	(2.8–7.0)	(0.5–3.5)	(2.0–3.1)	(2.1–6.1)
EC_{75}	30.0	30.0	83.0 [a]	2.0 [b]	6.0 [a]	17.3 [a]
	(12.0–57.0)	(20.0–56.0)	(60.0–113.0)	(1.2–3.0)	(3.0–7.3)	(9.1–33.3)
1 week paced						
EC_{25}	3.0	0.9	1.2	0.03 [b]	0.8 [a]	1.0 [a]
	(2.0–6.1)	(0.3–2.9)	(0.8–2.3)	(0.02–0.06)	(0.3–1.2)	(0.5–3.0)
EC_{50}	12.9	3.3 [a]	3.6 [a]	0.5 [b]	2.3 [a]	2.1 [a]
	(5.9–28.8)	(1.5–5.0)	(2.1–5.3)	(0.2–1.3)	(2.0–3.1)	(1.5–4.6)
EC_{75}	51.0	27.0	18.0	7.9 [b]	12.0	25.6
	(20.0–80.0)	(11.0–83.0)	(9.0–63.0)	(3.3–13.0)	(10.0–18.0)	(10.0–33.3)
End-stage						
EC_{25}	1.3	3.0 [a]	3.3 [a]	0.3 [b]	0.3	0.6
	(1.1–1.6)	(2.3–9.7)	(2.4–7.1)	(0.2–0.6)	(0.2–0.5)	(0.4–0.9)
EC_{50}	9.1	14.1	10.0	0.8 [b]	0.8	0.8
	(5.7–14.4)	(9.3–21.1)	(7.6–20.0)	(0.5–1.3)	(0.6–1.2)	(0.6–1.1)
EC_{75}	11.1	67.0 [a]	103.0 [a]	2.8 [b]	7.0	10.0 [a]
	(9.3–12.3)	(30.0–130.0)	(87.0–213.0)	(1.1–4.6)	(3.3–10.0)	(5.5–23.0)

Each value is the geometric mean with 95% confidence limits in parentheses for at least four dogs. [a] Significance versus no polymyxin and [b] significance for saphenous vein verses dorsal pedal artery. $P < 0.05$.

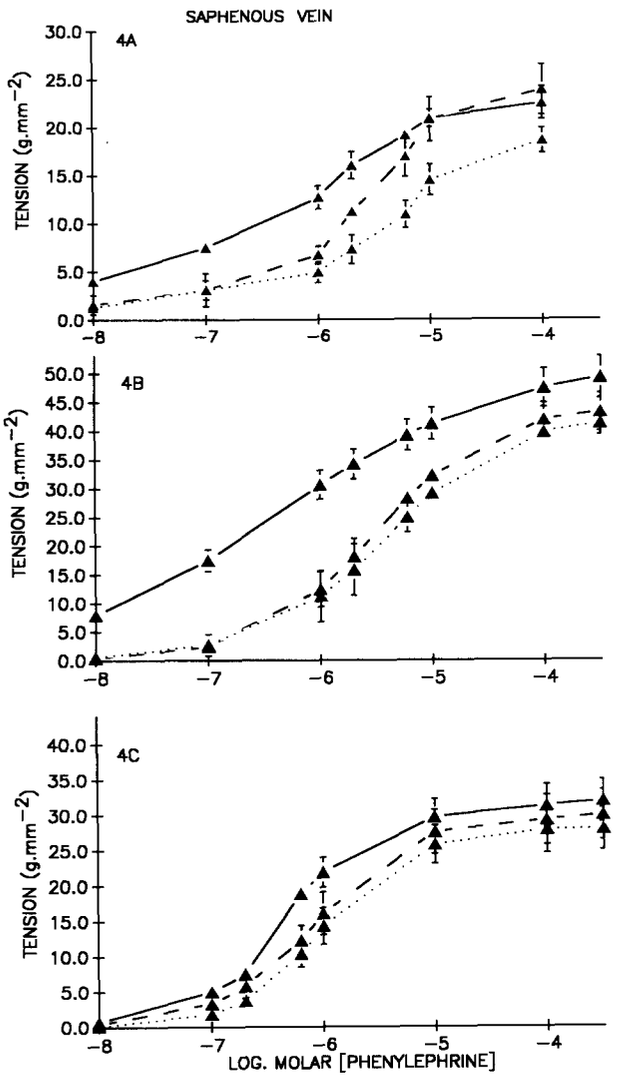

Fig. 4. Concentration-effect curves generated to phenylephrine in the absence and presence of polymyxin B on rings of saphenous vein. Panel A represents data derived at control, before heart failure, panel B, data derived after 1 week of pacing, and panel C, data derived at peak heart failure. Solid lines represent concentration-effect curves to phenylephrine in the absence of polymyxin B, dashed lines and dotted lines in the presence of 2×10^{-5} M and 10^{-4} M polymyxin B respectively. Each point is the mean ± S.E.M. from at least four dogs.

these agents prevent activation of the enzyme through phospholipid interaction (Kikkawa et al., 1989) and they may inhibit other protein kinases. However, polymyxin B in previous studies was found to be most effective and relatively selective for protein kinase C (Kuo et al., 1984). In support of this, polymyxin B does inhibit contractions of the saphenous vein in response to tumour-promoting phorbol esters (Forster et al., 1991). Therefore, it is likely that in the present study, polymyxin B is relatively selective for protein kinase C. However, polymyxin B did not significantly alter the maximal response to noradrenaline and phenylephrine, nor did it effect the EC_{50} in the dorsal pedal artery. Therefore, it might be concluded that a distinct protein kinase C involvement may be negligible in the α_1-adrenoceptor-mediated contractions of the dorsal pedal artery. It may be that, in the dorsal pedal artery where no inhibition was observed, *only* Ca^{2+} influx was responsible for the sustained tone by directly activating a Ca^{2+} channel independent of a protein kinase C mechanism. In support of this is that nifedipine inhibited noradrenaline and phenylephrine to different extents at the same three time points (Forster and Campbell, 1993).

In contrast, in the saphenous vein, the concentration-effect curves to noradrenaline and phenylephrine have distinctly different shapes as heart failure develops. After 1 week of pacing, the curves became non-classically broad. This indicates the involvement of multiple, contributing mechanisms or receptor subtypes. During developing heart failure, a distinct high affinity component is observed which is more sensitive to polymyxin B. This high affinity component is lost in the phenylephrine response at end-stage heart failure. The differential effects after 1 week pacing are difficult to reconcile, but may possibly be related to other phenomena rather than a direct protein kinase C activation. It may be assumed, for instance, that when noradrenaline interacts with an α_1-adrenoceptor there is activation of both inositol 1.4.5-trisphosphate and diacylglycerol. Both inositol 1.4.5-trisphosphate and diacylglycerol augment Ca^{2+} movements, which in turn would increase active force and produce an increase in developed tension. It is also possible that the high and low affinity components in the saphenous vein are due to distinct α_1-adrenoceptors (the saphenous vein is known to have multiple α-adrenoceptors (Hicks et al., 1991)). No such distinction could be seen in the dorsal pedal artery. Therefore, it may be concluded that the calcium influx pathway which is operative in the dorsal pedal artery (Forster and Campbell, 1993) is independent of diacylglycerol and may involve other α_1-adrenoceptors. These observations are based solely on the contractile response to noradrenaline and phenylephrine. It should be commented that both these agonists activate both α_1- and α_2-adrenoceptors. However, it is unlikely in the present study that an α_2-adrenoceptor was involved since yohimbine (an α_2-adrenoceptor antagonist) was present throughout. Furthermore, previous studies have indicated that both the dorsal pedal artery and the saphenous vein do not exhibit increased responsiveness to α_2-adrenoceptor stimulation during developing heart failure (Forster and Armstrong, 1990).

Notwithstanding, chronic exposure of cells to phorbol esters cause a down-regulation of protein kinase C (Ballester and Rosen, 1985). There may also be some negative effect on ionic movements and it has been shown, in cultured smooth muscle cells, that excessive

protein kinase C activation can lead to increased Ca^{2+} efflux (Colucci et al., 1986). This may represent a compensatory mechanism aimed to offset the detrimental effects of increased venous pressures in heart failure. In the long term, this higher venous pressure and/or stretch may be responsible for the greater in vitro responsiveness as seen in the saphenous vein following heart failure.

Acknowledgements

This study was supported by a Grant-In-Aid from the Ontario Heart and Stroke Foundation. I am grateful to the technical assistance of Ms Ana Albernaz and to Medtronic (Canada) for supplying the pacemaker generators and leads.

References

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.

Aub, D.L. and J.W. Putney, 1985, Properties of receptor controlled inositol triphosphate formation in parotid acinar cells, Biochem. J. 225, 263.

Ballester, R. and O.M. Rosen, 1985, Fate of immunoprecipitable protein kinase C in GH_3 cells treated with phorbol 12-myristate 13-acetate, J. Biol. Chem. 260, 15194.

Berridge, M.J., 1983, Inositol triphosphate and diacylglycerol as second messengers, Biochem. J. 212, 849.

Bolton, T.B., 1979, Mechanisms of action of transmitters and other substances on smooth muscle, Physiol. Rev. 59, 606.

Coleman, H.N., R.R. Taylor, P.E. Pool, G.H. Whipple, J.W. Covell, J. Ross Jr. and E. Braunwald, 1971, Congestive heart failure following chronic tachycardia, Am. Heart J. 81, 790.

Colucci, W.S., M.A. Gimbrone and R.W. Alexander, 1986, Phorbol diester modulates α-adrenergic receptor-coupled calcium efflux and α-adrenergic receptor number in cultured vascular smooth muscle cells, Circ. Res. 58, 393.

Danthaluri, N.R. and R.C. Deth, 1984, Phorbol ester-induced contractions of arterial smooth muscle and inhibition of α-adrenergic response, Biochem. Biophys. Res. Commun. 125, 1103.

Deth, R. and C. Van Breemen, 1974, Relative contributions of Ca^{2+} influx and cellular Ca^{2+} release during drug induced activation of the rabbit aorta, Pflüg. Arch. 348, 13.

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

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

Forster, C., P. Campbell and P.W. Armstrong, 1991, Activation of protein kinase C in developing pacing-induced heart failure, Pharmacologist 33, 48.

Forster, C., P.M. Campbell and P.W. Armstrong, 1992a, Temporal alterations in perpheral 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. Physiol. Pharmacol. 70, 1417.

Francis, G.S., 1985, Plasma catecholamine levels in patients with congestive heart failure, Cardiovasc. Rev. Rep. 6, 444.

Grima, E.A., G.W. Moe, R.J. Howard and P.W. Armstrong, 1994, Recovery of attenuated baroreflex sensitivity in conscious dogs after reversal of pacing induced heart failure, Cardiovasc. Res. 28, 384.

Han, C., P.W. Abel and K.P. Minneman, 1987, α_1-Adrenoceptor subtypes linked to different mechanisms for increasing intracellular Ca^{2+} in smooth muscle, Nature 329, 24.

Hicks, P.E., M. Barras, G. Herman, P. Mauduit, A.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.

Hori, M., K. Sato, K. Sakata, H. Ozaki, H. Takano-Ohmuro, T. Tsuchiya, H. Sugi, I. Kato and H. Karaki, 1992, Receptor agonists induce myosin phosphorylation-dependent and independent contractions in vascular smooth muscle, J. Pharmacol. Exp. Ther. 261, 506.

Khalil, R.A. and C. Van Breemen, 1988, Sustained contraction of vascular smooth muscle: calcium influx or C-kinase activation?, J. Pharmacol. Exp. Ther. 244, 537.

Kikkawa, U., A. Kishimoto and Y. Nishizuka, 1989, The protein kinase C family: heterogeneity and its implications, Annu. Rev. Biochem. 58, 31.

Kishimoto, A., Y. Takai, T. Mori and Y. Nishizuka, 1980, Activation of calcium and phospholipid-dependent protein kinase by diacylglycerol, its possible relation to phosphotidylinositol turnover, J. Biol. Chem. 255, 2273.

Kuo, J.F., R.L. Raynor, F.J. Mazzei, R.C. Schatzman, R.S. Turner and W.R. Kem, 1983, Cobra polypeptide cytotoxin I and marine worm polypeptide cytotoxin A-IV are potent and selective inhibitors of phospholipid- senstive Ca^{2+}-dependent protein kinase, FEBS Lett. 153, 183.

Kuo, J.F., R.C. Schatzman, R.S. Turner and G.J. Mazzei, 1984, Phospholipid-sensitive Ca^{2+}-dependent protein kinase: a major protein phosphorylation system, Mol. Cell. Endocrinol. 35, 65.

Main, J.S., C. Forster and P.W. Armstrong, 1991, Inhibitory role of the coronary arterial endothelium to α-adrenergic stimulation in experimental heart failure, Circ. Res. 68, 940.

Moe, G.W., C. Montgomery, R.J. Howard, E.A. Grima and P.W. Armstrong, 1993, Left ventricular myocardial blood flow, metabolism, and effects of treatment with enalapril: further insights into the mechanisms of canine experimental pacing-induced heart failure, J. Lab. Clin. Med. 121, 294.

Nishizuka, Y., 1984, The role of protein kinase C in cell surface signal transduction and tumour promotion, Nature 308, 693.

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

Parmley, W.W., 1978, Pathophysiology of congestive heart failure, Am. J. Cardiol. 55, 9A.

Rasmussen, H., J. Forder, I. Kojima and A. Scriabine, 1984, TPA-induced contraction of isolated rabbit vascular smooth muscle, Biochem. Biophys. Res. Commun. 122, 776.

Takuwa, Y., G. Kelley, N. Takuwa and H. Rasmussen, 1988, Protein

phosphorylation changes in bovine carotid artery smooth muscle during contraction and relaxation, Mol. Cell. Endocrinol. 60, 71.
Thomas, J.A. and G.H. Marks, 1978, Plasma norepinephrine in congestive heart failure, Am. J. Cardiol. 41, 233.
Tian, W.-N., S. Gupta and R.C. Deth, 1990, Species differences in chloroethylclonidine antagonism at vascular alpha-1 adrenergic receptors, J. Pharmacol. Exp. Ther. 253, 877.
Van Breemen, C., P. Aaronson and R. Loutzenhiser, 1979, Na-Ca interactions in mammalian smooth muscle, Pharmacol. Rev. 30, 167.
Yuan, S. and A.K. Sen, 1986, Characterization of the membrane-bound protein kinase C and its substrate proteins in canine cardiac sarcolemma, Biochim. Biophys. Acta 886, 152.