EFFECTS OF NORADRENALINE AND POTASSIUM CHLORIDE ON PERIPHERAL VESSELS IN ONE EXPERIMENTAL MODEL OF HEART FAILURE

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Abstract - We investigated contraction to noradrenaline (NA) and KCl and sensitivity of NA at the level of larger vessels (thoracic aorta and vena cava; left renal artery and left renal vein; lateral saphenous artery and lateral saphenous vein and finally central ear artery and marginal ear vein) in a model devised to mimic heart failure. The model presented here is the rabbit coronary ligation model in which myocardial infarction was produced in male New Zeland white rabbits (2.6 Kg-3.0 Kg) by ligation of the marginal branch of the left descending coronary artery. The development of chronic heart failure was allowed to proceed over eight weeks. Animals were killed by overdose with pentobarbitone sodium (i.v injection). Arteries and veins were carefully removed with as little connective tissue as possible and placed in cold physiological salt solution (PSS). The arterial and venous rings were mounted in 10 ml isolated organ baths, bathed in Kerbs maintained at 37 ° c with 95% O_2 plus 5% CO_2. The rings were then placed under different resting tensions. After initial application of tension, tissues were left to equilibrate for a 60 min period. Then all tissues were exposed to cumulative concentration of NA (1nM-300μM). Following complete washout, the preparations were left for 45 minutes to re-equilibrate. Then all preparations were contracted with KCl (Krebs solution, sodium free and high KCl, 125 mM) and allowed to contract for 5-10 min. Following complete washout with normal Krebs an additional 30 minutes equilibration period was allowed. Then cumulative concentration-response curves (CCRC) to NA obtained by increasing the concentration of the agonist in half-log increments. In contraction responses to NA aorta, ear artery and ear vein were the most sensitive preparations (pD_2 values: 9.96, 7.04 and 7.8 respectively). Renal artery and aorta had relatively very large maximum responses to NA among the arteries (6.7 and 4.3g respectively) and saphenous vein had greatest maximum response among the veins (2.9g). The results led to two major conclusions with respect to the model. First, vasoconstrictions to noradrenaline were unaltered. Second, contractions to KCl (125 mM) were preserved in large vessels (arteries and veins) in coronary ligated rabbits after 8 weeks compared with a normal control population.

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INTRODUCTION

Chronic heart failure is a clinical syndrome characterised by the inability of the heart to provide adequate nutrient supply to metabolically active tissues (1). Sudden cardiac death claims an estimated 350,000 lives per year in the United States and between 50,000 and 100,000 lives a year in the United Kingdom. There are numerous underlying diagnoses in patients suffering sudden cardiac death. In 75% of cases, the underlying pathology causing heart failure in patients with sudden cardiac death is coronary heart disease (2). It has been described as a condition of generalised neurohumoral excitation, characterised by activation of the sympathetic nervous and renin-angiotensin systems, increases in plasma vasopressin concentration, and parasympathetic withdrawal (3,4). Elevated plasma noradrenaline resulting from increased noradrenaline release and decresed noradrenaline reuptake can act as natural agonist on vascular α - adrenoceptors, which mediate vasoconstriction in vascular beds (5).

Several animal models of human congestive heart failure (CHF) have been developed in attempts to reproduce these features to study the pathogenic mechanisms involved in this disease. The coronary artery occlusion model of heart failure in the rat has been extensively studied. The model has been validated by the measurement of haemodynamic variables (6). Rapid ventricular pacing in the dog has been shown to fulfil the clinical, radiographic and haemodynamic definations of congestive heart failure (7). Pigs share similar cardiac anatomy and coronary vasculture to that of humans, which makes them suitable for the study of human disease processes (8). Cardiomyopathic male hamsters of the BIO TO-2 strain, a unique experimental model of CHF characterised by progressive myocytolytic necrosis of cardiac muscle are available in study of congestive heart failure (CHF) (9). In coronary ligation model it has become recognised that collateral flow is the most important determinant of the rate and extent of cell death within an ischaemic zone. Collateral flow in the rabbit has been shown to be

essentially very poor, similar to the human and pig (10). Since in 75% of cases, the underlying pathology causing heart failure in patients with sudden cardiac death is coronary heart disese this model produces similar circumstance to coronary heart disease. Several studies that investigated α - adrenoceptors in heart failure reported conflicting results in experimental heart failure. Some of them suggested downregulation of α-adrenoceptors probably due to receptor exposure to elevated catecholamine levels (11,12). Some other reporters found no difference in density and sensitivity of α - adrenoceptors between experimental heart failure and control groups (13,14). Therefore, the aim of our study was to investigate the possibility of changing density and sensitivity of α - adrenoceptors in this model of heart failure.

MATERIALS AND METHODS

The model was prepared by M. Hicks and co-workers in the Royal Infirmary, Glasgow. Myocardial infarction was produced in male New Zeland white rabbits (2.6 kg - 3.0 kg) by ligation of the marginal branch of the left descending coronary artery. The development of chronic heart failure was allowed to proceed over eight weeks. Sham operated animals underwent a similar procedure but no ligation was performed. Animals were killed by overdose with pentobarbitone sodium (i.v injection). Arteries and veins were carefully removed with as little connective tissue as possible and placed in cold physiological salt solution (PSS). Four paris of arteries and veins (thoracic aorta and vena cava; left renal artery and left renal vein; lateral saphenous artery and lateral saphenous vein and finally central ear artery and marginal ear vein) of the sham operated with mean ejection fraction of (70.5 ± 2.13) and coronary ligated rabbits with mean ejection fraction of (46.5 ± 4.4), as determined by echocardiography, were studied. The arterial and venous rings were mounted in 10 ml isolated organ baths, bathed in Krebs maintained at 37°c and gased with 95% O_2 plus 5% CO_2. Blood vessels were used immediately. The rings were then placed under different resting tensions which were deremined by contraction to NA (1 μM) from some preliminary experiments. After initial application of tension, tissues were left to equilibrate for a 60 min period. Then all tissues were exposed to cumulative concentrations of NA (1 nM - 300 μM). Following complete washout, the preparations were left for 45 minutes to re-equilibrate. Then all preparations were contracted with potassium chloride (KCl) (Krebs solution, sodium free and high KCl, 125 mM) and allowed to contract for 5-10 min. Following complete washout with normal Krebs, an additional 30 minutes equilibration period was allowed. Then cumulative concentration-response curves (CCBC) to NA obtained by increasing the concentration of the agonist in half-lig increments. Contraction to NA in each concentration allowed five minutes to reach maximum. Response to each contractile agonist is expressed as absolute tension (g). All data are given as mean ± SEM. Significance was always accepted at the 0.05 level of probablity.

Solutions and Drugs

The composition of the modified Krebs-Henselite solution was as follows: (in mM): NaCl 118.4, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.6, $MgSO_4$ 0.6, $CaCl_2$-2.5 and glucose 11. Na_2 EDTA (23 μM) was also included in the Krebs in all experiments to prevent degradative oxidation of NA and propranolol (1 μM) and cocaine hydrochloride (10 μM) were also included to inhibit β - adrenoceptors and neuronal uptake of NA respectively. The following compounds were used: (-) - noradrenaline bitrate (Sigma); propranolol HCl (Sigma); cocaine HCl (Mac Carthys). All drugs were dissolved in distilled water. All concentrations of the drugs used are expressed as final concentration in the organ bath.

RESULTS

Contraction to NA

Initialy all tissues were exposed to cumulative concentrations of NA (1 nM-300 μM). Aorta, ear artery and ear vein were the most sensitive preparations (pD_2 values: 9.96, 7.04 and 7.8 respectively). Renal artery and aorta had relatively very large maximum responses to NA among the arteries (6.7 and 4.3 g respectively) and saphenous vein had greatest maximum response among the veins. In vasoconstriction to NA, there was no difference between coronary ligated and sham operated rabbits after 8 weeks operation (Table 1,2).

Response to KCl

All preparations were contracted with KCl (Krebs solution, Na free and high KCl, 125 mM) and allowed to contract for 5-10 min. Contraction to KCl in the first or second test showed no significant difference and arteries except saphenous vein had greater contractions compared with corresponding veins. Renal artery, saphenous vein and aorta had the greatest maximum contraction (7,5 and 4.2 respectively). However, the response to KCl was similar in coronary ligated and sham operated rabbits after 8 weeks operation (Table 3).

Table 1. Comparison of noradreneline pD_2 in four paris of arteries and veins of the coronary ligated and sham operated rabbits after 8 weeks operation

Vessel	pD_2 1 st CCRC to NA sham	pD_2 1 st CCRC to NA sham	pD_2 1 st CCRC to NA sham	pD_2 1 st CCRC to NA sham
Aorta	6.96 ± 0.08	6.72 ± 0.28	7.09 ± 0.07	6.98 ± 0.25
Vena cava	6.23 ± 0.18	5.95 ± 0.15	6.33 ± 0.23	5.8 ± 0.22
Renal artery	6.07 ± 0.08	6.11 ± 0.16	6.34 ± 0.11	6.57 ± 0.22
Renal vein	6.46 ± 0.21	6.29 ± 0.17	6.51 ± 0.15	6.09 ± 0.1
Saphenous artery	6.43 ± 0.13	6.72 ± 0.18	6.46 ± 0.08	6.69 ± 0.11
Saphenous vein	6.86 ± 0.16	6.33 ± 0.17	6.44 ± 0.08	6.28 ± 0.15
Ear artery	7.04 ± 0.03	6.65 ± 0.2	6.34 ± 0.14	6.09 ± 0.13
Ear vein	7.8 ± 0.25	7.72 ± 0.13	7.89 ± 0.16	7.77 ± 0.14

pD_2 expressed as the - log of the EC_{50} (concentration producing 50% of the maximum response) of noradrenaline (NA). Initially all tissues were exposed to cumulative concentration of NA (1 nM - 300 μM). Following washout, all preparations were contracted with KCl (125 mM). Following washout preparations were exposed cumulatively to NA.
Each point represents mean ± s.e.mean (n=6).

Table 2. Comparison of maximum response expressed as tension (g) of noradreneline in four paris of arteries and veins of the coronary ligated and sham operated rabbits after 8 weeks operation

Vessel	Maximum response 1 st CCRC to NA sham	Maximum response 1 st CCRC to NA sham	Maximum response 1 st CCRC to NA sham	Maximum response 1 st CCRC to NA sham
Aorta	4.26 ± 0.36	5.75 ± 0.73	6.4 ± 0.54	6.44 ± 1.01
Vena cava	1.19 ± 0.33	1.56 ± 0.16	1.5 ± 0.3	1.74± 0.21
Renal artery	6.71 ± 0.61	6.65 ± 0.32	7.61 ± 0.62	7.07 ± 0.49
Renal vein	1.48 ± 0.19	1.6 ± 0.38	1.54 ± 0.26	2.08 ± 0.73
Saphenous artery	2.34 ± 0.34	2.91 ± 0.19	2.53 ± 0.41	3.35 ± 0.3
Saphenous vein	2.92 ± 0.34	3.1 ± 0.49	3.32 ± 0.43	3.63 ± 0.53
Ear artery	1.79 ± 0.29	1.37 ± 0.19	2.2 ± 0.34	1.72 ± 0.31
Ear vein	0.2 ± 0.04	0.31 ± 0.06	0.45 ± 0.07	0.46 ± 0.05

Initially all tissues were exposed to cumulative concentration of NA (1 nM - 300 μM). Following washout, all preparations were contracted with KCl (125 mM). Following washout preparations were exposed cumulatively to NA. Maximum response is expressed as tension (g).
Each point represents mean ± s.e.mean (n=6). Statiscally significant differences are represented by * $p < 0.05$, unpaired student's t-test.

Table 3. Comparison of contractions to KCl (125 mM) expressed as tension (g) in four paris of arteries and veins of the coronary ligated and sham operated rabbits after 8 weeks operation

Vessel	Maximum response (g) KCl sham	Maximum response (g) KCl Ligated
Aorta	3.61 ± 0.37	4.76 ± 0.71
Vena cava	1.07 ± 0.26	1.32 ± 0.12
Renal artery	6.34 ± 0.7	5.86 ± 0.42
Renal vein	1.23 ± 0.29	1.25± 0.15
Saphenous artery	2.31 ± 0.37	2.79 ± 0.19
Saphenous vein	5.17 ± 0.3	5.93± 0.45
Ear artery	1.76 ± 0.26	1.29 ± 0.37
Ear vein	0.32± 0.06	0.47 ± 0.03

Initially all tissues were exposed to cumulative concentration of NA (1 nM - 300 μM). Following washout, all preparations were contracted with KCl (125 mM). Contraction to KCl is expressed as tension (g).

Data are expressed as mean ± s.e.mean (n=6).

DISCUSSION

Response to KCl

Abnormal function of the contractile apparatus of vessels, can be assessed by contraction to KCl. In our study, high potassium chloride (125 mM) was used as a contractile agent. It causes direct smooth muscle depolarisation leading to smooth muscle contraction via calcium entry through voltage sensitive calcium channels.

We observed that contraction to KCl was not different from control. Our results agree with other reports (11,12,13) in dogs with pacing induced heart failure indicating that contraction to potassium chloride (125 mM) was unchanged. We suggest that there is no general change in contractile apparatus or smooth muscle responsiveness in this model of heart failure.

α - adrenoceptor activity

This study demonstrates that the physiological mixed α_1 / α_2 - agonist noradrenaline produced concentration-dependent contractions in arteries and veins from controls and rabbits with coronary ligation. Our study agree with some of the reports, in pacing-induced CHF in canine coronary arteries (15), in canine femoral artery (16) and in human with CHF (14,17) that showing vasoconstrictions to noradrenaline were unaltered in this model of heart failure.

It has been known that plasma noradrenaline levels are elevated in CHF patients and increased circulating catecholamines in chronic heart failure might be expected to lead to adrenergic down regulation. However in sharp contrast to β_1 - adrenoceptors down regulation in faling hearts, the α_1 - adrenoceptor population is maintained at normal levels in the failing human heart (18). In peripheral vessels some investigators found increased tissue sensitivity to adrenergic agents during CHF (11,12). Contrary to Forster and co-workers, Main and colleageus (16) found that in pacing -induced CHF in canine coronary arteries, the maximal contractile response to methoxamine was attenuated. The data suggest that α_1 - adrenoceptor - mediated constriction is diminished. Our results indicate that the vascular responsiveness to noradrenaline was preserved in this model of heart failure.

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