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VASODILATOR EFFECTS OF THE β-AGONIST ISOPRENALINE IN AN EXPERIMENTAL MODEL OF HEART FAILURE

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ABSTRACT

Heart failure is a clinical syndrome characterized by the inability of the heart to provide nutrient supply to tissues. In 75% of cases, the underlying pathology causing heart failure in patients with cardiac death is coronary heart disease. A rabbit model of heart failure with coronary ligation was produced to mimic coronary heart disease in humans. After producing the model, two arteries and two veins were investigated in the two groups (control and with coronary ligation). Arteries and veins were cut as rings and bathed in Krebs solution maintained at 37 °C, and gassed with 95% oxygen and 5% CO_2. Then all tissues were placed under different resting tensions and allowed to equilibrate for 1 hour. Then all the tissues were contracted with U-46619 (0.1 μM) nearly ten minutes before initial application of isoprenaline. When the U-46619 (0.1 μM)-induced contraction reached a plateau, concentration-response curves to isoprenaline were obtained. Isoprenaline was chosen as a vasodilator, it's effect resulting from stimulating beta receptors in blood vessels. Isoprenaline induced relaxation in all tissues, but the renal artery was the most sensitive and showed maximum relaxation.[20-26] Compared to acetylcholine, relaxation responses were small and maximum responses observed in the vena cava, aorta and renal vein were only 10 percent. In all tissues, relaxation responses to the vasodilator agent isoprenaline showed no significant difference between control and coronary ligated rabbits 8 weeks after operation.

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INTRODUCTION

Chronic heart failure is a clinical syndrome characterized 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 generalized neurohumoral excitation, characterized 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 decreased noradrenaline reuptake can act as a natural agonist on vascular α- and β-adrenoceptors, which mediate vasoconstriction via vascular α-adrenoceptors and vasorelaxation via vascular β-adrenoceptors.[5] Several studies that investigated β-adrenoceptors in heart failure reported conflicting results in experimental heart failure. Some of them suggested down-regulation of β-adrenoceptors, probably due to receptor exposure to elevated catecholamine levels.[6,7] Others found no difference in density and sensitivity of β-adrenoceptors between experimental heart failure and control groups.[8,9] 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 hemodynamic variables.[10] Rapid ventricular pacing in the dog has been shown to fulfill the clinical, radiographic and hemodynamic definitions of congestive heart failure. Pigs possess a cardiac anatomy and coronary vasculature similar to that of humans, which makes them suitable for the study of human disease processes.[11] Cardiomyopathic male hamsters of the BIO TO-2 strain, a unique experimental model of CHF characterized by progressive myocytolytic necrosis of cardiac muscle are available in the study of congestive heart failure (CHF).[12] The rabbit coronary ligation model is a relatively straightforward model of left ventricular dysfunction. In the coronary ligation model it has become recognized that collateral flow is the most important determinant of the rate and extent of cell death within an ischemic zone. Collateral flow in the rabbit has been shown to be essentially very poor, similar to the human and pig.[13] Since in 75% of cases, the underlying pathology causing heart failure in patients with sudden cardiac death is coronary heart disease, this model produces a state similar to coronary heart disease. Therefore, the aim of our study was to investigate the possibility of changing the density and sensitivity of β-adrenoceptors in this model of heart failure.

MATERIALS AND METHODS

Myocardial infarction was produced in male New Zealand white rabbits (2.6-3.0 kg) by ligation of the marginal branch of the left descending coronary artery. The left circumflex artery (which supplies a significant part of the left ventricle) was ligated with an Ethicon suture at the mid-point between the atrioventricular groove and the cardiac apex. 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. After eight weeks, four arteries and veins (thoracic aorta, vena cava, left renal artery and left renal vein) of the sham operated (with a mean ejection fraction of 70.5±2.13) and coronary ligated rabbits (with a mean ejection fraction of 46.5±4.4, as determined by echocardiography), were studied. 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 Krebs maintained at 37 °C and gassed with 95% O_2 plus 5% CO_2. The rings were then placed under different resting tensions: aorta (2-2.5g), renal artery (1.5g), vena cava (0.5g) and renal vein which were determined by contraction to noradrenaline (1μM) from some preliminary experiments. Isoprenaline was chosen as a beta adrenoceptor agonist, producing relaxation in vascular beds. After initial application of tension, tissues were left to equilibrate for a 60 min period, during which time the tension was readjusted to a set value which was maintained constant throughout the rest of the experimental day. Then all tissues were precontracted with U-46619 (0.1μM) nearly ten minutes before initial application of isoprenaline. U-46619 was chosen as precontractor since it has no effect on beta adrenoreceptors. This induced submaximal contraction in all vessels. When the U-46619-induced contraction reached a plateau, cumulative concentration-response curves (CCRC) to isoprenaline were obtained by increasing the concentration of isoprenaline in half-log increments. Relaxation to isoprenaline in each concentration reached a maximum after five minutes. All concentrations are expressed as the final concentration in the organ bath fluid. Results are expressed as mean ± standard error of mean (s.e. mean). Comparisons between the two groups were performed using unpaired Student's t-test with values. Comparisons among several groups were performed using one-way analysis of variance. A value of $p<0.05$ was taken as statistically significant.

Solutions and drugs

The composition of the modified Krebs-Henselite solution was as follo ws (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. Other substances consisted of noradrenaline bitrate (Sigma), U-46619 (Upjohn), and isoprenaline (Sigma). All drugs except U-46619 were dissolved in distilled water. U-46619 was initially dissolved in high-performance liquid chromatography-grade absolute ethanol, with subsequent dilutions made in distilled water.

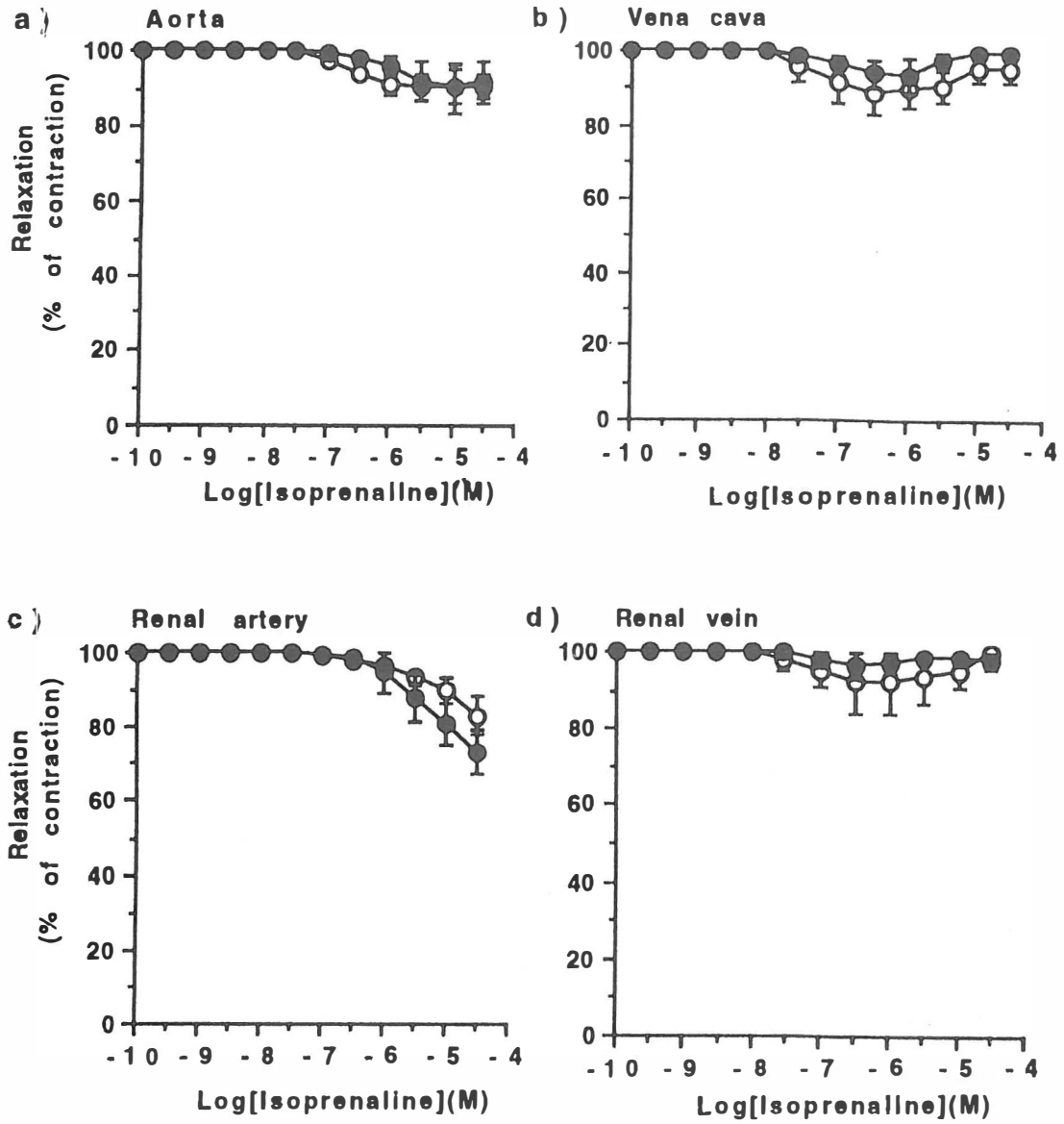

Fig. 1. Relaxation in response to isoprenaline in the four isolated arteries and veins of sham operated animals (○) with mean ejection fraction of (69.67±3.25) and coronary ligated rabbits (●) with mean ejection fraction of (49.83 ±2.7) 8 weeks after operation. Results are expressed as % of maximum response to U-46619 (0.1μM) used for inducing tone. Each point represents the mean ± s.e. mean (n= 6). Statistically, there is no significant difference between the two groups using unpaired Student's t-test and one way ANOVA.

RESULTS

U-46619 (9, 11-dideoxy 11a, 9a-epoxymethanoprostaglandin F2α) produced submaximal contraction in all vessels. Isoprenaline induced relaxations in both arteries and veins (Fig. 1). In every case the response obtained to isoprenaline within the range tested was smaller compared to ACh or SNP. The renal artery was the most sensitive and showed maximum relaxation (20-26%). Relaxation in response to the β-adrenoceptor agonist isoproterenol was not significantly different between coronary ligated and sham operated rabbits 8 weeks after operation.

DISCUSSION

Since circulating catecholamines exert a tonic vasodilatory effect on peripheral vessels via β-adrenoceptors, we investigated the role of β-adrenoceptors in coronary ligated rabbits. It is now well established that β-adrenoceptor

function is abnormal in the myocardium of patients with congestive heart failure (CHF).[6] In failing ventricles, there is a decreased number of β-adrenoceptors, reduced isoprenaline-stimulated adenylate cyclase activity, and depressed inotropic and chronotropic responsiveness to β-adrenoceptor agonists. Bristow and colleagues examined isolated cardiac tissue using radioligand-binding techniques and reported that β_1-adrenoceptor down-regulation occurred in the failing left ventricle, whereas there was no change in β_2-adrenoceptors.[7] The fact that noradrenaline has greater β_1- than β_2-adrenoceptor effects may explain the selective down-regulation of β_1-adrenoceptors. In spite of the known down-regulation of β_1-adrenoceptors in the failing left ventricle, few data in peripheral vessels are available. Since the trigger for β-adrenoceptor desensitization is thought to be chronically elevated circulating catecholamines,[7,14] this condition should also induce desensitization of peripheral β-adrenoceptors as well as those in the heart.

In the present study we found no change in isoprenaline-induced relaxation in coronary ligated rabbits compared with a normal control population. To explain the lack of change in β-adrenoceptors in vascular beds of the heart failure group, there are two main explanations. Down-regulation of β-adrenoceptors is more selective for cardiac β_1-adrenoceptors and vascular beds containing β_2-adrenoceptors.[15] Thus a β_2-adrenoceptor resistance to down-regulation could explain the absence of β_2-adrenoceptor changes, such as found in our study, and consistent with prior studies[8,9,16] investigating this problem which found no peripheral β-adrenoceptor desensitization or down-regulation in animal models of heart failure. An alternative explanation for the lack of β-adrenoceptor changes in our study is that these changes may occur over a long period of time in human heart failure. We allowed this model to develop over eight weeks similar to other animal models of heart failure.[12,17] Since in animal models the time period required for heart failure to develop is very short related to humans, the results obtained in our model of heart failure have to be interpreted and extrapolated to humans with caution.

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