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Prostanoid-mediated inotropic responses are attenuated in failing human and rat ventricular myocardium

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ABSTRACT

Prostanoid-modulatory approaches in heart failure patients have displayed effects which may seem to be mutually incompatible. Both treatment with prostanoids and inhibition of prostanoid synthesis have resulted in increased mortality in heart failure patients. Currently, it is unknown if prostanoids mediate contractile effects in failing human heart and if this can explain some of the clinical effects seen after prostanoid modulatory treatments. Therefore, the objectives of this study were to determine if prostanoids could elicit direct inotropic responses in human ventricle, and if so to determine if they are modified in failing ventricle. Contractile force was measured in left ventricular strips from non-failing or failing human and rat hearts. The ratio of phosphorylated to non-phosphorylated myosin light chain 2 (MLC-2) was measured by Western blotting in myocardial strips, and the levels of prostanoid FP receptor mRNA and protein were measured in rat by real-time RT-PCR and receptor binding assays. In non-failing human hearts, prostanoids evoked a positive inotropic effect and an increase of MLC-2 phosphorylation which was absent in failing human hearts. In failing rat heart, the prostanoid FP receptor-mediated inotropic response and prostanoid FP receptor-density was reduced by ~40–50% compared to non-failing rat heart. Prostanoids mediate a sustained positive inotropic response in non-failing heart, which appears to be down regulated in failing heart. The pathophysiological significance of changes in prostanoid-mediated inotropic support in the failing heart remains to be determined.

1. Introduction

Prostanoids have been thought to be beneficial in heart failure. Epoprostenol (PGI_2) is known to reduce pulmonary and peripheral vascular resistance (Patterson et al., 1995) and clinical studies with short term treatment with epoprostenol in patients with heart failure demonstrated an improvement in exercise tolerance (Sueta et al., 1995). But despite favorable acute and long-term hemodynamic effects (Haywood et al., 1995), long-term treatment of heart failure patients with epoprostenol was prematurely terminated due to increased mortality (Califf et al., 1997). Inhibition of prostanoid synthesis has also shown favorable effects in animal models. Pigs with cytokine-induced cardiopulmonary dysfunction showed improvement when treated with indomethacin, a nonselective inhibitor of the prostanoid producing enzymes cyclooxygenase (COX) 1 and 2 (Kruse-Elliott and Olson, 1993). Additionally, specific inhibition of COX-2 improved left ventricular function and lowered mortality in a mouse model of heart failure (Delgado et al., 2004). These findings suggest that inhibition of prostanoid production in heart failure patients might be beneficial to cardiac function and long-term survival. However, in clinical studies non-steroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors show deleterious effects in heart failure (Bleumink et al., 2003; Pitkala et al., 2002) and other cardiovascular diseases (Bresalier et al., 2005; Mamdani et al., 2004; Solomon et al., 2005).

Although the mechanisms causing the unfavorable effects of prostanoid-modulatory approaches in heart failure patients remain unknown, a possible contributing factor is the direct effect of prostanoids on contractility. Increased contractility in left ventricle due to stimulation of prostanoid FP receptor has been shown in animal models (Otani et al., 1988; Pönicke et al., 2000). Montalescot et al. (1998) demonstrated a positive inotropic response to prostacyclin (PGI_2) at therapeutic doses in heart failure patients, but were not able to determine whether the inotropic response was direct upon the heart or indirect due to activation of the sympathetic nervous system. Since prostanoids elevate cardiac cAMP levels in the heart (Metsa-Ketela, 1981) and positive inotropic agents elevating cAMP levels

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generally increase mortality in heart failure patients, stimulation with prostanoids might be suspected to increase mortality. However, if prostanoids provide tonic inotropic support through cAMP-independent mechanisms, inhibition of the prostanoid synthesis with subsequent loss of inotropic support may be an additional factor contributing to increased mortality, as found for treatment with alpha-adrenoceptor-antagonists in heart failure (ALLHAT Collaborative Research Group, 2000). A better understanding of whether and how prostanoids evoke inotropic responses in human cardiac ventricle is therefore needed.

The objectives of this study were 1) to determine if prostanoids could elicit direct inotropic responses in human cardiac left ventricle and, if so, elucidate the mechanism of action and 2) determine if prostanoid inotropic responses are modified in failing ventricle. To answer this, we measured contractility in non-failing human hearts and in terminally failing explanted human hearts. In addition, we studied rats with heart failure as a model to further investigate changes to the prostanoid-mediated inotropic responses. Our data show that prostanoids can evoke inotropic responses in non-failing human left ventricle that appear absent in failing human left ventricle.

2. Material and methods

Experiments done in rat conform with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and were approved by the Norwegian Animal Research Authority. The use of human myocardium conforms with the principles outlined in the Declaration of Helsinki. All subjects or next of kin gave written informed consent to participate in the study. The study was approved by the ethics Regional Ethics Committee in South-Eastern Norway Regional Health Authority (#S05172).

2.1. Preparation of human ventricular muscle strips

Human left ventricular muscle strips were obtained from eleven explanted hearts from patients with terminal heart failure undergoing heart transplantation at Oslo University Hospital, Rikshospitalet, Oslo, and 4 non-failing human hearts not suitable for implantation. Characteristics of patients are shown in Table 1. Strips from the patients were prepared from the explanted heart immediately after it was taken out from the patient and placed into relaxing solution (room temperature) containing (mM): NaCl (118.3), KCl (3.0), $CaCl_2$ (0.2), $MgSO_4$ (4.0), KH_2PO_4 (2.4), $NaHCO_3$ (24.9), glucose (10.0) and mannitol (2.2) and kept in this solution for 30–90 min until the strips were mounted in organ baths. The solution was equilibrated with 95% O_2/5% CO_2 (pH 7.4).

2.2. Measurement of ventricular strip contractility

Left ventricular strips from human and rat (1–1.5 mm diameter) were mounted in organ baths containing the solution described above, human at 37 °C and rat at 31 °C. After mounting, the relaxing solution was replaced with a high calcium solution of identical composition with the exception of $CaCl_2$ and $MgSO_4$ concentrations (human: 2.5 mM and 1.2 mM, respectively; rat: 1.8 mM and 1.2 mM, respectively) and NaCl adjusted accordingly. The muscles were driven electrically (field stimulation) at a frequency of 1 Hz with impulses of 5-ms duration and current about 20% above individual threshold (10–15 mA, determined in each experiment). The isometrically contracting muscles were stretched to the maximum of their length-tension curve (Skomedal et al., 1997). Maximal developed force (F_{max}), maximal development of force $(dF/dt)_{max}$, time to peak force (TPF), time to 80% relaxation (TR80) and relaxation time (RT; RT = TR80-TPF) were measured. Inotropic responses were expressed as increases in $(dF/dt)_{max}$ (Skomedal et al., 1997). The descriptive parameters at the end of the equilibration period were used as basal (control) values. The experiments were performed in the presence of blockers (added 90 min prior to agonist stimulation) of adrenergic (prazosin 1 μM, timolol 1 μM) and muscarinic cholinergic (atropine 1 μM) receptors. Other inhibitors, when used, were added to the muscles ~45 min before the agonist. Agonist was added cumulatively until the maximal response was obtained (concentration–response curves) or as a single bolus in the presence or absence of the inhibitors. Following force measurements, muscles were immediately frozen in liquid nitrogen.

2.3. Rat model of heart failure

Dahl salt-sensitive (DSS) rats (derived from the Sprague–Dawley rat) were used as the heart failure model. One subset of rats was given a high-salt diet (Altromin C-1051, 8% NaCl) after the age of 6 weeks (day 43). This diet caused a left ventricular hypertrophy at the age of 11 weeks and by 15–20 weeks of age, the rats displayed clinical symptoms of congestive heart failure (Failing) (Inoko et al., 1994; Kihara and Sasayama, 1997) with pulmonary congestion, tachypnea, pleural effusion and weight loss (Table 3). The rats were then anesthetized (2–3% isoflurane) and the hearts were harvested.

Table 1
Patient characteristics.

Human no.	Sex	Age	Etiology	CI (l/min/m^2)	Drug treatment
Non-failing 1	Female	66			
Non-failing 2	Male	80			
Non-failing 3	Female	66			
Non-failing 4	Female	42			Ac, Cant, Nitro
Failing 1	Male	52	CAD	2	Aa, Ac, Alda, ARB, βant, D, Dig, COX2, Lip
Failing 2	Female	9	CMP		ACEI, Ac, βant, D, Dig, K
Failing 3	Male	64	CAD	2	Ac, ACEI, Alda, Crv, Dig, D, EPO, FA, Fe, Lip, PPI
Failing 4	Male	64	CMP	1.6	Aa, ACEI, AD, Alda, D, Dig, La, PPI
Failing 5	Female	25	CMP	2.7	Ac, ACEI, D, Fe, Im, KCL, Lip, Pred
Failing 6	Male	55	CMP	3	Aa, Ac, ACEI, βant, Dig, Mg
Failing 7	Male	56	CMP	1.6	Ac, ACEI, Alda, Crv, D, Dig
Failing 8	Male	17	CMP		Ac, ACEI, Crv, D, KCL
Failing 9	Male	58	CAD	2.,3	Ac, ACEI, Alda,βant, D
Failing 10	Male	64	CAD	2.1	Aa, Ac, ACEI, Alda, βant, D, Lip
Failing 11	Female	55	CMP	0.9	Aa, Ac, ACEI, βant, D, PPI

Abbreviations: Aa, antiarrhythmic; Ac, anticoagulant; ACEI, ACE inhibitor; AD, antidepressant; Adip, antidiabetic; Alda, aldosterone antagonist; Ap, allopurinol; ARB, angiotensin II receptor antagonist; βant, β-antagonist; CAD, coronary artery disease; Cant, Calcium antagonist, Cip, ciproxin; CMP, cardiomyopathy, Crv, carvedilol; D, diuretic; Dig, digitoxin; EPO, erythropoetin; FA, folic acid supplement; Fe, iron supplement; Im, immunomodulatory; K, potassium supplement; KCl, potassium chloride; La, laxative; Lip, lipid-lowering drug; Mg, magnesium supplement; Nitro, Nitroglycerin; PPI, protonpump inhibitor; Pred, prednisolone.