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Rat strain-related differences in myocardial adrenergic tone and the impact on cardiac fibrosis, adrenergic responsiveness and myocardial structure and function

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Abstract

Sprague–Dawley (SD) rats have been reported to have a higher sympathetic activity than Wistar–Kyoto (WKY) rats. In the present study we sought to determine if these rat strain-related differences in sympathetic activity exist at a myocardial level and whether they translate into changes in cardiac fibrosis, contractile responsiveness to adrenergic agonists, and cardiac structure and function. Coronary effluent noradrenaline concentrations, as determined in isolated, perfused heart preparations, were higher in 5-month-old SD as compared to age-matched WKY male rats. This difference was accompanied by higher resting heart rates in SD rats as assessed *in vivo*. However, increases in myocardial noradrenaline release in SD rats did not translate into enhanced myocardial fibrosis, cardiac hypertrophy or remodeling, changes in basal ventricular systolic and diastolic function, or to down-regulation of inotropic responses to the β-adrenoreceptor agonists, noradrenaline, isoproterenol and dobutamine. Although age-matched male SD rats were heavier, no differences in absolute heart weights were noted between rat strains. Moreover, left ventricular (LV) posterior wall thickness as assessed by echocardiography, as well as cardiac myocyte dimensions as determined by laser scanning confocal microscopy were similar between rat strains. Furthermore, LV internal diameters as determined *in vivo*, as well as LV diastolic volume intercept determined in isolated, perfused heart preparations were similar between rat strains.

Increases in myocardial noradrenaline release in SD rats also did not translate into differences in LV systolic chamber and myocardial function as assessed *in vivo* (LV endocardial and midwall fractional shortening) and at controlled loads and heart rates *ex vivo* (the slope of the LV developed pressure–volume relation determined). Likewise, neither myocardial hydroxyproline content nor LV chamber stiffness as assessed by the slope of the LV end-diastolic pressure–volume relation were different in SD and WKY rats. In conclusion, rat strain-related differences in cardiac adrenergic tone do indeed exist, but in young animals these differences do not translate into cardiac phenotypes known to contribute to progressive cardiac dysfunction.

Keywords: Sprague–Dawley rats; Wistar–Kyoto rats; Adrenergic tone; Contractile function

1. Introduction

An increase in sympathetic activity is a neurohumoral response that accompanies heart failure irrespective of the cause [1,2]. Moreover, an increase in sympathetic activity in heart failure, by promoting myocardial apoptosis [3], reducing myocardial β-adrenoreceptor-cAMP signal transduction [4], inducing cardiac hypertrophy [5], and stimulating interstitial and hence chamber remodeling [6] is now thought to be a major determinant of progressive heart failure.

Mice with genetically enhanced excessive sympathetic activity progress to heart failure [7]. However, whether naturally occurring genetic differences in cardiac sympathetic activity promote the development or progression of cardiac dysfunction is still uncertain. Some [8], but not other [9] human studies suggest that genetic variation in myocardial β-adrenoreceptors (β-AR) modifies the progression of heart failure. Animal models of naturally occurring genetic differences in sympathetic activity may clarify whether genetic variation in sympathetic effects impact on cardiac function. In this regard, Sprague–Dawley (SD) and Wistar–Kyoto (WKY) rats represent two genetically distinct rat strains with evidence for differences in sympathetic activity.

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Out-bred SD rats have an increased resting heart rate [10,11], enhanced splanchnic sympathetic nerve activity [12], and higher plasma noradrenaline concentrations [13] as compared to inbred WKY rats. However, whether these differences occur at a cardiac level or translate into differences in cardiac structure or function has not been formally explored. The aim of the present study was therefore to determine whether differences in cardiac adrenergic tone as assessed by myocardial noradrenaline release exist between age and gender-matched SD and WKY rats and to assess whether these differences translate into cardiac phenotypes known to mediate adrenergic-induced progressive heart failure.

2. Materials and methods

The study complies with the Guide to the Care and Use of Experimental Animals, and was approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (clearance number: 2003/77/3). Male SD and WKY rats at ∼5 months of age were housed in individual cages, and exposed to light by means of an automated system from 07:00 to 19:00 h. All animals were allowed to acclimatize to the housing conditions with free access to food and tap water for at least 10 days prior to entry into the study.

2.1. Isolated, perfused heart preparations

In the present study, isolated, perfused heart preparations were used to assess myocardial noradrenaline release, left ventricular (LV) systolic and end-diastolic pressure–volume relations, and inotropic responses to β-AR agonists. Rats were anaesthetised with ketamine (75 mg kg^{-1}) and xylazine (15 mg kg^{-1}). The hearts were excised from the chest, mounted on Langendorff apparatus, and retrogradely perfused via the aorta at a constant flow with carefully filtered, warmed (37 °C) physiological saline solution (PSS) saturated with 95% O_2 and 5% CO_2 [14]. The solution contained (in mM) 118.0 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 25 $NaHCO_3$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 10.0 glucose, and had a pH of 7.4. The coronary flow rate was measured by collecting venous effluent, and adjusted to 10 ml min^{-1} g^{-1} of heart weight. The cardiac preparations were paced at a constant frequency of 300 beats min^{-1} via platinum wire electrodes attached to the right atrium and the apex of the LV. The voltage of pulses used for cardiac pacing was set to be 10% above threshold. To determine LV systolic and end-diastolic pressure–volume relations over a wide range of filling volumes, the left atrium was trimmed and a water-filled balloon-tipped cannula coupled to a micromanipulator and a Statham P23 pressure transducer was introduced through the atrioventricular orifice into the LV cavity.

2.2. Myocardial noradrenaline release

Isolated, perfused heart preparations of SD and WKY rats were allowed to stabilize for 15 min prior to assessing myocardial noradrenaline release. To exclude variations of myocardial noradrenaline release due to differences in chamber filling volumes, LV cavity volume was set at 0.18 ml in all heart preparations. Samples of coronary effluent were collected for one min from the hearts of SD and WKY rats whilst perfused at a constant flow rate. The coronary effluent was stabilized by adding Na_2EDTA and $HClO_4$ to 10 ml of coronary effluent to achieve a final concentration of 0.01 mol l^{-1} and 0.025%, respectively. Noradrenaline was immediately extracted from 1 ml of coronary effluent using alumina (Sigma) adsorption with a Tris buffer at pH 8.6 eluted with 0.1 M $HClO_4$, and then stored at −70 °C until determinations. Noradrenaline concentrations were measured using reversed phase, ion-exchange high performance liquid chromatography with electrochemical detection [15].

2.3. Load- and heart rate-independent measure of LV systolic and diastolic function

Baseline LV systolic and diastolic function as well as chamber dimensions were assessed by reconstructing LV systolic and diastolic pressure–volume relations from recordings obtained *ex vivo*. LV developed pressure was calculated as the difference between end-systolic and end-diastolic pressure and recorded on a Hellige polygraph. The maximum rate of LV pressure development (+d*P*/d*t*) was obtained using a differentiator (model 13-4616-71, Gould Instrument Systems, Valley View, OH) with a high-frequency cut-off set at 300 Hz. Using a micromanipulator, the LV balloon volume was gradually increased by increments of 0.01 ml starting from 0.16 ml, and LV end-diastolic and developed pressures were determined at as many multiple small increments in volume as were practically possible.

In our pilot experiments, we determined that similar changes in volume of the balloon kept out of the LV cavity result only in very small (1–3 mm Hg) increases of intraluminal pressure. These values were subtracted from recorded LV developed pressures prior to final statistical evaluation.

The slope of the linear portion of the LV developed pressure–volume relation (LV end-systolic elastance) was used as a load-independent measure of LV systolic chamber function [14,16]. The slope of the linear portion of the LV end-diastolic pressure–volume relation was used as an index of LV chamber stiffness [6]. The LV volume intercept (V_0) of the LV end-diastolic pressure–volume relationship was used as an index of LV chamber dilatation [6,14].

2.4. Pharmacological responses

Prior to pharmacological assessments, the LV volume in all heart preparations was set at 0.18 ml to elicit the approximate physiologically relevant (∼100 mm Hg) values of LV developed pressure normally recorded in the middle portion of LV systolic pressure–volume relation. The substances used in the experiments were dissolved in the same PSS used to perfuse the hearts and infused just proximal to the aortic cannula by means of a Harvard infusion pump (model 22M T/W). The rate of infusion was 0.3 ml min^{-1}, and the total duration of infusion of each concentration of each agent was 60 s. Concentration–response relations were constructed by assessing LV developed pressure