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How to

Assessing structural and functional responses of murine hearts to acute and sustained β-adrenergic stimulation in vivo

Sarah-Lena Puhl *, Kate L. Weeks, Antonella Ranieri, Metin Avkiran

Cardiovascular Division, King's College London British Heart Foundation Centre of Research Excellence, The Rayne Institute, St Thomas' Hospital, London SE1 7EH, United Kingdom

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ABSTRACT

Introduction: Given the importance of β-adrenoceptor signalling in regulating cardiac structure and function, robust protocols are required to assess potential alterations in such regulation in murine models in vivo.
Methods: Echocardiography was performed in naïve and stressed (isoprenaline; 30 μg/g/day s.c. for up to 14 days) mice, in the absence or presence of acute β-adrenergic stimulation (dobutamine 0.75 μg/g, i.p.). Controls received saline infusion and/or injection. Hearts were additionally analysed gravimetrically, histologically and biochemically.
Results: In naïve mice, acute β-adrenoceptor stimulation with dobutamine increased heart rate, left ventricular (LV) fractional shortening (LVFS), ejection fraction (LVEF) and wall thickness and decreased LV diameter ($p < 0.05$). In stressed mice, dobutamine failed to induce further inotropic and chronotropic responses. Furthermore, following dobutamine injection, these mice exhibited lower LVEF and LVFS at identical heart rates, relative to corresponding controls. Sustained isoprenaline infusion induced LV hypertrophy (increased heart weight, heart weight/body weight ratio, heart weight/tibia length ratio and LV wall thickness ($p < 0.05$)) by 3 days, with little further change at 14 days. In contrast, increases in LVEF and LVFS were seen only at 14 days ($p < 0.05$).
Discussion: We describe protocols for and illustrative data from the assessment of murine cardiac responses to acute and sustained β-adrenergic stimulation in vivo, which would be of value in determining the impact of genetic or pharmacological interventions on such responses. Additionally, our data indicate that acute dobutamine stimulation unmasks early signs of LV dysfunction in the remodelled heart, even at a stage when basal function is enhanced.

1. Introduction

Regulation of cardiac function by the sympathetic nervous system is achieved primarily through the stimulation of cardiac β_1-adrenergic receptors (β_1-adrenoceptors, β_1-AR), with acute stimulation inducing rapid and pronounced chronotropic, inotropic and lusitropic effects. Additionally, sustained β_1-adrenoceptor stimulation induces cardiac hypertrophy and remodelling, which may be physiological or pathological in nature depending on the magnitude and duration of stimulation (Booysen, Norton, Opie, & Woodiwiss, 2012; Kitagawa, Yamashita, Ito, & Takaki, 2004; Webb et al., 2010). Such effects of β_1-adrenoceptor stimulation are achieved principally through the activation of cAMP-dependent protein kinase (protein kinase A) isoforms, although activation of other cAMP effectors, such as members of the exchange protein directly activated by cAMP (Epac) family, may also contribute in some cases.

In view of the importance of β_1-adrenoceptor signalling pathways in regulating cardiac structure and function, it is important to have robust methods to determine how genetic manipulations (e.g. gene knock-out, knock-in or overexpression), pharmacological interventions (e.g. putative therapeutic drugs) and cardiac or systemic disease states (e.g. myocardial ischaemia, hypertension, diabetes) in mouse models may impact on such regulation. In this paper, we describe methods for the in vivo and ex vivo assessment of the effects of both acute and sustained β_1-adrenoceptor stimulation on cardiac structure and function in wild-type mice, which may be readily adapted for use in conjunction with such settings.

2. Materials and methods

2.1. Study population

Thirty-four male C57/Bl6J mice (8–10 weeks of age, from Charles River UK Ltd., Kent, UK) were included in this study and were housed

Abbreviations: AW, anterior wall; β-AR, β-adrenoceptor; d, end-diastolic; DOB, dobutamine; EF, ejection fraction; FS, fractional shortening; HW, heart weight; i.p., intraperitoneal; ISO, isoprenaline; IVS, interventricular septum; LV, left ventricular; LVAW, left ventricular anterior wall; LVID, left ventricular internal diameter; LVPW, left ventricular posterior wall; s, end-systolic; s.c, subcutaneous; TL, tibia length; vol, volume.

* Corresponding author at: Cardiovascular Division, King's College London, The Rayne Institute, St Thomas' Hospital, Westminster Bridge Road, London SE1 7EH, United Kingdom.
E-mail addresses: sarah_lena.puhl@kcl.ac.uk (S.-L. Puhl), kate.weeks@kcl.ac.uk (K.L. Weeks), antonella.ranieri@kcl.ac.uk (A. Ranieri), metin.avkiran@kcl.ac.uk (M. Avkiran).

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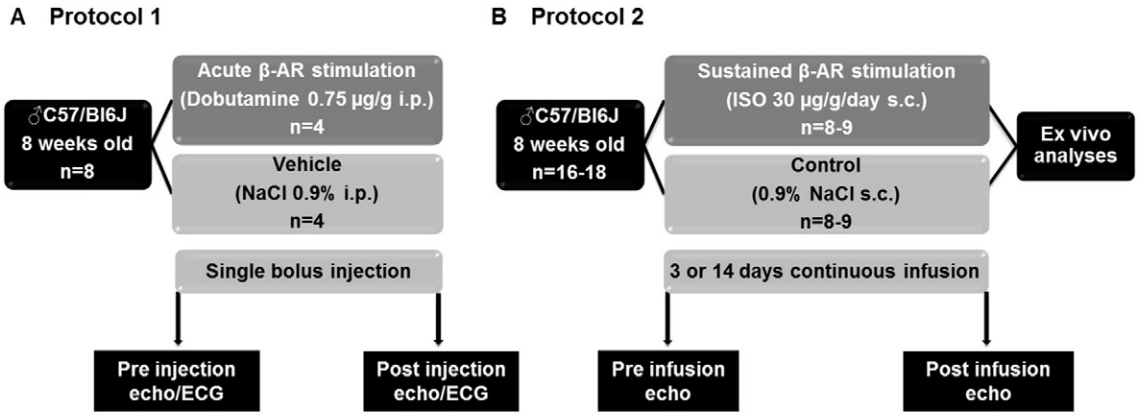
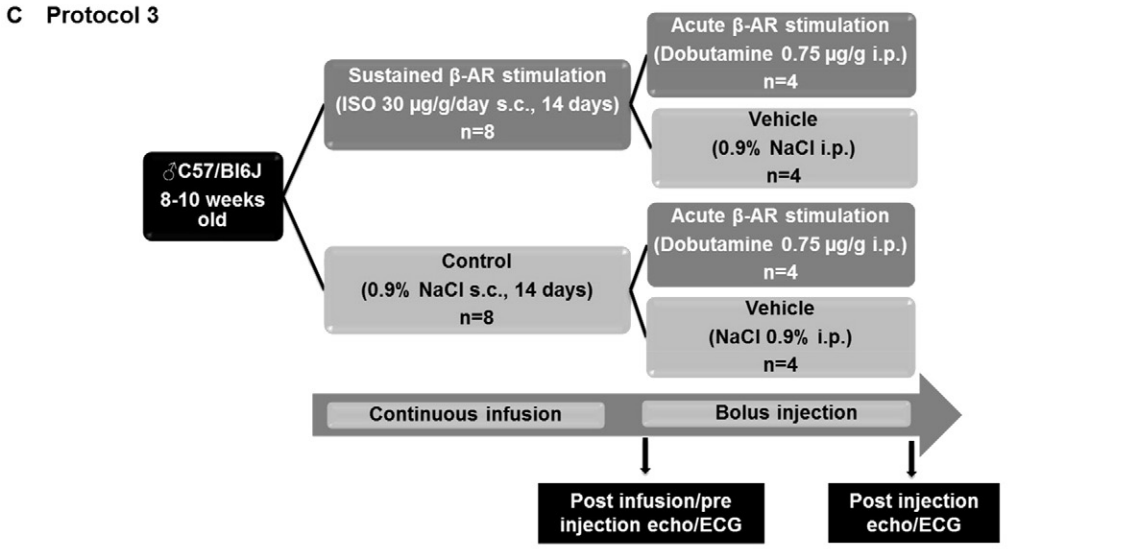
in groups of 4 in pathogen-free, individually ventilated cages with a 12-h light/12-h dark regime. Normal mouse chow containing 0.9% NaCl and water was provided ad libitum. The study was approved by the local Ethics Review Board and animal handling and experiments were performed according to the Home Office regulations, as detailed in the Home Office Guidance on the Operation of the Animals (Scientific Procedures) Act 1986, HMSO (London) and the Guide for Care and Use of Laboratory Animals by the US National Institute of Health (NIH Publication 8th edition).

2.2. Study design

Three different in vivo protocols were performed.





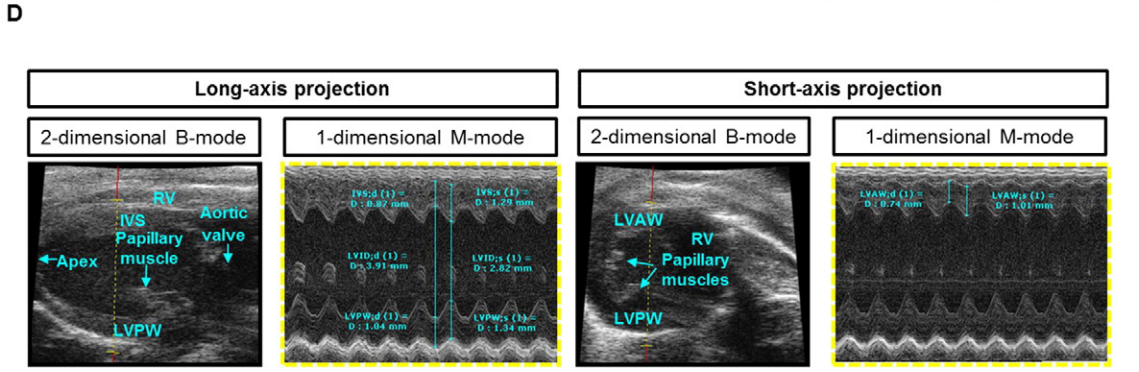


Fig. 1. Study population and design. Experimental protocols for assessment of cardiac and other responses to (**A**) acute β-adrenoceptor stimulation with DOB, (**B**) sustained β-adrenoceptor stimulation with ISO and (**C**) acute β-adrenoceptor stimulation with DOB following sustained β-adrenoceptor stimulation with ISO. (**D**) For echocardiographic analyses, 2-dimensional (B-Mode) images were recorded in parasternal long- (left panel) and short-axis (right panel) projections with guided one-dimensional M-mode recordings at the mid-ventricular level, apical of the posteriomedial papillary muscle in both views. Standard measurements of inter-ventricular septum (IVS), left ventricular internal diameter (LVID) and left ventricular posterior wall (LVPW) were performed in systole and diastole in parasternal long-axis projection. Left ventricular (LV) cavity size and wall thickness were measured during at least three beats from this projection and averaged. Left ventricular anterior wall (LVAW) thickness was determined during at least three beats in systole and diastole in short-axis projection.