Activated plasma coagulation β-Factor XII-induced vasoconstriction in rats

Peter C. PAPAGEORGIOU*†, Erik L. YEO†, Peter H. BACKX*†‡ and John S. FLORAS*†

*Heart and Stroke/Richard Lewar Centre of Excellence, University of Toronto, Toronto, ON, Canada, †Department of Medicine, University Health Network and Mount Sinai Hospital, University of Toronto, Toronto, ON, Canada, and ‡Department of Physiology, University of Toronto, Toronto, ON, Canada

MATERIALS AND METHODS

Calibration of LV RVU from echocardiographic volume indices

Male BN rats weighing 250–300 g ($n = 6$) were studied following isoflurane inhalation (0.75–1.25%; Halocarbon Laboratories) delivered with a mixture of oxygen (BOC Gases) using a Fluotec Mark 2 vaporizer (Cyprane) at a gas flow rate of 2 litres/min. A micro-tip pressure–volume catheter transducer (SPR-838; Millar Instruments) linked to a control unit (MPCU-200; Millar Instruments) and connected to a MacLab/8 data acquisition system (AD Instruments) driven by PowerLab Chart v.4.2 software (AD Instruments) was advanced retrogradely into the LV from the right common carotid artery. Baseline LVBP (mmHg) was derived from the BP waveform and volume (RVU) was derived from the volume waveform. These measurements were recorded continuously at 2 kHz sampling rate (Figure S1, right-hand panels).

Concurrently, an anatomical M-mode echocardiographic assessment with two-dimensional monitoring using a Vevo 770 high-resolution imaging system (Visual Sonics) with a cardiovascular scanhead transducer (model RMV 710B) was used to measure the short-axis LVIDs (LV internal diameter end-systole) and LVIDd (LV internal diameter end-diastole) in mm (Figure S1, left-hand panels). The Teichholz formula [1,2], which defines the relationship between blood volume (V) of the ventricle and the short axis inner diameter (D) as $V = (7D^3)/(2.4 + D)$, was used to calculate LVEDV and LVESV from LVIDd and LVIDs respectively (Vevo 770 Software System Version 2.2.3; Visual Sonics). After baseline BP and volume measurements were acquired, the IVC (inferior vena cava) was occluded by applying a cotton tip applicator (Figure S1, bottom panel). A minimum of five baseline and five responses to IVC occlusion was recorded per animal. Numerical data were fitted with a second-order polynomial trendline using the Sigma Stat program (version 2.03; SPSS). The relationship between RVU (y) and echocardiographic volume (x) could be described by the equation $y = -9 \times 10^{-5}x^2 + 0.0706x + 10.138$ (Figure S2).

The conductance catheter method (SPR-838; Millar Instruments) has been validated in the Sprague–Dawley rat [3], using trans-thoracic echocardiography as a reference. Bland–Altman analyses for quantification of average differences were, for LVEDV, $30 \pm 50 \, \mu$l; for LVESV, $-40 \pm 30 \, \mu$l and for SV, $3 \pm 20 \, \mu$l. Absolute values for these variables, derived using the conductance catheter in these experiments, were similar to baseline values in the present experiment.
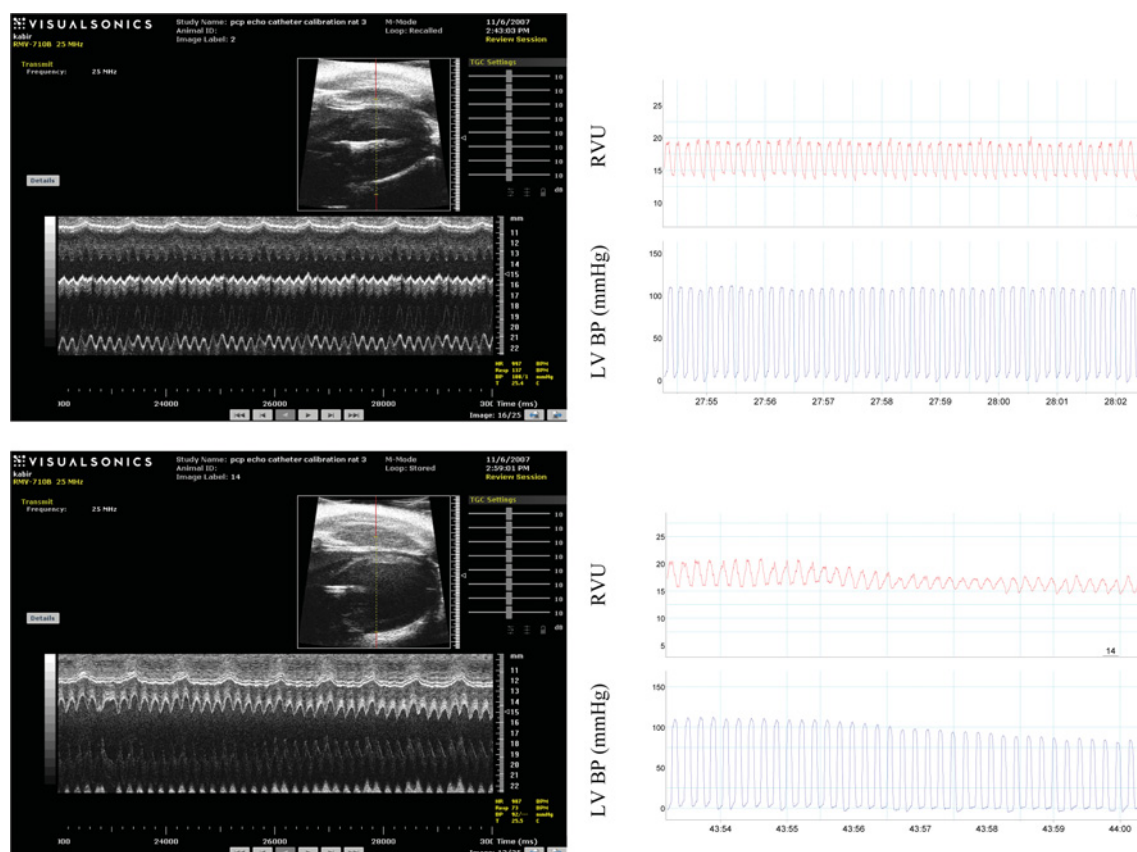
Correspondence: Dr John S. Floras (email john.floras@utoronto.ca).

Figure S1   Simultaneous acquisition of LV volume measurements by echocardiography and by micro-tip pressure–volume catheter transducer

Representative traces of echocardiographic assessment of the short axis LVIDs and LVIDd (left panels). LVEDV and LVESV were calculated from LVIDd and LVIDs using the Teichholz formula $V = (7D^3)/(2.4 + D)$. Concurrently, LVBP (mmHg) and RVU were recorded continuously using a micro-tip pressure–volume catheter transducer (right panels). Data were acquired at baseline (top panels) and during IVC occlusion (bottom panels).
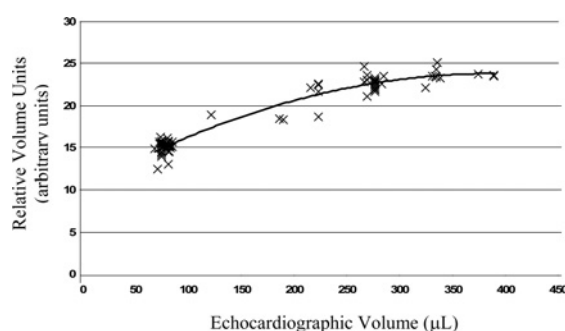

Figure S2   Derivation of RVU from echocardiographic volumes

LV volumes estimated from a micro-tip pressure–volume catheter transducer reported as RVU versus calculated volumes recorded concurrently from an anatomical M-mode echocardiographic assessment of the left ventricle. Numerical data were fitted with a second-order polynomial trendline. The relationship between RVU (y) and echocardiographic volume (x) could be described by the equation $-9\times10^{-5}x^2 + 0.0706x + 10.138$.

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