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

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A B S T R A C T

By inducing BK (bradykinin)-stimulated adrenomedullary catecholamine release, bolus injection of the β-fragment of activated plasma coagulation Factor XII (β-FXIIa) transiently elevates BP (blood pressure) and HR (heart rate) of anaesthetized, vagotomized, ganglion-blocked, captopril-treated bioassay rats. We hypothesized that intravenous infusion of β-FXIIa into intact untreated rats would elicit a qualitatively similar vasoconstrictor response. BN (Brown Norway) rats received for 60 min either: (i) saline (control; $n = 10$); (ii) β-FXIIa (85 ng/min per kg of body weight; $n = 9$); or (iii) β-FXIIa after 2ADX (bilateral adrenalectomy; $n = 9$). LV (left ventricular) volume and aortic BP were recorded before (30 min baseline), during (60 min) and after (30 min recovery) the infusion. TPR (total peripheral resistance) was derived from MAP (mean arterial pressure), SV (stroke volume) and HR. Saline had no haemodynamic effects. β-FXIIa infusion increased its plasma concentration 3-fold in both groups. In adrenally intact rats, β-FXIIa infusion increased MAP by 6% (5 ± 2 mmHg) and TPR by 45% (0.50 ± 0.12 mmHg/ml per min), despite falls in SV (−38 ± 8 μl) and HR [−18 ± 5 b.p.m. (beats/min)] (all $P < 0.05$). In 2ADX rats, β-FXIIa had no HR effect, but decreased SV (−89 ± 9 μl) and MAP (−4 ± 1 mmHg), and increased TPR by 66% (0.59 ± 0.15 mmHg/ml per min) (all $P < 0.05$). After infusion, adrenally intact rats exhibited persistent vasoconstriction (MAP, 10 ± 1 mmHg; TPR, 0.55 ± 0.07 mmHg/ml per min; both $P < 0.05$), whereas in 2ADX rats, MAP remained 5 ± 1 mmHg below baseline ($P < 0.05$) and TPR returned to baseline. End-study arterial adrenaline (epinephrine) concentrations in the three groups were 1.9 ± 0.6, 9.8 ± 4.1 and 0.6 ± 0.2 nmol/l respectively. Thus, in neurally intact lightly anaesthetized untreated rats, β-FXIIa infusion induces both adrenal catecholamine-mediated and adrenally independent increases in peripheral resistance.

INTRODUCTION

For decades after its initial discovery in 1955, FXIIa (activated Factor XII) was considered exclusively an element of the coagulation cascade [1]. However, it is now appreciated that FXIIa also engages the fibrinolytic system, RAS (renin–angiotensin system), complement system and KKS (kallikrein–kinin system) [2–4]. With evidence for a positive relationship between plasma FXIIa concentration and BP (blood pressure) now

Key words: adrenal gland, β-fragment of activated Factor XIIa (β-FXIIa), blood pressure, bradykinin, haemodynamics, vasoconstriction.

Abbreviations: 2ADX, bilateral adrenalectomy; BK, bradykinin; BN, Brown Norway; BP, blood pressure; b.p.m, beats/min; CO, cardiac output; DBP, diastolic BP; FXIIa, activated Factor XII; β-FXIIa, β-fragment of FXIIa; HR, heart rate; KKS, kallikrein–kinin system; LV, left ventricular; LVEDV, LV end-diastolic volume; LVESV, LV end-systolic volume; MAP, mean arterial pressure; PACAP, pituitary adenylate cyclase-activating polypeptide; RVU, relative volume unit; SBP, systolic BP; SV, stroke volume; TPR, total peripheral resistance.

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emerging from population studies [5–7], the question arises whether such interactions between FXIIa and peptides involved in cardiovascular regulation exert important haemodynamic effects.

Previous work from our institution has identified a novel physiological interaction between FXIIa and the KKS and sympatho-adrenal system, leading to changes in BP. Specifically, when injected into a rat bioassay, an acute bolus (0.3–1 μg/kg of body weight) of the β-FXIIa (β-fragment of FXIIa; which contains the serine protease catalytic region [2–4]) stimulates the release of adrenomedullary catecholamines [8,9], via a non-cholinergic peptidergic mechanism of action [10], resulting in marked, but transient, increases in both BP and HR (heart rate) [11]. BP also increases if trypsin-activated FXI (Factor XI)-deficient plasma is injected, but not if FXII-deficient plasma is injected [11].

These acute sympatho-adrenal and cardiovascular responses to β-FXIIa are augmented by pre-treatment with captopril or enalapril [8,11], are attenuated by pre-treatment with selective antagonists of BK (bradykinin) B_2 [12] and PACAP (pituitary adenylate cyclase-activating polypeptide) PAC-1 [13] receptors, are absent in both adrenal medullectomized rats and kininogen-deficient BN (Brown Norway) Katholiek rats [14], and are blocked by combined α- and β-adrenoceptor blockade [8]. Taken together, these several observations indicate that expression of this pressor response, which is specific to β-FXIIa, requires both an intact KKS and adrenal medullae.

Thus far, evidence for participation of plasma coagulation FXIIa in BP regulation has been derived from such bolus injection experiments involving a bioassay rat preparation characterized by low basal BP, HR and plasma catecholamine concentrations. Whether this coagulation factor, which is activated and which circulates in higher plasma concentrations chronically in several conditions characterized by vascular inflammation or injury [15–18], exerts functionally important haemodynamic effects under conditions more representative of normal circulatory physiology has yet to be determined. The objectives of the present study therefore were first, to confirm that the acute rise in BP observed in response to a bolus injection of FXIIa in an antagonized and vagotomized ACE (angiotensin-converting enzyme) inhibitor-treated rat is also evident if FXIIa is given by infusion to an innervated non-medicated rat; and secondly, to characterize the haemodynamic mechanisms responsible for any subsequent BP increase. On the basis of this prior literature, our hypothesis was that a 60-min infusion of FXIIa would increase plasma adrenaline (epinephrine) concentrations, and as a consequence, TPR (total peripheral resistance) and BP.

This study was presented in part at the 22nd Congress of the International Society on Thrombosis and Haemostasis (ISTH), 11–16 July 2009, Boston, MA, U.S.A. [18a]

MATERIALS AND METHODS

Experimental preparation

Male BN rats (*Rattus norvegicus*) weighing 250–350 g (Charles River Laboratories) were distributed into three experimental groups: (i) saline infusion (control, $n = 10$); (ii) β-FXIIa infusion ($n = 9$); and (iii) β-FXIIa infusion following 2ADX (bilateral adrenalectomy, $n = 9$). Animals were cared for and studied in accordance with the Canadian Council on Animal Care principles and guidelines. All experimental protocols were approved by the Advisory Committee on Animal Services of the Division of Comparative Medicine, Faculty of Medicine, University of Toronto. All animals were administered isoflurane anesthetic (2–5 %; Halocarbon Laboratories) with a mixture of oxygen (BOC Gases) using a Fluotec Mark 2 vaporizer (Cyprane) at a gas flow rate of 3 litres/min until adequate depth of anaesthesia was attained. 2ADX was performed prior to infusion only in the 2ADX group. Briefly, through a ventral midline incision, both adrenal glands were located superior to each kidney and isolated from surrounding adipose and connective tissue, and excised. The abdomen was then closed and sutured (3-0 Dexon; Ethicon) and the skin incisions closed with 9 mm autoclips (Becton Dickinson).

The right common carotid artery of all rats was cannulated by a micro-tip pressure–volume catheter transducer (SPR-838; Millar Instruments) advanced retrograde into the left ventricle to record LV (left ventricular) pressure and estimated volume throughout the cardiac cycle [19]. To record arterial BP, the right femoral artery was cannulated with an ultra-miniature micro-tip pressure catheter transducer (SPR-671; Millar Instruments) advanced ~12 cm towards the descending aorta, above the renal arteries. To administer saline or coagulation β-FXIIa infusions, the left femoral vein was cannulated by a polyethylene tube that was attached to a 1 ml syringe connected to an infusion pump. Core body temperature was monitored and maintained at 37 °C using a homeothermic heating pad (HB-101-402; VWR). The dose of isoflurane inhalation was reduced to maintain light anaesthesia (0.75–1.25 %; Halocarbon Laboratories). Only rats that had sustained <1.5 ml blood loss during surgery and exhibited stable BP and HR for at least 10 min after surgery were studied. To collect blood for plasma β-FXIIa and catecholamine determinations, on completion of the experimental protocol, the pressure–volume catheter was replaced by a second polyethylene tube.

Human coagulation β-FXIIa

As described previously [9,10], highly purified human coagulation β-FXIIa (30 kDa, Lot β-FXIIa 1000P and 2070P; Enzyme Research Laboratories) was dissolved in heparinized saline (0.9 % NaCl with 20 units/ml heparin) to achieve a final concentration of 10 ng/μl and divided into 50 μl aliquots. All solutions of β-FXIIa were stored

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at $-80\,^{\circ}$C until use and kept on ice during experiments to retard degradation.

Experimental protocol and haemodynamic measurements

Data were acquired continuously over three consecutive periods: baseline (0–30 min), femoral venous infusion (β-FXIIa at 85 ng/min per kg of body weight at 10 μl/min or physiological saline control at 10 μl/min; 30–90 min) and recovery (90–120 min). Assuming that a rat has ~15 ml of blood containing ~40 μg/ml of FXII zymogen [20] (600 μg for a 300 g rat), the total dose of β-FXIIa infused over 60 min (5.1 μg/kg of body weight; 1.53 μg for a 300 g rat), which represents ~0.26 % of its total FXII zymogen, can be considered to lie well within its physiological range. Alternatively, based on the Beer–Lambert law, where concentration is proportional to absorbance, the circulating FXIIa levels (0.86 ± 0.05 A_{550} units) were approximately <0.63 % of the total FXII zymogen content (1.37 ± 0.09 A_{550} units; RAT*a* diluted 1:100) [21].

The micro-tip pressure and pressure–volume catheter transducers were each linked to a control unit (MPCU-200; Millar Instruments) connected to a MacLab/8 data acquisition system (AD Instruments) driven by PowerLab Chart v.4.2 software (AD Instruments). Arterial SBP (systolic BP) and DBP (diastolic BP) were derived from the BP waveform and are given in mmHg. MAP (mean arterial pressure) was calculated using the equation MAP = DBP + 1/3 (SBP − DBP) and is given in mmHg. HR was derived from the BP waveform by cyclic variable analysis and is given in b.p.m. (beats/min). LVESP (LV end-systolic pressure) and LVEDP (LV end-diastolic pressure) were derived from the LV pressure waveform and are given in mmHg. LV RVU (relative volume units, arbitrary units) for systole (RVU_{min}) and diastole (RVU_{max}) were derived from the volume waveform. In a separate set of rat experiments, as described in the Supplementary Online material (available at http://www.clinsci.org/cs/122/cs1220581add.htm), a second-order polynomial trend-line ($y = -9\times10^{-5}x^2 + 0.0706x + 10.138$) was derived to permit conversion of RVU (y) into absolute LV volume measurements (x; μl). SV (stroke volume) was then calculated as LVEDV (LV end-diastolic volume)–LVESV (LV end-systolic volume) and is given in μl. CO (cardiac output; ml/min) was calculated as the product of SV and HR. TPR was derived from MAP/CO and is given in mmHg/ml per min.

Plasma coagulation β-FXIIa and catecholamine measurements

At the end of the recovery period, arterial blood (500 μl samples) were collected in 3.8 % buffered citrate solution (Vacutainer, blue-top; Becton Dickinson) for β-FXIIa determinations, and in K_2EDTA (365973, Microtainer, lavender-top; Becton Dickinson) for catecholamine assay. Samples were centrifuged immediately at 2000 ***g*** for 20 min at 4 °C. Plasma was separated and stored at $-80\,^{\circ}$C in approximately 100 μl aliquots for analysis. Plasma β-FXIIa was measured using a novel FXIIa ELISA that employs mAb (monoclonal antibody) 201/9 which binds to conformational epitopes on the heavy chain of β-FXIIa and exhibits no cross-reactivity with FXII zymogen or FXIIa–inhibitor complexes [21]. Adrenaline and noradrenaline concentrations were determined using HPLC with electrochemical detection [22].

Statistical analysis

Numerical data are presented as means ± S.E.M. Statistical comparisons were analysed using Sigma Stat program (version 2.03; SPSS). These investigations were structured as if two separate studies, with findings in the experimental group (β-FXIIa) compared first with its saline control, and subsequently to the group into which β-FXIIa was infused subsequent to 2ADX. Thus Student's *t* test was used to compare baseline values, plasma β-FXIIa and plasma catecholamine concentrations between the β-FXIIa experimental group and these two specific control reference groups (β-FXIIa compared with control; β-FXIIa compared with 2ADX). As shown in Figure 1, the haemodynamic data acquired over the entire experiment were averaged over 5 min intervals. To obtain means for subsequent analysis, 0–30 min data were averaged to obtain steady-state baseline values, and for comparisons against baseline, 40–90 min data were averaged to obtain steady-state infusion period values, and 100–120 min data were averaged to obtain steady-state recovery period values. Steady-state values within-treatment groups (baseline compared with infusion compared with recovery) and between-treatment groups (β-FXIIa compared with control; β-FXIIa compared with 2ADX) were compared by two-way ANOVA, applying the Student–Newman–Keuls *post hoc* test for pairwise multiple comparisons. A value for $P < 0.05$ was required for statistical significance.

RESULTS

Table 1 presents for the experimental group, and for the two respective control groups, mean baseline data for each of the haemodynamic variables studied. Apart from TPR, there were no differences in baseline haemodynamic values between the β-FXIIa and saline control group. Removal of the adrenal gland resulted in significantly lower baseline BP, HR and TPR, yet significantly higher SV, as compared with the β-FXIIa experimental group.

Figure 1 illustrates BP, HR, LVEDV and LVESV, CO and TPR at 5 min intervals before, during and after each of the three infusions. Figure 2 presents the haemodynamic changes from pre-infusion baselines (i.e. the mean of 0–30 min) during both the steady-state infusion periods (i.e. the mean of 40–90 min) and the steady-state recovery periods (i.e., the mean of 100–120 min). Saline infusion

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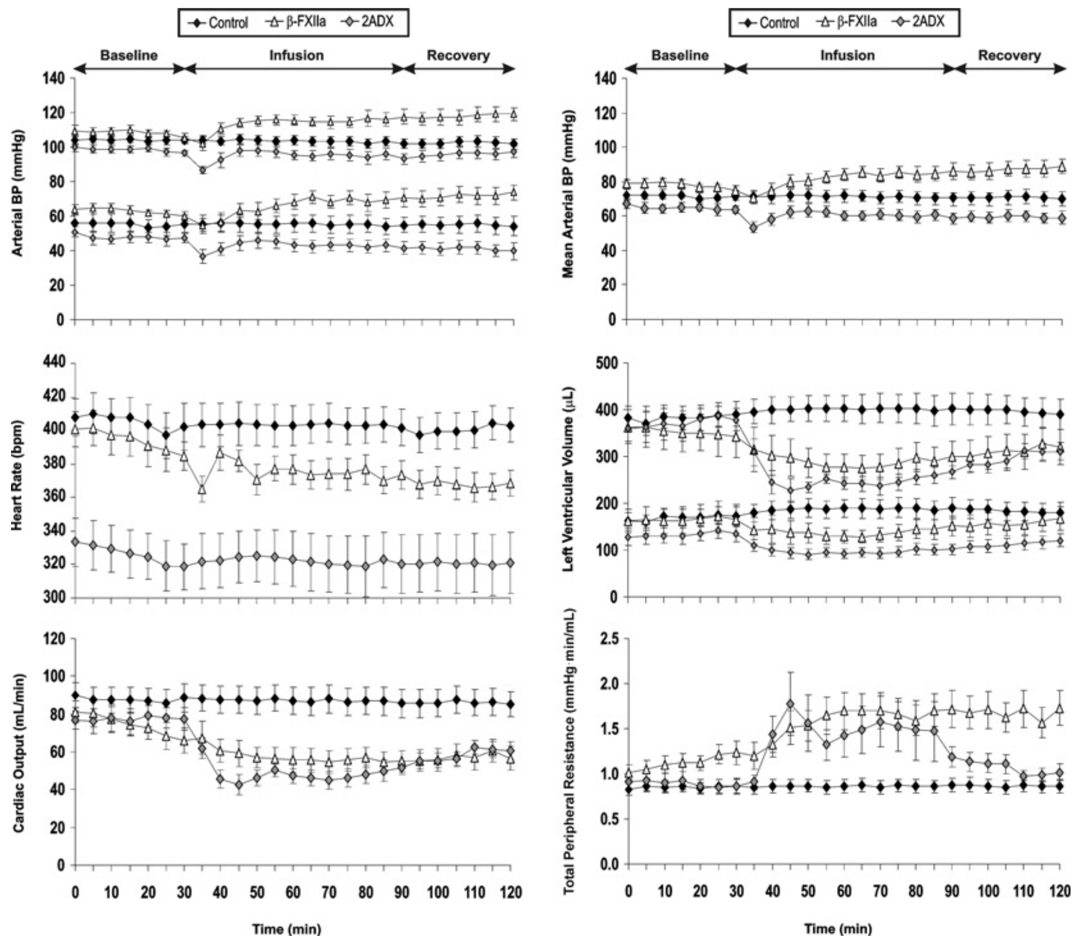

Figure 1 Time course of changes in arterial BP, MAP, HR, LV volume, CO and TPR before infusion, during infusion and over a 30 min post-infusion recovery period in the control, β-FXIIa and 2ADX groups

Values are means ± S.E.M. Baseline was from time 0–30 min. Infusions (10 μl/min for 60 min) were with saline (Control), or human coagulation β-FXIIa (85 ng/min per kg of body weight) into either BN rats with intact adrenal glands (β-FXIIa) or into bilateral adrenalectomized BN rats (2ADX). In the arterial BP plot, the upper values are the SBP and the lower values are the DBP. In the LV volume plot, the upper values are the LVEDV and the lower values are the LVESV.

(control group) did not change any haemodynamic variable at any time (Figures 1 and 2).

Table 1 Haemodynamics at baseline

Values are means ± S.E.M. ($n = 9$ or 10; see text). $^{*}P < 0.05$ for the β-FXIIa group compared with control group; $^{\dagger}P < 0.05$ or $^{\dagger\dagger}P < 0.01$ for the β-FXIIa group compared with the 2ADX group.

Haemodynamic variable	Control	β-FXIIa	2ADX
SBP (mmHg)	104 ± 3	108 ± 3	98 ± 2†
DBP (mmHg)	55 ± 6	63 ± 3	48 ± 3††
MAP (mmHg)	72 ± 4	78 ± 3	65 ± 3††
LVEDV (μl)	383 ± 26	353 ± 36	372 ± 26
LVESV (μl)	169 ± 17	165 ± 26	133 ± 16
SV (μl)	215 ± 14	188 ± 10	239 ± 17†
HR (b.p.m.)	405 ± 11	394 ± 10	326 ± 15††
CO (ml/min)	88 ± 7	74 ± 6	77 ± 5
TPR (mmHg/ml per min)	0.85 ± 0.07*	1.12 ± 0.08	0.89 ± 0.07†

Haemodynamic changes during β-FXIIa infusion

In the intact rats, β-FXIIa infusion caused a rapid and sustained decrease in HR, a gradual reduction in LVEDV and LVESV, with only the latter returning to baseline, and a gradual and sustained reduction in CO. Because β-FXIIa infusion increased BP above baseline values, these cardiac responses were offset by progressive and greater vasoconstriction (Figure 1). At steady-state, β-FXIIa infusion decreased HR by 18 ± 5 b.p.m., LVEDV by 65 ± 10 μl, LVESV by 27 ± 7 μl, SV by 38 ± 5 μl and CO by 24 % (− 18 ± 2 ml/min) from their corresponding pre-infusion baseline values (all $P < 0.05$). By contrast, TPR increased by 45 % (0.50 ± 0.12 mmHg/ml per min; $P < 0.05$). The net effect was an increase in steady-state SBP (7 ± 2 mmHg), DBP (4 ± 1 mmHg) and MAP (by 6 %, or 5 ± 2 mmHg; all $P < 0.05$) above corresponding baseline values (Figure 2).

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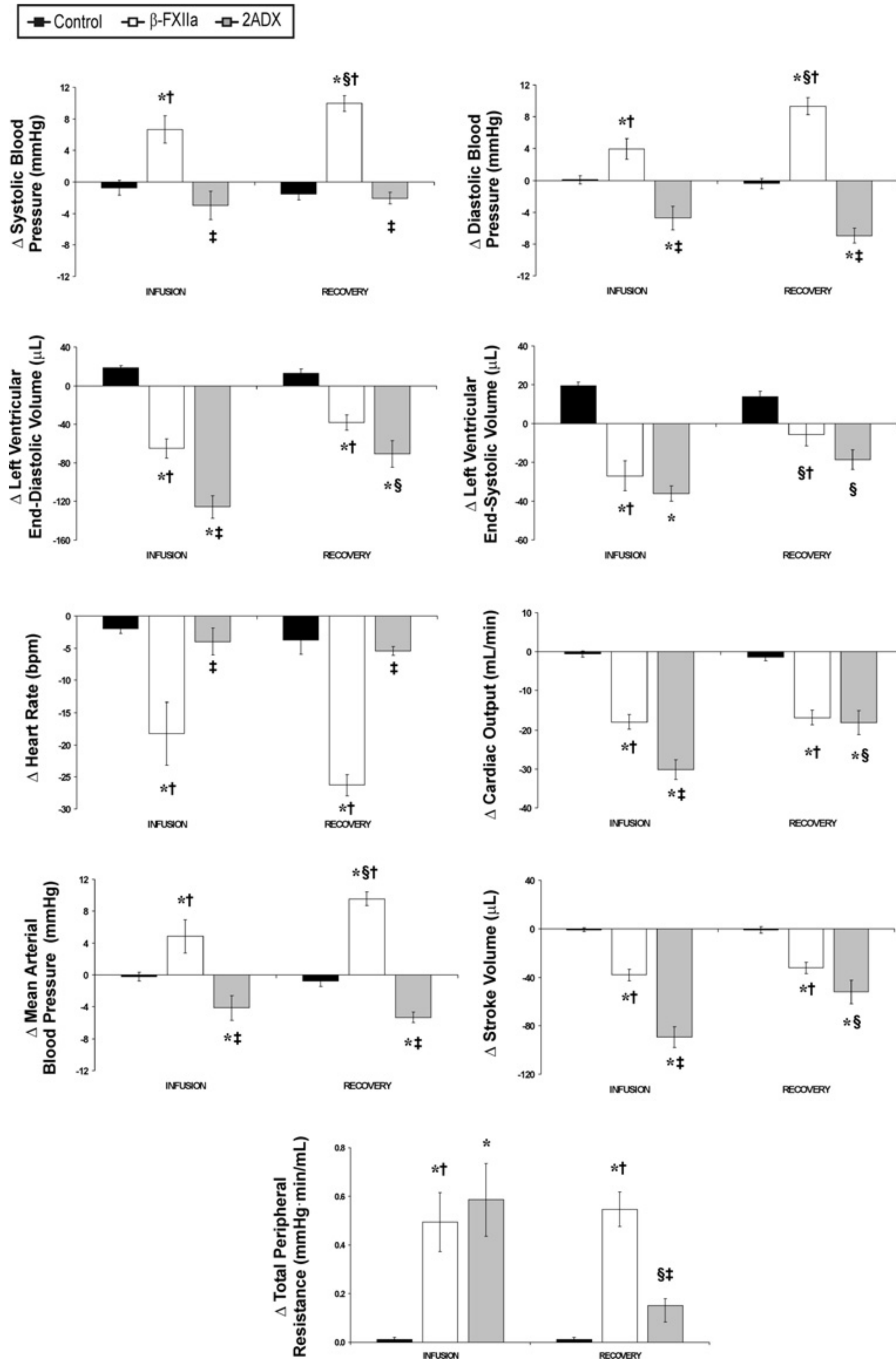

Figure 2 Within-group steady-state changes in each haemodynamic variable from pre-infusion baseline (time 0–30 min) values during the infusion (time 40–90 min) and recovery (time 100–120 min) periods

Values are means ± S.E.M. Saline control, black bar; human coagulation β-FXIIa infusion, open bar; human coagulation β-FXIIa infusion following 2ADX, grey bar steady-state. Within-group comparisons: $^{*}P < 0.05$ for infusion or recovery compared with baseline values; $\S P < 0.05$ for recovery compared with infusion values. Between-group comparisons: $\dagger P < 0.05$ for infusion or recovery values in the β-FXIIa group compared with corresponding saline control infusion or recovery values; $\ddagger P < 0.05$ for the infusion or recovery values in the β-FXIIa group compared with the corresponding 2ADX group infusion or recovery values.

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In the 2ADX group, β-FXIIa infusion caused an early BP dip with recovery, but had no HR effect beyond the profound reduction subsequent to adrenalectomy. Infusion caused marked reductions in LVEDV, LVESV and CO but increased TPR (Figure 1). At steady-state, β-FXIIa infusion did not change HR, but decreased LVEDV by $126 \pm 11\ \mu$l, LVESV by $36 \pm 4\ \mu$l, SV by $89 \pm 9\ \mu$l and CO by 39 % (-30 ± 3 ml/min) from their corresponding pre-infusion baseline values (all $P < 0.05$). By contrast, TPR rose by 66 % (0.59 ± 0.15 mmHg/min per ml; $P < 0.05$). The net effect was no significant change in steady-state SBP, but reductions in both DBP (-5 ± 1 mmHg) and MAP (-4 ± 1 mmHg, or 6 %; both $P < 0.05$) below corresponding baseline values (Figure 2).

Importantly, there was no difference in absolute terms in the systemic vasoconstrictor response to β-FXIIa infusion (TPR) between the two groups (0.50 ± 0.12 compared with 0.59 ± 0.15 mmHg/ml per min), but there were significant differences between adrenally intact and 2ADX rats with respect to the effects of β-FXIIa infusion on SBP (7 ± 2 compared with -3 ± 2 mmHg), DBP (4 ± 1 compared with -5 ± 1 mmHg), MAP (5 ± 2 compared with -4 ± 1 mmHg), LVEDV (-65 ± 10 compared with $-126 \pm 11\ \mu$l), SV (-38 ± 5 compared with $-89 \pm 9\ \mu$l), HR (-18 ± 5 compared with -4 ± 2 b.p.m.) and CO (-18 ± 2 compared with -30 ± 3 ml/min) (all $P < 0.05$) (Figure 2).

Haemodynamic changes after β-FXIIa infusion

In intact rats, the β-FXIIa induced reductions in HR, LVEDV and CO, and increases in TPR and BP were sustained throughout the 30 min recovery period (Figure 1). Post-β-FXIIa infusion, there were persistent reductions during the steady-state analysis period (100–120 min) with respect to HR (-26 ± 2 b.p.m.), LVEDV ($-38 \pm 8\ \mu$l), SV ($-32 \pm 5\ \mu$l) and CO (-17 ± 2 ml/min, or -23 %) below their respective baseline values (all $P < 0.05$), while LVESV was no longer different from baseline. However, TPR remained significantly elevated by 49 %, or 0.55 ± 0.07 mmHg/ml per min, above its pre-infusion value ($P < 0.05$). The net effect was an increase in post-infusion steady-state SBP (10 ± 1 mmHg), DBP (9 ± 1 mmHg) and MAP (10 ± 1 mmHg or 13 %) (all $P < 0.05$) above their corresponding baseline values (Figure 2).

In the 2ADX group, the β-FXIIa-induced reductions in LVEDV, LVESV, SV and CO were sustained through the 30 min recovery period, TPR returned to baseline, and DBP and MAP remained below their respective pre-infusion values (Figure 1). At steady-state, post-β-FXIIa infusion, HR remained unchanged, but there were persistent reductions in LVEDV ($-71 \pm 14\ \mu$l), LVESV ($-19 \pm 5\ \mu$l), SV ($-52 \pm 10\ \mu$l) and CO (-18 ± 3 ml/min or -23 %) below their respective baseline values (all $P < 0.05$). However, TPR returned to pre-infusion values. The net effect was a decrease in post-infusion steady-state DBP (-7 ± 1 mmHg) and MAP (-5 ± 1 mmHg or -8 %, both $P < 0.05$) below their respective baselines (Figure 2).

In contrast with the infusion steady-state, during the post-β-FXIIa infusion (recovery) period in absolute terms the increase in TPR above baseline values was significantly greater in adrenally intact rats than in 2ADX rats (0.55 ± 0.07 compared with 0.15 ± 0.07 mmHg/ml per min; $P < 0.05$), as were SBP (10 ± 1 compared with -2 ± 1 mmHg), DBP (9 ± 1 compared with -7 ± 1 mmHg), MAP (10 ± 1 compared with -5 ± 1 mmHg) and HR (-26 ± 2 compared with -5 ± 1 b.p.m.) (all $P < 0.05$). By contrast, changes in baseline with respect to LVEDV, LVESV, SV and CO did not differ between the two groups (Figure 2).

Plasma coagulation FXIIa and catecholamine concentrations

Post-recovery plasma coagulation FXIIa absorbance, which was 0.26 ± 0.04 A_{550} units in the saline control group, was increased 3-fold by the β-FXIIa infusion (0.86 ± 0.05 A_{550} units; $P < 0.05$; Figure 3). Similar absorbance values were achieved by its infusion into adrenalectomized rats (0.90 ± 0.04 A_{550} units). Plasma adrenaline and noradrenaline concentrations were determined to confirm previous observations indicating that pressor and vasoconstrictor responses to β-FXIIa infusion were mediated through increases in adrenally derived catecholamines. In saline-infused rats, plasma noradrenaline and adrenaline concentrations measured at the end of the protocol were 2.5 ± 1.5 and 1.9 ± 1.2 nmol/l respectively (Figure 4). Corresponding values for β-FXIIa-infused adrenally intact rats were noradrenaline, 3.6 ± 1.4 nmol/l, and adrenaline, 9.8 ± 4.1 nmol/l ($P > 0.05$ and $P < 0.05$ respectively compared

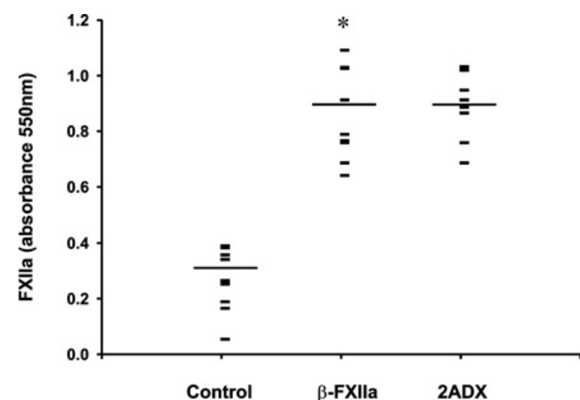

Figure 3 Individual plasma coagulation FXIIa absorbance measurements obtained at the end of the post-infusion (recovery) period in the control, β-FXIIa and 2ADX groups
Horizontal bar represents the mean value. $^*P < 0.05$ for the β-FXIIa compared with control group values. β-FXIIa and 2ADX group values did not differ.

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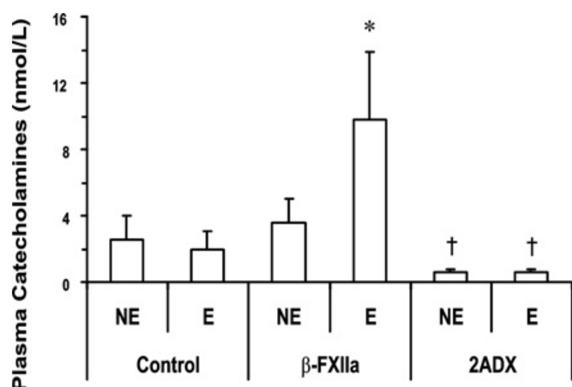

Figure 4 Plasma noradrenaline (NE) and adrenaline (E) concentrations obtained at the end of the post-infusion (recovery) period in the control, β-FXIIa, and 2ADX groups
Values are means ± S.E.M. $^{*}P < 0.05$ for the β-FXIIa group compared with control group. $^{\dagger}P < 0.05$ for the β-FXIIa group compared with the 2ADX group.

with saline). By comparison, post-infusion concentrations of noradrenaline and adrenaline adrenalectomized rats were 0.6 ± 0.2 and 0.6 ± 0.2 nmol/l respectively (both, $P < 0.05$ compared with corresponding values in the intact adrenal β-FXIIa group).

DISCUSSION

To our knowledge, this is the first characterization of the sympatho-adrenal and haemodynamic responses to a continuous intravenous infusion of plasma coagulation β-FXIIa. Our key finding was that coagulation β-FXIIa infusion into neurally intact, non-medicated, BN rats elicited a significant vasoconstrictor response that, with adrenals present, was sustained, along with elevated plasma adrenaline concentrations and BP, for 30 min beyond the administration period.

When activated by a conformational change, endothelial-cell bound FXIIa can be cleaved to release and circulate in plasma as the β-FXIIa fragment that retains proteolytic capacity [2–4]. Plasma β-FXIIa activates kallikrein rapidly, which in turn activates additional FXII and further digests FXIIa to β-FXIIa in a positive feedback cycle of protease activation that generates β-FXIIa and kallikrein from their respective zymogen substrates, each of which is present at 1000-fold higher titres [2–4, 20]. Kallikrein then generates BK-(1–9) from its plasma substrate kininogen [2,23]. Because increases in plasma β-FXIIa concentration were detected following its infusion, we can conclude that C1-INH (C1 inhibitor), the main endogenous inhibitor of both β-FXIIa and kallikrein, does not inhibit fully this feedback activation once an endogenous titre threshold has been reached.

In the extensively studied rat bioassay, bolus injection of human β-FXIIa elevates plasma BK acutely by ∼2-fold [10,14]. In turn, BK, acting upon B_2 receptors located on adrenal chromaffin cells, is a potent catecholamine secretagogue, which in the rat releases preferentially adrenaline [12,14,24]. In previous bioassay experiments, the selective BK B_2 antagonist HOE 140 attenuated catecholamine (plasma adrenaline by 77 %, plasma noradrenaline by 85 %), HR and BP responses to bolus injections of coagulation β-FXIIa [12]. In addition, in the kininogen-deficient BN Katholiek rat, a bolus injection of β-FXIIa did not increase plasma catecholamine concentrations even though adrenomedullary responsiveness to exogenous BK was retained, indicating that an intact KKS is required for β-FXIIa to stimulate adrenomedullary catecholamine release [14]. These observations suggest that haemodynamic responses observed during β-FXIIa in the present experiments represent the summation and interaction of acute peptide (primarily BK, but also PACAP), adrenal catecholamine and reflex or autonomic responses to infusion of this coagulation factor, whereas, because of the short plasma BK half-life [23], haemodynamic responses observed after its infusion represent the summation and interaction of catecholamine, non-BK-peptide (e.g. PACAP) and reflex-mediated effects. Our objective in performing adrenalectomy was to expose the catecholamine-independent haemodynamic effects of β-FXIIa infusion in this non-captopril-treated, neurally intact rat preparation.

Although BK is commonly considered a vasodilator, when infused continuously into rats at doses between 5 and 80 μg/min per kg of body weight it has been shown to cause an acute dose-dependent increase in MAP that is abolished by pre-treatment with the selective BK B_2 receptor antagonist HOE 140 [25,26]. Hoagland et al. [25,26] concluded that this BP rise is mediated via BK B_2 receptor-activated sympatho-excitatory pathways. Indeed, there is evidence that BK can activate afferent nerve endings eliciting a sympatho-excitatory reflex [27], and that the increase in amplitude of excitatory junction potentials is eliminated by HOE 140 [28]. Intrarenal infusions of BK of 0.5 and 1 μg/min for 5 min [29] or 5–80 μg/min per kg of body weight for 2 min [26], which increase MAP acutely in a dose-dependent manner, are eliminated by renal denervation [29] and abolished by HOE 140 [26], indicating that one set of such afferent sympatho-excitatory nerve endings originates within the kidneys. In perfused rat mesenteric arteries pre-infused with noradrenaline, BK infusions (10^{-6} to 10^{-4} M) elicit a dose-dependent vasoconstrictor response independent of the endothelium [30]. In conscious rats, intrarenal infusions of BK (0.5 and 1 μg/min for 5 min) cause increases in mesenteric arterial resistance by 20 % and 33 % respectively from baseline which are eliminated by renal denervation [29]. The increase in TPR during β-FXIIa infusion in both adrenally intact and 2ADX rats is consistent with stimulation, by BK, of one or more of these vasoconstrictor mechanisms.

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TPR increased significantly during β-FXIIa infusion in both experimental groups, but β-FXIIa infusion decreased LVEDV, LVESV, SV and CO. Each of these cardiac haemodynamic effects was significantly more profound in the 2ADX group. These findings probably represent BK-induced venodilation that was unopposed by BK-stimulated catecholamine release in the 2ADX group and therefore augmented. Indeed, as illustrated in Figure 1, in the 2ADX group β-FXIIa infusion elicited an immediate transient fall in BP. BK causes endothelium-dependent venodilation via BK B_2-receptor-mediated liberation of NO [31]. Venodilation, in turn lowers LVEDV (pre-load), an observed effect that was doubled, in the present experiments, by adrenalectomy (-126 ± 11 compared with -65 ± 10 μl, $P < 0.05$). Of note, mice lacking the adrenaline-synthesizing enzyme phenylethanolamine *N*-methyltransferase, have 22 % lower LVEDV compared with wild-type controls [32], confirming that plasma adrenaline is important in maintaining pre-load. An increase in capillary endothelial permeability will also reduce pre-load. In rats, BK-(1–9) infusion increases rapidly mesenteric capillary permeability (measured as the albumin leakage index) from 5 % to 95 % via BK B_1 and B_2 receptor-dependent mechanisms [33].

β-FXIIa infusion also reduced LVESV in both groups of rats. The consequent reduction in SV was augmented by adrenalectomy (-89 ± 9 μl compared with -38 ± 5 μl, $P < 0.05$), likely due to the combination of decreased end-diastolic myocyte stretch, and diminished inotropy due to the profound reduction in circulating catecholamines. In both groups, CO (the product of SV and HR) fell during β-FXIIa infusion; in intact rats, this was due to a decrease in both components, whereas in adrenalectomized rats this reflected the more profound reduction in SV.

Adrenalectomy caused a marked reduction in baseline HR (Figure 1). β-FXIIa infusion had no additional effect, whereas in rats with intact adrenals, β-FXIIa infusion elicited a significant fall in HR. This chronotropic response would be anticipated from prior studies in conscious rats; a 90 min intravenous infusion of adrenaline increased plasma concentrations 90-fold, by 18.4 nmol/l, and increased MAP significantly, but caused only a transient, non-significant decrease in HR [34]. In the anaesthetized, ganglion-blocked, captopril-treated bioassay preparation 1 μg/kg intravenous β-FXIIa injection evoked a 170-fold increase in plasma adrenaline and increased HR from 315 to 408 b.p.m. [14].

Since endogenous NO has been shown to augment vagal tone, one explanation for the slower HR observed *in vivo* again may be BK-induced NO generation [35–37]. BK also acts directly on the sino-atrial node, via the BK B_2 receptor, to lower HR [37]. In BK B_2 receptor knockout mice basal HR is elevated compared with littermate controls [38], and pharmacological blockade by the BK B_2 receptor antagonist HOE 140 increases HR in both rats [39] and humans [40]. A second potential explanation for the slower HR observed in rats with intact adrenal glands is that β-FXIIa induced increases in PACAP [13], which lowers HR by liberating acetylcholine from postganglionic parasympathetic nerves [41]. Importantly, a significant increase in SBP (7 ± 2 mmHg), which would reduce HR rate reflexively by stimulating the arterial baroreceptor reflex, was observed only in the adrenally intact group. Each or a combination of these several HR modulating pathways could contribute to the observed negative chronotropic response to β-FXIIa.

Once the β-FXIIa infusion ended, TPR reverted promptly to baseline in the 2ADX group, but remained significantly elevated, along with BP, in the intact group of rats. The time course over which this occurred in the 2ADX is consistent with its rapid degradation in plasma [23], with dissipation in parallel with its sympatho-excitatory effects. By contrast, any BK-induced reductions in pre-load due to vasodilation or increased capillary permeability would be sustained throughout the recovery period. The present findings indicate that the vasoconstrictor after effects of coagulation β-FXIIa require a functioning adrenal gland, and are mediated in part by plasma adrenaline, a potent vascular α-adrenergic-receptor-mediated peripheral vasoconstrictor in the rat [34,42].

However, because the plasma half-life of catecholamines in the rat is 90 s or less [43], haemodynamic responses to BK B_2 receptor-stimulated catecholamine release would be expected to dissipate rapidly. Thus, other vasoconstrictor mechanisms must be invoked to account for the sustained vasoconstriction observed in the present experiments. One attractive candidate is PACAP, a neurotransmitter that can both increase splanchnic sympathetic nerve activity [44] and also stimulate the adrenal chromaffin membrane-associated PACAP PAC-1 receptor [45] to release adrenaline preferentially into plasma [13,46]. In rats, the adrenal gland is the organ with the second highest concentration of PACAP [47]. Previous bioassay rat studies, using a pharmacological inhibitor, demonstrated that a significant proportion of the acute BP effect of bolus β-FXIIa is mediated via the PAC-1 receptor [13]. This effect is durable: plasma adrenaline concentrations in rats remain 233 % above baseline 60 min after a 10 min PACAP infusion (50 μM at 3 μl/min) [48].

To summarize, in the present experiments, we demonstrate that short-term intravenous infusion of plasma coagulation β-FXIIa exerts significant haemodynamic effects, which require an intact adrenal gland for their full expression. These include an increase in peripheral vasoconstriction that exceeds reductions in pre-load and CO resulting overall in a significant increase in BP. Such vasoconstriction is, in part, adrenal-independent, but in rats with adrenals *in situ* vasoconstriction persists for at least 30 min after β-FXIIa infusion.

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It is now appreciated that plasma coagulation FXIIa concentrations are elevated, in some instances more than 10-fold, in many patients with primary essential hypertension [5], diabetes [49], coronary heart disease [6,7] and chronic renal failure [49–52], conditions associated with increased risk of stroke and myocardial infarction and often characterized by vascular inflammation, are increased more than 5-fold after thrombolytic therapy [53] and remain elevated for 6 weeks following myocardial infarction [18].

In such states, chronic coagulation β-FXIIa elevation may disturb further already abnormal BP regulatory mechanisms, and increase the risk of adverse cardiovascular events. With FXIIa inhibitors currently under development [54], there may indeed be an opportunity to exploit this pathway as therapy of hypertension in otherwise difficult-to-treat conditions characterized by increased FXIIa in plasma, such as end-stage renal disease.

AUTHOR CONTRIBUTION

Peter Papageorgiou conducted the experiments; Peter Papageorgiou, Erik Yeo, Peter Backx and John Floras designed the experiments and prepared the paper for publication.

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SUPPLEMENTARY ONLINE DATA

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

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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 ± 50 μl; for LVESV, -40 ± 30 μl and for SV, 3 ± 20 μl. Absolute values for these variables, derived using the conductance catheter in these experiments, were similar to baseline values in the present experiment.

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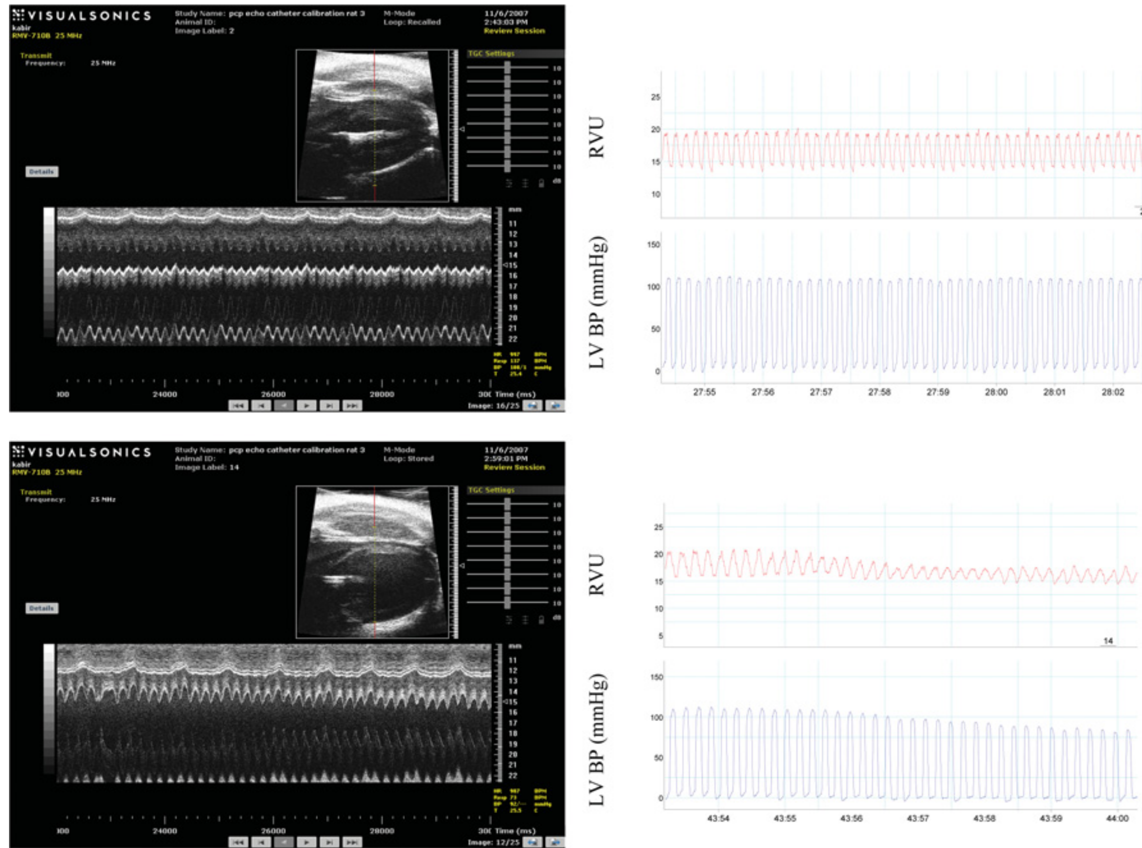

Figure S1 Simultaneous acquisition of LV volume measurements by echocardiography and by micro-tip pressure–volume catheter transducer

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).

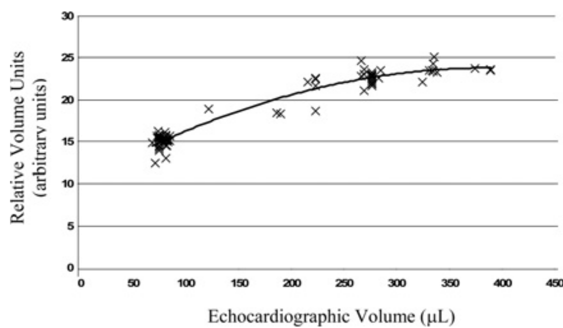

Figure S2 Derivation of RVU from echocardiographic volumes

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 $-9 \times 10^{-5}x^2 + 0.0706x + 10.138$.

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