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RESEARCH ARTICLE

Cold-impaired cardiac performance in rats is only partially overcome by cold acclimation

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SUMMARY

The consequences of acute hypothermia include impaired cardiovascular performance, ultimately leading to circulatory collapse. We examined the extent to which this results from intrinsic limitations to cardiac performance or physiological dysregulation/autonomic imbalance, and whether chronic cold exposure could ameliorate the impaired function. Wistar rats were held at a 12 h:12 h light:dark (L:D) photoperiod and room temperature (21°C; euthermic controls), or exposed to a simulated onset of winter in an environmental chamber by progressive acclimation to 1 h:23 h L:D and 4°C over 4 weeks. *In vivo*, acute cold exposure (core temperature, T_b=25°C) resulted in hypotension (approximately –20%) due to low cardiac output (approximately –30%) accompanying a bradycardia (approximately –50%). Cold acclimation (CA) induced only partial compensation for this challenge, including increased coronary flow at T_b=37°C (but not at T_b=25°C), maintenance of ventricular capillarity and altered sympathovagal balance (increased low:high frequency in power spectral analysis, PSA), suggesting physiological responses alone were insufficient to maintain cardiovascular performance. However, PSA showed maintenance of cardiorespiratory coupling on acute cold exposure in both groups. *Ex vivo* cardiac performance revealed no change in intrinsic heart rate, but a mechanical impairment of cardiac function at low temperatures following CA. While CA involved an increased capacity for β-oxidation, there was a paradoxical reduction in developed pressure as a result of adrenergic down-regulation. These data suggest that integrated plasticity is the key to cardiovascular accommodation of chronic exposure to a cold environment, but with the potential for improvement by intervention, for example with agents such as non-catecholamine inotropes.

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Key words: β-oxidation, capillarity, heart rate variability, hypotension, hypothermia.

INTRODUCTION

Mammals are generally sensitive to hypothermia, with the first pathological symptoms occurring at a core temperature (T_b) as high as 34°C in humans. Further drops in T_b lead to impaired ventilation and circulatory decline, in turn leading to hypoxaemia and acidosis (Cossins and Bowler, 1987). The normal limit is around 25°C, after which ventilation may cease and ventricular fibrillation leads to circulatory arrest, but even moderate cooling can lead to significant pathology if prolonged. Epidemiological studies have shown an increase in acute myocardial infarctions in colder weather, probably involving elevated haemostatic risk factors, hypertension and sustained tachycardia. Cold acclimation (CA) reduces sensitivity to the adrenergic pressor response, but does not elicit any change in heart rate (f_H) (Budd et al., 1993), although the pattern of haemodynamic responses may vary between cold acclimation and cold exposure (De Lorenzo et al., 1999). Other mammals, however, accommodate more direct exposure to extreme and varying thermal environments, though surprisingly little is known about cardiovascular adaptation to low temperatures (Gordon, 1993). One challenge is to identify which of the suite of presumed adaptive responses to cold exposure reflects species-specific characteristics, and which are capable of physiological modification.

In response to cooling, cardiac performance is impaired as a result of alteration in sinoatrial pacemaker activity, adrenergic sensitivity, myocyte calcium handling and, hence, contractility. For example, a 6-fold decrease in guinea pig papillary muscle contractile strength was noted after 3 weeks at 5°C, but a 30% increase in time to peak tension (Takagi et al., 1999). Rat left ventricle was significantly weaker (greater reduction in maximum developed pressure generated against a balloon) and more slowly contracting (reduced peak dP/dt) when acutely cooled, compared with that of hibernators such as ground squirrel (Caprette and Senturia, 1984). Depressed cardiac sarcoplasmic reticulum calcium transporter activity at low temperature may lead to high cytosolic free Ca^{2+} in cold-sensitive species, contributing to the loss of cardiac function at low temperature (Liu et al., 1997). Previously, we have shown that during progressive levels of acute hypothermia (T_b=37 to >31 to >25°C), mean arterial blood pressure (MABP) of rats was maintained until 31°C, then displayed a reversible hypotension, whereas f_H showed a more linear bradycardia, suggesting a compensation to preserve cardiac output up to a critical temperature (Sabharwal et al., 2004a). It remains unclear to what extent impaired cardiovascular performance results from inadequate plasticity of extrinsic (i.e.

integrative or physiological) or intrinsic (i.e. cardiomyocyte phenotypic) regulators of cardiac function.

Prolonged exposure of rats to low environmental temperatures typically results in reduced skeletal muscle growth but increased heart mass, due to differential rates of *in vivo* protein synthesis and degradation (McAllister et al., 2000; Samuels et al., 1996). In guinea pigs, myocardial hypertrophy following CA-induced volume overload is compensated by increases in capillarity (Kayar and Banchero, 1985). Chronic cold exposure (4 weeks at 6°C) increased resting f_H and systolic, diastolic and mean blood pressures in unanaesthetised, unrestrained rats compared with those of controls maintained at 25°C (Fregly et al., 1989). Resting and β-adrenergic stimulated f_H of CA rats increased over that of controls (Barney et al., 1980), suggesting enhanced catecholamine sensitivity *in vivo*, although acute cold exposure increased noradrenaline turnover in cardiac muscle but CA was without further effect (Dulloo et al., 1988). Similarly, the increase in coronary muscle blood flow during β-agonist (isoproterenol) infusion was comparable in room temperature and CA rats (2.9- and 2.6-fold above control values, respectively) (Wickler et al., 1984), suggesting that coronary reserve is non-adaptive despite increased responsiveness to β-adrenergic stimulation.

In both control and CA rats, acute hypothermia (T_b=25°C) reversibly shifted the baroreflex–renal sympathetic nerve activity curve left and downwards with decreases in the setpoint pressure and maximal gain, whereas it markedly impaired the baroreflex–f_H curve characterised by decreases in response range, minimum response and maximum gain from that at T_b=37°C (Sabharwal et al., 2004a). The marked attenuation of the baroreflex control of f_H during acute hypothermia was probably due to an impairment of both central and peripheral components of the reflex arc, including a delay in conduction, which was non-adaptive on chronic cold exposure. However, recent data suggest that autonomic neural drive, previously assumed to have a commonality of purpose among tissues, may show target organ-dependent control (Ootsuka and McAllen, 2006), which suggests our current understanding of cold adaptation is limited, and analysis of a wider integrative response is required.

We therefore exploited the anaesthetised instrumented rat and Langendorff-perfused heart preparation to examine the integrative (extrinsic) and phenotypic (intrinsic) responses, respectively, comparing the degree of cardiovascular compensation in euthermic control and cold-acclimated rats exposed to acute cooling.

MATERIALS AND METHODS

All animals were used in accordance with the UK Animals (Scientific Procedures) Act 1986, and had access to water and food *ad libitum*. Male Wistar rats (Charles River, Margate, Kent, UK) of ~370 g body mass (M_b) (*in vivo* studies) or ~280 g M_b (*ex vivo* studies) at experimentation were randomly assigned to control and cold acclimation (CA) groups. All reagents were supplied by BDH or Sigma (Poole, Dorset, UK) unless otherwise stated, and all inorganic chemicals were of ANALAR quality.

Cold acclimation

Control rats were held under ambient conditions of photoperiod and temperature (12 h:12 h light:dark, L:D, and 21°C). Animals subjected to CA were held in an environmental chamber with light-tight ventilation and a low energy time-programmable light source; they were allowed 3 days to adapt to these initial settings, after which the photoperiod was reduced by 1 h every other day to 1 h:23 h L:D, and the temperature reduced 1.5°C every other day to 4°C by day 25. These conditions were maintained for 4–6 days, then animals were entered into experiments. For logistic reasons associated with sample preparation, three sets of cold-acclimated rats and body mass-matched euthermic controls were used: set 1, for *in vivo* measurement of haemodynamic parameters; set 2, for measurement of *ex vivo* cardiac performance; and set 3 for measurement of capillary supply and enzyme activity. While small differences in age and M_b were accommodated by matched animals in each group, comparison of responses between groups may be quantitatively (though not qualitatively) modulated.

Surgical preparation

Methods were similar to those used previously (Sabharwal et al., 2004a). Briefly, animals were initially anaesthetised with isofluorane (Fluothane, ICI; 3–4% in oxygen) while the left jugular vein was cannulated, patency being maintained with heparinised saline (20 U ml^{-1}); isofluorane anaesthesia was then replaced with an infusion of α-chloralose and urethane (165 mg and 2.5 g in 10 ml saline i.v.). A tracheal cannula was used to maintain a patent airway, allow spontaneous breathing and attachment to a spirometer. The left femoral vein and artery were cannulated to allow infusion of drugs and measurement of arterial blood pressure, respectively. An incision was made lateral to the midline of the thorax to allow the positive electrocardiogram (ECG) electrode to be inserted into the chest wall at approximately position V4, with the negative electrode in the right forepaw, and a reference electrode in the right hindpaw. After surgery, animals were transferred to a thermostatically controlled stage (see Sabharwal et al., 2004b), the head and paws being secured to minimise any movement. Once on the thermoplate, physiological saline solution (0.9% NaCl) was given by continuous infusion (1.25 ml h^{-1}; Harvard Apparatus, Holliston, MA, USA) to maintain fluid balance. A J-type thermistor was inserted deep into the oesophagus to measure T_b (°C) throughout the experimental protocol.

In vivo cardiac performance

Set 1 animals (Table 1) were allowed to stabilise for 90 min then recordings were taken for 5 min at T_b≈37 and 25°C. After cooling to the low T_b (at a rate of ~1°C every 10 min) the animals were allowed to stabilise for 30 min and further readings were then taken before the animal was rewarmed to T_b=37°C. At T_b≈37 and 25°C some animals received radiolabelled microspheres (^{113}Sn, ^{46}Sc; DuPont NEN, Mechelen, Belgium) delivered into the left ventricle *via* a right carotid arterial cannula. Reference flow was obtained by sampling from one brachial artery using a precision withdrawal pump (Braun, Melsungen, Germany), and MABP was measured using a catheter in the other brachial artery (for details, see Egginton et al., 1998).

In other animals, the baroreflex control of f_H was assessed by pharmacological manipulation of MABP, again at T_b≈37 and 25°C. MABP was increased by giving a bolus injection *via* the femoral vein of ~10 μg phenylephrine hydrochloride (PE), and decreased using ~10 μg sodium nitroprusside (SNP), at a dose of 1 mg kg^{-1}, given in random order. Higher concentrations were used if a 6.65 kPa (50 mmHg) change in MABP was not elicited. ECG and MABP were recorded during and after the bolus injections; all variables were stable for 5 min prior to each bolus. The influence of vagal tonus on cardiac cycle duration was examined using high-resolution ECG recordings in animals with intact vagi, and again following bilateral vagotomy.

ECG signals were amplified and recorded using a bioamplifier and Chart software (AD Instruments, Oxford, UK). Optimal settings for well-defined R waves at 37°C were as follows: range 1 mV, high pass

Table 1. Body mass and haemodynamic indices for euthermic rats (control) and rats exposed to cold acclimation (CA) under normothermic (37°C) or hypothermic (25°C) conditions

	Normothermia		Hypothermia	
	Control (12)	CA (11)	Control (11)	CA (10)
Body mass (g)	365±7	358±5	388±3	377±3
Systolic pressure (kPa)	17.8±0.5	18.5±0.8	16.5±0.9	16.2±1.5
Diastolic pressure (kPa)	14.6±0.5	15.6±0.9	11.8±0.8†	10.8±1.1†
Stroke volume (ml)	0.58±0.12	0.61±0.09	0.73±0.11	0.94±0.12†
LVBF (ml min^{-1} 100 g^{-1})	207±22	305±20*	429±52††	374±47
LVC (ml min^{-1} 100 g^{-1} kPa^{-1})	12.9±1.6	18.7±2.9	32.9±3.9††	36.5±10.8
Cardiac index (ml min^{-1} 100 g^{-1})	108±19	78±12	60±16	45±6†
CMW (J kg^{-1})	682±127	703±87	516±92	645±130
Stroke work (J kg^{-1})	4.23±0.83	3.17±0.54	2.55±0.41	2.33±0.69
TPR (kPa min ml^{-1})	0.11±0.02	0.09±0.02	0.11±0.02	0.08±0.01

LVBF, left ventricular blood flow; LVC, left ventricular conductance; CMW, cardiac minute work; TPR, total peripheral resistance.
Means ± s.e.m. (number of rats), set 1 animals. *P<0.01 *vs* control; $^{\dagger}P$<0.05, $^{\dagger\dagger}P$<0.001 *vs* normothermia.

0.3 Hz, low pass 50 Hz, sampling rate 1 kHz. The trace was used to calculate f_H (beats min^{-1}) and R–R intervals (ms), as well as the relative duration of the cardiac cycle components (sampled at 10 kHz). The tracheal cannula was fitted to a respiratory flow head attached to a spirometer (ML140, AD Instruments), and ventilation frequency (f_V, breaths min^{-1}) measured online. Minute ventilation (ml min^{-1}) was calculated offline by integration of the respiratory cycle using f_V and tidal volume (V_T, ml). The femoral artery cannula was attached to a pressure transducer (SP844 Pressure transducer; Memscap, Skoppum, Norway) to record arterial blood pressure (kPa).

Ex vivo cardiac performance

Set 2 animals (Table 4) were prepared surgically as outlined previously (Hauton et al., 2001). Briefly, anaesthesia was induced with pentobarbital (60 mg kg^{-1} i.p. in saline) and, following thoracotomy, hearts, excised with lungs and thymus *in situ*, were immersed in ice-cold Krebs–Hensleit medium. Excess tissue was dissected, and the aorta was trimmed at the level of the carotid artery branches and cannulated (16 gauge cannula). Hearts were perfused in retrograde fashion as outlined previously (Hauton and Ousley, 2009). An incision was made in the right ventricle and the left atrial appendage was removed. A small flexible non-elastic balloon was inserted into the left atrium through the mitral valve and into the left ventricle. Fluid-filled ventricular balloons were constructed using Saran^{TM} wrap polythene film attached to a fine plastic catheter, and connected to a pressure transducer (Memscap) and a graduated Hamilton syringe (0–1000 µl: Hamilton, NV, USA). Hearts were maintained at 37°C and perfused at a constant pressure (100 cm H_2O) with a Krebs–Hensleit crystalloid medium supplemented with glucose (10 mmol l^{-1}) and $CaCl_2$ (1.3 mmol l^{-1}) gassed with oxygen/CO_2 (95:5). Developed pressure was measured following isovolumetric contraction of the fluid-filled balloon and recorded to computer using a digital interface (AD Instruments).

The initial balloon volume was adjusted until recorded diastolic pressure was 0 kPa and the developed pressure (systolic pressure–diastolic pressure) was <1.33 kPa (10 mmHg). The balloon volume was increased in incremental steps (50 µl) after stabilisation of diastolic pressure, and developed pressure was recorded in real time until the peak systolic pressure exceeded 26.6 kPa (200 mmHg). The balloon was then deflated and the process repeated. Coronary flow was estimated from timed collections of a known volume of perfusate, and expressed as volume/unit mass of cardiac tissue. Ventricular performance was calculated off-line. Heart rate, systolic pressure, diastolic pressure and hence developed pressure were measured. Rate of change of pressure (+dP/dt) was calculated from the maxima of the first-order derivative of the pressure trace. Rate–pressure product (RPP, kPa min^{-1}) was calculated at each balloon volume as heart rate × developed pressure. End-diastolic volume was estimated from linear regression of the diastolic performance curve for values greater than zero at the point at which the regression line bisected the balloon volume at zero diastolic pressure.

Cardiac histochemistry and metabolism

Set 3 animals (Figs 4 and 5) were acclimated as above, and killed by cervical dislocation. Hearts were blotted dry and weighed, atria and connective tissue discarded, and a transverse midline incision was made. The lower portion (apex) of the heart was mounted onto cork discs in Tissue-Tek OCT compound (Sakura, Torrance, CA, USA) before being frozen in liquid nitrogen-cooled isopentane. Cryostat sections (10 µm) were cut and capillaries visualised using an alkaline phosphatase method (Deveci and Egginton, 2002) using nitroblue tetrazolium reagent, generating an insoluble formazan pigment. Capillary density was quantified (magnification ×200) in three fields per section, and expressed as capillary number per unit cross-sectional area of myocardium.

For enzyme analyses, muscle samples were homogenised (Polytron; Kinematica, www.kinematica-inc.com) in 30 volumes (w/v) of ice-cooled 2 mmol l^{-1} $MgCl_2$, 1 mmol l^{-1} EDTA, 50 mmol l^{-1} imidazole-HCl, pH 7.4 at 20°C using 2× 10 s intervals with cooling on ice. Enzyme activity was determined using a dual beam spectrophotometer with a thermostatically controlled cuvette holder. Optimal conditions (pH, substrate/cofactor concentrations) when activity was proportional to the amount of homogenate added were determined in pilot experiments. Enzyme activity was measured at 25±0.1°C using substrate deletion as a control for background activity, monitored by following the oxidation of the pyridine nucleotide NADH at 340 nm (using a millimolar extinction coefficient, $\varepsilon_{mmol\,l^{-1}}$=6.22) or reduction of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) at 412 nm ($\varepsilon_{mmol\,l^{-1}}$=13.6), with the results expressed as µmol product formed min^{-1} g^{-1} wet mass. Reaction media followed those used elsewhere (Egginton and Hudlicka, 1991); preliminary experiments showed assay conditions for maximal enzyme activities to be as follows.

Lactate dehydrogenase (EC 1.1.1.27; LDH)

LDH was assayed as pyruvate reductase in a medium consisting of 1.5 mmol l^{-1} NADH, 50 mmol l^{-1} imidazole-HCl buffer, pH 7.4. The

reaction was initiated by addition of 3 mmol l^{-1} sodium pyruvate to the medium. (The pyruvate concentration that gave the highest LDH activity was determined for each tissue.)

Phosphofructokinase (EC 2.4.1.1; PFK)

PFK activity was determined in the presence of 1.5 mmol l^{-1} NADH, 7 mmol l^{-1} $MgCl_2$, 2 mol l^{-1} AMP, 2 U ml^{-1} α-glycerophosphate dehydrogenase, 0.5 U ml^{-1} aldolase and 75 mmol l^{-1} triethanolamine-HCl buffer, pH 8.2. The reaction was initiated by addition of ATP and D-fructose 6-phosphate to the medium.

Citrate synthase (EC 4.1.3.7; CS)

The assay medium consisted of 2.5 mmol l^{-1} DTNB, 5.0 mmol l^{-1} oxaloacetic acid (OAA), 3.0 mmol l^{-1} *S*-acetyl coenzyme A (CoA), 50 mmol l^{-1} triethanolamine-HCl buffer, pH 8.0. CS activity was determined at 420 nm after the addition of 0.3 mmol l^{-1} acetyl CoA (sodium salt), following subtraction of background deacylase activity from activity in the presence of OAA.

3-Hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35; HAD)

HAD activity was determined in the presence of 1.5 mmol l^{-1} NADH, 1 mmol l^{-1} EDTA and 50 mmol l^{-1} imidazole buffer, pH 7.4. The reaction was initiated by addition of 2.0 mmol l^{-1} acetoacetyl CoA (sodium salt).

Data analysis

Heart rate variability (HRV) was analysed with HRV extension software (AD Instruments) using 512 consecutive beats (R waves), a Hann window to remove any noise interference to the power spectrum and a 2/3 overlap to minimise spectral leakage due to any baseline instability. The component bandwidths were set as very low frequency (VLF, 0–0.04 Hz), low frequency (LF, 0.04–1 Hz) and high frequency (HF, 1–3 Hz) (Kuwahara et al., 1994; Sabharwal et al., 2004a). A fast Fourier transform (FFT) algorithm was used to calculate the power spectrum (power spectral analysis, PSA), and the boundaries for ectopic beats and artefacts were defined using Poincare plots.

ECG and blood pressure traces, obtained during the pharmacological manipulation of MABP, were used to analyse the baroreflex control of f_H. Systolic and diastolic pressure readings were used to calculate MABP, the timing of SP was used to confirm R-waves in the ECG trace, and the mean of the next three consecutive R–R intervals was used to determine instantaneous f_H for each temperature. f_H was normalised by converting values into a change from baseline (%), which was then plotted against MABP (DeltaGraph, Red Rock Software Inc., Salt Lake City, UT, USA). A sigmoid logistic function equation (Kent et al., 1972) was used to fit a baroreflex curve to the plotted data at T_b=37°C and T_b=25°C for each animal:

$$f_H = A / \{ 1 + \exp [B (\mathrm{MABP} - C)] \} + D , \quad (1)$$

where A is the response range for f_H, B is the gain, C is the midpoint pressure at the midrange of the curve and D is the minimum response for f_H. From these parameters other parameters such as the maximum response, saturation pressure for MABP (P_{sat}), threshold pressure for MABP (P_{thr}), maximal gain and operating range can be calculated (Miki et al., 2003; Sabharwal et al., 2004a).

To calculate vagal tone from R–R intervals, the following equation was used (Campbell et al., 2004):

$$\%\mathrm{Chol} = \frac{(\mathrm{R-R})_{IR} - (\mathrm{R-R})_{VR}}{(\mathrm{R-R})_{IR}} \times 100 , \quad (2)$$

where %Chol is percentage cholinergic tone, $(R–R)_{IR}$ is the intact resting control R–R interval (high cholinergic tonus:low adrenergic tonus), and $(R–R)_{VR}$ is the vagotomised resting R–R interval (no cholinergic tonus, low adrenergic tonus).

All data are expressed as means ± s.e.m., unless otherwise stated. Statistical evaluation was performed using factorial and repeated measures analysis of variance (ANOVA), as appropriate, with Fisher's PLSD to estimate the *post hoc* significance (StatView, SAS Institute, Cary, NC, USA). Statistical significance was accepted at P<0.05.

RESULTS

Throughout cold acclimation all animals remained active and healthy; post-mortem body mass was unaffected by chronic exposure to a cold environment with a short photoperiod (Tables 1 and 4).

Haemodynamics

There was little evidence for a hyperdynamic cardiovascular system on chronic cold exposure, with only a modest though consistent

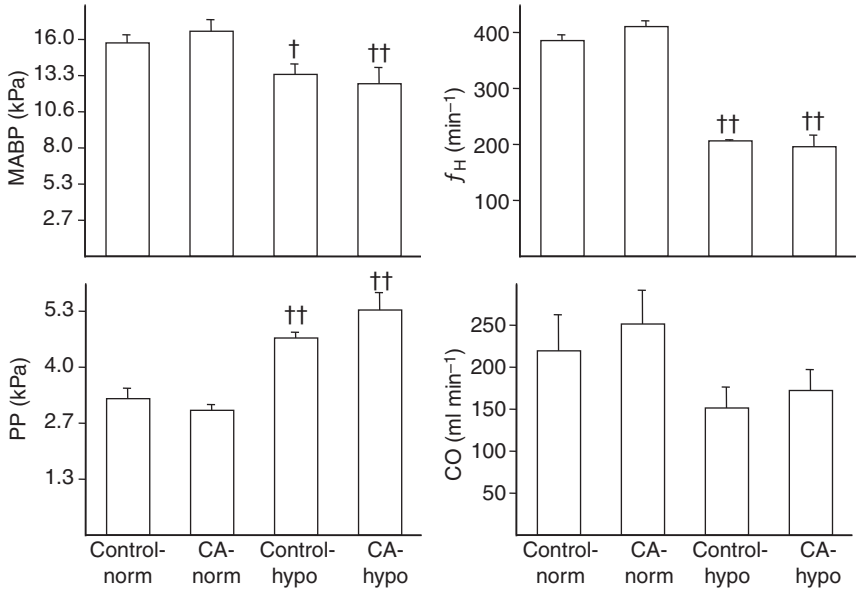

Fig. 1. Estimates of *in vivo* cardiovascular performance of rats in euthermic (Control) and cold acclimation (CA) groups under normothermic (norm) and hypothermic (hypo) conditions. MABP, mean arterial blood pressure; PP, pulse pressure; CO, cardiac output; f_H, heart rate. Means + s.e.m. (set 1 animals, N=10–12, see Table 1). *P<0.01 *vs* control; †P<0.05, ††P<0.001 *vs* normothermic.

Table 2. Values of HRV in the frequency domain at different T_b, during cooling and rewarming

	37°C	25°C	R37°C
LF (Hz)			
Control	0.26±0.01	0.10±0.01*	0.23±0.01
CA	0.11±0.01	0.05±0.01*	0.14±0.01
LF power absolute (ms^2)			
Control	0.08±0.02	0.06±0.01	0.07±0.01
CA	0.15±0.01†	0.06±0.02*	0.09±0.03
LF power normalised (%)			
Control	10.13±3.10	4.09±1.50*	10.61±3.30
CA	35.33±8.50†	16.69±2.02*,†	30.76±7.40
LF power CCV (%)			
Control	1.74±0.22	1.10±0.06*	1.57±0.16
CA	4.16±0.34†	1.19±0.11*	2.67±0.45
HF (Hz)			
Control	1.18±0.05	0.74±0.04**	1.16±0.05
CA	1.02±0.054	0.71±0.09*	1.04±.04
HF power absolute (ms^2)			
Control	1.07±0.18	1.12±0.33	1.02±0.13
CA	0.82±0.24	0.35±0.09†	0.79±0.11
HF power normalised (%)			
Control	87.86±3.36	92.26±2.30	86.38±3.64
CA	62.99±9.90†	77.58±6.70†	68.26±4.40
HF power CCV (%)			
Control	6.93±0.73	6.56±0.62	6.69±0.63
CA	5.43±0.74	2.84±0.36†	4.62±0.85
Total power (ms^2)			
Control	1.14±0.17	0.84±0.34*	1.07±0.13
CA	1.34±0.19	0.46±0.07**,†	1.06±0.10
LF/HF ratio			
Control	0.070	0.053	0.068
CA	0.179	0.175	0.110

T_b, core temperature; LF, low frequency; HF, high frequency; R37°C, rewarmed to 37°C.
Means ± s.e.m.; control rats, N=10; CA rats, N=7; set 1 animals. *P<0.05, **P<0.01 *vs* 37°C. †P<0.05 *vs* control rats at same T_b (ANOVA).

trend for elevation of MABP, f_H, cardiac output (CO), cardiac minute work (CMW), stroke work, etc. (Fig. 1, Table 1) in anaesthetised rats. There was an acute hypothermic hypotension, again with only a modest influence of thermal adaptation: the uncompensated bradycardia (to ~200 beats min^{-1}; P<0.001) resulted in a similar MABP in both control and cold-acclimated rats (Fig. 1), but ~2-fold greater hypotension relative to normothermic values on cooling in the CA group, with a proportionally greater effect on diastolic pressure (P<0.05) than on systolic pressure (n.s.; Table 1). Cardiac output was reduced by ~45% at 25°C in both groups, with partial compensation for low f_H by increased pulse pressure as a result of greater stroke volume, which was enhanced with CA (all P<0.001; Fig. 1, Table 1). Cardiac performance tended to be reduced in the cold, though few indices reached statistical significance. Intriguingly, blood flow and conductance were increased on cooling in control (P<0.01), but this was less pronounced in CA rats (n.s.; Table 1).

HRV

On acute cooling control rats show lower peak frequencies following power spectral analysis (PSA), consistent with the observed bradycardia, and a much reduced beat-to-beat variability (Table 2). The LF peak showed a modest reduction in frequency but a marked reduction in spectral power (both P<0.05), while the HF peak showed a more pronounced reduction in frequency (P<0.01) but no change in power. CA rats had a lower LF but higher HF power than control animals (P<0.05). Consequently, CA animals had a greater LF/HF ratio (an index of sympathovagal tone) than control at both T_b=37 and 25°C, but were much less affected by an acute change in T_b (Table 2).

Blood pressure variability (supplementary material Table S1) behaved in a similar manner to HRV, except HF was little changed on cooling, and LF/HF was higher than for the corresponding f_H analysis at 37°C, suggesting a high degree of autocorrelation. This was demonstrated by cross-spectral analysis (supplementary material Table S2), showing both a phase shift and increased coherence between R–R and MABP signals on cooling, the latter being slightly greater in CA rats. A similar pattern was seen for R–R and ventilation (data not shown); hence, little change was noted in the relationship between MABP and f_V.

Vagal tone

Bilateral sectioning of the vagus nerve provides an intermediate step between the *in vivo* and *ex vivo* preparations, showing responses in the absence of autonomic control but in the presence of humoral factors. The data suggest little vagal tone at 37°C in anaesthetised rats, with prolongation of all measured intervals at 25°C (P<0.001; Table 3). Vagal tone was estimated to be 3.9% at 37°C and 2.1% at 25°C, suggesting that influence of cooling on the cardiac cycle was a direct temperature (Q_{10}) effect – i.e. of chronotropic not inotropic origin – that was not driven by higher vagal tone (Table 3).

Intrinsic myocardial function

A separate group of animals were used for Langendorff preparations to act as an *ex vivo* comparison with *in vivo* cardiac work and HRV. Under anaesthesia at euthermic T_b, f_H and RPP were similar in the two groups of rats, although MABP was significantly greater in CA

Table 3. Influence of core temperature on ECG intervals (ms) in euthermic control rats with and without bilateral vagotomy

	P–R	QRS	S–T	Q–T	R–R
T_b=37°C					
Intact	46.3±0.7	11.7±0.2	6.3±0.3	31.1±3.7	128.0±2.7
Vagotomised	47.3±0.5	10.8±0.3	6.9±0.1	30.5±1.8	133.0±3.5
T_b=25°C					
Intact	72.1±0.7**	15.5±0.4**	9.9±0.5**	41.5±0.5**	211.5±3.2**
Vagotomised	72.7±0.5**	18.2±0.2**,†	13.7±0.4**,†	42.1±0.9**	216.0± 1.6**
T_b=rew37°C					
Intact	48.3±1.1	11.9±0.4	6.0±0.4	32.1±1.4	129.9±3.0
Vagotomised	47.1±0.3	9.6±0.5	6.3±0.2	29.8±0.6	131.0±2.1

ECG, electrocardiogram.
Means ± s.e.m. (number of rats); intact euthermic rats, N=6; euthermic rats with bilateral vagotomy, N=7; set 1 animals. *P<0.05, **P<0.001 *vs* 37°C; †P<0.05 *vs* intact euthermic rats at the same T_b (ANOVA).

Table 4. *In vivo* and *ex vivo* values for perfused hearts at 37°C from control and CA rats

	Control (6)	CA (6)
In vivo		
M_b (g)	281±5	286±9
Heart mass (g)	1.51±0.05	1.82±0.09*
Heart/M_b (%)	0.54±0.01	0.64±0.03**
f_H (beats min^{-1})	430±15	418±18
MABP (kPa)	14.1±0.7	17.8±0.5**
RPP (kPa min^{-1})	4118±309	3898±204
Ex vivo		
f_H (min^{-1})	308±11	268±17
Estimated EDV (μl)	313±11	377±6***
Developed pressure at 300 μl Balloon volume (kPa heart^{-1})	13.0±0.8	9.8±0.8
Developed pressure at EDV (mmHg)	13.0±0.8	9.4±1.3*
RPP at EDV (kPa min^{-1} heart^{-1})	3582±98	2258±318**

f_H, heart rate; MABP, mean arterial blood pressure; M_b, body mass; RPP, rate–pressure product; EDV, end diastolic volume.

Means ± s.e.m. (number of rats); set 2 animals. *P<0.05, **P<0.01, ***P<0.001 *vs* control rats at the same T_b/perfusate temperature (ANOVA).

rats (P<0.01; Table 4). Heart mass was significantly increased following CA (P<0.05), showing a 20% increase relative to M_b (P<0.01; Table 4). Following removal of the heart, *ex vivo* HRV showed no effect of CA when measured at 37°C. For example, R–R interval, NN5 (R–R intervals with a greater than 5 ms difference from the prior R–R interval), total spectral power and LF/HF ratio were 212.9±7.8/217.9±12.2 ms, 6.0±2.5/3.6±1.9%, 2.41±0.61/2.26±0.57 ms^2 and 0.20±0.04/0.27±0.03 for control/CA hearts, respectively (N=6, all n.s.).

As T_b does not significantly change in non-hibernating mammals on chronic cold exposure (Deveci and Egginton, 2007), subsequent data for perfused hearts probably reflect the consequences of cardiac remodelling, with the lower intrinsic f_H revealing a positive chronotropic autonomic influence *in vivo* of ~35%. Both f_H and peak developed pressure were unaffected by CA; however, developed pressure at end diastolic volume (EDV) was decreased in CA hearts *ex vivo* (P<0.05; Table 4). Moreover, RPP estimated at EDV was 40% less than control following CA (P<0.01; Table 4).

For both control and CA rat hearts, left ventricular developed pressure increased with increasing balloon volume (Fig. 2A). For CA rats, the diastolic performance was displaced to the right of the control curve, indicative of an increased diastolic volume required to generate an equivalent DP. Indeed, EDV estimated from linear regression of the DP curve was increased by 20% in CA rats (P<0.001; Table 4). Developed pressure reached a peak at a balloon volume of 250 and 350 μl for control and CA rats, respectively, with the former developing significantly greater peak pressure than the latter (P<0.01; Fig. 2B). The rate of change of pressure (+dP/dt) reached a peak at 250 and 400 μl balloon volume for control and CA hearts, respectively (both close to the respective EDV; Fig. 3A). At all points measured, RPP was lower in hearts from CA than from control rats. While peak RPP was achieved at the estimated EDV, for CA hearts this was one-third lower than that of control rat hearts (P<0.001; Fig. 3B).

Cardiac morphometrics and metabolism

Accompanying an increased heart mass, a trend for a cold-induced increase in capillary density was suggested in the epicardium (P<0.09), but not endocardium or papillary (control 2110±40 *vs* CA 2159±40 mm^{-2}) muscle (Fig. 4). However, the changes were modest overall (1880±47 *vs* 1950±50 mm^{-2} for control and CA hearts, respectively, n.s.) and had no significant effect on the calculated diffusion distances: 11.9±0.7 *vs* 11.5±0.1 μm (epicardium), 11.4±0.4 *vs* 11.2±0.1 μm (endocardium) and 10.8±0.3 *vs* 10.7±0.5 μm (papillary) for control and CA hearts, respectively. In contrast, elevated coronary flow (Table 1) suggests that potential oxygen delivery is increased on hypothermia following CA, possibly reflecting a longer diastolic interval.

In vitro maximal enzyme activities showed no change in overall oxidative capacity (CS), but an increase in the capacity for fatty acid metabolism (P<0.05; HAD) and reduced glycolytic capacity (P<0.05; PFK) on cold acclimation (Fig. 5). Consequently, the relative anaerobic *vs* aerobic capacity (PFK:CS ratio) was decreased (P<0.05), and the potential contribution of β-oxidation to aerobic metabolism (HAD:CS) was increased (P<0.01) for CA hearts (data not shown). LDH showed no change (Fig. 5), resulting in a LDH:PFK ratio of 8.43±0.91 and 15.36±1.47 for control and CA hearts, respectively (P<0.01).

DISCUSSION

Preservation of haemodynamic and cardiac performance at core temperatures different from the normal hypothalamic set point may offer a selective advantage for animals to which such excursions are unavoidable. However, the majority of mammals, including the rat, defend T_b and hence must maintain cardiovascular performance to permit essential activity such as foraging. We demonstrate that in the rat, acute cooling of T_b depresses cardiovascular performance

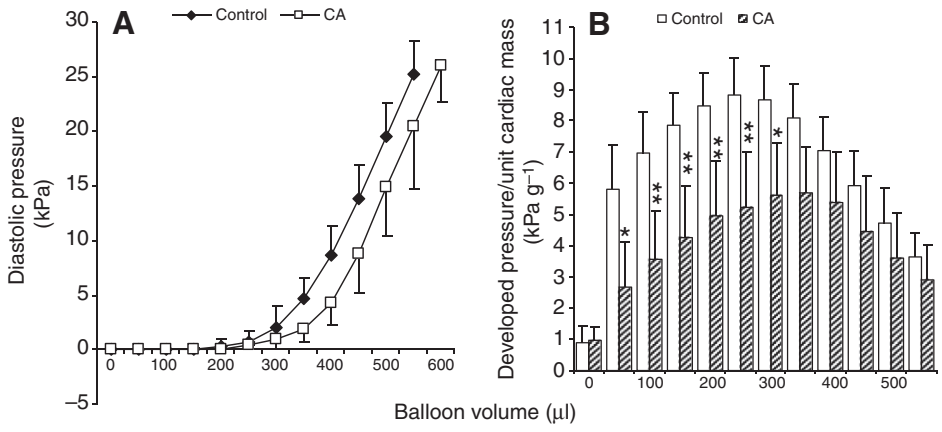

Fig. 2. Developed pressure (A) and cardiac performance (B) estimated at a fixed coronary perfusion pressure of 100 cm H_2O at 37°C, developed against a fluid-filled intra-ventricular balloon (set 2 animals, N=6). *P<0.05, **P<0.01 *vs* 0 μl balloon volume.

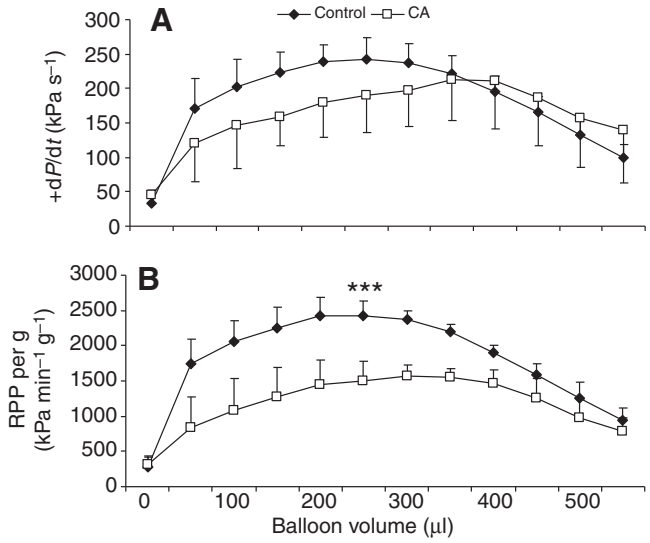

Fig. 3. Cardiac contractility (+d*P*/d*t*) and rate–pressure product (RPP) for *ex vivo* hearts at 37°C from control and CA rats. Means ± s.d. (set 2 animals, *N*=6). ***P<0.001 *vs* 0 μl balloon volume.

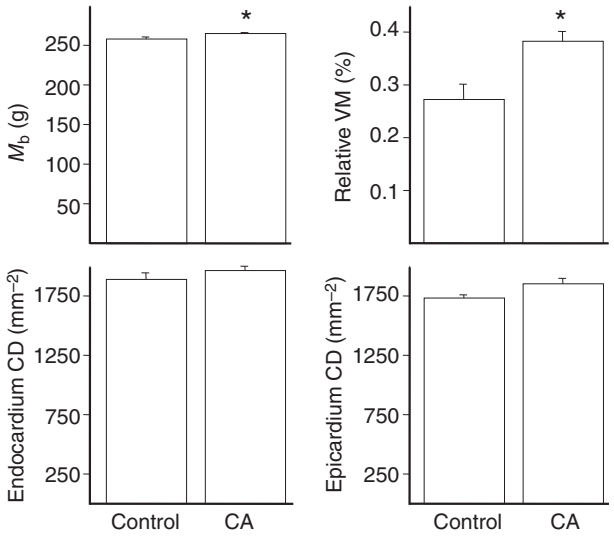

Fig. 4. Changes in heart mass and ventricular capillary density (CD) in control and CA rats. VM, ventricular mass; Endo, endocardium; Epi, epicardium. Means ± s.e.m. (set 3 animals, *N*=6). *P<0.001 *vs* control.

in vivo, despite eliciting additional autonomic drive, and that this is ameliorated to only a limited extent by cold acclimation. Moreover, performance of the isolated perfused heart suggests that hypertrophy of cardiac muscle on CA provides incomplete compensation for the enhanced demand.

Physiological responses to acute hypothermia

The observed *in vivo* hypothermic hypotension of euthermic rats on acute cooling to T_b=25°C may be expected from impaired myocardial performance and/or vascular tone at low temperatures. Consequently, the reduced cardiac output parallels a change in f_H that was only partially compensated by a Frank–Starling-mediated increase in stroke volume. This probably reflects the small decrease in cardiac work on acute cold exposure. An increased pulse pressure in response to acute cooling was largely due to a decreased diastolic pressure, consistent with a generalised decrease in vascular tone leading to decreased total peripheral resistance (TPR). However, there was no change in TPR in the current study, possibly because of an elevated catecholamine titre (Barney et al., 1980; Depocas and Behrens, 1978), suggesting that impaired myocardial relaxation underlies poor performance. An increased coronary blood flow without any adjustment in TPR implies an active feedback to improve oxygen/substrate delivery during an acute thermal challenge, a response that would be aided by the prolonged diastolic period, allowing greater coronary perfusion.

A reduction in both frequency and power of f_H power spectra is consistent with the known depressive effects of cooling on biological rate functions, identifying an accommodation of, rather than central defence against, hypothermia. The decreased LF power, associated with a decrease in total power, is not paralleled by changes in HF power, leading to a 25% reduction in the LF/HF ratio. This index of sympathovagal balance shows that the relative preservation of HF in the cold results from a differential maintenance of parasympathetic *vs* sympathetic drive. ECG waveform analysis shows that impaired cardiac performance in the cold is unlikely to be due to altered signal conductance, with a ~50% decrease in f_H being matched by 30–60% increase in all cardiac intervals measured, and must therefore be due to either poor electrical excitability of cardiac myocytes or reduced contractility.

A reflex bradycardia is usually associated with greater vagal tone and increased HRV, but vagotomy prior to acute cooling was without significant effect on heart rate *in vivo*, or elements of the cardiac cycle identified from the ECG, confirming the findings of low vagal tone in anaesthetised rats (Fewell et al., 2007; Matthew et al., 2002). However, vagotomy enhanced the prolongation of QRS and S–T intervals, implicating a vagal contribution to ventricular repolarisation during a hypothermic challenge.

The absence of compensation in cardiac performance following acute cold exposure is therefore unlikely to result from an inability to remove the minimal, inhibitory chronotropic influence of the vagus. This preparation does not allow us to eliminate the effects of anaesthesia completely, although previous studies imply minimal effects on vagal tone following chloralose–urethane anaesthesia

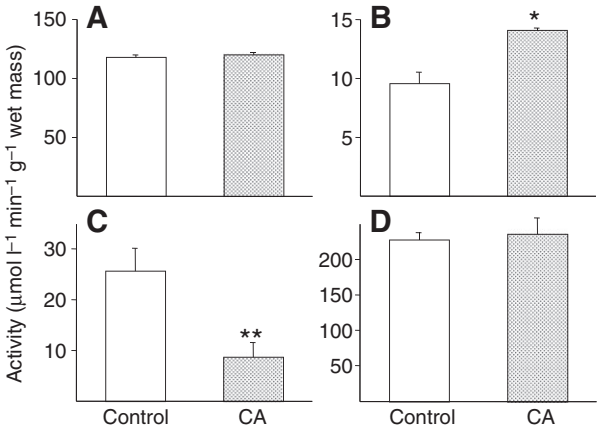

Fig. 5. Changes in activity of enzymes of intermediary metabolism in ventricles from control and CA rats: specific activity of (A) citrate synthase (CS), (B) 3-hydroxyacyl coenzyme A dehydrogenase (HAD), (C) phosphofructokinase (PFK), (D) lactate dehydrogenase (LDH). Means + s.e.m. (set 3 animals, *N*=6). *P<0.05, **P<0.01 *vs* control.

(Halliwill and Billman, 1992). In addition, examination of HRV, and particularly preservation of the HF component, supports the minimal vagal contribution to f_H control in the rat (Kuo et al., 2005). This is at odds with data from the decerebrate ferret, which show a modest vagal-induced bradycardia on cooling (Zheng et al., 1996), suggesting a species-specific response. This lack of compensation probably represents a failure to sufficiently increase sympathetic stimulation, as previous investigations report high levels of sympathetic activity for the mouse (Ishii et al., 1996) and rat (Hashimoto et al., 1999), whilst for the guinea pig (Akita et al., 2002) and vole (Ishii et al., 1996) parasympathetic activity predominates.

Effects of CA

CA only slightly increased MABP and f_H in rats at T_b=37°C, confirming previous observations (Fregly et al., 1989), although a hyperdynamic cardiovascular system was more evident in smaller (set 2) than in larger (set 1) animals. This may result from e.g. elevated cardiac contractility (stroke volume) or increased vascular tone (TPR). Catecholamine release following cold exposure may lead to increased cardiac contractility and leads to a short-lived improvement in cardiac contraction, yet over prolonged periods it leads to decrements in cardiac performance (Engelhardt et al., 1999), suggesting that both components may be involved in the maintenance of CO. However, the decrease in MABP on acute cooling was similar for control and CA rats, in agreement with our previous results (Deveci and Egginton, 2007; Sabharwal et al., 2004a; Sabharwal et al., 2004b), although the changes in f_H and stroke volume were greater following acclimation (f_H –88 *vs* –110%, stroke volume +25 *vs* +54%, respectively). This indicates a limited adaptive capacity to accommodate extra cardiac demand. Interestingly, cooling led to a loss of the relative hypertension noted for CA rats at normothermic T_b, suggesting a degree of autonomic realignment following CA.

The decreased diastolic pressure, coupled with preserved TPR and decreased f_H, might suggest prolonged aortic outflow. This is supported by the increased stroke volume in CA rats following acute cooling as a result of enhanced ventricular filling but is at odds with previous observations suggesting cooling causes increased TPR (Kondratiev and Tveita, 2006). However, this discrepancy may reflect the relatively high, but clinically relevant, set point (25°C) used for our experiments compared with previous studies, e.g. 15°C (Kondratiev and Tveita, 2006).

The decrease in cardiac index is similar among groups (70–80%), although CMW and stroke work are rather less affected following CA (–32% *vs* –9%, –66% *vs* –36%, respectively). Nevertheless, the integrated cardiovascular response shows surprisingly poor thermal adaptation, with the bradycardia on acute cooling giving rise to a similar hypotension in both control and CA rats. The relatively greater depressive effect of cold on diastolic pressure (–24% control, –44% CA) than on systolic pressure (–8% and –14%, respectively) is consistent with a compensatory increase in contractility, consistent with a rise in stroke volume, but could also be elicited by a reduced cardiac afterload. Indeed, a reduced TPR may result from an expansion of the vascular bed following CA (Deveci and Egginton, 2002).

At normothermic temperature coronary perfusion (left ventricular blood flow, LVBF; and to a lesser extent left ventricular conductance, LVC) is enhanced after cold acclimation. However, the small increase is attenuated on acute cooling, suggesting a limited capacity for vascular dilatation. The 5-fold lower hyperaemia in CA rats on acute cooling is in part a reflection of the higher flow at 37°C, which is not consistent with an attenuated depression of CMW, suggesting additional feedback control. Intriguingly, the increased LVBF in response to acute cooling in control rats, most likely reflecting an impaired vasoconstriction, was attenuated in CA rats, indicating a partial recovery of vasomotor tone with acclimation (Brown et al., 1993), probably reflecting tissue-specific decreases in vascular resistance (conductance is increased by 155% and 95%, respectively). Interestingly, TPR is ~15% lower after CA, and drops a similar amount on cooling, such that hypothermic CA rats had a TPR some 30% lower than that of euthermic controls. This suggests that coronary perfusion is being actively moderated, analogous to functional hyperaemia in skeletal muscle.

However, the sympathovagal balance is altered in CA animals (increased LF/HF), suggesting an adaptive realignment of central cardiovascular drive towards a relatively greater sympathetic tonus that may aid a reduced thermal sensitivity (lower Q_{10}) during acute cold exposure. Our data support the presence of a high degree of sympathetic tone in the rat that is unaltered following CA at T_b=37°C, while acute cooling reveals a degree of compensation. A profound decrease in sympathetic tone was noted for control rats at T_b=25°C, which was attenuated in CA rats, implying better preservation of autonomic control on acute cooling, as also indicated by the relatively preserved LF/HF ratio for CA *vs* control rats. Although LF/HF is readily accepted as a measure of sympathovagal balance, we do acknowledge that under different physiological conditions vagal modulation could significantly alter LF (Fazan et al., 2005; Jokkel et al., 1995; Chapleau and Sabharwal, 2011). However, in this study the changes in LF/HF are primarily the result of a reduction in T_b, as on rewarming the ratio of LF/HF was returned to pre-cooling levels; this is further supported by blood pressure variability estimates showing similar restoration. Indeed, the decrease in blood pressure variability on cooling is blunted in CA rats, suggesting partial adaptive compensation. Cardiorespiratory integration is maintained at 25°C, confirming previous observations that this is a safe T_b from which to elicit recovery, as evident by the return to baseline values on rewarming in both groups. This response indicates a lack of differential thermal sensitivity among cardiac and ventilatory control in an animal that defends T_b, but this may not be the case in animals that habitually experience a reduction in T_b (Deveci and Egginton, 2007).

We document increased ventricular mass but preservation of capillary density, an observation not noted for pathological models of hypertrophy (Bishop et al., 1996; Rakusan et al., 1992). We cannot exclude angiogenesis as a contributing factor to the preservation of capillary density in the face of increased heart mass, but without data for capillary:fibre ratio or endothelial proliferation markers this cannot be proved, although the current data are consistent with an elongation of pre-existing muscle fibres with preservation of capillary perfusion distances (Bishop et al., 1996; Kayar and Weiss, 1992). This is also supported by increases in estimated EDV, suggesting elongation of ventricular myocytes rather than concentric hypertrophy, which is a proven stimulus for angiogenesis (Hudlicka et al., 1992). While there is little influence of remodelling on intracardiac diffusion distances, an increased coronary blood flow associated with a prolonged diastolic period may be sufficient to increase substrate delivery, consistent with a greater oxidative capacity.

LVBF was increased in CA rats despite preserved myocardial capillary density, which therefore represents a decreased vascular resistance in the heart not revealed by estimates of TPR. This implies a decreased coronary flow reserve (greater proportion of capillaries perfused in CA hearts) (Tsagalou et al., 2008) in order to

accommodate metabolic demands of the myocardium. Previous work has shown that *ex vivo* fatty acid oxidation for perfused hearts is unaffected by CA (Cheng and Hauton, 2008), whereas HAD activity was higher in hearts from CA than from control rats. However, HAD activity is derived from optimised reaction conditions to give an estimate of maximal capacity for β-oxidation. That this is not realised *ex vivo* suggests the rate-limiting step for β-oxidation is upstream of this point, at the outer mitochondrial membrane, and regulated through malonyl-CoA-mediated inhibition of carnitine palmitoyl-transferase 1 (CPT1). Western blot analysis has previously documented that, despite hypertrophy, cardiac CPT1 levels are unchanged by CA (Cheng and Hauton, 2008), confirming the importance of fatty acid flux into the mitochondria rather than individual enzyme activities.

The role of circadian variation in setting levels of sympathetic activity may be a confounding factor in this. Telemetry in rats revealed that sympathetic tone is elevated at night (Hashimoto et al., 1999), and as CA involved increasing the duration of darkness in the acclimation process, we cannot exclude the influence of photoperiod on our observations. Nevertheless, this presumably represents a normal adaptation to the winter for which day length is one of multiple contributing factors, and previous studies that have not accounted for this may report a temperature effect that is not physiologically relevant. An additional consideration is the combined effect of cold and short daylength, as experienced in nature. Reduced photoperiod has been shown to increase sarcoplasmic reticulum calcium content in a hibernator, Siberian hamster (Dibb et al., 2005), although whether the lack of such adaptive changes in the non-hibernator rat represents a component of the incomplete adaptation remains to be clarified.

Intrinsic *vs* extrinsic factors responsible for CA

We show that there is a physiological response to acute cooling, and this to a limited extent is affected by tissue remodelling and autonomic drive. To examine the extent to which this also reflects phenotypic changes in the myocardium, *ex vivo* cardiac performance was determined.

The modest (~15%) slowing of intrinsic f_H is unlikely to play a significant role in *in vivo* cardiovascular performance, but may become important after prolonged CA, e.g. in hibernators. *In vivo* HRV in the rat is low and preserved despite cooling to a new set point (T_b=25°C), with similar observations for isolated perfused hearts (T_b=27°C) (Langer et al., 1999), suggesting this may be an intrinsic characteristic of the sinoatrial node rather than a physiological response to cooling. Supporting this contention, HRV following Langendorff perfusion was entirely derived from the sinoatrial node and extremes of preload or afterload were without effect on HRV for the perfused heart (data not shown), demonstrating that periodicity of sinoatrial node discharge was maintained at low temperatures.

The preservation of both CO and f_H implies that stroke volume is preserved, despite the modest increase in estimated EDV. Ventricle stiffness, inferred from the gradient of the diastolic performance curve, appears to be unchanged by CA, and therefore decreases in developed pressure/RPP are likely to be a consequence of reduced acto-myosin cross-links for increased length sarcomeres. This will preserve stroke volume at the expense of greater residual volume in the ventricle but result in reduced force development; hence, a diminished RPP:CA led to a reduced developed pressure that was only partially recovered by β-adrenergic stimulation (dobutamine), suggesting a down-regulation of adrenoreceptor reserve (Cheng and Hauton, 2008) through prolonged catecholamine exposure (Benjamin et al., 1989; Matthews et al., 1996). The poor developed pressure, however, is in contrast to the elevated MABP seen *in vivo*, suggesting that intrinsic weakness of the myocardium is compensated for physiologically *in situ*. Part of this response includes elevated coronary flow, i.e. hearts are relying on increased delivery by perfusion but not diffusion, yet this was not preserved in the *ex vivo* perfused heart (data not shown), which may be the result of the 'constant pressure' perfusion technique used.

That coronary flow reserve was not preserved in *ex vivo* perfused hearts (data not shown) may also explain the inability to further increase LVBF on *in vivo* cooling in control rats, as previously noted for other examples of cardiac hypertrophy (Knaapen et al., 2008). For the *ex vivo* heart, specific coronary flow ($ml\,g^{-1}$ tissue) was preserved when perfused at constant pressure, although these experimental conditions rely on the interplay between local humoral factors to control perfusate flow rather than the integrated feedback system operating *in vivo*. Interestingly, perfused rat hearts retain the ability to auto-regulate coronary perfusion pressure at low temperature (17°C) yet are more sensitive to temperature change compared with a hibernator, Richardson's ground squirrel (Burlington et al., 1989).

Estimates of *ex vivo* performance suggest that CA led to cardiac hypertrophy characterised by a large, mechanically inefficient heart (when parameters are expressed relative to heart mass), showing an increased EDV indicative of the 'volume overload' form of hypertrophy (Heroux and Pierre, 1957; Roukoyatkina et al., 1999). Previous studies with CA noted enhanced catecholamine release (Depocas and Behrens, 1978), coupled with increased diurnal activity and raised f_H (Chambers et al., 2000; Ishii et al., 1996), as potential initiators of hypertrophy. Preservation of developed pressure at EDV, indicative of the operating characteristic of hearts under basal contraction (Fletcher et al., 1981), suggests an adaptation to cold for the volume-dilated myocardium (Cheng and Hauton, 2008). This is further supported by use of angiotensinogen protein-null (Sun et al., 2003) and the angiotensin I receptor-null mice (Sun et al., 2005), where MABP was diminished for both transgenes exposed to chronic cold, but cardiac hypertrophy was attenuated. However, this hypertrophy may be an overall neutral effect as RPP is preserved *in vivo*, implying that although intrinsic cardiac performance may be limiting in CA rats, it is sufficient for the cardiovascular demands during cold exposure so long as a normal T_b (37°C) is preserved.

Concluding remarks

These data suggest that the integration of plasticity, rather than the adaptive response of component elements, is the key to cardiovascular accommodation of chronic exposure to a cold environment. The poor mechanical performance of the isolated *ex vivo* perfused heart demonstrates an incomplete adaptation that preserves adequate cardiovascular performance during euthermia, but would offer little additional advantage if adopting a new, lower thermal set point. In addition, the apparent modest increases in EDV led to diminished cardiovascular performance when estimated for rats at rest, and this will be further compromised by inclusion of additional stressors such as locomotion. This is critical for the rat as defence of T_b would promote the ability to forage in a time of food shortage. Yet, enhanced sympathetic activity is necessary to facilitate even this degree of accommodation, as myocardial remodelling alone is insufficient to preserve cardiac performance. By extrapolation, these findings imply that multi-component therapy is required for rescue from induced or accidental hypothermia, possibly including non-catecholamine inotropes such as calcium sensitisers. In addition, hypothermic

preconditioning has recently been shown to improve post-ischaemic haemodynamic recovery, as a result of reduced accumulation of reactive oxygen species (Khaliulin et al., 2007), suggesting that such changes may have adaptive benefit with inadequate thermoregulation.

LIST OF ABBREVIATIONS

CA	cold acclimation
CMW	cardiac minute work
CO	cardiac output
CoA	coenzyme A
CS	citrate synthase
ECG	electrocardiogram
EDV	end diastolic volume
f_H	heart rate
f_V	ventilation frequency
HAD	3-hydroxyacyl coenzyme A dehydrogenase
HF	high frequency
HRV	heart rate variability
LDH	lactate dehydrogenase
LF	low frequency
LVBF	left ventricular blood flow
LVC	left ventricular conductance
MABP	mean arterial blood pressure
M_b	body mass
P	pressure
PFK	phosphofructokinase
PSA	power spectral analysis
P_{sat}	saturation pressure for MABP
P_{thr}	threshold pressure for MABP
RPP	rate–pressure product
t	time
T_b	core temperature
TPR	total peripheral resistance
VLF	very low frequency
V_T	tidal volume

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