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Functional properties and responses to ischaemia–reperfusion in Langendorff perfused mouse heart

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Despite minimal model characterisation Langendorff perfused murine hearts are increasingly employed in cardiovascular research, and particularly in studies of myocardial ischaemia and reperfusion. Reported contractility remains poor and ischaemic recoveries variable. We characterised function in C57/BL6 mouse hearts using a ventricular balloon or apicobasal displacement and assessed responses to 10–30 min global ischaemia. We examined the functional effects of pacing, ventricular balloon design, perfusate filtration, [Ca^{2+}] and temperature. Contractility was high in isovolumically functioning mouse hearts (measured as the change in pressure with time (+d*P*/d*t*), 6000–7000 mmHg s^{-1}) and was optimal at a heart rate of ~420 beats min^{-1}, with the vasculature sub-maximally dilated, and the cellular energy state high. Post-ischaemic recovery (after 40 min reperfusion) was related to the ischaemic duration: developed pressure recovered by 82 ± 5%, 73 ± 4%, 68 ± 3%, 57 ± 2% and 41 ± 5% after 10, 15, 20, 25 and 30 min ischaemia, respectively. Ventricular compliance and elastance were both reduced post-ischaemia. Post-ischaemic recoveries were lower in the apicobasal model (80 ± 4%, 58 ± 7%, 40 ± 3%, 32 ± 7% and 25 ± 5%) despite greater reflow and lower metabolic rate (pre-ischaemic myocardial O_2 consumption ($\dot{V}_{O_2,myo}$) 127 ± 15 *vs.* 198 ± 17 μl O_2 min^{-1} g^{-1}), contracture, enzyme and purine efflux. Electrical pacing slowed recovery in both models, small ventricular balloons (unpressurised volumes < 50–60 μl) artificially depressed ventricular function and recovery from ischaemia, and failure to filter the perfusion fluid to < 0.45 μm depressed pre- and post-ischaemic function. With attention to these various experimental factors, the buffer perfused isovolumically contracting mouse heart is shown to be stable and highly energised, and to possess a high level of contractility. The isovolumic model is more reliable in assessing ischaemic responses than the commonly employed apicobasal model. *Experimental Physiology* (2001) **86.6**, 703–716.

Perfused rodent hearts are an invaluable tool in cardiovascular research. Recently the mouse has become a popular model owing to developments in murine transgenic technology. Due to its small size the perfused mouse heart is technically challenging, and validated and improved models for assessment of phenotypic outcomes of transgenic manipulations are essential. While other isolated heart models have been extensively characterised (contributing to their value), there has been no adequate characterisation of the isovolumic Langendorff perfused mouse heart. It is not surprising that such fundamental parameters as intrinsic heart rate, left ventricular systolic pressure, ±d*P*/d*t* and coronary flow are all unacceptably variable. For example, estimates of contractility are quite low in previous studies (Galinanes & Hearse, 1990; Chen *et al.*. 1998; Kameyama *et al.*. 1998; Sato *et al.*. 1998; Bartfay *et al.*. 1999; Flogel *et al.* 1999; Hampton *et al.* 2000). Even in recent studies in isolated murine hearts, levels of ventricular contractility have been reported to be below 25–30% of *in situ* values (Pazos-Moura *et al.* 2000; Tardiff *et al.* 2000; Zhao *et al.* 2000; MacGowan *et al.* 2001). Greater efforts are essential to define the normal range of cardiac function in different murine models (Kass *et al.* 1998), and to develop improved models with which to study cardiovascular physiology, pharmacology and biochemistry.

In terms of ischaemia and reperfusion, excessively variable responses have also been reported in different murine preparations (Galinanes & Hearse, 1990; Marber *et al.* 1995; Plumier *et al.* 1995; Radford *et al.* 1996; Li *et al.* 1997; Sumeray & Yellon, 1998; Xi *et al.* 1998; Flogel *et al.* 1999; Kang *et al.* 1999; Headrick *et al.* 2000*a*; Sumeray *et al.* 2000; Yoshida *et al.* 2000; Zhao *et al.* 2000). Only one group has attempted to characterise the effects of ischaemia, and the relation between ischaemic duration and tissue injury, in the mouse heart (Sumeray & Yellon, 1998). It is unfortunate that these investigators employed unloaded empty hearts with

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function assessed via apicobasal displacement, and up to 8-fold differences in functional recoveries (following identical ischaemic periods) have been reported in this particular preparation (Marber *et al.* 1995; Plumier *et al.* 1995; Yoshida *et al.* 1996, 2000; Sumeray & Yellon, 1998; Xi *et al.* 1998; Sumeray *et al.* 2000). This contrasts with the perfused rat heart, for example, which has been extensively characterised and generally displays highly reproducible responses (De Leiris *et al.* 1984; Galinanes & Hearse, 1990).

Due to the variability in baseline function and ischaemic responses, and since it is essential that perfused mouse heart models are effectively characterised, we wished to document and optimise functional properties of the Langendorff perfused isovolumically contracting mouse heart, and to study the effects of varying periods of global ischaemia and reperfusion. We compared ischaemic responses with those for hearts functionally assessed via apicobasal displacement (a more commonly employed variant).

METHODS

Langendorff perfused murine heart model

The following investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). Hearts were isolated from 7- to 14-week-old wild-type C57/BL6 mice (26.0 ± 1.2 g, body weight; 136 ± 8 mg, wet heart weight; $n = 168$) anaesthetised with 60 mg kg^{-1} sodium pentobarbital administered intraperitoneally. A thoracotomy was performed and hearts were excised into ice-cold perfusion fluid. The aorta was cannulated under magnification and the coronary circulation perfused at 80 mmHg with modified Krebs-Henseleit buffer containing (mM): NaCl 120, $NaHCO_3$ 25, KCl 4.7, $CaCl_2$ 2.5, Mg_2SO_4 1.2, KH_2PO_4 1.2, glucose 15 and EDTA 0.5. Buffer was equilibrated with 95% O_2–5% CO_2 at 37 °C, giving a pH of 7.4 and $P_{O_2} \geq 600$ mmHg at the aortic cannula over a 1–5 ml min^{-1} flow range. Buffer was initially passed through a 5.0 μm filter on preparation, and all perfusate delivered to hearts was filtered via an in-line 0.45 μm Sterivex-HV filter (Millipore, Bedford, MA, USA) to continuously remove microparticulates. The left ventricle was vented with a polyethylene apical drain and hearts were instrumented for functional measurements as outlined below. Hearts were then immersed in warmed perfusate in a jacketed bath maintained at 37 °C. Temperature of the perfusion fluid was continuously assessed by a needle thermistor at the entry into the aortic cannula, and the temperature of the water bath was assessed using a second thermistor probe. Temperatures were recorded using a 3-channel Physitemp TH-8 digital thermometer (Physitemp Instruments Inc., Clifton, NJ, USA).

In some hearts myocardial O_2 consumption ($\dot{V}_{O_2,myo}$) was calculated from arterial (perfusate) and venous (pulmonary arterial) fluid samples obtained in a gas-tight syringe and analysed for O_2 content on a Corning 278 blood-gas analyser, as described by us previously (Headrick *et al.* 1991, 1998*b*). $\dot{V}_{O_2,myo}$ (ml O_2 min^{-1} g^{-1}) was calculated as:

$$\dot{V}_{O_2,myo} = (P_{O_2,a} - P_{O_2,v}) \times \text{coronary flow} \times c/760,$$

where $P_{O_2,a}$ and $P_{O_2,v}$ are the partial pressures of O_2 in the arterial and venous effluent samples, respectively, and c is the solubility of O_2 in Krebs-Henseleit solution (22.7 μl O_2 atm^{-1} ml^{-1} at 37 °C).

Measurement of isovolumic contraction via intraventricular balloon

For assessment of isovolumic function fluid-filled balloons were constructed of readily available pliable polyvinyl chloride plastic film (Saran wrap) tied with a 4–0 suture onto a short length of polyethylene tubing. The plastic film used was initially stretched over a syringe tip to give a spherical form to the balloon. Balloons were ~9 mm in length when deflated, and only balloons yielding pressures less than 2 mmHg at an inflated volume of 60 μl were employed (unless otherwise stated). Deflated balloons, connected to a P23 XL pressure transducer (Viggo-Spectramed, Oxnard, CA, USA) by fluid-filled polyethylene tubing, were inserted into the left ventricle via the atrium to provide measurement of ventricular pressure (Headrick *et al.* 2000*a*; Peart & Headrick, 2000). Balloon volume was controlled by a calibrated Hamilton 500 μl threaded plunger syringe (Hamilton Co, Reno, NV, USA) and was initially increased to give an end-diastolic pressure of 5 mmHg. Coronary flow rate was monitored via a Doppler flow probe (1N probe; Transonic Systems Inc., Ithaca, NY, USA) in the aortic perfusion line, connected to a T206 Transonic flowmeter. Functional data were recorded at 1 kHz on a 4-channel MacLab data acquisition system (ADInstruments, Castle Hill, Australia) connected to an Apple computer. Ventricular pressure was digitally processed to yield peak systolic pressure, diastolic pressure, $\pm dP/dt$ and heart rate. The rate–pressure product was calculated as the product of left ventricular developed pressure × heart rate (Peart & Headrick, 2000).

We assessed the undamped natural frequency and damping coefficient of the ventricular balloon, tubing and transducer by measuring the transient step response, as described by Peura (1998). This gave a damping coefficient of 0.51, slightly less than the optimal value of 0.64, and a high undamped natural frequency of 110 Hz, indicating a flat frequency response to 96.8 Hz with accurate measurement of ventricular pressures to 9.7 Hz heart rate (Grossman, 1986).

Measurement of contractile function via apicobasal displacement

In hearts with function assessed by apicobasal displacement, the left ventricle was vented with a polyethylene drain and a fine stainless steel hook attached to the ventricular apex and connected to a Grass FT03C strain gauge (Grass Instruments, Quincy, MA, USA), as described by us recently (Matherne *et al.* 1997; Morrison *et al.* 2000). Transducer position was adjusted using a micromanipulator to give a diastolic tension of 1.0 g. Coronary flow was measured as described above and all functional data (apicobasal displacement, perfusion pressure and coronary flow) were recorded at a sampling rate of 1 kHz on a 4-channel MacLab data acquisition system. Apicobasal displacement was digitally processed to yield peak systolic tension, diastolic tension, change in tension with time ($\pm dT/dt$) and heart rate. The rate–tension product was calculated as the product of developed tension × heart rate (Matherne *et al.* 1997; Morrison *et al.* 2000).

Left ventricular pressure–volume relationships

End-diastolic and systolic pressure–volume relationships (EDPVRs and ESPVRs, respectively) for the left ventricle were determined as indicators of ventricular compliance and elastance according to the methods outlined by Kameyama *et al.* (1998) for mouse hearts and Wannenburg *et al.* (1992) for rat hearts. Briefly, measurements of ventricular pressures were made at varying balloon volumes in stabilised mouse hearts ($n = 6$). Balloon volume was increased until contractile function could be

detected. This value was set as zero volume. Balloon volume was increased from this value in 5.3 μl increments (5.3 μl per revolution using the calibrated syringe) until left ventricular developed pressure plateaued and then began to decline. Functional parameters were measured after 2 min at each volume, and the ESPVRs and EDPVRs determined. The slope of the ESPVR normalised to ventricular mass was calculated as an indicator of myocardial contractility or elastance (E_{max}, mmHg g ml^{-1}), as described previously (Wannenburg *et al.* 1992; Kameyama *et al.* 1998). Slope was determined by non-linear regression of raw data from individual experiments, using the equation provided by Wannenburg *et al.* (1992), and also by linear regression (for comparison). Linear and non-linear curve-fitting routines in the Statistica software package (Statsoft, Tulsa, OK, USA) were employed. The inverse of the slope of the EDPVR, normalised to heart mass, was calculated as an indicator of ventricular compliance or diastolic stiffness. At the end of the experiments hearts were removed, the atria dissected away and ventricular myocardium carefully dissected under magnification into left and right ventricles. Blotted weights for each ventricle were then measured. It is noted that left ventricular contractility assessed via the 'steady-state' analysis described above may slightly exceed that measured using single perturbed beat techniques, owing to changes in cell stretch- and length-dependent activation in the seconds to minutes immediately after a volume change. Nonetheless, the technique as used here and elsewhere (Wannenburg *et al.* 1992; Kameyama *et al.* 1998) permits comparison of ventricular elastance and stiffness at different times within the same heart (i.e. pre- *versus* post-ischaemia).

Measurement of myocardial metabolites

For determination of intracellular metabolic status in normoxic heart, myocardial ATP, ADP, 5′-AMP, phosphocreatine and creatine levels were determined in hearts (n = 6) stabilised for 30 min before being freeze-clamped in tongs cooled in liquid N_2. Powdered frozen tissue was extracted in 0.6 M perchloric acid and neutralised samples were analysed by HPLC as outlined by us in detail previously (Harrison *et al.* 1998; Headrick *et al.* 1998*a*,*b*). Concentrations were calculated per gram wet weight and converted to millimolar based upon an estimated myocardial cytosolic volume of 0.47 ml g^{-1} (Headrick *et al.* 1998*b*). Free cytosolic [ADP] was determined from the creatine kinase equilibrium based on an intracellular pH of 7.1 and a free [Mg^{2+}] of 0.5 mM (Headrick *et al.* 1998*a*), as described by us in detail previously (Harrison *et al.* 1998; Headrick *et al.* 1998*a*,*b*). The adenylate energy charge is a commonly employed indicator of energy state and was also calculated from total tissue nucleotide levels:

$$\text{Adenylate energy charge} = \frac{0.5(2\text{ATP} + \text{ADP})}{(\text{ATP} + \text{ADP} + \text{AMP})}.$$

Measurement of enzyme efflux and purine moiety washout

To assess cell disruption, venous levels of lactate dehydrogenase (LDH) and creatine kinase (CK) were measured prior to and following 20 min ischaemia and 40 min reperfusion. LDH efflux was also measured in hearts subjected to 10, 15, 25 and 30 min ischaemia. To assess adenine nucleotide depletion venous adenosine, inosine and hypoxanthine levels were measured prior to and following 20 min ischaemia and 40 min reperfusion. Coronary effluent samples were collected from isovolumically and apicobasally contracting mouse hearts (n = 7 in all groups) and were stored at −80 °C until analysis. Commercially available spectrophotometric assays for LDH and CK (Sigma) were optimised for sensitivity. Venous levels of adenosine, inosine and hypoxanthine levels were assessed by HPLC as described by us previously (Harrison *et al.* 1998; Headrick *et al.* 1998*a*,*b*). Normoxic enzyme or purine effluxes were calculated as the product of coronary flow (ml min^{-1} g^{-1}) × effluent concentrations (U or nmol ml^{-1}, respectively). Total post-ischaemic efflux was calculated as the product of concentration reached in the reperfusion effluent (U or nmol ml^{-1}) and the total reperfusion volume (ml (g wet wt)$^{-1}$).

Experimental protocols

Stabilised hearts in which coronary flow was greater than 5 ml min^{-1} (normally due to an aortic tear), displayed poor contractile function (<100 mmHg developed pressure after stabilisation), were bradycardic (heart rate < 320 beats min^{-1}) or unacceptably arrhythmic, were excluded from the study. A series of characterisation experiments were initially undertaken in order to assess baseline functional properties of the murine model, and its response to ischaemia and reperfusion.

Baseline function and model stability was compared in groups of isovolumically and apicobasally contracting mouse hearts (n = 8 and n = 7, respectively) perfused under aerobic conditions for 120 min. Changes in heart rate, coronary flow and left ventricular developed pressure or tension over this period were determined relative to measurements made immediately after instrumentation. To examine the potential impact of perfusate filtration, baseline functional parameters were also determined in a sub-set of mouse hearts (n = 7) in which the perfusion buffer was not filtered through 5.0 and 0.45 μm filters.

Ca^{2+} sensitivity of left ventricular contractile function was assessed in hearts (n = 8) stabilised for 30 min with perfusate containing 2.0 mM free Ca^{2+} (i.e. 2.5 mM total calcium and 0.5 mM EDTA). Hearts were then switched to perfusion with perfusate containing 4.50, 3.50, 1.75, 1.25, 1.00, and 0.75 mM total Ca for periods of 5 min. The Ca^{2+} concentration, in the presence of 0.5 mM EDTA, equates to 4.0 mM, 3.0 mM, 1.25 mM, 0.75 mM, 0.5 mM, and 0.25 mM free Ca^{2+} levels, respectively, in the perfusate. Hearts were electrically paced at 400 beats min^{-1} during these experiments. The Ca^{2+} concentration required to produce 50% maximal systolic pressure (EC_{50}) was determined from individual concentration–response data (expressed in absolute units) by fitting the following 3-parameter logistic equation to data from each experiment:

$$\text{Developed pressure} = A - A/1 + ([Ca^{2+}]/EC_{50})^{\text{slope factor}},$$

where A is the maximal response (at infinite [Ca^{2+}]). The equation was fit to raw data using the Statistica data analysis program (Statsoft, Tulsa, OK, USA), and individual EC_{50} values derived from each fit.

Temperature dependence of cardiac function was assessed in hearts maintained at temperatures of 35, 36, 37 and 38 °C (n = 7 per temperature). Finally, the rate dependence of contractile function was assessed in normoxic hearts (n = 7) subjected to 2 min periods of pacing at varying heart rates, as described by Lim *et al.* (1999). In these hearts the right atrium was excised to reduce the intrinsic beating rate, and the hearts were stabilised for 30 min at a rate of 390 beats min^{-1}. Heart rate was then increased to 510 beats min^{-1} and decreased to 480, 450, 420, 360, and 330 beats min^{-1}. Contractile function was assessed after 3 min at each rate, when function was found to be stable (Lim *et al.* 1999).

To examine coronary reflow during early reperfusion, coronary flow and ventricular diastolic pressures were correlated during the initial 3 min of reperfusion in isovolumically contracting hearts subjected to 20 min ischaemia. A second group of hearts

Table 1. Baseline function in normoxic Langendorff perfused mouse hearts

	Isovolumic (n = 38)	Unfiltered (n = 7)	Apicobasal (n = 14)
Heart rate (beats min^{-1})	391 ± 11	345 ± 17 *	388 ± 12
Diastolic pressure/tension (mmHg or g)	5 ± 1	9 ± 2 *	1.0 ± 0.1
Developed pressure/tension (mmHg or g)	153 ± 5	117 ± 14 *	1.6 ± 0.1
+dP/dt/+dT/dt (mmHg s^{-1} or g s^{-1})	6620 ± 224	4270 ± 394 *	52.3 ± 4.5
−dP/dt/−dT/dt (mmHg s^{-1} or g s^{-1})	4958 ± 216	4008 ± 365 *	47.0 ± 4.6
$\dot{V}_{O_2,myo}$ (μl O$_2$ min^{-1} g^{-1})	196 ± 15	172 ± 19	129 ± 18 *
Coronary flow (ml min^{-1} g^{-1})	21.3 ± 2.4	14.5 ± 1.0*	17.5 ± 2.4

Functional parameters measured after 30 min of aerobic perfusion in un-paced hearts. Values are means ± S.E.M. * $P < 0.05$ *vs.* isovolumic mouse hearts.

Table 2. Baseline enzyme and purine efflux in normoxic Langendorff perfused mouse hearts

	Adenosine efflux (nmol min^{-1} g^{-1})	ΣAdo+Ino+ Hypo efflux (nmol min^{-1} g^{-1})	LDH efflux (U (30 min)$^{-1}$ g^{-1})	CK efflux (U (30 min)$^{-1}$ g^{-1})
Isovolumic (n = 7)	1.4 ± 0.2	3.6 ± 0.5	1.2 ± 0.4	0.3 ± 0.1
Apicobasal (n = 7)	1.8 ± 0.3	3.0 ± 0.4	1.1 ± 0.3	0.3 ± 0.1

Venous adenosine efflux and the sum of adenosine, inosine and hypoxanthine efflux (ΣAdo+Ino+Hypo efflux) were measured at the end of 30 min of aerobic perfusion. Venous efflux of enzymes was measured as the total accumulated over 30 min of normoxic perfusion prior to ischaemia. Values are means ± S.E.M. * $P < 0.05$ *vs.* isovolumic mouse hearts.

(n = 6) received 0.1 μM 2-chloroadenosine, a maximal dilatory concentration (Headrick *et al.* 2000*b*), during the initial 3 min of reperfusion in order to assess the impact of vasodilatation on the relation between flow and diastolic pressure.

To highlight the functional impact of intraventricular balloon design, a group of hearts (n = 8) was instrumented with balloons which were smaller than those normally employed. These balloons were only ~6 mm in length, yielding pressures of 4–5 mmHg at an inflated volume of 60 μl, as used elsewhere (Kameyama *et al.* 1998; Hampton *et al.* 2000). Baseline function was measured, pressure–volume relationships acquired, and the functional response to 20 min ischaemia and reperfusion assessed and compared with that for control hearts.

To examine the impact of electrical pacing on functional outcome, stabilised hearts (n = 8 for isovolumic hearts, n = 7 for apicobasal hearts) were switched to electrical pacing via silver wires attached to the right ventricle and aortic cannula. The hearts were paced using a Grass SD9 stimulator (Grass Instruments, Quincy, MA, USA) at a rate of 397 ± 8 beats min^{-1} (ventricular pacing at 20% above threshold, typically 2–3 V amplitude, 2 ms duration square pulses). Following a further 10 min of normoxic perfusion at this heart rate functional parameters were recorded and global normothermic ischaemia was produced by clamping the aortic perfusion line for 20 min followed by 40 min of reperfusion. Pacing was resumed after 2 min reperfusion (Headrick *et al.* 1998*a*, 2000*a*; Morrison *et al.* 2000). To highlight the effects of electrical pacing through the ischaemic insult itself, pacing was maintained throughout the entire experimental period in a sub-set of isovolumically functioning hearts (n = 6).

Finally, the relation between ischaemic duration and functional outcome was assessed by comparing responses to 10 (n = 7 for isovolumic hearts, n = 6 for apicobasal hearts), 15 (n = 8 for isovolumic hearts, n = 7 for apicobasal hearts), 20 (n = 8 for isovolumic hearts, n = 7 for apicobasal hearts), 25 (n = 8 for isovolumic hearts, n = 7 for apicobasal hearts) or 30 min (n = 6 for isovolumic hearts, n = 8 for apicobasal hearts) of global normothermic ischaemia followed by 40 min of aerobic perfusion.

Analysis of data

All results are expressed as means ± S.E.M. Functional parameters under baseline conditions were statistically analysed by a one-way analysis of variance. Functional responses to ischaemia–reperfusion in the different groups were analysed by multi-way analysis of variance for repeated measures. When significance was detected a Tukey's HSD *post hoc* test was employed for individual comparisons. Significance was accepted for $P < 0.05$.

RESULTS

Functional stability, baseline function and energy state

Baseline functional variables are shown in Table 1. In hearts in which the buffer was not filtered to 0.45 μm, baseline function was depressed (Table 1). Heart rate, systolic pressure and coronary flow were all reduced, and diastolic pressure elevated (Table 1). Functional stabilities for isovolumically and apicobasally contracting mouse hearts were similar. After 120 min of perfusion left ventricular developed pressure fell significantly to 74 ± 3% of baseline in isovolumic mouse heart and to 82 ± 6% in the apicobasal mouse heart. Heart rate and coronary flow did not change significantly in either model over this period.

Total intramyocardial concentrations of ATP, ADP and 5′-AMP were 9.1 ± 0.7, 2.8 ± 0.3 and 0.6 ± 0.1 mM, respectively. The phosphocreatine (PCr)/ATP ratio was 1.9 ± 0.2, and the free cytosolic [ADP] and adenylate energy

charge were calculated to be 61 ± 8 μM and 0.85 ± 0.07, respectively. Venous concentrations of adenosine after 30 min stabilisation were 60–70 nM, and normoxic purine effluxes were similar in the two isolated heart models (Table 2).

Functional effects of volume, Ca^{2+}, temperature and pacing rate

Functional dependence on ventricular volume is shown in Fig. 1*A*. Elevations in volume from zero to ~60 μl resulted in a steep rise in systolic and developed pressure. Non-linear regression analysis of the ESPVR yielded an E_{max} of 1468 ± 87 mmHg g ml^{-1}. Linear regression of the ESPVR yielded a lower E_{max} of 897 ± 112 mmHg g ml^{-1}. Ventricular diastolic compliance was calculated to be 28.7 ± 3.9 μl mmHg g ml^{-1}. The relation between developed pressure and diastolic pressure shows optimal force development at 4–6 mmHg diastolic load (Fig. 1*B*).

In hearts instrumented with balloons smaller than those normally employed baseline contractile function was artificially reduced. Developed pressure was only 118 ± 11 mmHg at a diastolic pressure of 6 ± 1 mmHg, and $+dP/dt$ was 4323 ± 542 mmHg s^{-1}. Figure 1*A* shows ESPVRs and EDPVRs for these hearts. Systolic pressure is artificially lower at each balloon volume and the EDPVR is steeper, increasing significantly at relatively low balloon volumes. E_{max} was calculated to be 845 ± 91 mmHg g ml^{-1} (compared with ~1500 mmHg g ml^{-1} in control hearts), and diastolic compliance was reduced to only 9.6 ± 1.3 μl mmHg g ml^{-1} (compared with ~29 μl mmHg g ml^{-1} in control hearts).

Mouse hearts were highly sensitive to changes in perfusate $[Ca^{2+}]$, with peak pressure achieved at 2–3 mM Ca^{2+} (Fig. 2*A*). Analysis of individual curves yielded an apparent EC_{50} of 1.0 ± 0.3 mM. Heart rate and ventricular pressure development were both sensitive to temperature, increasing by 32 beats min^{-1} $°C^{-1}$ and 25 mmHg $°C^{-1}$, respectively (Fig. 2*B*). Analysis of the effects of rate on contractile function revealed a positive staircase phenomenon in hearts paced at 300 to 450 beats min^{-1}, whereas contractile function declined at higher rates (Fig. 2*C*). The relation between heart rate and developed pressure in hearts perfused at different temperatures shows that effects of temperature on contractile function are only partially accounted for by associated rate changes (Fig. 2*C*).

Response to ischaemia in isolated perfused mouse hearts

Global normothermic ischaemia abolished contractile function in isovolumically and apicobasally contracting models within 2–3 min. While diastolic pressure rose consistently through ischaemia in isovolumic hearts, an initial decline in diastolic tension was observed in the apicobasal model. Times to increase diastolic pressure to 20% and 50% of peak ischaemic contracture in isovolumic hearts were ~210 and 320 s, respectively, *versus* ~700 and 850 s, respectively, in the apicobasal model (Fig. 3). The latter values were significantly longer than those for isovolumic hearts. Peak ischaemic contracture was ~75 mmHg in isovolumic hearts and ~2.0 g in the apicobasal model. Although contracture development was not altered by electrical pacing prior to but not during ischaemia (Fig. 3), studies in a sub-set of isovolumic hearts revealed that pacing during ischaemia itself markedly exaggerated contracture development. Under these conditions peak contracture was increased to 102 ± 15 mmHg, and the times to 20% and 50% of peak contracture were shortened to only 135 ± 18 and 214 ± 16 s, respectively.

Functional recovery from ischaemia in isolated perfused mouse hearts

Diastolic pressure or tension remained elevated during reperfusion following 20 min ischaemia, and contractile function was depressed in both models (Figs 4*A* and *B*).

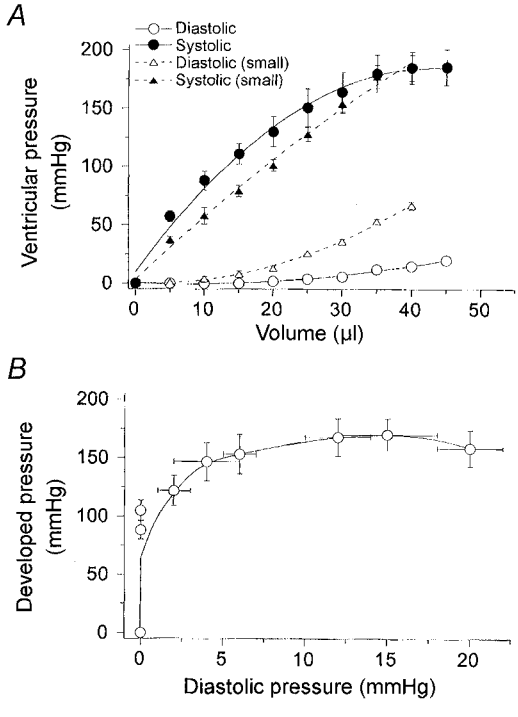

Figure 1

A, effects of variations in ventricular volume on left ventricular systolic (filled symbols) and diastolic pressures (open symbols) (n = 8). ESPVRs and EDPVRs were plotted according to non-linear regression. *B*, the relationship between diastolic pressure and left ventricular developed pressure. All values shown are means ± S.E.M.

Diastolic tone progressively fell through reperfusion to a value 15% higher than pre-ischaemia in the apicobasal model. Diastolic pressure in isovolumic hearts gradually fell to a value remaining markedly higher than pre-ischaemia (~20 mmHg) (Fig. 4*A*). Left ventricular developed pressure and tension displayed similar patterns of recovery during reperfusion, both peaking at 2–3 min of reperfusion before a transient decline at ~5 min followed by progressive recovery (Fig. 4*B*). Ultimate recovery of contractile function (expressed as a percentage of the pre-ischaemic value) was higher in isovolumically contracting hearts (~70%) *versus* apicobasally contracting hearts (~40%).

Analysis of the slopes of the ESPVRs and EDPVRs in hearts subjected to 20 min ischaemia and 40 min reperfusion showed a decline in E_{max} from a control value of ~1500 mmHg g ml^{-1} to 657 ± 85 mmHg g ml^{-1} after ischaemia ($P < 0.05$ *vs.* control). The slope of the EDPVR increased indicating a decline in ventricular compliance (or increased diastolic stiffness). Compliance declined from ~29 μl mmHg^{-1} g^{-1} in control hearts to 15.2 ± 2.6 μl mmHg^{-1} g^{-1} ($P < 0.05$ *vs.* control).

Post-ischaemic diastolic dysfunction increased, and recovery of left ventricular developed pressure or tension reduced, in paced *versus* un-paced hearts (Figs 4 and 6). It is interesting that electrical pacing during the ischaemic period itself further reduced post-ischaemic recovery. Left ventricular pressure development in this sub-set of hearts recovered to only 44 ± 5% of the pre-ischaemic value after 20 min ischaemia and 40 min reperfusion ($P < 0.05$ *vs.* hearts not paced during ischaemia). Balloon design also had an impact on apparent functional responses to ischaemia–reperfusion. In hearts instrumented with small balloons, times to 20% and 50% peak contracture were prolonged to 333 ± 19 and 409 ± 12 s, peak contracture was unaltered, and recovery of ventricular function was reduced. Ventricular developed pressure recovered to only 53 ± 5% or 60 ± 7 mmHg after reperfusion, lower than recovery determined for control hearts.

Coronary flow responses to reperfusion differed between models (Fig. 4*C*). While pronounced hyperaemia was

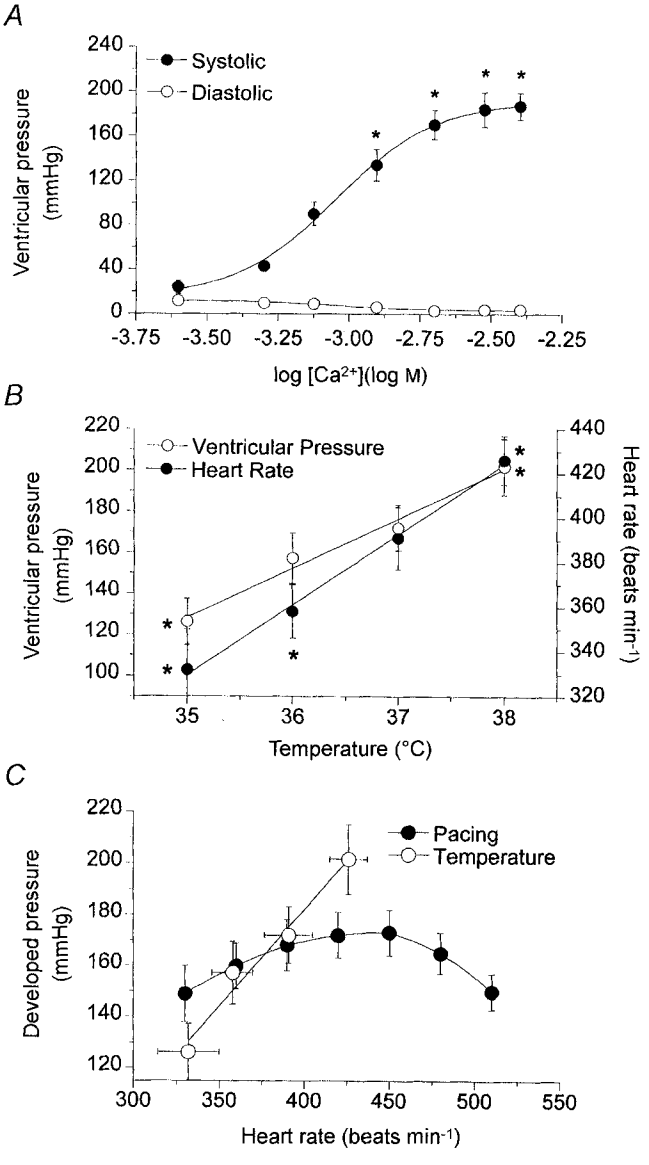

Figure 2

Functional effects of Ca^{2+}, temperature and rate. *A*, ventricular pressures are shown for hearts perfused with varying perfusate [Ca^{2+}] (n = 8). *B*, heart rate and left ventricular developed pressure are shown for hearts perfused at varying temperatures (n = 7). *C*, left ventricular developed pressure is shown in hearts paced at varying heart rates (n = 7). Also shown is the relation between ventricular pressure and rate for hearts perfused at varying temperature (from *B*). All values shown are means ± S.E.M. * $P < 0.05$ *vs.* values under control conditions (2.0 mM free [Ca^{2+}], 37 °C).

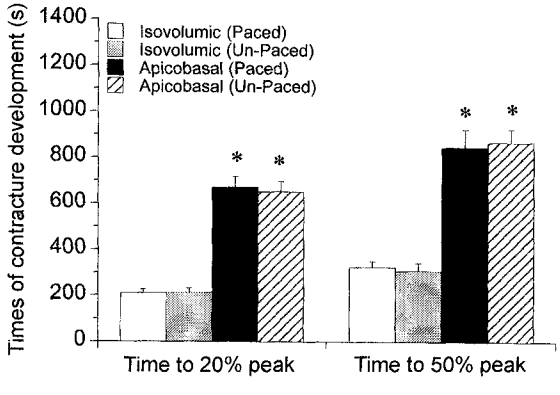

Figure 3

Times to ischaemic contracture in mouse hearts. Times were determined in isovolumically contracting C57/BL6 mouse hearts allowed to beat at the intrinsic rate (n = 8) or electrically paced (n = 8), and in C57/BL6 hearts with contractile function assessed via apicobasal displacement and which were permitted to beat at the intrinsic rate (n = 7) or were electrically paced (n = 7). Times to contracture were calculated as the times to reach 20% and 50% of the maximum ischaemic contracture. Values are means ± S.E.M. * $P < 0.05$ apicobasal *vs.* isovolumic hearts.

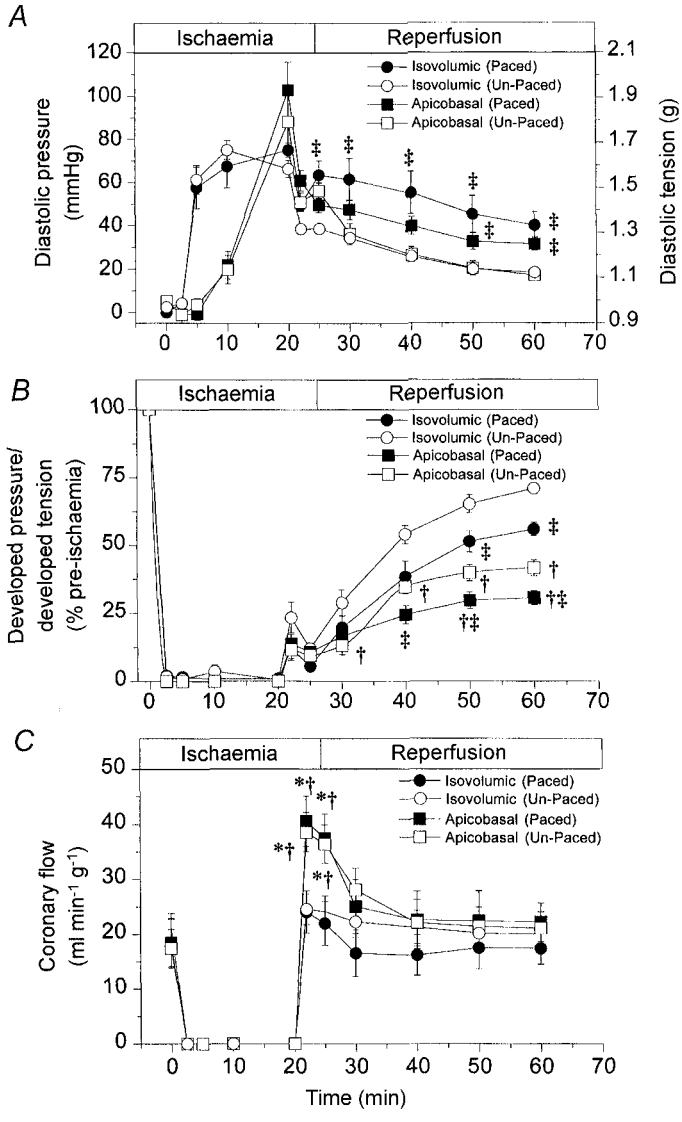

Figure 4

Functional responses to 20 min global ischaemia and 40 min reperfusion in isovolumically contracting mouse hearts allowed to beat at the intrinsic rate (n = 8) or electrically paced (n = 8), and in hearts with contractile function assessed either via apicobasal displacement and allowed to beat at the intrinsic rate (n = 7) or electrically paced (n = 7). Responses for left ventricular diastolic pressure or tension (A), ventricular developed pressure or tension (B), and coronary flow (C) are shown. Values are means ± S.E.M. Note that all values during reperfusion for diastolic and developed pressure differ significantly from pre-ischaemic values ($P < 0.05$). * $P < 0.05$ *vs.* pre-ischaemic values; † $P < 0.05$ apicobasal *vs.* isovolumic hearts; ‡ $P < 0.05$ paced *vs.* un-paced hearts.

observed in the apicobasal model (flow initially increasing to more than 200% of pre-ischaemic values), hyperaemia was modest in isovolumic hearts (flow less than 150% of pre-ischaemic values). After initial hyperaemia, coronary flow declined to comparable levels in both groups, and was unaltered by pacing (Fig. 4*C*). Lower reflow in the isovolumic model suggests that vascular compression due to contracture in the presence of an intraventricular balloon may significantly reduce coronary reperfusion. This possibility was examined, and it was found that a linear correlation existed between diastolic pressure and coronary reflow during the initial minutes of reperfusion (Fig. 5). Coronary resistance was calculated to be ~2.3 mmHg ml^{-1} min^{-1} g^{-1}, equating to maximal coronary dilatation (Headrick *et al.* 2000*b*). A coronary vasodilator (0.1 μM 2-chloroadenosine) had no effect on initial reflow or the relation between coronary flow and pressure (Fig. 5). Regression lines determined for the two data sets did not differ significantly.

It seems paradoxical that while indicators of functional recovery from ischaemia were greater in isovolumically

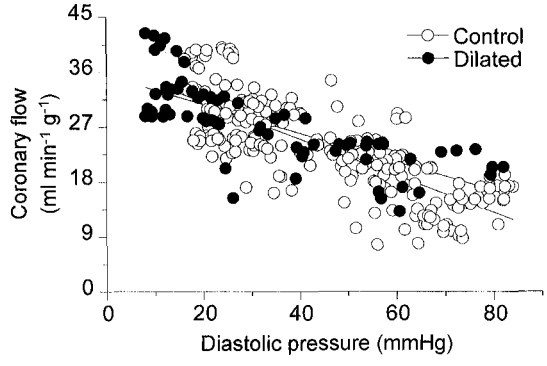

Figure 5

Relationships between coronary flow and diastolic pressure during the initial 3 min of reperfusion following 20 min ischaemia. The relationships were assessed in control hearts (n = 8) and in hearts treated with 0.1 μM 2-chloroadenosine to maximally activate vasodilatory adenosine receptors (n = 6). The lines shown are linear regression lines fit to the raw data.

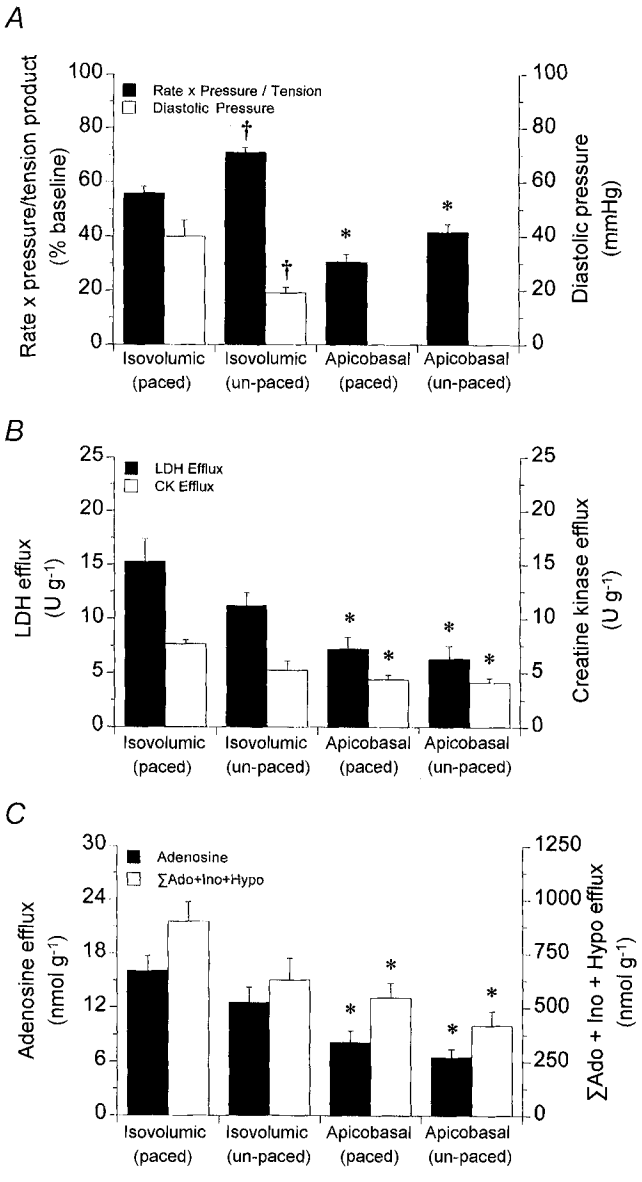

Figure 6

Post-ischaemic injury following 20 min ischaemia and 40 min reperfusion, assessed as percentage recovery for the rate–pressure or rate–tension product and absolute recovery of diastolic pressure (*A*); LDH and CK efflux (*B*); adenosine and Σadenosine + inosine + hypoxanthine efflux (*C*). Data are shown for isovolumically contracting C57/BL6 mouse hearts allowed to beat at the intrinsic rate (n = 8) or electrically paced (n = 8), and in C57/BL6 mouse hearts with function assessed via apicobasal displacement and allowed to beat at the intrinsic rate (n = 7) or electrically paced (n = 7). Values shown are means ± S.E.M. Note that all values measured during reperfusion differed significantly from pre-ischaemic values ($P < 0.05$). * $P < 0.05$ apicobasal *vs.* isovolumic hearts; † $P < 0.05$ un-paced *vs.* paced hearts.

contracting hearts (Fig. 6*A*), the degree of tissue damage and de-energisation, indicated by LDH, CK and purine efflux, was also greater. Total LDH and CK efflux during 30 min reperfusion were both almost 2-fold higher in isovolumic hearts (either paced or un-paced) (Fig. 6*B*), as was purine efflux (Fig. 6*C*).

Relation between ischaemic duration and myocardial injury

The deficit in post-ischaemic function was related to the duration of ischaemia (Fig. 7). Recovery of diastolic pressure was reduced as ischaemic duration increased (Fig. 7*A*). Similarly, ventricular developed pressure recovered to 82, 73, 68, 57 and 41% of pre-ischaemic values after 40 min reperfusion following 10, 15, 20, 25 and 30 min of ischaemia, respectively. Expressed relative to non-ischaemic time-matched hearts, developed pressure recovered to 98, 86, 71, 66 and 52% of control values after 40 min reperfusion, respectively. Developed tension recovered to 80, 58, 40, 32 and 25% of pre-ischaemic values following 40 min reperfusion after 10, 15, 20, 25 and 30 min of ischaemia, respectively (Fig. 7*B*). Heart rate and flow both recovered to pre-ischaemic values after all ischaemic durations (data not shown). The extent of functional recovery in the apicobasal perfused heart model was lower after each ischaemic duration compared with isovolumically contracting hearts (Fig. 7).

Post-ischaemic enzyme leakage was unaltered by ischaemic durations up to 15 min (Fig. 7*C*). After this period efflux increased markedly. A linear relation between enzyme leakage (indicating tissue necrosis) and contractile recovery was observed above ischaemic durations of 15 min (Fig. 8). With 10–15 min of ischaemia there was no necrosis, as indicated by enzyme leakage. Nonetheless, contractile recovery was incomplete after these ischaemic periods.

DISCUSSION

The isolated perfused heart is a valuable model, allowing for detailed physiological analyses which can now be coupled with molecular genetic approaches to delineate mechanisms of injury during ischaemia and reperfusion. Although the rat heart has been the most widely employed isolated model in studies of ischaemia–reperfusion (De Leiris *et al.* 1984; Galinanes & Hearse, 1990), the mouse has been rapidly adopted. It is unfortunate that increased use has occurred despite minimal model characterisation, inevitably leading to unacceptable variations in baseline function between laboratories, in addition to highly variable responses to ischaemia–reperfusion. We therefore undertook a thorough characterisation of this model.

Normoxic function and stability

A fundamental property of isolated hearts is that they are functionally deteriorating. This is multifactorial in origin, involving absence of key blood-borne elements, and progressive tissue oedema. It is therefore necessary to examine functional stability. Functional stabilities in the present models are acceptable and greater than in previous reports (Galinanes & Hearse, 1990). It is interesting to note that Galinanes & Hearse (1990) have observed that functional

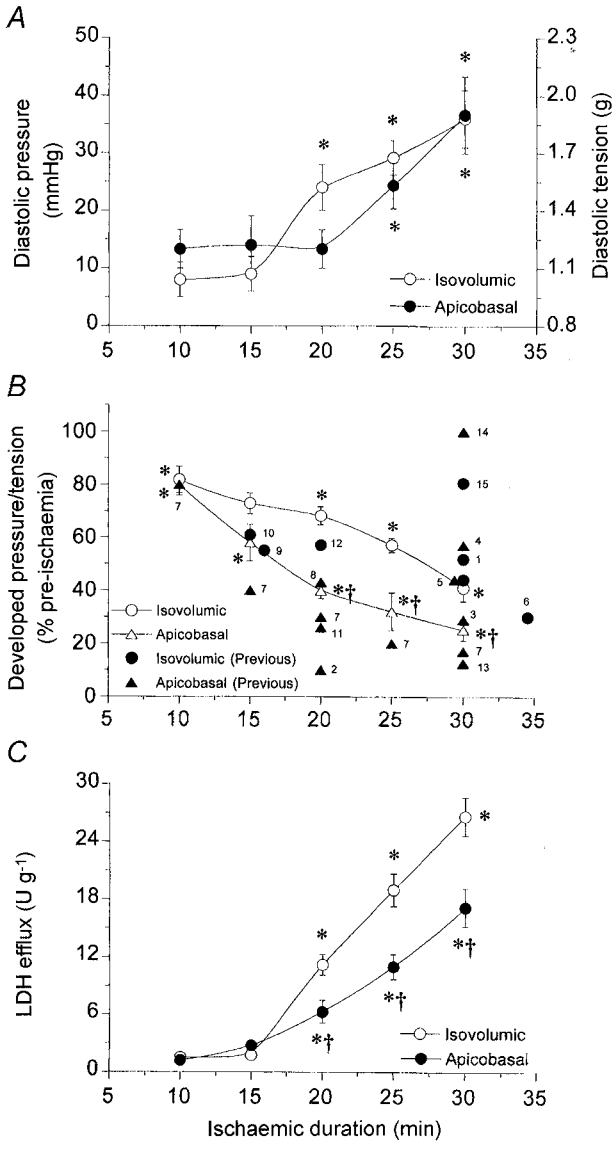

Figure 7

Impact of ischaemic duration on post-ischaemic diastolic pressure or tension (*A*), percentage recovery of left ventricular developed pressure or tension (*B*), and total post-ischaemic LDH efflux (*C*). Mouse hearts were subjected to 10 (n = 7 for isovolumic hearts, n = 6 for apicobasal hearts), 15 (n = 8 for isovolumic hearts, n = 7 for apicobasal hearts), 20 (n = 8 for isovolumic hearts, n = 7 for apicobasal hearts), 25 (n = 8 for isovolumic hearts, n = 7 for apicobasal hearts) or 30 min (n = 6 for isovolumic hearts, n = 8 for apicobasal hearts) of global ischaemia followed by 40 min aerobic reperfusion. Values are means ± S.E.M. *B*, values from the literature for recoveries of developed pressures or tensions are shown (reference numbers provided). Data is shown for: 1, Galinanes & Hearse, 1990; 2, Marber *et al.* 1995; 3, Plumier *et al.* 1995; 4, Yoshida *et al.* 1996; 5, Matherne *et al.* 1997; 6, Chen *et al.* 1998; 7, Sumeray & Yellon, 1998; 8, Xi *et al.* 1998; 9, Flogel *et al.* 1999; 10, Hampton *et al.* 2000; 11, Morrison *et al.* 2000; 12, Peart *et al.* 2000; 13, Sumeray *et al.* 2000; 14, Yoshida *et al.* 2000; 15, Zhao *et al.* 2000. Note that recoveries for Yoshida *et al.* (1996), Hampton *et al.* (2000), Chen *et al.* (1998) and Galinanes & Hearse (1990) were determined after 20, 20, 45 and 60 min reperfusion respectively. * $P < 0.05$ *vs.* pre-ischaemic values; † $P < 0.05$ apicobasal *vs.* isovolumic hearts.

stability varies considerably between species, with reductions in left ventricular function over 120 min ranging from only 18% in rabbit up to 40% in guinea-pig.

Estimates of left ventricular developed pressure and +dP/dt for isolated mouse hearts vary from as low as 50–60 mmHg and 1000–3000 mmHg s^{-1}, respectively (Chen *et al.* 1998; Kameyama *et al.* 1998; Sato *et al.* 1998; Gyurko *et al.* 2000; Pazos-Moura *et al.* 2000; Zhao *et al.* 2000; MacGowan *et al.* 2001), to greater than 110 mmHg and 4000–6000 mmHg s^{-1}, respectively (Brooks & Apstein, 1996; Headrick *et al.* 2000*a*; Peart & Headrick, 2000; Sam *et al.* 2000). These values compare with *in situ* left ventricular pressures of ~110 mmHg (Georgakopoulos *et al.* 1998), and +dP/dt values ≥8000 mmHg s^{-1} (Hoit *et al.* 1997; Georgakopoulos *et al.* 1998). Prior estimates of contractility are therefore as low as 10% of *in situ* values. In our hands developed pressure (with 1.75–2.0 mM Ca^{2+}) normally exceeds 120 mmHg at a diastolic pressure of ~5 mmHg (Table 1), and ±dP/dt ranges from 5000–6000 mmHg s^{-1} at 37 °C (Table 1) to more than

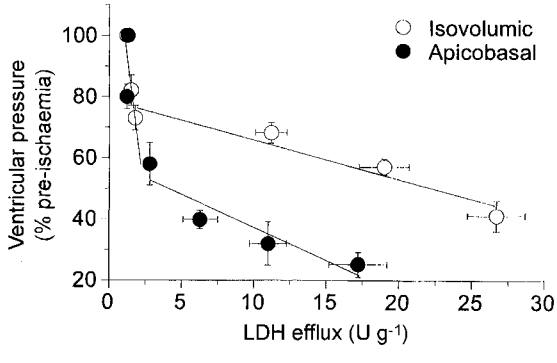

Figure 8

Relation between LDH leakage and function (% pre-ischaemic values) in post-ischaemic Langendorff perfused mouse hearts (after 10, 15, 20, 25 and 30 min ischaemia). Efflux is also shown for non-ischaemic hearts (at 100% pre-ischaemic function). All values are means ± S.E.M..

7000 mmHg s^{-1} at 38 °C (data not shown). Considering unavoidable differences between *in vitro* and *in situ* models, the contractile properties obtained under the present conditions are in reasonable agreement with *in situ* parameters, and are superior to previous estimates.

Intrinsic heart rates for Langendorff perfused mouse hearts range from as low as 225–280 beats min^{-1} (Yoshida *et al.* 1996, 2000; Chen *et al.* 1998) up to 490 beats min^{-1} (Galinanes & Hearse, 1990). Hearts beating at 225–280 beats min^{-1} are operating at less than 45% of resting *in situ* values (based upon 550–600 beats min^{-1} *in situ*) (Uechi *et al.* 1998). This is a key point since both physiological responses and responses to injury are dependent upon metabolic rate. Low rates may contribute to unusually high levels of recovery from ischaemia in some studies (Yoshida *et al.* 2000). We obtain intrinsic rates of 380–390 beats min^{-1} at 37 °C. As shown in Fig. 2*B*, heart rate is highly temperature dependent and the extent of perfusate filtration also has an impact on rate (Table 1). The latter may stem from impaired perfusion of nodal tissue. Low beating rates in earlier studies may therefore result from low temperature and/or inadequate perfusion fluid filtration. Additionally, since heart rate is sensitive to oxygenation and locally released factors including adenosine, low heart rates may also reflect inadequate tissue oxygenation.

Tissue oxygenation and energy state are two key determinants of stability and contractility. In an acceptable model the vasculature should be sub-maximally dilated and responsive to physiological and pathophysiological stimuli. The coronary circulation in the current model operates at ~40% of the peak flow of 45 ml min^{-1} g^{-1} (Headrick *et al.* 2000*b*), and is responsive to varied stimuli. Although it has been suggested that crystalloid buffer may not adequately oxygenate murine hearts and that added erythrocytes are required (Brooks & Apstein, 1996), the model exhibits the hallmarks of O_2-sufficient tissue: ventricular pressure, heart rate and contractility are all high; the vasculature is sub-maximally dilated; substantial dilatation occurs during β-adrenoceptor or A_2 adenosine receptor stimulation (Headrick *et al.* 2000*b*); cellular energy state is high; myocardial purine and enzyme effluxes are low (Table 2). The high [PCr]/[ATP] ratio and energy charge, and low cytosolic [ADP] all indicate well-oxygenated and energised myocardium. Employing recently measured inorganic phosphate (P_i) levels (Headrick *et al.* 1998*a*), we estimate a ΔG_{ATP} (the Gibbs free energy of ATP hydrolysis) of −61.4 kJ mol^{-1}, exceeding the values for rat myocardium (Harrison *et al.* 1998; Headrick *et al.* 1998*b*), and reflecting efficient oxidative ATP generation at the expense of ADP and P_i.

Functional effects of preload, balloon design, perfusate [Ca^{2+}], temperature and heart rate

The pressure–volume relationship for mouse shows a plateau in ventricular pressure development at a volume giving a diastolic pressure of 4–6 mmHg (Fig. 1*B*). Thus, optimal ventricular function is obtained at a 'preload' equivalent to a diastolic pressure of 5 mmHg. In terms of accurate measurement of ventricular pressure development, it is also crucial that balloons with unpressurised volumes significantly exceeding the ventricular volume are employed. Several groups have employed small ventricular balloons, some as low as 3–4 mm in length (Kameyama *et al.* 1998; Hampton *et al.* 2000) *versus* 8–9 mm in the present study. Smaller balloons, whilst easier to place within the murine ventricle, lead to spurious measurements since the compliance of the inflated balloon influences the apparent diastolic and systolic pressures. We show that balloons only marginally smaller than those used here reduce apparent pressure development, +d*P*/d*t*, and the ESPVR, and increase the slope of the EDPVR (Fig. 1*A*). The artificially reduced levels of elastance and contractile function, and enhanced diastolic stiffness measured with these smaller balloons are consistent with values measured previously (Brooks & Apstein, 1996; Kameyama *et al.* 1998; Pazos-Moura *et al.* 2000; Sam *et al.* 2000). Thus, low contractility in some prior studies may reflect the effects of inadequate balloon design.

The perfusate [Ca^{2+}] in isolated mouse hearts ranges from 1.2–1.3 mM (Galinanes & Hearse, 1990; Chen *et al.* 1998; Bartfay *et al.* 1999; Gyurko *et al.* 2000; Hampton *et al.* 2000) up to 2.0–2.5 mM (Brooks & Apstein, 1996; Radford *et al.* 1996; Kameyama *et al.* 1998; Xi *et al.* 1998; Flogel *et al.* 1999; Peart & Headrick, 2000; MacGowan *et al.* 2001). We show that the mouse heart is highly sensitive to 0.5–2.5 mM Ca^{2+}, with an EC_{50} of ~1.0 mM extracellular Ca^{2+} (Fig. 2*A*). Providing hearts with 1.2–1.3 mM Ca^{2+} results in ventricular pressures 40–50% lower than in hearts supplied with 2.0–2.5 mM Ca^{2+}, contributing to low ventricular contractility in those studies employing low [Ca^{2+}] (Galinanes & Hearse, 1990; Chen *et al.* 1998; Bartfay *et al.* 1999; Schulze *et al.* 1999; Gyurko *et al.* 2000; Hampton *et al.* 2000) but not those using 2.0–2.5 mM Ca^{2+} (Kameyama *et al.* 1998; Luo *et al.* 1998; Sato *et al.* 1998; Zhao *et al.* 2000; MacGowan *et al.* 2001).

Contractility is also extremely sensitive to temperature (Fig. 2*B*), consistent with reductions in contractility during hypothermia in other species (Stowe *et al.* 1999). While the mechanism is not fully understood, it will stem in part from associated changes in heart rate (Khoury *et al.* 1996; Hoit *et al.* 1997). As shown in Fig. 2*C*, temperature-dependent changes in heart rate can account for some but not all of the change in contractile function during alterations in temperature. The remaining temperature dependence probably resides in activation of myocardial enzymes; most myocardial enzymes exhibit 50% changes in activity per 10 °C (Belzer & Southard, 1988). This may improve the speed and efficiency of Ca^{2+} handling, cross-bridge cycling and myofibrillar tension development.

We observe a positive staircase phenomenon in mouse hearts paced at rates from 330 to 450 beats min^{-1}, beyond which function falls as rate increases (Fig. 2*C*). This 'biphasic' dependence has been observed in mouse hearts supplied with erythrocyte-enriched perfusion fluid (Lim *et al.* 1999). Since contractility is optimal at 420–450 beats min^{-1}, it is possible that low contractility in prior studies may also stem from low (Yoshida *et al.* 1996, 2000; Chen *et al.* 1998) or high heart rates (Galinanes & Hearse, 1990; Flogel *et al.* 1999).

Responses to ischaemia–reperfusion: isovolumic contraction *versus* apicobasal displacement

Owing to a general paucity of background information, and the technical challenge of assessing contractile function via intraventricular balloon in small hearts, the majority of studies of ischaemia–reperfusion in mouse have been undertaken in unloaded empty hearts with function assessed via apicobasal displacement (Marber *et al.* 1995; Plumier *et al.* 1995; Yoshida *et al.* 1996, 2000; Li *et al.* 1997; Matherne *et al.* 1997; Sumeray & Yellon 1998; Xi *et al.* 1998; Kang *et al.* 1999; Morrison *et al.* 2000; Sumeray *et al.* 2000). Fewer studies have been performed in isovolumically contracting hearts (Galinanes & Hearse, 1990; Chen *et al.* 1998; Flogel *et al.* 1999; Headrick *et al.* 2000*a*; Peart & Headrick, 2000; Zhao *et al.* 2000). We predicted greater tolerance to ischaemia in the apicobasal model, due to reduced metabolic rate or 'demand' in unloaded hearts (Headrick *et al.* 1991), reduced contracture and vascular compression. It seems paradoxical that ventricular function recovered to a lesser extent in the apicobasal model (Figs 4, 6 and 7) despite reduced enzyme and purine efflux (both 50–60% of values for isovolumic hearts). Values for the recovery from ischaemia of isovolumic hearts agree reasonably with values published previously (Galinanes & Hearse, 1990; Chen *et al.* 1998; Flogel *et al.* 1999; Headrick *et al.* 2000*a*; Peart & Headrick, 2000), and data in Fig. 7 demonstrate that a more consistent relation exists between functional recovery and ischaemic duration in the isovolumic *versus* the unloaded apicobasally contracting model.

In the apiscobasal model, metabolic rate is only 65% of that for isovolumically contracting hearts (Table 1), in agreement with the greater cost of isovolumic work (Headrick *et al.* 1991). Ischaemic contracture was less pronounced and coronary reflow was greater in the apicobasal model, probably due to reduced vascular compression. These factors should all contribute to an improved functional outcome from ischaemia in the apicobasal model. The dissociation of contractile recovery from reflow and tissue injury (assessed by enzyme and purine efflux measurements) probably reflects the inherent inaccuracy of apex-to-base contraction as a measure of global ventricular mechanics. The method assesses long-axis shortening with no measure of circumferential shortening. Since there are shifts in longitudinal *versus* circumferential function on transition to ischaemia (Villarreal *et al.* 1991) and during reperfusion (Brunvand *et al.* 1995), apicobasal displacement will not accurately reflect ventricular mechanics.

In addition to variable functional recoveries, infarct development is remarkably erratic in the apicobasal model. Sumeray & Yellon (1998) report ~10% infarction after 20 min ischaemia while others report 25–45% (Marber *et al.* 1995; Xi *et al.* 1998) and up to 60% infarction (Morrison *et al.* 2000). Ischaemia for 30 min results in as little as 8% (Yoshida *et al.* 1996) or 25% (Yoshida *et al.* 2000) to as much as 60% infarction (Sumeray & Yellon, 1998). Others report 45–55% infarct development after 50 min ischaemia (Li *et al.* 1997; Kang *et al.* 1999). Although some variability may stem from methods of normalisation to area at risk, these outcomes are suggestive of inherent problems with the model, which clearly is not robust in terms of the responses to ischaemia–reperfusion.

In relation to post-ischaemic reflow, it has been suggested that microvascular compression is the major determinant of post-ischaemic coronary flow (Nevalainen *et al.* 1986; Manciet *et al.* 1994). The unloaded and isovolumically contracting hearts presented an interesting opportunity to test this notion. As noted above, reflow is much greater in unloaded hearts which exhibit reduced vascular compression. Moreover, our data reveal an inverse linear dependence of post-ischaemic flow on diastolic pressure in isovolumic hearts (Fig. 5). The vasculature is maximally dilated during reperfusion, and unresponsive to an added dilatatory stimulus. These findings confirm the importance of vascular compression as a determinant of early reflow.

Post-ischaemic enzyme leakage

Enzyme and purine leakage under normoxic conditions (Table 2) is minimal and comparable to values published for mouse and rat (Li *et al.* 1997; Harrison *et al.* 1998; Headrick *et al.* 1998*b*; Kang *et al.* 1999; Morrison *et al.* 2000). Following ischaemia substantial cellular damage is reflected in enhanced leakage of both LDH and CK (Figs 6 and 8). Enzyme efflux tends to match functional outcome, being lowest in hearts displaying greater functional recovery. Consistently higher LDH *versus* CK efflux was observed, in agreement with our previous observations in the rat (Harrison *et al.* 1998). Enzyme efflux data reveals an absence of significant tissue necrosis with 10–15 min of ischaemia in both isovolumic and apicobasally contracting models, with a pronounced increase in tissue damage as the period of ischaemia is increased to ≥20 min (Fig. 8). Since contractile recovery is significantly impaired after 10 and 15 min ischaemia, these periods appear to be appropriate for inducing and consequently studying reversible contractile dysfunction (stunning) in the absence of significant necrosis. It should be noted that errors are evident in enzyme leakage calculated for post-ischaemic murine hearts in prior studies. Total myocardial creatine kinase activity is ~10 000 U (g dry wt)$^{-1}$ in mouse (Galinanes & Hearse, 1990), that is, approximately 2000 U (g wet wt)$^{-1}$. Nonetheless, Plumier *et al.* (1995) and Yoshida *et al.* (2000) report post-ischaemic CK leakages of more than four times the total tissue content. Such discrepancies further emphasise the need to better characterise the perfused murine model.

Experimental design issues

From the viewpoint of experimental design, we show that a range of variables have a significant impact on function and responses to ischaemia–reperfusion. As predicted, use of ventricular balloons with low unpressurised volumes artificially depresses normoxic function and estimated contractility, and also depresses the apparent recovery from ischaemia (expressed either as percentage of the pre-ischaemic values or in absolute units). We also show that lack of

perfusion fluid filtration depresses normoxic function and coronary flow (Table 1), and markedly depresses functional recovery and reflow following ischaemia and reperfusion. Suspended micro-particulates greater than 0.8 μm have been previously shown to reduce contractile function in a time-dependent manner in rat hearts (Robinson *et al.* 1985). Effects of micro-particulates may be exaggerated in small murine hearts, and this may be an additional factor in producing variations in functional parameters in prior murine studies. Some investigators fail to employ filtration (Kameyama *et al.* 1998; Luo *et al.* 1998; Bartfay *et al.* 1999; Schulze *et al.* 1999), do not report degree of filtration, or use large filter cut-offs (Marber *et al.* 1995; Brooks & Apstein, 1996). We also show that electrical pacing, often employed to normalise rate between experimental groups and following insult, significantly impairs functional tolerance to ischaemia (Figs 4 and 6). Furthermore, we also found that pacing during the ischaemic insult itself exaggerated contracture development and consequently reduced functional recovery. These varied factors should all be considered when designing experiments, and particularly when comparing findings between different laboratories.

Conclusions

In the present study we have extensively characterised functional and metabolic properties of the isolated perfused murine heart, and assessed functional responses to ischaemia–reperfusion in variants of the Langendorff perfused model. We find that with attention to perfusate filtration, balloon design and heart rate, the isovolumically contracting Langendorff perfused mouse heart is stable and possesses high levels of contractility and energy. With respect to ischaemia–reperfusion, the apicobasally contracting (unloaded) heart does not accurately reflect changes in post-ischaemic ventricular mechanics, and contractile recovery is paradoxically depressed despite enhanced reflow, and reduced metabolic rate, contracture, and tissue injury (assessed by LDH, CK and purine effluxes). While the isovolumically contracting mouse heart should prove valuable in assessing phenotypic outcomes from transgenic manipulations, the factors noted above should be considered carefully when designing experiments. With increased attention to such issues the existing variability in function found in mouse heart studies may be reduced to levels associated with better characterised models.

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