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Protection of hearts from reperfusion injury by propofol is associated with inhibition of the mitochondrial permeability transition

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Abstract

Objective: Diminishing oxidative stress may protect the heart against ischaemia–reperfusion injury by preventing opening of the mitochondrial permeability transition (MPT) pore. The general anaesthetic agent propofol, a free radical scavenger, has been investigated for its effect on the MPT and its cardioprotective action following global and cardioplegic ischaemic arrest. **Method:** Isolated perfused Wistar rat hearts were subjected to either warm global ischaemia (Langendorff) or cold St. Thomas' cardioplegia (working heart mode) in the presence or absence of propofol. MPT pore opening was determined using [^{3}H]-2-deoxyglucose-6-phosphate ([^{3}H]-DOG-6P) entrapment. The respiratory function of isolated mitochondria was also determined for evidence of oxidative stress. **Results:** Propofol (2 μg/ml) significantly improved the functional recovery of Langendorff hearts on reperfusion (left ventricular developed pressure from 28.4±6.2 to 53.3±7.3 mmHg and left ventricular end diastolic pressure from 52.9±4.3 to 37.5±3.9 mmHg). Recovery was also improved in propofol (4 μg/ml) treated working hearts following cold cardioplegic arrest. External cardiac work on reperfusion improved from 0.42±0.05 to 0.60±0.03 J/s, representing 45–64% of baseline values, when compared to controls ($P<0.05$). Propofol inhibited MPT pore opening during reperfusion, [^{3}H]-DOG-6P entrapment being 16.7 vs. 22.5 ratio units in controls ($P<0.05$). Mitochondria isolated from non-ischaemic, propofol-treated hearts exhibited increased respiratory chain activity and were less sensitive to calcium-induced MPT pore opening. **Conclusion:** Propofol confers significant protection against global normothermic ischaemia and during cold cardioplegic arrest. This effect is associated with less opening of mitochondrial MPT pores, probably as a result of diminished oxidative stress. Propofol may be a useful adjunct to cardioplegic solutions in heart surgery.

Keywords: Cardioplegia; Free radicals; Ischaemia; Mitochondria; Reperfusion; Ventricular function

1. Introduction

Propofol is an anaesthetic agent frequently used during cardiac surgery and in postoperative sedation [1]. There are reports that propofol can protect hearts from the damage that occurs during oxidative stress [2] or on reperfusion after a period of ischaemia (reperfusion injury) [3,4]. It has

Abbreviations: AF, aortic flow-rate; AP, peak aortic pressure; CF, coronary flow-rate; CO, cardiac output; DOG, 2-deoxyglucose; DOG-6P, 2-deoxyglucose-6-phosphate; ECW, external cardiac work; EGTA, ethylene glycol-bis-(2-aminoethyl)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HR, heart rate; KHS, Krebs–Henseleit solution; LAP, left 'atrial' pressure; LVDP, left ventricular developed pressure; LVEDP, left ventricular end diastolic pressure; MPT, mitochondrial permeability transition; NTA, nitrilotriacetic acid; TMPD, *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylendiamine; RPP, rate-pressure product

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been proposed that this action of propofol may be mediated by its ability to act as a free radical scavenger [5–8] or through inhibition of plasma membrane calcium channels [9,10]. Both oxidative stress and elevated cytosolic calcium concentrations are known to be associated with reperfusion injury and are probably responsible, either directly or indirectly, for damaging the myocyte [11–14]. This may be caused by oxidation/peroxidation of proteins and lipids, and activation of proteases and phospholipases. Another major cause of reperfusion injury is thought to be the mitochondrial permeability transition (MPT) [13,14]. This is associated with the opening of non-specific pores in the inner mitochondrial membrane under conditions of high matrix [Ca^{2+}], especially when this is accompanied by oxidative stress, low matrix concentrations of ADP and ATP, and high concentrations of Pi [14]. Such conditions exactly match those occurring during reperfusion of the ischaemic heart. Work from our laboratory has provided direct evidence that MPT pores do open during reperfusion, but not during ischaemia [15–17], and that the extent of functional recovery of a heart is inversely correlated with pore opening [16–18]. The greatly depressed ATP/ADP ratio and elevated AMP concentration found in hearts that do not recover during reperfusion [15–17] supports this conclusion. A critical role for the pore in ischaemia–reperfusion injury is also implied by the observation that the immunosuppressant cyclosporin A (CsA), which inhibits the MPT in isolated mitochondria [19,20], protects isolated rat hearts and cardiac myocytes against ischaemia–reperfusion damage [15–17,21].

The MPT causes damage to the heart because it uncouples mitochondria, causing them to hydrolyse rather than synthesise ATP. When this occurs, the myocyte is no longer able to repair any damage caused during ischaemia and is destined to die by necrosis [14]. Even if the MPT were to occur transiently, it is now thought that the resulting mitochondrial swelling will cause sufficient cytochrome c release to induce cell death by apoptosis [14,18,22–24]. As such, it has been suggested that the MPT may act as a cell death switch, determining not only whether a cell lives or dies, but also the pathway by which death occurs, i.e. apoptosis or necrosis [22–24]. Indeed, it has been shown that apoptosis rather than necrosis occurs in those areas of the reperfused heart that experience a less severe insult; for example cells that surround the necrotic area of an infarct [25–28]. Such apoptotic heart cells exhibit activated caspase-3, and inhibitors of caspases can provide hearts with some protection from reperfusion injury [29,30].

The ability of propofol to act as a free radical scavenger and to reduce cytosolic [Ca^{2+}] suggests that inhibition of the MPT may be an additional mechanism by which it protects hearts from reperfusion injury. Indeed, there are reports that propofol can inhibit the MPT in isolated mitochondria [31,32], although the concentrations used in these studies were considerably greater than those employed clinically. In this paper we confirm the protective effects of propofol against reperfusion injury in the heart, whether or not cardioplegic solution is utilised. Furthermore, we have used mitochondrial entrapment of [^{3}H]-2-deoxyglucose-6-phosphate (DOG-6P) to demonstrate that this is associated with an inhibition of the MPT [16–18]. In the isolated mitochondria, increased respiratory chain activity was observed in the presence of propofol. These data imply propofol is reducing the exposure of mitochondria to oxidative stress. Our data suggest that, in addition to its use in anaesthesia, propofol may be a beneficial additive to the cardioplegic solution used in cardiac surgery.

2. Methods

This study 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).

2.1. Langendorff heart perfusion

The procedures were essentially the same as described previously [15,16]. Hearts (about 0.7 g) were removed from male Wistar rats (235–280 g) and immediately arrested in ice-cold buffered Krebs–Henseleit solution (KHS). The aorta was rapidly cannulated and perfused in the Langendorff mode using Krebs–Henseleit buffer containing (mM) NaCl 118, $NaHCO_3$ 25, KCl 4.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 11 and $CaCl_2$ 1.2. The buffer was bubbled with 95% O_2/5% CO_2 at 37°C (pH 7.4) and perfusion performed at a constant flow-rate of 10–12 ml/min. It was confirmed that this flow-rate was in excess of that required to maintain maximal rates of oxygen uptake. A water-filled balloon was inserted into the left ventricle for continuous monitoring of developed pressure. Balloon volume was set to give an initial left ventricular end diastolic pressure of 2.5–5 mmHg. Global normothermic ischaemia was induced after a total pre-ischaemic period of 60 min by switching off the pump and immersing the heart in buffer maintained at 37°C. In all experiments, the ischaemic period was 30 min (see Fig. 1a). When present, propofol was added 10 min before the onset of ischaemia and throughout the reperfusion phase. The final concentration used was 2 μg/ml, which was achieved by the addition of 'Diprivan' (Zeneca Pharma, UK) which contains 10 mg/ml of propofol in an intralipid emulsion. Control hearts were treated with an equivalent concentration of 10% Intralipid (Pharmacia and Upjohn Ltd.) alone. Retrograde delivery flow-rate was maintained at 10–12 ml/min during reperfusion, which was continued for 30 min without a significant change in aortic pressure, implying that perfusion remained homogeneous.

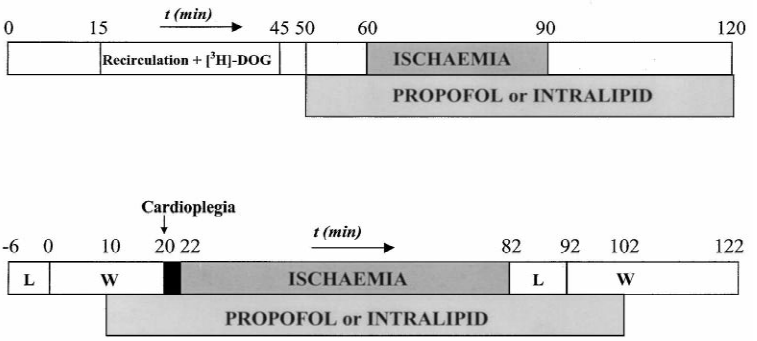

Fig. 1. Perfusion protocol for Langendorff (a) and working heart preparations (b). L, Langendorff mode; W, working heart mode.

2.2. Measurement of the MPT using [^{3}H]-DOG-6P entrapment by mitochondria

2-Deoxyglucose (DOG) enters the myocyte on the glucose transporter and is metabolised to DOG-6P which remains entrapped in the cytosol. It does not enter the mitochondria unless the MPT pore opens. We have previously defined optimal conditions under which hearts can be perfused with [^{3}H]-DOG to load them with [^{3}H]-DOG-6P without any effect on their function. Removal of unmetabolised [^{3}H]-DOG is achieved by a subsequent flow through perfusion in the absence of DOG. The extent to which the [^{3}H]-DOG-6P enters mitochondria of such loaded hearts can then be used as an indicator of the number that have undergone the MPT [16–18]. Measurement of the mitochondrial [^{3}H]-DOG-6P requires rapid isolation of the mitochondria in sucrose buffer containing EGTA, which immediately closes the MPT pores and thus traps the [^{3}H]-DOG-6P within the matrix. In outline, the experimental protocol was as follows (Fig. 1a). After a 15-min flow-through period for stabilisation, hearts were perfused in recirculating mode with 40 ml of KHS containing 0.5 mM [^{3}H]-DOG (0.1 μCi/ml) for 30 min. Perfusion was then returned to flow-through (non-recirculating) mode with normal buffer for 5 min and then 10 min with propofol or intralipid alone. Ischaemia was then induced as described above and maintained for 30 min before reperfusion with buffer containing either propofol or intralipid alone.

Following the perfusion protocol, the ventricles were rapidly cut away, weighed and homogenised with a Polytron homogeniser in 5 ml of ice-cold sucrose buffer (mM: sucrose 300, Tris–HCl 10, EGTA 2; pH 7.4). The volume was made up to 40 ml with buffer containing 5 mg/ml bovine serum albumin (BSA) and a sample retained for measurement of total [^{3}H] after protein precipitation by addition of 5% (w/v) perchloric acid (PCA). Heart mitochondria were prepared from the remainder of the homogenate by centrifugation for 2 min at 2000×*g* in a bench-top centrifuge to remove cell debris, followed by centrifugation of the supernatant at 10 000×*g* for 5 min to sediment the mitochondria. The pellet was then washed in 2×40 ml at 10 000×*g* in BSA-free sucrose buffer and finally resuspended in 0.5 ml of sucrose buffer. A 100-μl sample of the suspension was retained for assay of citrate synthase to correct for mitochondrial recovery, whilst to the remainder an equal volume of 5% PCA was added to release the entrapped [^{3}H]. Protein was precipitated by centrifugation at 10 000×*g* for 2 min, and the resultant supernatant was assayed for radioactivity in 10 ml scintillant (Packard Emulsifier-Safe). Mitochondrial entrapment of [^{3}H]-DOG-6P was calculated as previously described [16–18] and is expressed as 10^5×[mitochondrial [^{3}H] dpm per unit citrate synthase]/[total heart [^{3}H] dpm/g wet wt]. Citrate synthase was used to correct for variation in recovery of mitochondria [16] and total homogenate [^{3}H] to correct for differential loading of hearts with [^{3}H]-DOG-6P. However, it was confirmed that the presence of propofol had no effect on either parameter (data not shown).

2.3. Measurement of the permeability transition and respiration in isolated heart mitochondria

Isolated hearts were Langendorff perfused for 15 min in flow-through mode, 30 min in recirculation mode with 0.5 mM DOG, 5 min normal perfusion and 10 min perfusion with intralipid (control) or 2 μg/ml propofol (see Fig. 1a). Well coupled, Percoll-purified heart mitochondria were then prepared using a standard procedure that utilises a Dounce Potter homogeniser rather than the rapid Polytron technique used to determine [^{3}H]-DOG-6P entrapment [33]. Measurement of mitochondrial respiration was performed at 30°C in a Clark-type oxygen electrode [33]. The buffer (pH 7.2) contained (mM): KCl 125, MOPS 20, Tris 10, EGTA 0.5, KPi 2, and was supplemented with the required substrate (2.5 mM 2-oxoglutarate+1 mM L-malate, 2.5 mM L-glutamate+1 mM L-malate or 2.5 mM succinate+1 μM rotenone). Rates of respiration were measured in the absence (state 2) and presence (state 3) of 1 mM ADP. At the end of each run, 0.5 μM antimycin A and 10 mM ascorbate+0.3 mM *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylendiamine (TMPD) were added and the new rate of respiration measured.

For measurement of the MPT, swelling of de-energised mitochondria at a range of calcium concentrations was determined by monitoring the decrease in light scattering at 520 nm as described previously [20,34]. Mitochondria were incubated at 25°C and 0.5 mg of protein/ml in 3.5 ml buffer (pH 7.2) containing (mM) KSCN 150, MOPS 20, Tris 10 and nitrilotriacetic acid 2, supplemented with 0.5 μM rotenone, 0.5 μM antimycin, 2 μM A23187. Nitrilotriacetic acid was present to allow buffered calcium concentrations of 50–500 μM to be employed, whilst the calcium ionophore A23187 was added to ensure complete equilibration of Ca^{2+} across the mitochondrial inner membrane under de-energised conditions.

2.4. Working heart perfusion

Hearts were isolated from male Wistar rats (300–350 g) and divided into three comparison groups: control ($n=12$), intralipid ($n=12$), and propofol ($n=14$). Following aortic cannulation and immediate retrograde perfusion (Langendorff mode) using standard oxygenated KHS (pH 7.4, 37±0.5°C) at 12 ml/min, the left atrium was cleaned of tissue debris and cannulated. Antegrade perfusion (working heart mode) was commenced and retrograde perfusion discontinued. The preload and afterload column heights were set at 15 and 80 cm, respectively. The aortic outflow was recirculated 5 min after changes to perfusate content or delivery mode, but the coronary effluent was not recirculated. Once in the working mode, the heart was allowed a 5-min stabilisation period before data acquisition, and was perfused for 20 min before induced cardioplegic arrest. With the atrial feeding and aortic outflow lines occluded, the heart was arrested by a 2-min retrograde infusion of a modified St. Thomas' I cardioplegia containing (mM): NaCl 147, KCl 20, $MgCl_2$ 16 and $CaCl_2$ 2, at pH 5.8 and pre-cooled to 10±1°C. It was then suspended in the cardioplegic solution at 20±1°C for a total 'ischaemic' time of 60 min. Initial reperfusion in Langendorff mode for 10 min was followed by a further 30 min of working heart mode before termination of the experiment (see Fig. 1b). When present, intralipid±propofol (4 μg/ml) was added to the perfusate 10 min before initiating arrest, and was present in the arresting media throughout ischaemia and in the reperfusion buffer until 10 min after switching back into the working heart mode. KHS alone was then used to wash out the test agents. During the experiment, heart function was assessed while in the working heart mode and data were collected at 5-min intervals for analysis. An inline flow meter allowed constant visualisation of aortic flow-rate (AF). Coronary flow-rate (CF) was obtained by timed collections of coronary effluent. Pressure transducers attached to the atrial feeding and aortic outflow lines were connected to Bridge Amp-MacLab/4s System (ADInstruments Pty. Ltd., Australia) which allowed for measurements of peak 'aortic' pressure (AP), left 'atrial' pressure (LAP) and pressure tracing derived heart rate (HR). Cardiac output (CO) was derived by adding aortic and coronary flow-rates. Rate-pressure product (RPP), expressed as mmHg.beats/min was calculated (AP×HR). The external cardiac work (ECW), expressed as J/s, represents the product of cardiac output and peak aortic pressure (CO×AP). For analysis, 'baseline' data is a specific term and refers to measurements taken immediately before the addition of test agents, at 10 min after the initiation of the working mode. Similarly, 'preischaemic' data refers to measurements taken just before the induction of cardioplegic arrest, at 20 min after the initiation of the working mode. 'Preischaemic' data can also be represented as a percentage (%) *of* the baseline values and as a '% *change*' *from* the baseline values (i.e. 100%−% of baseline value). 'End reperfusion' data are considered to be the measurements taken at the last recorded time point of the perfusion protocol, at 30 min after re-initiating the working heart mode during reperfusion.

2.5. Analysis of results

Data are expressed as mean±S.E.M. Statistical analyses were performed with the aid of Statview v. 5 for Windows (SAS Institute Inc., NC, USA). The statistical differences between groups were calculated by two-tailed Student's *t*-test as appropriate. For the working heart model, heart function data were compared using two-way (time and treatment factors) analysis of variance (ANOVA) for repeated measurements. Where 'preischaemic' data were expressed as % *change from* baseline values, one-way ANOVA was used to detect any significant inter-group differences. Fisher's protected least-significant difference (PLSD) post-hoc test was used for multiple comparisons if significant *F*-values were obtained. Differences were considered to be statistically significant when $P<0.05$.

3. Results

3.1. Propofol increases functional recovery from ischaemia of Langendorff perfused hearts

Table 1 presents data on the effects of 2 μg/ml propofol on the functional recovery of Langendorff perfused hearts subjected to 30 min global ischaemia at 37°C. When compared to control hearts treated with intralipid, the presence of propofol had no effects on the preischaemic heart rate, left ventricular developed pressure (LVDP) or left ventricular end diastolic pressure (LVEDP). It was confirmed that intralipid alone also had no significant effects (data not shown). During the ischaemic phase, the presence of propofol significantly delayed the time taken for contracture to be initiated and decreased the maximum ischaemic contracture. Recovery of LVDP was significantly improved by propofol after 30 min reperfusion and

Table 1
Effect of propofol (2 μg/ml) on the functional recovery of Langendorff hearts and the MPT following ischaemia and reperfusion[a]

	n	Preischaemia				Ischaemia		Reperfusion				
		LVDP (mmHg)	LVEDP (mmHg)	HR (bt/min)	LVDP×HR (mmHg·bt/min)×10^3	Ischaemic contracture (mmHg)	Time to contracture (min)	LVDP (mmHg)	LVEDP (mmHg)	HR (bt/min)	LVDP×HR (mmHg·bt/min)×10^3	DOG Ratio (units)
Intralipid control	10	78.1±2.9	1.65±0.70	277±36	21.6±1.1	53.8±3.9	12.8±0.7	28.4±6.2	52.9±4.3	209±24	5.9±1.8	22.5±1.8
Propofol	8	78.5±4.3	1.06±0.52	257±16	20.2±1.7	39.5±3.5**	15.6±1.1*	53.3±7.3*	37.5±3.9**	223±15	11.9±1.7*	16.7±1.2**

[a] The perfusion protocol and method for determining mitochondrial [^{3}H]-DOG-6P entrapment are described under Methods and schematically in Fig. 1a. Data are presented as means±S.E. of the number of experiments shown. The [^{3}H]-DOG-6P entrapment of control hearts subjected to 120 min perfusion without ischaemia were 8.3±1.3 and 5.0±0.9 ratio units (n=4) in the absence and presence of propofol. Values for the LVDP and LVEDP in these experiments were not significantly different from data reported for preischaemic hearts (data not shown), although the heart rate was significantly reduced by propofol from 279±5 to 242±11 beats per minute (P<0.02). Abbreviations used: as listed in the text.

Statistical significance of propofol-treated versus intralipid-only controls * P<0.05; ** P<0.025.

the LVEDP was less elevated. Indeed, there was an inverse correlation between the recovery of LVDP and the LVEDP (r=−0.82; P<0.001).

3.2. Propofol decreases mitochondrial [^{3}H]-DOG-6P entrapment during reperfusion

Table 1 also presents data on the mitochondrial entrapment of [^{3}H]-DOG-6P as an estimate of the extent of the MPT. As described under Methods, data are expressed as ratio units (10^5×[mitochondrial [^{3}H] dpm per unit citrate synthase]/[total heart [^{3}H] dpm/g wet wt]). If all mitochondria undergo the MPT the predicted value for the mitochondrial [^{3}H]-DOG-6P entrapment is 110 units. Control hearts were perfused for 15 min before loading with [^{3}H]-DOG by recirculation for 30 min and then perfused in flow-through mode for a further 60 min. Mitochondrial [^{3}H]-DOG-6P entrapment was 8.3±1.3 and 5.0±0.9 ratio units in the absence and presence of propofol, respectively. In hearts that experienced 30-min ischaemia followed by 30-min reperfusion the values increased to 22.5±1.8 and 16.7±1.2 units, respectively. Thus, propofol gave a significant (P<0.025) decrease in mitochondrial pore opening coincident with its protection of heart function.

3.3. Propofol treatment of hearts improves respiration and inhibits MPT pore opening by isolated mitochondria

The [^{3}H]-DOG-6P entrapment data suggest that propofol protects mitochondria from undergoing the MPT. This protection could involve a direct effect on the MPT mechanism as has been demonstrated previously by others [31,32]. However, in these earlier experiments, concentrations of propofol much greater than used clinically were employed. In contrast, when we added propofol at 2 μg/ml (in intralipid) to isolated mitochondria, we were not able to demonstrate any protective effect (see dotted line on Fig. 2a). However, mitochondria isolated from propofol-treated hearts did show less pore opening at the same [Ca^{2+}] than those from control hearts as shown in Fig. 2a. This was true at a range of [Ca^{2+}], but the effect was less as [Ca^{2+}] increased (Fig. 2b). It should be noted that the conditions we have used for measuring the MPT are designed to eliminate effects of propofol on the MPT that might be secondary to calcium loading of the mitochondria or differences in membrane potential, both of which are potent modulators of the MPT [14,34]. Since the MPT is greatly sensitised to [Ca^{2+}] by oxidative stress [14,34], the inhibitory effect of propofol-treatment on the MPT that we observe may reflect the established antioxidant properties of propofol. This conclusion is strengthened by the data of Table 2 which presents rates of ADP-stimulated respiration by mitochondria from control and propofol-treated hearts. For oxidation of substrates entering the respiratory chain at complex I (L-malate in the presence of glutamate or 2-oxoglutarate) or complex II (succinate) values for mitochondria from propofol treated hearts were significantly greater than for those from control hearts, whilst for ascorbate oxidation (enters at cytochrome *c*) there was no significant stimulation. Rates of respiration in the presence of uncoupler (0.1 μM carbonylcyanide *p*-trifluoromethoxyphenylhydrazone which gives maximal stimulation of respiration) were also determined and found to be the same as rates stimulated by ADP. This implies that respiration is limited by electron flow through the respiratory chain rather than ADP translocation or ATPase activity under these conditions, and thus that propofol-treatment is stimulating electron flow from NADH and succinate to cytochrome *c*. Inhibition of complex 1 and complex 3 activity is characteristic of oxygen free radical damage to mitochondria [12,16] and thus these data are also consistent with propofol reducing oxidative stress even in mitochondria from control hearts.

3.4. Propofol improves the functional recovery of the working rat heart from ischaemia with cold cardioplegic arrest

The effects of propofol and intralipid on the function of the preischaemic isolated perfused working rat heart are reported in Table 3. ANOVA analysis of the raw data of all groups together (preischaemia vs. baseline: effect of time and treatment), revealed significant interaction with the

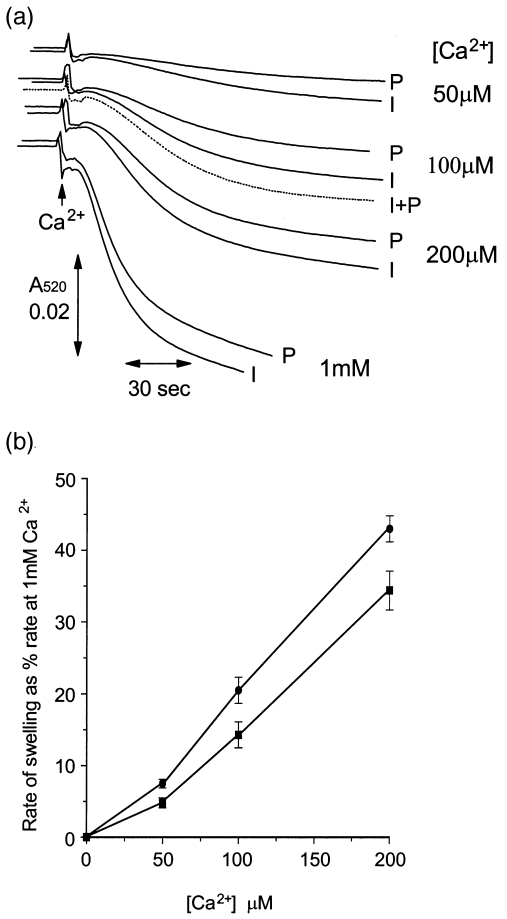

Fig. 2. The effects of propofol (2 μg/ml) treatment of perfused rat hearts on the Ca^{2+} sensitivity of the MPT in subsequently isolated mitochondria. The perfusion protocol and mitochondrial preparation was as described in Table 2. Measurement of the MPT under de-energised conditions was performed by monitoring the calcium-induced decrease in light scattering (A_{520}) as described under Methods. In (a) original traces are shown for one mitochondrial preparation derived from either intralipid (I) or propofol-treated (P) hearts, with swelling induced by the free concentration of Ca^{2+} shown. The dotted line at 100 μM [Ca^{2+}] shows the effects of 2 μg/ml propofol added directly to the cuvette on the swelling of mitochondria from intralipid treated hearts. In (b), mean data (±S.E. as error bars) are shown for five separate mitochondrial preparations. Rates of swelling are expressed relative to the maximum rate determined at 1 mM [Ca^{2+}] and were determined by differentiation of the traces shown to obtain the maximum rate of change of A_{520}. This value was unchanged by propofol treatment being 0.0059±0.0007 and 0.0062±0.0005 A_{520} units per second for intralipid controls (solid circles) and propofol (solid squares) treated hearts, respectively.

effect of time (significant F-values, $P<0.05$ for HR, AP, RPP, AF, CO and ECW) but not with treatment alone. When each group was analysed separately for the interaction of time, only within the propofol group was the preischaemic heart performance found to be significantly depressed when compared to baseline. Furthermore, when the raw data were transformed and represented as percentages of baseline values (data not shown), 'preischaemic' heart performance was shown by ANOVA to be significantly different among groups; significant F-values for the interaction of both time and treatment were obtained for HR, AP, RPP, AF, CO and ECW. These data suggested that propofol (4 μg/ml) exerted a significant depressive effect on heart pump function when added to perfusion media before cardioplegic arrest. Likewise, when the preischaemic data (HR, AP, RPP, AF, CO and ECW) were expressed as % *change* from baseline values, inter-group comparison also suggested that the observed depression by propofol was significantly greater than any observed changes in the control and intralipid groups. For coronary flow, ANOVA did not reveal any significant inter-group differences in raw data prior to cardioplegic arrest, but when expressed as a percentage of baseline, inter-group differences were shown (significant F-value, $P<0.001$). In the intralipid group, an unexplained significant 6% drop in preischaemic coronary flow was observed ($P<0.01$ vs. control and propofol treated hearts, see Fig. 3c). At the same time, the 7% increase in CF of the propofol group fell just short of statistical significance when compared with control hearts ($P=0.052$).

All hearts recovered after ischaemic cardioplegic arrest. In Fig. 3a–d, raw data for CO, ECW, CF and LAP are plotted as means at consecutive 5-min intervals during working heart mode before and after cardioplegic arrest. Statistically significant effects of propofol on recovery of the heart at the end of the reperfusion protocol were detected by repeated measurements ANOVA (significant F-values, $P<0.05$ for effect of group interaction). Thus, inter-group comparisons of the raw data and data expressed as percentage recovery (i.e. percentage of baseline) revealed that the propofol treated hearts had superior functional recovery in measured AP, AF, CO and ECW. Mean CF was also consistently higher on reperfusion in the propofol group (see Fig. 3c). Mean LAP was elevated in all the hearts after cold cardioplegic arrest but hearts of the propofol group showed better recovery towards baseline upon reperfusion ($P=0.02$ and 0.055 for propofol vs. control and intralipid, respectively, when analysed as % recovery).

4. Discussion

Our results confirm data of others that propofol protects isolated perfused rat hearts from ischaemia–reperfusion injury [3,4]. The concentrations of propofol used in the present study are lower than in other experiments and more typical of concentrations employed in clinical anaesthesia [1,35,36]. This is important because higher concentrations of propofol have been reported to impair oxidative phosphorylation by isolated mitochondria [32,37,38] which may account for reports that propofol has deleterious effects on reperfusion injury in dog and pig hearts [39,40]. The present study has confirmed that propofol at 4 μg/ml used in combination with cold cardioplegia confers significant additional protection from ischaemia–reperfusion

Table 2
The effect of propofol treatment of perfused rat hearts on the respiration of their isolated mitochondria[a]

Treatment of hearts	Respiratory substrate	Rate of respiration (nmol O_2/min per mg of protein)		
		State 2	State 3	Ascorbate
Intralipid	Glutamate	80±4	132±8	1124±107
	2-Oxoglutarate	77±5	246±32	1070±94
	Succinate	166±3	250±4	1015±50
Propofol	Glutamate	81±4	164±8*	1263±80
	2-Oxoglutarate	85±6	359±31*	1156±45
	Succinate	208±9**	338±20**	1193±101

[a] Hearts were perfused for 15 min in flow-through mode, 30 min in recirculation mode with 0.5 mM DOG and then 15 min in flow through mode, the final 10 min of which was in the presence of intralipid (as controls) or 2 μg/ml propofol. Preparation of mitochondria and measurement of rates of respiration were performed as described under Methods. Respiratory substrates used were 2.5 mM 2-oxoglutarate+1 mM L-malate, 2.5 mM L-glutamate+1 mM L-malate or 2.5 mM succinate+1 μM rotenone. Rates of respiration were measured in the absence (state 2) and presence (state 3) of 1 mM ADP and then uncoupler (0.1 μM carbonylcyanide *p*-trifluoromethoxyphenylhydrazone), but the latter were found to be the same as ADP stimulated rates and are not included. At the end of each run, 0.5 μM antimycin A and 10 mM ascorbate+0.3 mM *N,N,N′,N′*-tetramethyl-*p*-phenylendiamine (TMPD) were added and the new rate of respiration measured. Data are presented as means±S.E. of five separate mitochondrial preparations for each perfusion condition.
* $P<0.05$; ** $P<0.005$ vs. intralipid control.

injury in the working heart model. However, at 2 μg/ml propofol no additional protection was observed in this system (data not shown) although it did offer protection in the ischaemic reperfused Langendorff preparation without cardioplegic arrest. Because cold cardioplegic arrest itself protects the heart, a higher concentration of propofol may be required to demonstrate an additional margin of protection.

In the propofol group, heart performance indices such as AP, RPP, AF, CO and ECW were all shown to have greater

Table 3
The effect of propofol (4 μg/ml) on working heart performance before and after cold cardioplegic arrest[a]

	n	HR (bt/min)	AP (mmHg)	RPP (mmHg·bt/min) (x10^3)	AF (ml/min)	CO (ml/min)	ECW (J/s)	CF (ml/min)	LAP (mmHg)
Baseline									
Control	12	278±8	103±1	28.5±0.8	52±1	68±2	0.93±0.03	16.3±0.6	8.8±0.3
Intralipid	12	274±10	103±2	28.2±1.2	51±1	67±2	0.93±0.04	15.9±0.5	9.0±0.2
Propofol	14	282±9	104±2	29.2±1.0	53±2	69±2	0.96±0.04	15.7±0.7	8.9±0.2
Preischaemic									
Control	12	279±9	101±2	28.1±1.0	50±2	66±2	0.89±0.03	16.5±0.5	9.1±0.3
Intralipid	12	270±11	103±2	27.7±1.3	51±1	66±2	0.91±0.04	14.9±0.5†	9.0±0.2
Propofol	14	260±9†	99±2†	25.8±1.0†	46±3†	63±3†	0.84±0.05†	16.8±0.8	9.2±0.3
% *Change*									
Control	12	0±2	−2±1	−2±2	−4±1	−3±1	−5±1	2±2	3±2
Intralipid	12	−2±1	0±0	−2±1	0±1	−2±1	−2±1	−6±1$^{\#}$	0±0
Propofol	14	−7±1*	−4±1*	−11±1*	−14±3*	−9±2*	−13±3*	7±2**	3±1
End reperfusion									
Control	12	280±12	83±2†‡	23.2±1.1†‡	27±4†‡	38±4†‡	0.42±0.05†‡	11.1±0.7†‡	11.0±0.3†‡
Intralipid	12	261±12†	83±2†‡	21.9±1.3†‡	28±3†‡	39±3†‡	0.44±0.05†‡	11.0±0.8†‡	11.1±0.4†‡
Propofol	14	280±15 ‡	89±1†‡*	24.9±1.4†	37±2†‡*	50±2†‡*	0.60±0.03†‡*	13.4±1.2†‡	10.1±0.3†‡
% *Recovery*									
Control	12	101±3	81±1	81±3	51±6	55±6	45±5	68±4	126±5
Intralipid	12	95±3	81±2	78±3	55±5	58±5	49±5	69±5	123±4
Propofol	14	99±3	86±2	85±3	70±3*	73±2*	64±3*	84±5*	114±2$^{\#}$

[a] The perfusion protocol is described schematically in Fig. 1b. Baseline data are read immediately before the addition of test agents, at 10 min into working heart mode. Preischaemic data that are read just before initiating cardioplegic arrest at 20 min, are also presented as a percentage (%) change from baseline values. Data collected at the end of the reperfusion protocol are also expressed as % recovery relative to baseline data. Data are presented as mean±S.E. of the three heart preparations: control, intralipid-only and propofol treated groups. Please see text for explanation of abbreviations and statistical methods used.
† $P<0.05$ vs. baseline; ‡ $P<0.05$ vs. preischaemia; * $P<0.05$ vs. both control and intralipid; $^{\#}$ $P<0.05$ vs. control; ** $P<0.05$ vs. intralipid.

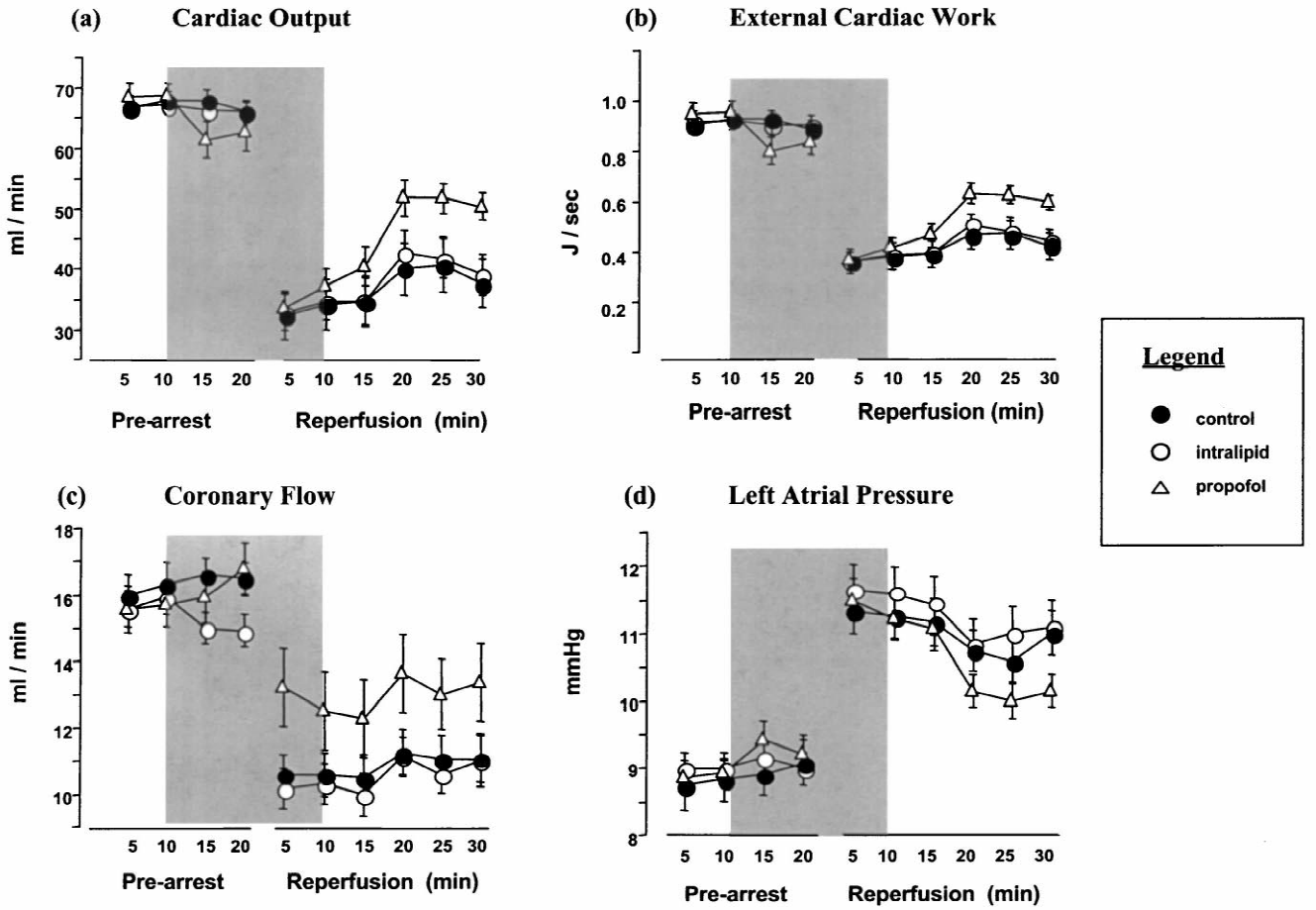

Fig. 3. Cardiac function of the isolated working rat heart subjected to cold cardioplegic arrest and subsequent reperfusion. The perfusion protocol is described under Methods and Fig. 1b. Propofol (4 μg/ml dissolved in intralipid emulsion) or intralipid alone was added 10 min after initiation of the working heart mode and washed out after 10 min into working heart reperfusion. Data are plotted as means±S.E. (as error bars) for the separate heart preparations: control, intralipid and propofol (n=12,12 and 14, respectively). The shaded area represents the period when test agents were present. The break in the plots represents 60 min cold cardioplegic arrest and 10 min initial Langendorff reperfusion.

recovery following ischaemic insult when compared with the control and intralipid groups (Table 3). In this study, propofol depressed work performance before ischaemia and increased the mean preischaemic CF which may increase oxygen availability to the cardiomyocytes. The propofol treated hearts showed greater recovery in CF after ischaemia (propofol 84% vs. control 68% at 30 min working heart reperfusion, $P<0.03$), even after the agent had been washed out following the critical and most damaging early reperfusion period (see Fig. 3c). The effect of propofol on coronary vasculature in ischaemia–reperfusion injury is uncertain but may involve factors other than free radicals and endothelial cell function [41,42]. In our model, with a constant preload driving force set at 15 cm fluid column height, changes in the measured 'atrial pressure' reflects the 'resistance' to flow as a result of heart pump dysfunction. Therefore, as a surrogate, higher recorded atrial pressures may reflect the ventricular dysfunction (elevated end diastolic pressures, reduced ventricular filling and a fall in cardiac output) characteristic of ischaemia–reperfusion injury [43–47]. In the propofol treated group, consistent with its protective effects, there was a trend towards lower mean atrial pressures during reperfusion when compared with the control and intralipid groups (Fig. 3d).

This is the first study showing the cardioprotective effects of propofol during cold cardioplegic arrest and is clinically relevant in the setting of cardiac surgery. Furthermore, the Langendorff initial reperfusion mimics the situation in clinical practice where, following the removal of the proximal aortic cross-clamp at the end of cold cardioplegic arrest, the heart is provided with continuing circulatory support as the patient is gradually weaned from cardiopulmonary bypass.

Our data show that one mechanism by which propofol exerts its protective effects is through preservation of mitochondrial function during reperfusion. In particular, protection of the mitochondria from the permeability transition may be important. Thus we have shown that significantly less opening of the MPT pores occurs during

reperfusion in the presence of propofol (Table 1). Furthermore, the permeability transition in mitochondria isolated from hearts treated with propofol is less sensitive to [Ca^{2+}] than control mitochondria (Fig. 2b). Others have demonstrated that direct addition of propofol to mitochondria can inhibit the MPT, but the concentrations used in these studies were about 10-fold higher than used in the present experiments [31,32]. At such high concentrations propofol has a range of effects on mitochondria, probably reflecting non-specific effects on membrane fluidity [32,37,38]. In our own experiments using 2 μg/ml propofol, we found no inhibition of the MPT when added directly to isolated de-energised mitochondria (Fig. 2a). Thus the protective effect of propofol on mitochondria in situ may not be through a direct effect on the MPT, but through indirect mechanisms. These probably include the well-documented ability of propofol to act as a free radical scavenger, a property which will lessen the oxidative stress experienced on reperfusion [5–8,48]. In isolated mitochondria such anti-oxidative effects have been observed at propofol concentrations as low as 1 μM [5]. Oxidative stress is known to modify thiol groups on the adenine nucleotide translocase causing an increase in the Ca^{2+}-sensitivity of the MPT [14,34]. It has also been shown to damage the respiratory chain complexes and so inhibit respiration. Our observation of greater respiratory chain activity found in the propofol-treated hearts (Table 2) is consistent with propofol diminishing such oxidative damage even under normal perfusion conditions. Our data also provide some evidence for a decrease in MPT pore opening by propofol under these basal conditions since the [^{3}H]-DOG-6P entrapment was reduced from 8.3±1.3 and 5.0±0.9 ratio units (n=4; P=0.08). We have previously suggested [16–18] that there may be transient MPT pore opening even in control hearts, and our present data are consistent with this reflecting the presence of reactive oxygen species in control hearts, whose production is reduced by propofol treatment. Another possible mechanism of action of propofol would be to reduce the calcium overload that occurs during ischaemia–reperfusion through the reported inhibitory effects on calcium channels [9,10]. This might also account for the decrease in both the time to contracture and maximal extent of contracture observed in the presence of propofol, and also the lower LVEDP during reperfusion (Table 1).

In conclusion, the present study provides evidence that propofol, when present at clinically relevant doses, can protect the heart from ischaemia–reperfusion injury with or without cardioplegia. The inclusion of propofol was associated with a significant reduction in the opening of MPT pores, suggesting its cardioprotective actions are, in part at least, mediated by its effect on the mitochondria. This effect is likely to be indirect, through its action as a free radical scavenger. Propofol may therefore be a useful adjunct to the cardioplegic solutions, as well as being an anaesthetic agent used in cardiac surgery.

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