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Functional comparison of anaesthetic agents during myocardial ischaemia–reperfusion using pressure–volume loops

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Background. Left ventricular (LV) pressure–volume (PV) loops were used to compare the functional effects that accompany the cardioprotection seen with desflurane, sevoflurane, and propofol in a rabbit preparation of coronary ischaemia–reperfusion (IR).

Methods. Male New Zealand White rabbits (n=48) were anaesthetized with propofol (70 mg kg^{-1} h^{-1}), desflurane (8.9%), or sevoflurane (3.8%) and randomized to receive IR or non-ischaemic time-matched (TC) perfusion protocol. IR groups (desIR, propIR, and sevIR) underwent 30 min of left anterior descending coronary artery occlusion and then 120 min of reperfusion. TC groups (desTC, propTC, and sevTC) were anaesthetized for 150 min without ischaemia. Haemodynamic endpoints included mean arterial pressure, heart rate, cardiac index, systemic vascular resistance index, preload-recruitable stroke-work, time constant of relaxation (τ), and end-diastolic PV relationship (EDPVR). Ventricles in the IR groups were excised and stained with 2,3,5-triphenyl-tetrazolium chloride in order to measure infarct size.

Results. Myocardial infarction size was greater in the propIR group [35.74 (SD 11.32)%] compared with the desIR [13.44 (3.09)%] and sevIR [17.96 (6.63)%] groups ($P<0.001$). EDPVR deteriorated in the sevIR and propIR groups compared with their TC groups, sevTC (P=0.03) and propTC (P=0.044), respectively. There was no difference in any haemodynamic endpoints for the desIR group compared with its TC control (desTC).

Conclusions. During ischaemia, all anaesthetics provide haemodynamic stability and preservation of LV contractility, whereas propofol and sevoflurane, but not desflurane, caused increased LV diastolic stiffness. Desflurane and sevoflurane provide superior cardioprotection compared with propofol.

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The anaesthetic agents desflurane, sevoflurane, and propofol are known to provide protection against ischaemia in the myocardium.[1 2] The mechanisms by which the volatile anaesthetics protect the myocardium against ischaemia have been demonstrated at a molecular level and involve the preservation of mitochondrial electron transport power.[1] Propofol has also been shown to reduce post-ischaemic myocardial injury by attenuating indices of oxidative stress in a dose-dependent fashion.[3 4] It remains unclear as to which of these agents provides greater protection to the heart during ischaemia–reperfusion (IR).

In certain types of surgery, such as cardiac surgery or surgery where temporary vascular occlusion occurs, IR may be a predictable occurrence. To date, there has been no study that investigates the functional properties of anaesthetic agents upon the myocardium when administered during IR. If specific anaesthetic agents can be shown to have superior organ protective properties, then

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the anaesthetic itself may reduce the extent of organ damage and facilitate earlier and more complete functional organ recovery. As most anaesthetic agents have both myocardial depressant and vasodilator properties, it can be difficult to separate myocardial from vascular effects using traditional ejection phase indices of systolic function (such as cardiac output or ejection fraction). Load-independent measurements of contractility and diastolic function, such as pressure–volume (PV) loop technology,[5 6] allow measurement of myocardial function separate from systemic vascular effects, but as they are highly invasive measurements, data in humans are rare.

The aim of this study was to utilize PV loops to compare the functional effects of the common anaesthetic agents desflurane, sevoflurane, and propofol when delivered to a rabbit preparation undergoing acute coronary IR.

Methods

The study was approved by the Animal Experimental Ethics Committee at the University of Melbourne which is compliant with the Guide for the Care and Use of Laboratory Animals according to the US National Institutes of Health (A5031-01) and was carried out in accordance with the guidelines of the National Health and Medical Research Council of Australia. Forty-eight male New Zealand White rabbits, weighing between 2 and 4 kg, were randomly assigned to receive anaesthesia using desflurane, sevoflurane, or propofol. Within each anaesthetic group, rabbits were further randomized to receive either an IR or a non-ischaemia perfusion time-matched (TC) control protocol. This resulted in six groups: desflurane IR (desIR), sevoflurane IR (sevIR), propofol IR (propIR), and their TC controls (desTC, sevTC, and propTC).

Anaesthesia

After s.c. infiltration with lidocaine 1%, a 22 G catheter was inserted into the left ear vein and anaesthesia was induced with propofol (Fresofol 1%, Pharmatel Fresenius Kabi, Austria) 5–10 mg kg^{-1} i.v. Rabbits were intubated and mechanically ventilated (small animal ventilator 7025, Ugo Basile, Camoerio, Italy). Tidal volume was initially set at 6 ml kg^{-1} and rate at 60 bpm. Rabbits then received a 1 MAC equivalent of either desflurane 8.9% (Baxter, IL, USA), sevoflurane 3.8% (Abbott, IL, USA), or propofol (70 mg kg^{-1} h^{-1}) as the sole anaesthetic agent with oxygen 100% according to the randomization protocol previously described.[7 8] MAC is the minimal anaesthetic concentration to prevent movement to a surgical stimulus in 50% of animals, and is a measure of anaesthetic equivalence between different agents.[9] We have previously shown in rabbits that a propofol infusion rate of 70 mg kg^{-1} h^{-1} results in a mean whole-blood propofol concentration of 8.8 mg $litre^{-1}$ after 1 h of infusion.[6] Rabbits anaesthetized with desflurane and sevoflurane received saline 0.9% (7 ml kg^{-1} h^{-1}) to match the volume of infusion in the propofol group. In order to ensure that depth of anaesthesia was sufficient during surgical preparation, anaesthesia concentration was adjusted in anticipation of painful events such as incision or sternotomy. Therefore, depending upon group selection, either the volatile anaesthetic was increased or additional boluses of propofol were administered in order to prevent movement. Anaesthesia continued at the prescribed levels when there was no surgical stimulation. Adequate depth of anaesthesia was assessed in animals at regular intervals by deep paw pinch and for the presence of apnoea when ventilation was intermittently ceased during surgical access to the heart. The absence of gross purposeful movement and spontaneous ventilation indicated that rabbits were adequately anaesthetized by this technique.

Surgery

Body temperature was maintained at 39°C using a regulated surface heating blanket (Harvard Apparatus, Holliston, MA, USA). Saline-filled catheters were inserted into the right internal carotid artery and into the right atrium through the right internal jugular vein. The heart was exposed via a median sternotomy and a Doppler flow probe was placed around the ascending aorta in order to record cardiac output and heart rate (HR) (T206, Transsonic Systems Inc., Ithaca, NY, USA). Right atrial pressure (RAP), cardiac output, HR, and both phasic and mean arterial pressure (MAP) were recorded on a PowerLab data acquisition system (8SP, AD Instruments, Sydney, NSW, Australia). A 3 Fr combined micromanometer pressure and dual-field conductance catheter (SPR-877, Millar Instruments, TX, USA) was inserted into the left ventricle (LV) via an apical stab. The conductance catheter contains a 10-electrode dual field system that has a flat frequency response up to 10 kHz and allows simultaneous high-fidelity recording of LV pressure and volume. Calibration of the catheter involved determination of parallel conductance of the surrounding myocardium (Vc) by the introduction of saline 10% (1 ml) solution into the right atrial catheter with simultaneous recording of PV calibration loops to produce a Vc calibration file. Five millilitres of blood were withdrawn to determine specific blood resistance (ρ). In addition, during subsequent acquisition of each PV loop, the cardiac output measured by the conductance catheter was then compared with the cardiac output as determined by the aortic flow probe in order to produce a constant of proportionality (α) that was utilized later during the offline analysis. A silicone sling was placed around the inferior vena cava in order to perform acute preload reduction. Both phrenic nerves were divided in the unlikely event that respiratory effort was to interfere with PV loop acquisition. Arterial blood gas was sampled before the start of the perfusion protocol and then repeated every hour. Ventilation

parameters were adjusted to maintain pH=7.4, Pa_{CO_2}=40–45 mm Hg, and Pa_{O_2}>100 mm Hg. After surgery, rabbits received a 30 ml bolus of i.v. NaCl 0.9% solution and a period of 30 min was then allowed for the preparation to stabilize before baseline recordings.

Perfusion protocol

For rabbits in the time-control groups, anaesthesia was maintained at 1 MAC for the duration of the experiment (150 min). In the animals that were subjected to the IR protocol, a silk suture was ligated around the proximal left anterior descending (LAD) coronary artery, for 30 min, at a point one-third of the distance from the origin of the LAD to the apex of the heart. Confirmation of LAD occlusion was evident by distal blanching of the myocardium. After 30 min, the ligature was released to permit reperfusion for a further 120 min.

Data collection

The raw haemodynamic variables, HR, MAP, RAP, and cardiac output, and also raw PV loop data were recorded at baseline and then at 2, 5, 10, 20, and 30 min during induced ischaemia, and then at 2, 5, 10, 20, 30, 40, 50, 60, 80, 100, and 120 min during the reperfusion phase. Rabbits in the non-ischaemic-matched time-control groups had the same data recorded at identical corresponding time intervals. All haemodynamic and PV loop data were digitally stored for subsequent analysis (Chart v5.5.1, AD Instruments, and Conduct NT v2.8, CD Leycom, Zoetermeer, The Netherlands), respectively. During offline PV loop analysis, data were corrected with the previously determined values for ρ, Vc, and α to produce absolute values for stroke volume, end-systolic volume, and end-diastolic volume during each cardiac cycle. Data analysis produced three families of haemodynamic endpoints: cardio-circulatory variables included MAP, HR, cardiac index (CI), and systemic vascular resistance index (SVRI); LV diastolic function variables included the end-diastolic pressure–volume relationship (EDPVR), and the time constant of relaxation (τ); and LV systolic function, measured by the preload-recruitable stroke-work (PRSW), which is the linear regression of stroke-work against end-diastolic volume. The PRSW and EDPVR were calculated offline using the first 10–15 PV loops immediately after acute preload reduction, and an r^2 value >0.9 was used to determine acceptable regression. Cardiac output was indexed to body weight to calculate CI; SVRI was calculated by 80×(MAP−RAP)/CI. An example of an original registration of a PV loop and the changes that occur during occlusion of the vena cava is illustrated in Figure 1.

A random selection of rabbits (n=6) in the volatile IR groups had a separate catheter placed in an ear artery before induction of anaesthesia from which blood was assayed to measure the decline in propofol concentration after induction. Whole-blood propofol assays were performed post-induction at 10 min intervals in the first hour and then every 30 min until conclusion of the IR protocol. Blood samples were collected in the heparinized tubes and refrigerated at 4°C. Propofol blood concentrations decrease at <0.2% per week at 4°C. They were subsequently analysed using a high-performance liquid chromatography assay, modified from the method of Plummer.[10] This assay is linear to at least 20 mg litre^{-1} and has a detection limit of 0.025 mg litre^{-1} and a coefficient of variation of 4.1% at 2 mg litre^{-1}.[11]

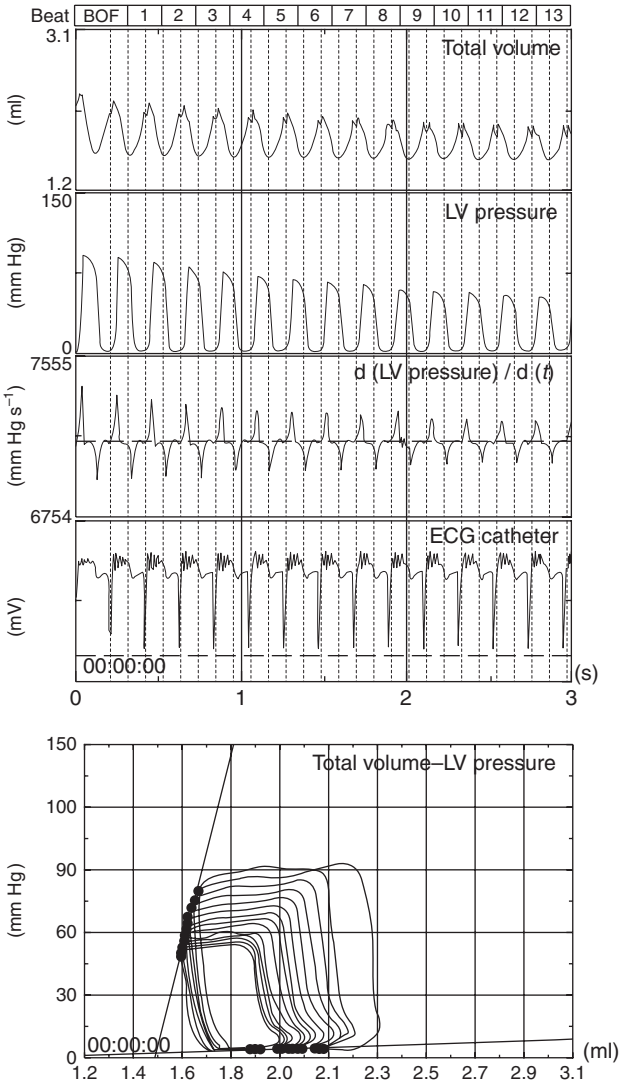

Fig 1 An original registration of a family of PV loops that occurs during acute preload reduction. The parameters in the first four panels are plotted against time. The first panel is instantaneous LV volume. The second panel is instantaneous LV pressure. The third panel is the first derivative of LV pressure (dp/dt). The fourth panel is the electrocardiograph. The last panel is the LV pressure plotted simultaneously against the corresponding LV volume (PV loop). The line intersecting the dots at the base of the family of PV loops is the EDPVR, and the steeper line to the left is the end-systolic PV relationship (measure of contractility, not used in this experiment). The individual area of each PV loop (stroke-work) when plotted against the corresponding end-diastolic pressure gives a straight-line relationship. The gradient of this line is the PRSW (not shown).

Determination of myocardial tissue damage

After data collection, animals that had received the IR perfusion protocol were administered heparin (1000 IU heparin i.v.) and the LAD artery was ligated once again. Rabbits were then killed with sodium pentobarbital (140 mg kg^{-1}) and the heart was excised *en bloc* with surrounding great vessels. The aortic root was cannulated and directly injected with Evans Blue dye 2% (1 ml). This resulted in blue staining of all the myocardium except for the area supplied distal to the ligated LAD (area at risk). The LV was then separated from the rest of the heart and frozen. The LV was weighed and then sectioned parallel to the atrioventricular groove into 2 mm thick slices. The unstained portion of the LV, the area at risk, was separated from the blue-stained portion, the area not at risk, and weighed. The area at risk heart slices were then incubated in 2,3,5-triphenyl 2H-tetrazolium chloride (TTC) in phosphate buffer (pH 7.4) at 37°C for 15 min.[12] All portions of the LV were stored in formaldehyde 4% for 1–2 days before area at risk and infarct size were measured. TTC stains the co-enzyme NADH that is present in viable tissue, brick-red.[13] Infarcted tissue can therefore be easily differentiated as it takes on a pale bleached appearance. Digital photographs were recorded and the area of infarction and the area at risk on both sides of the tissue slices were measured using ImageJ v10.2 software (National Institutes of Health, USA). The area at risk was then calculated by the sum of the ratios of the weights of the non-stained sections to total weight of each LV slice. Similarly, the infarct to area at risk ratio was calculated by dividing the area of the pale tissue by the area at risk.

Statistical analysis

The degree of myocardial tissue damage was expressed as the infarct size/area at risk ratio % (SD). Statistical analysis was performed on raw data. Cardio-circulatory and PV loop data are presented as the change from baseline *vs* time in the IR and time-control group comparisons, while comparisons between IR groups are presented as absolute values. All raw data can be found in Supplementary Tables S1–S7. Each haemodynamic endpoint *vs* time was then compared between IR and respective TC controls for each anaesthetic agent. In addition, all haemodynamic endpoints *vs* time were compared between IR groups. Continuous data are presented as mean (SEM). Repeated-measures analysis of variance (RM-ANOVA), allowing for multisampling asphericity by applying the Greenhouse–Geisser correction, was used to compare changes between the groups over time.[14] Differences in baseline values for all variables, area at risk, and infarct size were compared with one-way ANOVA. If significance was indicated for comparisons between more than one variable, then a Tukey's *post hoc* analysis was performed. Comparisons within groups of families were adjusted for multiple hypotheses with the Ryan-Holm step-down procedure for the Bonferroni inequality (P'). Probability values of P or $P'<0.05$ were considered statistically significant. Statistical analysis was performed using SPSS version 16 (SPSS Inc., IL, USA). The authors had full access to the data and take responsibility for its integrity.

Results

Animal characteristics

The weight of rabbits in each group was similar. The distribution of the weights in the IR groups was 3.3 (0.41) kg and in the time-control groups was 3.3 (0.48) kg [mean (SD)] ($P=0.797$).

Tissue damage

There was no difference in the size of the areas at risk between groups of animals that received different anaesthetic agents and were subjected to myocardial ischaemia, propIR=53.2 (5.5)%, desIR=53.3 (7.7)%, sevIR=45.7 (7.6)% ($P=0.348$). In the same animals, myocardial infarction size in the area at risk was greater in the group that received propofol compared with animals that received either sevoflurane or desflurane, propIR=35.7 (4.0)%, sevIR=18.0 (2.3)%, desIR=13.4 (1.1)%, the propIR group compared with the sevIR group ($P<0.001$), the propIR group compared with the desIR group ($P<0.001$), the desIR group compared with the sevIR group ($P=0.489$). This indicates that the volume of myocardium subjected to ischaemia was similar for each group and that the differences in infarct size were not due to differences in the sizes of the ischaemic insult.

Cardio-circulatory endpoints

There was no difference in the profile of HR, MAP, CI, or SVRI with time for each anaesthetic agent during IR when compared with TC controls, indicating no change in traditional ejection phase indices was seen during IR (Figs 2–4).

LV systolic function

There was no difference in the profile of PRSW with time for each anaesthetic agent during IR when compared with TC controls, indicating no change in LV systolic function was seen during IR (Figs 2–4).

LV diastolic function

There was no difference in τ with time during IR when compared with TC controls for each anaesthetic agent. EDPVR increased with time during IR in the sevIR and propIR groups when compared with their TC controls, sevTC ($P=0.03$) and propTC ($P=0.044$), respectively. This indicates that during IR, there was an increase in the

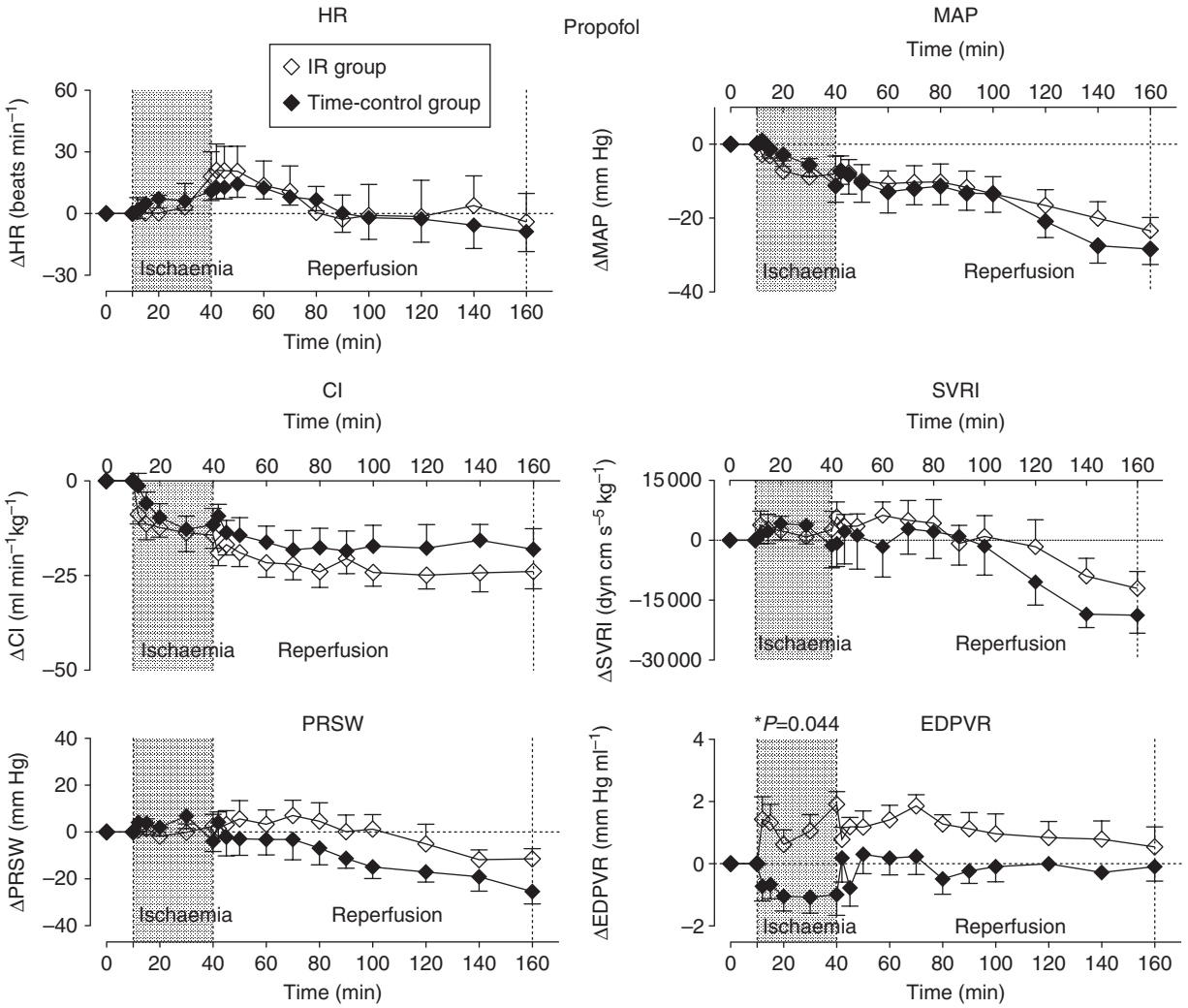

Fig 2 Cardio-circulatory, LV systolic and diastolic function endpoints, comparing the IR and the time-control groups for propofol. Data are presented as mean (SEM). *P=0.044 for the group×time interaction for EDPVR, otherwise P=NS for all other group×time interactions (RM-ANOVA with the Greenhouse–Geisser correction). HR, heart rate; MAP, mean arterial pressure; CI, cardiac index; SVRI, systemic vascular resistance index; PRSW, preload-recruitable stroke-work (contractility); EDPVR, end-diastolic PV relationship (diastolic stiffness).

LV chamber stiffness and a deterioration in LV diastolic function with time in these two groups when compared with their TC controls. There was no difference in EDPVR with time during IR when compared with the TC control group for desflurane, indicating that desflurane preserves LV diastolic function during IR (Figs 2–4).

Comparison of haemodynamic endpoints in IR groups

All the cardio-circulatory variables, PRSW, and EDPVR were compared between each IR group over time to establish whether changes in any of these variables may have contributed to the differences in myocardial infarction size or changes in diastolic function. There was no difference in the profile for HR (P=0.285), CI (P=0.904), PRSW (P=0.328), or EDPVR (P=0.337) between the IR groups (Fig. 5). The profile of MAP with time remained higher in the propIR group compared with the sevIR group (P'=0.012); however, all other comparisons of MAP in the other IR groups were not different, propIR *vs* desIR (P'=0.106) and sevIR *vs* desIR (P'=0.148). SVRI changed over time for the propIR group compared with the sevIR group (P'=0.036); however, other comparisons of SVRI were not different, propIR *vs* desIR (P'=0.154) and sevIR *vs* desIR (P'=0.656) (Fig. 5).

Serum propofol assays

Whole-blood propofol concentrations were measured in six rabbits. At 2 h after induction of anaesthesia, propofol concentrations approached zero, and remained negligible throughout the remainder of the experiment. The whole-blood propofol concentration at the commencement of ischaemia in these rabbits was 0.37 (0.165) mg litre^{-1} [mean (SD)].

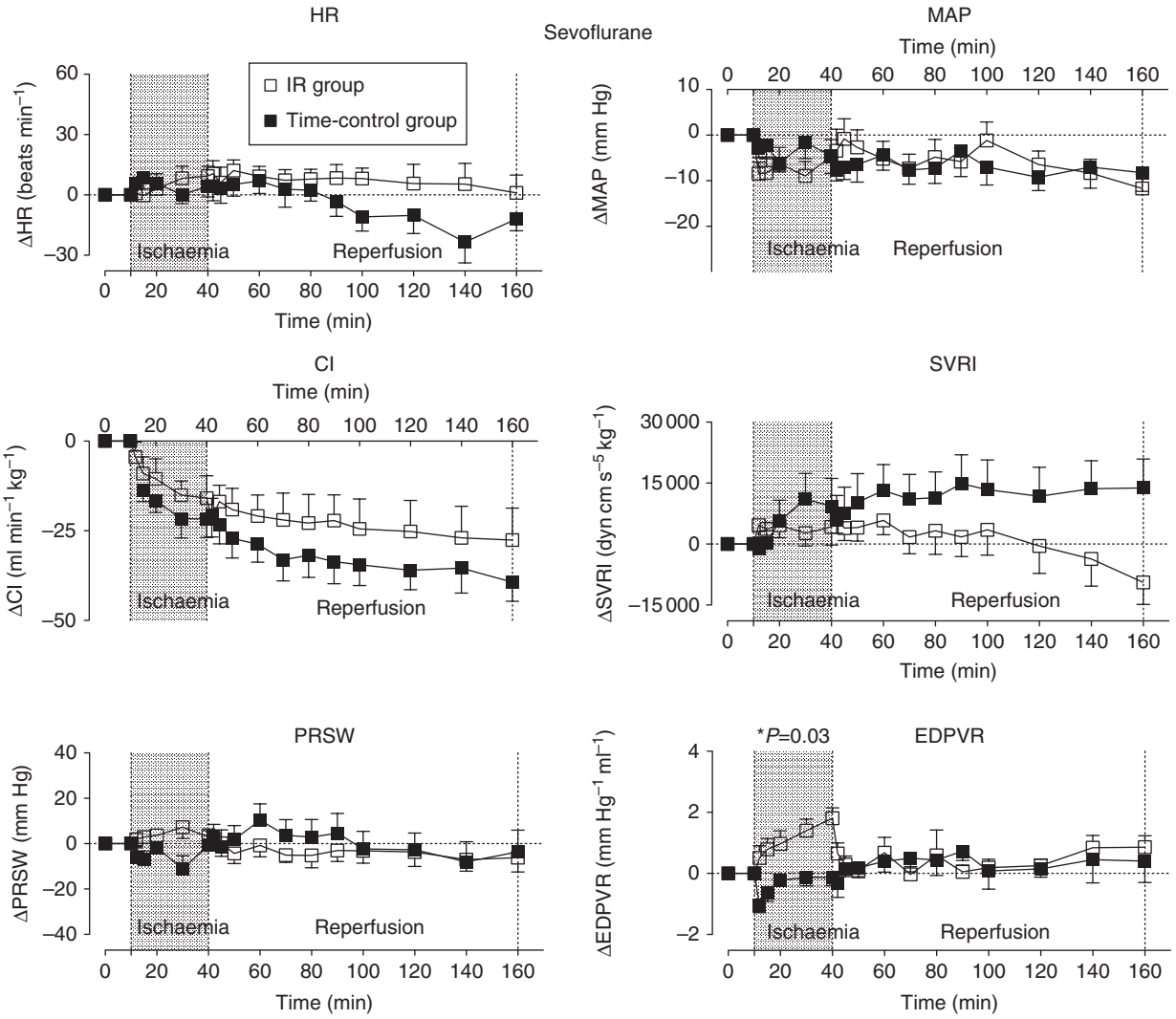

Fig 3 Cardio-circulatory, LV systolic and diastolic function endpoints, comparing the IR and the time-control groups for sevoflurane. Data are presented as mean (SEM). *P=0.03 for the group×time interaction for EDPVR, otherwise P=NS for all other group×time interactions (RM-ANOVA with the Greenhouse–Geisser correction). HR, heart rate; MAP, mean arterial pressure; CI, cardiac index; SVRI, systemic vascular resistance index; PRSW, preload-recruitable stroke-work (contractility); EDPVR, end-diastolic PV relationship (diastolic stiffness).

Discussion

The novel findings of this study indicate that in an open-chest rabbit model of IR, the administration of desflurane, sevoflurane, or propofol preserves LV systolic function and haemodynamic endpoints, despite large areas of induced myocardial ischaemia (>40% of the LV). At the same time, myocardial stiffness, as determined by EDPVR, is significantly impaired by propofol and sevoflurane but is unchanged with desflurane. In addition, this study confirms that the volatile anaesthetic agents sevoflurane and desflurane provide superior cardiac protection during IR, as evident by the induction of a significantly smaller infarct size when compared with the i.v. anaesthetic propofol. The clinical corollary of this is that during the conduct of surgery, it may not be possible for anaesthetists to detect occult ischaemia by relying on traditional clinical monitoring based solely upon pressure and flow. Moreover, the choice of anaesthetic administered during IR may determine the subsequent extent of cell death and long-term organ function.

The absence of changes in cardio-circulatory variables and LV contractility seen during substantial ischaemia in this experiment was counterintuitive. In particular, the functional change in PRSW, which reflects LV systolic function, was not seen to correlate with the extent of myocardial infarction. In addition, despite an area of significant infarction in the area at risk in the propIR group, CI remained unchanged compared with the propTC group. On the basis of the data from this study, we were unable to explain this observation fully. However, stable PRSW on PV loop analysis suggests that the functional loss related to the myocardial infarction may be compensated by improved contractility and regional function in the non-ischaemic areas of the heart. Species differences reveal

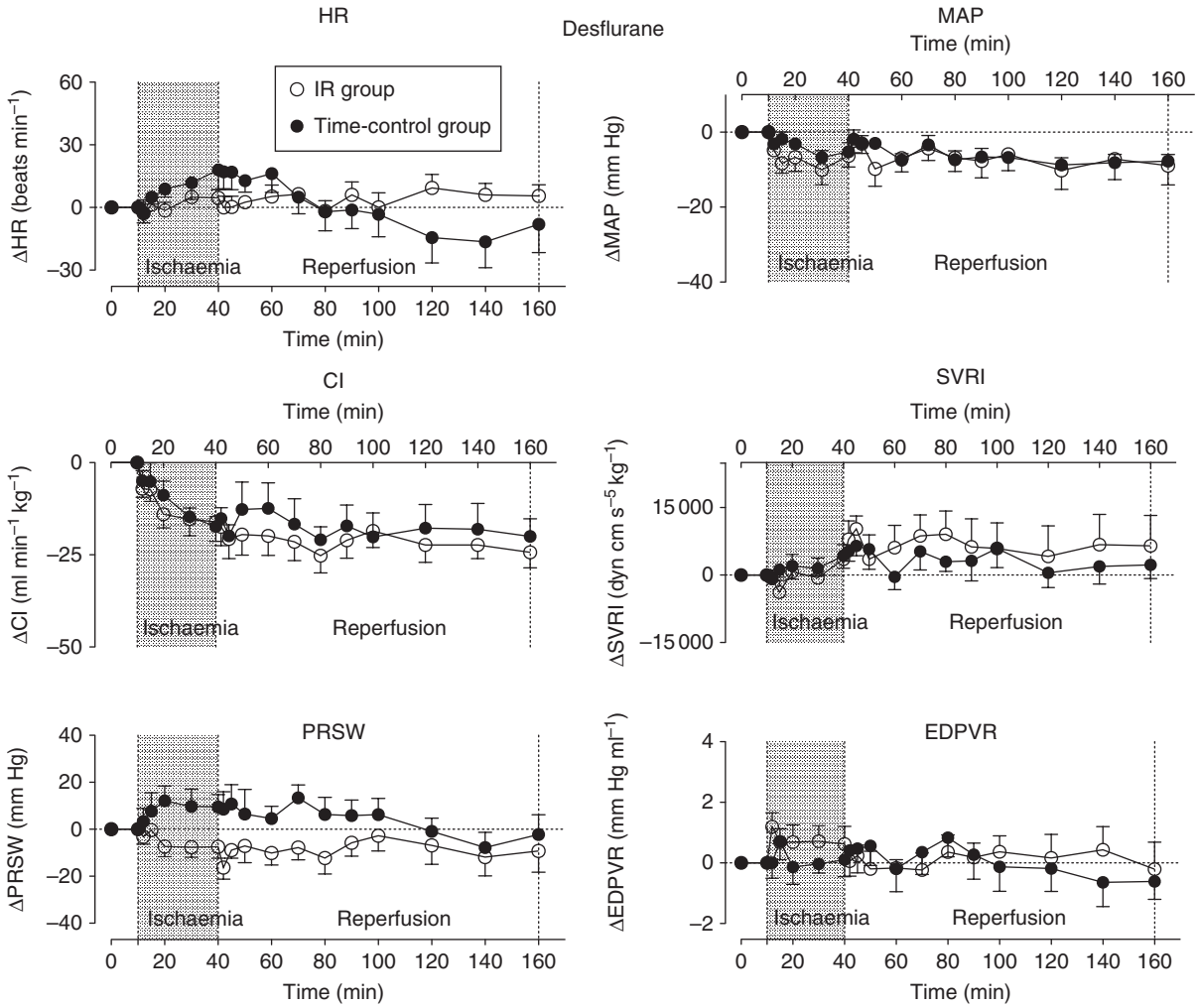

Fig 4 Cardio-circulatory, LV systolic and diastolic function endpoints, comparing the IR and the time-control groups for desflurane. Data are presented as mean (SEM). *P*=NS for all group×time interactions (RM-ANOVA with the Greenhouse–Geisser correction). HR, heart rate; MAP, mean arterial pressure; CI, cardiac index; SVRI, systemic vascular resistance index; PRSW, preload-recruitable stroke-work (contractility); EDPVR, end-diastolic PV relationship (diastolic stiffness).

that a direct relationship exists between body mass, cardiac output, and stroke volume and an inverse relationship relates body mass and HR; this indicates that compared with humans, cardiac output in the rabbit is allometrically rate-dependent and hence less affected by changes in stroke volume.[15] A stable HR, which was seen to occur in all groups, would therefore account for the corresponding minimal changes observed in CI between IR and time-control groups.

The cardio-circulatory parameters in this study with the exception of HR and SVRI (desflurane groups) were seen to decrease over time even in the non-ischaemic time-controls. As this study was designed to investigate the functional interaction of the anaesthetic agents with IR, any parameter that progressively increased or decreased jointly over time in both the IR and the time-control groups would not have produced a between-group effect that differed as a consequence of IR. This is particularly evident with propofol. Propofol anaesthesia resulted in significant decreases in SVRI, MAP, CI, and PRSW over time; however, these changes were seen to occur equally in both the IR and the time-control groups. This produced haemodynamic changes that were most likely related to a property of propofol anaesthesia itself and not secondary to the interaction of propofol with IR. This has been demonstrated in earlier studies that investigated rabbit anaesthesia. For instance, the net result of i.v. propofol infusions administered to rabbits is progressive hypotension, caused by a substantial decrease in SVRI over time.[16 17] In contrast, the study reveals that volatile anaesthesia produces more stable anaesthesia in rabbits, as illustrated by less severe simultaneous reductions from baseline in SVRI and MAP for both the IR and the time-control groups in the desIR, desTC, sevIR, and sevTC groups, respectively.

The graphical profiles of CI and SVRI for sevoflurane and PRSW for desflurane, respectively, appear divergent. However, statistically this is not the case. This is due to

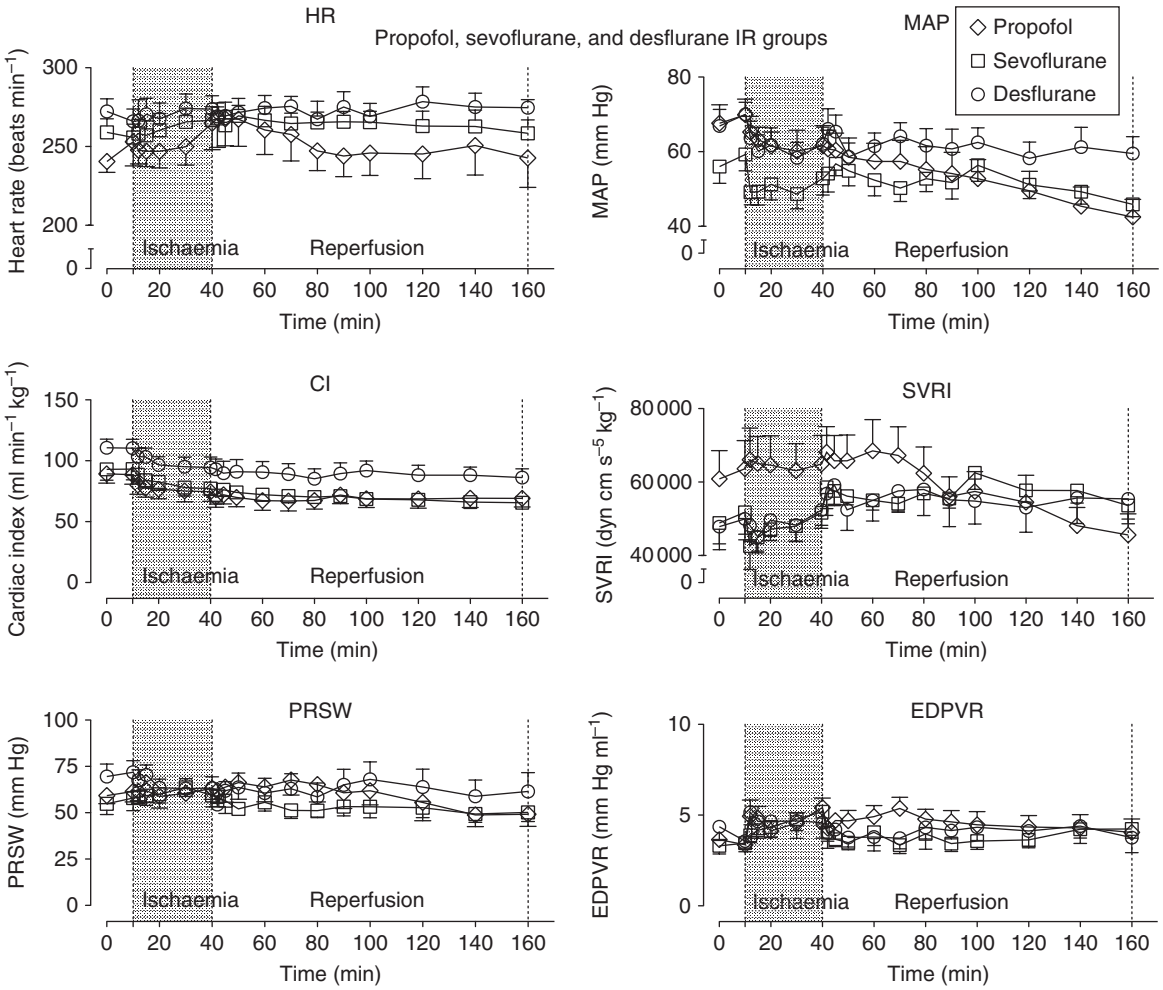

Fig 5 Cardio-circulatory, LV systolic and diastolic function endpoints, comparing the IR groups for propofol, sevoflurane, and desflurane. Data are presented as mean (SEM). Significant differences were detected when individual comparisons were made between IR groups for group×time interaction for propofol and sevoflurane with respect to MAP and SVRI (RM-ANOVA with the Greenhouse–Geisser correction and correction for multiple comparisons, sevIR *vs* propIR for MAP $P'=0.012$ and sevIR *vs* propIR for SVRI $P'=0.036$). Otherwise, P=NS for all other group×time interactions. HR, heart rate; MAP, mean arterial pressure; CI, cardiac index; SVRI, systemic vascular resistance index; PRSW, preload-recruitable stroke-work (contractility); EDPVR, end-diastolic PV relationship (diastolic stiffness).

the consequences of making multiple sequential measurements of a biological variable in the same group of subjects. Observations are not independent in a statistical sense, but are to a greater or lesser extent correlated. The risk of type I error therefore increases with the greater number of within-group observations, which was up to 18 for each parameter in this study. As a consequence, the risk of making false-positive inferences from such an experiment can be exaggerated.[14] In order to prevent the acceptance of a false scientific hypothesis, we choose to apply the Greenhouse–Geisser adjustment for significance to accommodate for such multisampling asphericity. The Greenhouse–Geisser conservative adjustment has been reported to overprotect against type I error, sometimes so effectively that the analysis has little power; however, it never inflates the risk of type I error for the within-group time effect, or for the combined interaction between groups and time.

Diastolic function as measured by EDPVR was the only parameter that was observed to differ between rabbits anaesthetized with and without IR and then only in the propofol and sevoflurane groups. This functional result has not been reported before and can be considered secondary to an interaction of these specific agents with the effects of ischaemia, reperfusion, or both. The changes in diastolic relaxation seen in the rabbits anaesthetized with propofol during IR can be explained by the disordered high-energy phosphate metabolism that accompanies an area of ischaemic myocardium. Levels of ATP in border-zone myocardium surrounding infarcted tissue have been shown to decrease to 42% of normal during ischaemia and these marked metabolic perturbations often cannot support normal cardiac function.[18] This directly impairs myocardial relaxation[19] and is consistent with the effects seen in the propIR group. In this group of rabbits, the larger infarct size was coupled to sustained abnormal diastolic

relaxation as measured by an increase in EDPVR over time. The initial increase in this parameter commenced during induced ischaemia, lasted well into the reperfusion phase and functionally correlated with the observed permanent tissue injury.

In contrast, the abnormal diastolic function that was seen during IR in the rabbits subjected to sevoflurane was associated with only modest myocardial tissue damage. Furthermore, the sevoflurane-induced increase in EDPVR occurred predominantly during ischaemia and was followed by a subsequent renormalization during reperfusion. This also correlates with the smaller amount of tissue injury that was seen in the sevoflurane group during IR. Hence although sevoflurane causes myocardial stiffness to acutely increase during ischaemia, the diastolic stunning of the heart improves during reperfusion along with the recovery in tissue injury. This was reflected by a smaller histological infarct size induced by sevoflurane during IR when compared with propofol.

Animals anaesthetized with desflurane had no changes in diastolic function during ischaemia or reperfusion. In keeping with this finding, Pagel and colleagues[20] revealed that desflurane blunts the ischaemia-induced increases in τ and regional chamber stiffness in both ischaemic and non-ischaemic zones in dogs. Although from a histological point of view, sevoflurane may protect against overt cell death to a similar degree as desflurane, from a functional standpoint, the PV loop data here indicate that during IR sevoflurane is not as effective as desflurane in preventing ischaemic diastolic dysfunction. This may be because desflurane offers more complete cardiac protection when compared with sevoflurane as has been shown when the preconditioning effects of these two agents were investigated previously in the rabbit myocardium.[21] An alternative explanation is that sevoflurane and desflurane may have primarily dissimilar effects upon diastolic function itself. For example, sevoflurane causes greater diastolic dysfunction in comparison with desflurane when administered to chronically instrumented dogs with induced heart failure.[22] However, if this were the case, abnormal diastolic function would be expected to have occurred in the sevTC group as well. Given that the sevTC group produced a stable EDPVR profile in our study, this would indicate that the abnormal diastolic function seen in the sevIR group was a result of an interaction of sevoflurane with IR.

The reduction in tissue damage seen in the rabbits anaesthetized in the volatile groups that underwent IR is in keeping with previous studies that report brief exposure, followed by washout, of volatiles before an ischaemic event also leads to a smaller infarct size.[21 23] It is not unreasonable to assume that the same protective effects would be brought into play if the volatile agent in question were utilized for the entire anaesthesia.[24]

The results of this study demonstrated a greater infarct size in the area at risk in rabbits anaesthetized with propofol. Cope and colleagues[25] describe similar effects when comparing the older volatiles isoflurane, enflurane, and halothane to propofol in rabbits subjected to IR. Although propofol has been shown to have anti-ischaemic properties and is believed to be cardioprotective, its mechanism of protection differs fundamentally from that seen with volatile anaesthesia and is related to its action as an oxygen free radical scavenger. Propofol has been shown to offer superior protection against IR when compared with isoflurane in humans;[4] however, this effect has not been consistently replicated when compared with sevoflurane or desflurane.[24 26–28] In clinical studies, the cardioprotective effects from propofol can be demonstrated at concentrations of 5 mg litre^{-1}; however, Shao and colleagues[29] did not elicit cardioprotection in rats with propofol until concentrations of 9–18 mg litre^{-1} were achieved.[4] The infusion rate of 70 mg kg^{-1} min^{-1} propofol used in this study is based upon a previous study in which we demonstrated this same infusion rate in rabbits produces whole-blood propofol concentrations of 8.8 mg litre^{-1}.[6] The anaesthetic concentration of propofol in this study was not sufficient to produce comparable cardioprotection to that which can be induced with an equivalent 1 MAC anaesthetic dose of sevoflurane or desflurane. Clinically, this is consistent with the effects of volatile anaesthetic agents demonstrated at the genetic level, in which sevoflurane has been shown to induce a protective metabolic phenotype that does not occur with propofol.[30]

The profiles of MAP and SVRI were shown to be significantly different between the propIR and sevIR groups only. Differences in infarct size in this study could not be attributed to hypotension as perfusion pressure was higher for the first half of the perfusion protocol in the propIR group, after which it was similar to that seen in the sevIR group (Fig. 5).

The limitations of this study are as follows: using propofol for induction of anaesthesia in the two volatile IR groups could, in theory, result in added benefit to the heart. In our study, on average, the reperfusion in each rabbit commenced ~150 min after induction of anaesthesia. The whole-blood propofol concentration in the random selection of six rabbits undergoing volatile anaesthesia at this time point was 0.37 (0.165) mg litre^{-1} and decreased further with time following this. On this basis, any additional cardioprotection secondary to oxygen free radical scavenging from propofol in the volatile groups would be expected to mirror blood levels at the time of reperfusion. Although it may be possible that propofol has preconditioning properties, this has not been reported in the literature.

The propofol infusion in this study appears high in relative terms when compared with that administered clinically in humans. This is due to different pharmacokinetics of propofol in rabbits. The clearance of propofol in rabbits is 340 ml kg^{-1} min^{-1} whereas in humans is 20–30 ml kg^{-1} min^{-1}; hence, these animals require a much higher infusion rate in order to achieve similar whole-blood propofol concentrations.[31 32] This study used published values

of MAC for sevoflurane and desflurane in rabbits and an estimate for equi-anaesthetic maintenance levels for propofol based on three previous rabbit studies[33–35] and also data previously published by our laboratory.[6] We only examined the effects of 1 MAC anaesthesia; it is possible that there is a dose–response curve for the functional effects of all the agents during IR and that at higher concentrations of sevoflurane or propofol, there may be effects similar to that observed with 1 MAC desflurane.

This study compared the functional effects of three anaesthetic agents during IR relative to each other. Although propofol did not appear to offer the same level of cardioprotection to that seen with the volatile agents, it has nonetheless been shown to exhibit cardiac protection. Therefore, the addition of a fourth arm comparing all three agents against a drug not recognized to offer cardioprotection could possibly reveal further additional functional properties that would be hidden by the design of this experiment.

In conclusion, this study reveals that in an anaesthetized, open-chest, IR rabbit preparation, desflurane, sevoflurane, and propofol provide haemodynamic stability and preservation of LV contractility during IR. An increase in LV diastolic stiffness seen with sevoflurane and propofol, but not desflurane, indicates that desflurane offers greater protection against ischaemic diastolic dysfunction when compared with the other agents, while histologically, the volatile anaesthetics desflurane and sevoflurane provide superior cardioprotection when compared with the i.v. anaesthetic propofol. This study suggests that under anaesthesia, haemodynamic conditions are well preserved during ischaemia for all anaesthetics tested, but the choice of anaesthetic can modulate the extent of diastolic dysfunction seen during the ischaemia phase and the overall cell death that subsequently occurs during reperfusion.

Supplementary material

Supplementary material is available at *British Journal of Anaesthesia* online.

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