Beneficial effect of concomitant administration of isoflurane and nicorandil

V. PIRIOU, S. ROSS, D. PIGOTT, R. EVANS AND P. FOEX

Summary
In common with halogenated anaesthetics, nicorandil, a new KATP channel opener, has been shown to have cardioprotective and vasodilator effects. Recent studies have also suggested that the vasodilator and protective effects of halogenated anaesthetics are mediated partly via KATP channel opening. This study examined the effects of concurrent administration of nicorandil and isoflurane on haemodynamic state and ventricular function before, during and after 15 min of ischaemia. We studied left ventricular function in 40 anaesthetized rabbits using ultrasonomicrometry. Measurements were obtained before, during and after 15 min of regional ischaemia. Regional ventricular function was assessed in terms of systolic shortening (SS%) and preload recruitable work area (PRWA, the area beneath the regional stroke work vs end-diastolic length relationship) during reperfusion. Four groups were studied: group F (n=10) received a bolus dose of fentanyl 100 μg kg⁻¹ and then 400 μg kg⁻¹ h⁻¹ throughout; group I (n=10) received 2.05% end-tidal concentration of isoflurane (1 MAC); group FN (n=10) received fentanyl, a bolus dose of nicorandil 100 μg kg⁻¹ and then 25 μg kg⁻¹ min⁻¹, 15 min before occlusion; and group IN (n=10) received nicorandil and fentanyl. Isorandil decreased left ventricular systolic pressure and ventricular contractility (+dP/dmax, slope of preload recruitable stroke work, and SS%). Nicorandil increased +dP/dmax in group FN. Post-ischaemic regional left ventricular contractility in group I did not differ from that in group F, however, groups receiving nicorandil recovered to a greater extent. Group IN showed better recovery compared with all other groups when ventricular contractility was assessed by PRWA normalized to pre-occlusion values (mean 99.3 (SEM 10.5) % vs 73.4 (7.5) %, 50.2 (5.8) % and 52.4 (3.7) % at 120 min reperfusion in groups FN, I and F, respectively). Tissue ATP and lactate contents did not differ between groups. We conclude that concurrent administration of nicorandil and isoflurane enhanced post-ischaemic recovery compared with isoflurane anaesthesia or nicorandil and fentanyl administration. (Br. J. Anaesth. 1997; 79: 68–77.)

Key words

Nicorandil, a nicotidamide ester, is a potassium channel opener used in clinical practice for antianginal therapy. This drug exhibits several actions, including vasodilatation, mediated by both nitrate radical and opening of KATP channels, and a cardioprotective effect, attributed mainly to opening of KATP channels.

Isoflurane is a vasodilator and also a cardio-depressant and protective agent. Recent investigations have shown that isoflurane-induced coronary vasodilatation and its cardioprotective effect are antagonized by glibenclamide, and therefore may be mediated partly by KATP channels.

With the possibility that the number of angina patients on long-term nicorandil treatment under-going surgery and general anaesthesia will increase in the future, this study was designed to investigate the potential effects of concurrent administration of isoflurane with nicorandil and of fentanyl with nicorandil. Haemodynamic state and regional myocardial function before ischaemia, and the time course of functional regional recovery after 15 min of regional ischaemia were studied in an anaesthetized, open chest rabbit model.

Materials and methods

SURGICAL PREPARATION OF ANIMALS
The study conformed to UK Animals Acts (Scientific Procedures, 1986). We studied 73 New Zealand White rabbits of both sexes (2.5–3.5 kg) premedicated with xylazine 1 mg kg⁻¹ i.m. and anaesthetized with ketamine HCl 75 mg kg⁻¹ i.m. Adequate depth of anaesthesia was ensured before any surgical procedure by absence of pedal and palpebral reflexes. The marginal ear vein was cannulated (20-gauge cannula) for administration of fluids (hetastarch 3 ml kg⁻¹ h⁻¹) and drugs. The central ear artery was also cannulated (18-gauge cannula) and connected to a pressure gauge cannula) and connected to a pressure...
transducer (Druck Ltd, Groby, Leicester, UK) for measurement of arterial pressure during surgery. All catheters were flushed with heparinized saline 10 u. ml⁻¹ to prevent clotting during the experiment. A tracheotomy was performed (tracheal tube size 4 mm od) and the lungs of the rabbits were ventilated mechanically (Servo ventilator 900B, Siemens-Elema, Sweden) with 100% oxygen. Tidal volume was set at 15 ml kg⁻¹ and ventilatory frequency at 35 bpm. Ventilation was adjusted to maintain P\textsubscript{CO₂} in the physiological range. End-tidal gas concentrations were measured continuously (gas analyser M1025A, Hewlett Packard, Bracknell, UK). Body temperature was recorded using the thermistor of a 7F pulmonary artery catheter (Swan-Ganz, American Edwards Laboratories, Anasco, Puerto Rico) inserted orally into the oesophagus and maintained at 39.0–40.5 °C. Limb lead II of the electrocardiogram was monitored continuously using subcutaneous needle electrodes. Anaesthesia was maintained with a bolus dose of fentanyl 100 \mu g kg⁻¹ followed by an infusion of 400 \mu g kg⁻¹ h⁻¹ during surgery and the stabilization periods. The heart was exposed via a left thoracotomy and suspended in a pericardial cradle. A 5-0 Dexon suture was passed around the major marginal branch of the left coronary artery approximately halfway between the apex and base and the suture ends threaded through a small vinyl tube to make a coronary snare. Where possible we tried to avoid snaring coronary veins but in some experiments this was not possible. A thin piece of rubber tubing was placed around the inferior vena cava to perform caval occlusions. Pressure in the left ventricle was measured by inserting a 16-gauge cannula into the apex of the left ventricle and connecting this to a micromanometer-tipped 8F catheter (Millar, Houston, TX, USA) through a tight adapter. This device was compared, in our laboratory, during \textit{in vivo} simultaneous pressure measurements with an 8F micromanometer-tipped catheter placed directly in the cavity of the left ventricle. The time lag between the two devices did not exceed 2 ms and the differences in pressure values were <1%. \textit{In vitro} measurements showed an undamped natural frequency of 66.6 Hz and a damping coefficient of 0.141. This 16-gauge cannula was used to minimize impairment in regional myocardial function. Two piezoelectric crystals (0.8 mm diameter, Triton, San Diego, CA, USA) for ultrasonic measurement of segmental lengths were implanted in the endocardium in the apical region supplied by the snared marginal coronary artery parallel to the minor axis. These were implanted approximately 8 mm apart through epicardial stab wounds. At the end of the experiment the heart was excised rapidly and tissue samples obtained from the non-ischaemic region (above the coronary snare) and the ischaemic region (between the pair of crystals). These samples were frozen rapidly in a Wollenberger clamp cooled in liquid nitrogen and stored in liquid nitrogen for analysis of adenine nucleotides and lactate content.

**MEASUREMENT OF TISSUE NUCLEOTIDES AND LACTATE CONCENTRATION**

The frozen tissue samples were ground in liquid nitrogen and extracted with perchloric acid (6% w/v). The neutralized extracts were assayed for ATP\textdegree, ADP\textdegree, AMP\textdegree and lactate\textdegree using standard enzymatic techniques with a spectrophotometer (Lambda 3 UV/VIS spectrophotometer, Perkin Elmer Ltd, Beaconsfield, Bucks, UK).

Energy charge potential was calculated as follow\textsuperscript{10}:

\[
\frac{[\text{ATP}]+0.5[\text{ADP}]}{[\text{ATP}]+[\text{ADP}]+[\text{AMP}]}\]

**STUDY PROCEDURE**

Four groups of animals were studied (fig. 1) and all experiments were performed randomly. In all groups after surgery there was a stabilization period of 30 min, after which baseline measurements were obtained under fentanyl anaesthesia. After baseline measurements, recordings were obtained at 30, 45 and 60 min. The measurement at 60 min was taken as the pre-occlusion value used for normalization of contractility indices. One hour after baseline measurements, regional ischaemia was imposed for 15 min, followed by 120 min of reperfusion. During ischaemia, measurements were obtained at 5 and 15 min and during reperfusion at 5, 10, 15, 30, 60, 90 and 120 min.

In all groups, surgery and baseline measurements were performed under fentanyl anaesthesia. In the fentanyl group (group F, \(n=10\)), fentanyl was continued throughout the experiment. In the isoflurane group (group I, \(n=10\)) infusion of fentanyl was stopped after baseline measurements and 1 MAC of
isoflurane (2.05% end-tidal concentration) introduced and continued for the duration of the experiment. In the fentanyl and nicorandil group (group FN, n = 10) infusion of fentanyl was continued throughout the experiment and a bolus dose of nicorandil 100 μg kg⁻¹ followed by infusion of 25 μg kg⁻¹ min⁻¹ were started 15 min before coronary occlusion and continued for the duration of the experiment. In the isoflurane and nicorandil group (group IN, n = 10) infusion of fentanyl was stopped after baseline measurements and 1 MAC of isoflurane introduced and continued for the duration of the experiment. A bolus dose of nicorandil 100 μg kg⁻¹ followed by infusion of 25 μg kg⁻¹ min⁻¹ were started 15 min before coronary occlusion and continued for the duration of the experiment.

To produce ischaemia, the coronary snare was tightened by pulling the silk through the tubing. A bolus dose of lignocaine 0.7 mg kg⁻¹ was infused just before coronary occlusion to prevent ventricular arrhythmias during ischaemia and reperfusion. Ischaemia was confirmed by changes in the shape of the pressure-length loop (reduction in systolic shortening, increase in post-systolic shortening and/or systolic bulging) (figs 2 and 3). Visually, myocardial ischaemia was confirmed by the appearance of regional myocardial surface cyanosis distal to the snare, and akinesia or bulging in this area. Experiments where these changes did not occur were discarded (n = 8). After 15 min, the snare was released and reperfusion begun for a
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period of 2 h. Reperfusion was confirmed by changes in the pressure–length loop (increase in systolic shortening, reductions in post-systolic shortening and systolic bulging). Visually, reperfusion was confirmed by the appearance of hyperaemia. Animals that did not exhibit return of flow and change in ischaemic loop shape were excluded \((n=5)\).

Measurements consisted of (1) steady state, and (2) caval occlusion obtained by pulling on the rubber tubing around the vena cava. Steady state variables were recorded for 5 s and caval occlusion for 15 s. All measurements were taken during expiratory apnoea to obtain an identical respiratory phase.

Nicorandil was dissolved in NaCl 0.9% (w/v). The dissolved nicorandil was infused in the marginal ear vein using an infusion pump.

DATA COLLECTION

ECG, pressures (central ear artery, left ventricular) and regional dimension signals were converted using an analogue-to-digital converter (AT-MIO-16, National Instruments Corporation, Austin TX, USA), and displayed continuously on an IBM AT personal computer using the real-time mode of software designed in this department. Data were sampled at a frequency of 500 Hz and stored on the hard disk. Pressure–length loops were displayed continuously on an oscilloscope.

DATA ANALYSIS

Data analysis was performed on an IBM AT personal computer using the play back mode of the above mentioned software. Peak positive and peak negative left ventricular \(dP/dt\) were obtained by electronic differentiation of the left ventricular pressure signal. End-diastole was defined as the first upward (positive) deflection of the left ventricular \(dP/dt\) signal. End-systole was defined as occurring at peak negative \(dP/dt\).\(^{12}\)

Length measurements were normalized to an initial value of end-diastolic length of 10 mm at control. The following definitions were used to quantify regional wall motion: EDL = end-diastolic length; ESL = end-systolic length; \(l_{\text{max}}\) = maximum length during systole; \(l_{\text{min}}\) = minimum segmental length during diastole. Systolic shortening fraction \((%SS)\), systolic bulging \((%SB)\) and post-systolic shortening \((%PSS)\) were defined as follows:

\[
\%SS = \frac{(EDL-ESL)}{EDL} \times 100
\]

\[
\%SB = \frac{(l_{\text{max}}-ESS)}{EDL} \times 100
\]

\[
\%PSS = \frac{(ESL-l_{\text{min}})}{ESL} \times 100
\]

Systolic shortening was expressed in absolute values and as a percentage of pre-occlusion values to assess functional recovery during reperfusion. Area under the curve of systolic shortening \(\times\) time was also calculated during the reperfusion period.

Preload recruitable work area (PRWA) was calculated by the method devised by Glover and colleagues\(^\text{13}\); segmental stroke work (SW) was calculated by electronic integration of left ventricular pressure \((P)\) and segmental length over the entire cardiac cycle:

\[
\text{SW} = \int P \times dl
\]

SW and end-diastolic segment length (EDL) were fitted using linear regression to the equation:

\[
\text{SW} = \text{Msw} \times (\text{EDL} - \text{Lw})
\]

where \(\text{Msw} = \text{slope and Lw} = x\)-intercept.

PRWA was calculated from the equation:

\[
\text{PRWA} = \frac{\text{Msw}}{2} \times (1.2l_{\text{wmax}} - \text{Lw})^2
\]

where \(l_{\text{wmax}} = \text{maximal } x\)-intercept value obtained for a given myocardial segment over the entire experiment.

PRWA was used to assess left ventricular function during the reperfusion period; values are expressed as a percentage of the pre-occlusion value. Area under the curve of PRWA \(\times\) time was also calculated during reperfusion.

STATISTICAL ANALYSIS

All values are expressed as mean (SEM). Statistical analyses were performed using two-way analysis of variance (ANOVA) with repeated measures on one factor. If ANOVA indicated significant differences between means, further comparisons were performed using Fisher’s test. Statistical significance was assumed if \(P<0.05\). Within-group comparisons were performed at times before ischaemia. Area under the curve and metabolic assays were analysed using one-way ANOVA followed by Fisher’s test where appropriate.

Results

Of the 73 rabbits included in this study, 33 did not complete the experiment for the following reasons: death during surgery \((n=4)\), crystals out of ischaemic area \((n=8)\), lack of reperfusion after releasing the coronary snare \((n=5)\), lethal ventricular fibrillation \((n=3\) in group \(F), n=4\) in group \(I), n=5\) in group FN and \(n=4\) in group IN). Therefore, there were 10 animals in each group.

EFFECTS OF NICORANDIL OR ISOFLURANE, OR BOTH, BEFORE ISCHAEMIA

Isoflurane decreased left ventricular systolic pressure (LVSP) \((73 (2)\) to \(51 (1)\) mm Hg) while heart rate remained unchanged. Both \(+dP/dmax\) and \(-dP/dmin\) decreased with isoflurane suggesting a decrease in global contractility and lusitropy (table 1). Regional contractility was depressed in both groups receiving isoflurane, as indicated by the decrease in systolic shortening \((27.3 (2.0)\) to \(22.0\) \((1.4)\%\) in group I and \(28.7 (2.0)\) to \(21.1\) \((1.8)\%\) in group IN) and the slope of preload recruitable stroke work (Msw) \((79.9 (11.1)\) to \(42.7\) \((5.2)\) mm Hg in group I and \(77.7\) \((10.4)\) to \(43.0\) \((5.9)\) mm Hg in group IN) (table 2). Nicorandil infusion increased
Table 1: Global haemodynamic state before, during and after ischaemia (n = 10 per group (mean (SEM). F = Fentanyl group, I = isoflurane group, FN = fentanyl and nicorandil group, IN = isoflurane and nicorandil group. HR = Heart rate, LVSP = left ventricular systolic pressure, LVEDP = left ventricular end-diastolic pressure. *P < 0.05 compared with fentanyl group, /P < 0.05 compared with isoflurane group, #P < 0.05 compared with fentanyl and nicorandil group, P < 0.05 compared with isoflurane and nicorandil group. *P < 0.05 compared with previous time during pre-ischaemic period. Baseline measurements were recorded during fentanyl anaesthesia.

<table>
<thead>
<tr>
<th>HR (beat min⁻¹)</th>
<th>Pre-occlusion (min)</th>
<th>Occlusion (min)</th>
<th>Reperfusion (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-30</td>
<td>-15</td>
<td>0</td>
</tr>
<tr>
<td>Heart rate, LVSP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>231 (9)</td>
<td>232 (9)</td>
<td>227 (10)</td>
</tr>
<tr>
<td>LVSP (mm Hg)</td>
<td>72 (6)</td>
<td>70 (5)</td>
<td>72 (4)</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>4.5 (0.9)</td>
<td>4.9 (1.0)</td>
<td>4.4 (0.8)</td>
</tr>
<tr>
<td>dP/dtmax (mm Hg s⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>3000 (318)</td>
<td>3321 (388)</td>
<td>3628 (446)</td>
</tr>
<tr>
<td>I</td>
<td>3041 (123)</td>
<td>1557 (59)</td>
<td>1644 (50)</td>
</tr>
<tr>
<td>FN</td>
<td>3164 (162)</td>
<td>3119 (168)</td>
<td>3276 (293)</td>
</tr>
<tr>
<td>IN</td>
<td>3050 (171)</td>
<td>1419 (70)</td>
<td>1452 (63)</td>
</tr>
<tr>
<td>dP/dtmin (mm Hg s⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>3423 (504)</td>
<td>3223 (394)</td>
<td>3451 (347)</td>
</tr>
<tr>
<td>I</td>
<td>3255 (189)</td>
<td>1555 (55)</td>
<td>1742 (78)</td>
</tr>
<tr>
<td>FN</td>
<td>2999 (252)</td>
<td>3189 (296)</td>
<td>3116 (376)</td>
</tr>
<tr>
<td>IN</td>
<td>3401 (153)</td>
<td>1557 (62)</td>
<td>1658 (102)</td>
</tr>
</tbody>
</table>
Table 2  Regional indices of mechanical function before, during and after ischaemia (n = 10 per group) (mean (SEM)). F = Fentanyl group, I = isoflurane group, FN = fentanyl and nicorandil group, IN = isoflurane and nicorandil group. EDL = End-diastolic length, SS = systolic shortening (actual values), Msw = slope of preload recruitable stroke work, PSS = post-systolic shortening and SB = systolic bulging. *P<0.05 compared with fentanyl group, †P<0.05 compared with isoflurane group, ‡P<0.05 compared with fentanyl and nicorandil group. 'P<0.05 compared with isoflurane and nicorandil group, *P<0.05 compared with previous time during pre-ischaemic period. Baseline measurements were recorded during fentanyl anaesthesia.

<table>
<thead>
<tr>
<th></th>
<th>Pre-occlusion (min)</th>
<th>Occlusion (min)</th>
<th>Reperfusion (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>-30 -15 0</td>
<td>5 15</td>
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<td></td>
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<tr>
<td>EDL (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>10.0 (0.0)</td>
<td>10.0 (0.3)</td>
<td>10.1 (0.2)</td>
</tr>
<tr>
<td>I</td>
<td>10.0 (0.0)</td>
<td>10.1 (0.3)</td>
<td>10.2 (0.3)</td>
</tr>
<tr>
<td>FN</td>
<td>10.0 (0.0)</td>
<td>10.1 (0.2)</td>
<td>9.9 (0.2)</td>
</tr>
<tr>
<td>IN</td>
<td>10.0 (0.0)</td>
<td>10.1 (0.1)</td>
<td>10.0 (0.2)</td>
</tr>
<tr>
<td>SS</td>
<td>F</td>
<td>27.7 (2.5)</td>
<td>29.5 (3.0)</td>
</tr>
<tr>
<td>I</td>
<td>27.3 (2.0)</td>
<td>22.0 (1.4)†</td>
<td>22.2 (1.8)</td>
</tr>
<tr>
<td>FN</td>
<td>32.1 (3.6)</td>
<td>33.5 (4.0)†</td>
<td>33.6 (4.2)†</td>
</tr>
<tr>
<td>IN</td>
<td>28.7 (2.0)</td>
<td>21.1 (1.6)†</td>
<td>22.0 (1.6)</td>
</tr>
<tr>
<td>Msw (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>80.9 (12.7)</td>
<td>84.9 (12.9)†</td>
<td>86.2 (17.0)†</td>
</tr>
<tr>
<td>I</td>
<td>79.9 (11.1)</td>
<td>42.7 (5.2)†</td>
<td>45.9 (6.5)</td>
</tr>
<tr>
<td>FN</td>
<td>74.0 (6.2)</td>
<td>75.6 (7.2)†</td>
<td>74.5 (7.3)†</td>
</tr>
<tr>
<td>IN</td>
<td>77.7 (10.4)</td>
<td>43.0 (5.9)†</td>
<td>40.8 (4.2)</td>
</tr>
<tr>
<td>PSS (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>1.1 (0.6)</td>
<td>1.0 (0.6)</td>
<td>1.2 (0.6)</td>
</tr>
<tr>
<td>I</td>
<td>0.4 (0.4)</td>
<td>0.9 (0.8)</td>
<td>1.1 (0.7)</td>
</tr>
<tr>
<td>FN</td>
<td>1.0 (0.6)</td>
<td>1.0 (0.7)</td>
<td>1.5 (0.8)</td>
</tr>
<tr>
<td>IN</td>
<td>4.0 (1.0)bc</td>
<td>2.6 (1.9)</td>
<td>3.1 (1.9)</td>
</tr>
<tr>
<td>SB (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>1.0 (0.6)</td>
<td>1.0 (0.3)</td>
<td>1.0 (0.3)</td>
</tr>
<tr>
<td>I</td>
<td>0.5 (0.2)</td>
<td>0.4 (0.2)</td>
<td>0.2 (0.1)</td>
</tr>
<tr>
<td>FN</td>
<td>1.3 (0.5)</td>
<td>1.6 (0.8)</td>
<td>1.5 (0.6)</td>
</tr>
<tr>
<td>IN</td>
<td>2.2 (0.5)bc</td>
<td>1.5 (0.5)</td>
<td>1.2 (0.4)</td>
</tr>
</tbody>
</table>

Interaction of isoflurane and nicorandil
Table 1 Comparison of area under the curve between groups (n=10 per group) (mean (SEM)) F = Fentanyl group, I = isoflurane group, FN = fentanyl and nicorandil group and IN = isoflurane and nicorandil group. SS = Systolic shortening (normalized) and PRWA = preload recruitable work area (normalized). *P<0.05 compared with fentanyl group, †P<0.05 compared with isoflurane group, ‡P<0.05 compared with fentanyl and nicorandil group.

Table 4 ATP, lactate and energy charge levels in reperfused and non-ischaemic tissue (μmol g⁻¹ wet weight). F = Fentanyl group, I = isoflurane group, FN = fentanyl and nicorandil group and IN = isoflurane and nicorandil group. *P<0.05 compared with non-ischaemic tissue.
Discussion

K<sub>ATP</sub> channels are present in several tissues, including cardiac muscle and vascular smooth muscle. Their gating is metabolically dependent; a decrease in intracellular ATP or increase in ADP or protons may induce a K<sup>+</sup> outward current which hyperpolarizes the cell. Glibenclamide, a K<sub>ATP</sub> channel blocker, increases basal coronary tone, suggesting that K<sub>ATP</sub> channels play an important physiological role in coronary vasoregulation. K<sub>ATP</sub> channels have also been implicated in the cardio-protection produced by agents such as nicorandil<sup>15</sup> and isoflurane.<sup>6,10</sup>

Nicorandil is a K<sub>ATP</sub> channel opener; it induces vasodilatation because of cell membrane hyperpolarization resulting in decreased calcium entry through T- and L-type calcium channels. Vasodilatation is also attributed to the presence of a nitrate radical. Small artery dilatation seems to be mediated by a nitrate-like effect.<sup>17</sup> Experimental studies in dogs<sup>18</sup> have shown that nicorandil enhances cardiac function. This could be attributed to altered loading status,<sup>19</sup> increased coronary flow, or both.<sup>20</sup>

Isoflurane causes dose-related vasodilatation. Patch clamp studies have shown that it acts by suppressing calcium currents.<sup>21</sup> Recently, Cason, Shubayev and Hickey<sup>5</sup> demonstrated that isoflurane-induced coronary vasodilatation in vivo is antagonized by the K<sub>ATP</sub> channel blocker glibenclamide and concluded that this vascular effect is mediated mainly by K<sub>ATP</sub> channel modulation; similar results have been obtained with halothane.<sup>22</sup> To our knowledge, only one study has investigated the potential haemodynamic effects of the combination of nicorandil and isoflurane: Tanaka,<sup>23</sup> using 0.75% and 1.5% isoflurane in an open chest anaesthetized dog model investigated the global haemodynamic effects of bolus doses of nicorandil 100 μg kg<sup>−1</sup> and 300 μg kg<sup>−1</sup>. They showed that the hypotensive effects of both nicorandil doses disappeared after 5 min at both isoflurane concentrations. Cardiac output was slightly increased during the first minute at both doses, as there was a transient decrease in vascular resistance.

The cardiac depressant properties of isoflurane are mediated by inhibition of trans-sarcolemmal calcium influx by T- and L-calcium voltage-dependent channels.<sup>24</sup> This results in decreased calcium availability for myocardial contraction. Contractile myofibrils also exhibit decreased calcium sensitivity. Compared with other halogenated anaesthetics, isoflurane seems to have less effect on the sarcoplasmic reticulum. In this study, isoflurane was set at 1 MAC (rabbit) end-tidal concentration, equivalent to 2.05%,<sup>11</sup> a dose that causes marked hypotension and cardiodepression in this model.

In our model, nicorandil showed no hypotensive effects 15 min after the bolus dose of 100 μg kg<sup>−1</sup> and continuous infusion of 25 μg kg<sup>−1</sup> min<sup>−1</sup>, with fentanyl or isoflurane anaesthesia. However, +dP/dtmax was increased significantly after administration of nicorandil with fentanyl but not isoflurane anaesthesia, possibly because of the magnitude of induced vasodilatation and cardiodepression with isoflurane. Using a model similar to ours, Iwamoto and colleagues<sup>15</sup> showed a cardio-protective effect without hypotensive action with infusion of nicorandil 10 μg kg<sup>−1</sup> min<sup>−1</sup>.

Mechanisms underlying transient post-ischaemic myocardial dysfunction are still not fully understood. The more favoured causes are cytosolic calcium overloading (“calcium paradox”), formation of free radicals during reperfusion and decrease in myofibril sensitivity to calcium. During ischaemia, intracellular ATP decreases and ADP, lactate and acidosis increase in the cardiac myocyte. These intracellular metabolic alterations induce efflux of potassium ions through K<sub>ATP</sub> channels shortening action potential duration. Contractility in ischaemic myocardium is reduced and metabolic demand decreases. The protective effects of nicorandil on stunned myocardium have been studied extensively in a chronically instrumented dog model<sup>25</sup> and in a pentobarbitone-anaesthetized open chest dog model.<sup>23</sup> These studies on stunned myocardium used similar doses of nicorandil as in this study. In these investigations myocardial function was assessed by regional systolic shortening (SS%)<sup>1</sup>. Several studies have also reported a decrease in infarct area after longer periods of ischaemia using nicorandil. The cardioprotective effect has been attributed to the dual properties of nicorandil: potassium channel opener<sup>2</sup> and vasodilator<sup>20</sup>; although the role of the latter is still debated. Compared with equi-hypotensive doses of nitrates, nicorandil showed improved recovery.<sup>3</sup> However, intracoronary infusion of nicorandil<sup>26</sup> and systemic infusion of aprikalim, a pure potassium channel opener, after ischaemia,<sup>27</sup> did not show a demonstrable beneficial effect. Other properties may be involved in the protective effect of nicorandil, including free radical scavenging activity<sup>28</sup> and antiplatelet aggregatory function.<sup>29</sup>

Halogenated anaesthetics also have cardio-protective properties. Both halothane and isoflurane have been shown to improve post-ischaemic recovery in several animal models<sup>4,10–12</sup> but the mechanisms remain unclear; decreased myocardial contractility and oxygen demand do not appear to play major roles.<sup>31</sup> Halogenated anaesthetics are more likely to exert their effects by decreasing the intracellular calcium transient, thereby protecting the heart against calcium overloading. The free radical scavenging properties of isoflurane<sup>32</sup> may also be involved. Kersten and colleagues<sup>6</sup> have recently shown that isoflurane-induced cardio-protection in stunning is partially blocked by glibenclamide in a chronic dog model, suggesting a role for K<sub>ATP</sub> channels.

We have been unable to show a protective effect on myocardial recovery for isoflurane compared with fentanyl. The low perfusion pressure displayed by the groups receiving 1 MAC of isoflurane may have affected recovery, and coronary steal may have been induced,<sup>36</sup> although this phenomenon is still debated.<sup>37</sup> In our study, we demonstrated enhanced recovery from stunning in animals receiving both
isoﬂurane and nicorandil. The group receiving isoﬂurane and nicorandil showed signiﬁcant enhancement (+26%) in PRWA compared with the group receiving fentanyl and nicorandil. PRWA is a good index of systolic function in the post-ischaemic period; compared with systolic shortening, it is sensitive and relatively load independent.13 By expressing contractility as systolic shortening in absolute values, the isoﬂurane and nicorandil group recovered less compared with the fentanyl and nicorandil group. This may be attributed to the cardiodepressive activity of isoﬂurane, as shown by the similar recovery when systolic shortening in the isoﬂurane group was normalized to a pre-occlusion value.

Our study demonstrated signiﬁcant depletion in tissue ATP content in the previously ischaemic area in all groups; this was not, however, reﬂected in decreased energy charge in the same areas. Assessment of cellular energy status may include other indices not measured here, such as phosphorylation potential ([ATP]/[ADP] + Pi), phosphocreatine and glycogen contents. However, it is likely that turnover and ﬂux rates of energy substrates and intermediary metabolites are more important than absolute concentrations. Another relevant factor in this context is compartmentation of metabolites between subcellular sites. Measurements of whole tissue concentrations cannot account for localization of energy substrates within diﬀerent organelles. Indeed, it has been suggested that whole tissue myocardial ATP content is not related to functional recovery after ischaemia,38,39 an observation conﬁrmed by this report.

Tissue lactate concentrations were also unchanged after 2 h of reperfusion in all groups, conﬁrming the metabolic integrity implied by the energy charge status and suggesting myocardial stunning with good biochemical recovery at 2 h of reperfusion rather than overt tissue infarction.

LIMITATIONS

Using this model we cannot state unequivocally if the diﬀerences in functional recovery were related to better functional recovery in stunned myocardium or to diminished infarct size. Weisel40 suggested a time of 10–20 min for stunning in rabbits. We did not measure collateral circulation, but radiomicrosphere studies41 have shown that collateral circulation in rabbits is poor (2%) compared with dogs (16%). This is one major advantage of using the rabbit model to study stunning. As we used an acute model, this study did not examine contractile function in reperfused areas after recovery from anaesthesia; a similar study with a chronic model would be necessary to establish the extent of recovery without anaesthesia. Our model did not allow us to measure coronary ﬂow to determine if there was further enhancement of coronary ﬂow with the combination of isoﬂurane and nicorandil, which may induce coronary steal. However, if this was the case recovery would be expected to be less in this group compared with the fentanyl and nicorandil group. Systolic pressures were very low in the isoﬂurane groups, however further experiments carried out in this department using 0.5 MAC of isoﬂurane have shown the same degree of contractile recovery with systolic pressures greater than 70 mm Hg (unpublished observations). Plasma concentrations of nicorandil were not measured, therefore we cannot be certain that plasma concentrations were stable during the experiment. Moreover, the cardiovascular system of the rabbit appears to be more sensitive to isoﬂurane. Consequently, caution must be exercised before relating this work to a clinical situation.

In summary, we have shown better recovery of stunned myocardium with the addition of nicorandil to isoﬂurane or fentanyl anaesthesia in acutely instrumented anaesthetized rabbits. The mechanisms underlying the beneﬁts of the combination of isoﬂurane and nicorandil are unclear. Enhancement of the cardioprotection afforded by nicorandil and isoﬂurane may involve KATP channel opening.

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References

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