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Colchicine Inhibits Isoflurane-induced Preconditioning

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Background: **When administered before prolonged myocardial ischemia and reperfusion, isoflurane exerts potent cardioprotective effects similar to those inferred by ischemic preconditioning. To determine whether an intact cytoskeleton is critically important in isoflurane-induced preconditioning, the authors used a rabbit model in which isoflurane-induced myocardial preconditioning decreases myocardial infarct size (IS) substantially. In this model, the authors tested whether the microtubule depolymerizing agent, colchicine, would inhibit isoflurane-induced myocardial preconditioning.**

Methods: **Myocardial IS was measured in four groups of propofol-anesthetized rabbits, each subjected to 30 min of anterolateral coronary occlusion followed by 3 h of reperfusion. Groups differed only in the pretreatments given, and only the control group received no pretreatment. An isoflurane-preconditioned group was pretreated with 15 min of end-tidal isoflurane, 1.1%, and then 15 min of washout. An isoflurane-plus-colchicine group was administered 2 mg/kg colchicine intravenously before isoflurane pretreatment. A colchicine-control group was administered 2 mg/kg colchicine but no isoflurane pretreatment. Myocardial IS and area at risk (AR) were defined by staining. Data were analyzed by analysis of variance or covariance.**

Results: **Infarct size, expressed as a percentage of AR (IS:AR) was 33.6% ± 8.8% (SD) in the control group. Isoflurane preexposure reduced myocardial IS:AR significantly, to 11.8% ± 9.1%. Colchicine pretreatment eliminated the preconditioning-like effect of isoflurane (IS:AR = 32.6% ± 8.7%). Colchicine alone did not alter IS (IS:AR = 27.6% ± 7.1%; *P* = not significant).**

Conclusions: **Colchicine abolished the preconditioning effect of isoflurane but did not increase IS when administered alone. An intact microtubular cytoskeleton is critically important in the process of volatile anesthetic-induced preconditioning. (Key words: coronary occlusion; ischemia; reperfusion.)**

WHEN administered before prolonged myocardial ischemia and reperfusion, isoflurane can exert a potent cardioprotective effect, reducing myocardial infarct size (IS).[1,2] This cardioprotective effect persists beyond the time of isoflurane administration and has therefore been termed anesthetic- or isoflurane-induced preconditioning to emphasize the similarity between this isoflurane-induced cardioprotection and ischemic preconditioning, the cardioprotection conferred by transient, sublethal myocardial ischemia.[3] Indeed, although the mechanisms underlying isoflurane-induced preconditioning are not completely known, studies to date have shown that isoflurane-induced preconditioning shares several cellular mechanisms with ischemic preconditioning. Mechanisms that seem to participate in both ischemic preconditioning and isoflurane-induced preconditioning include opening of sarcolemmal and mitochondrial adenosine triphosphate–sensitive potassium channels,[1,4–6] an adenosine receptor-mediated pathway,[4] and a protein kinase C (PKC)-mediated pathway.[7]

Another cellular component that apparently plays a role in classical ischemic preconditioning, but has not been studied in an isoflurane-preconditioning model, is the cytoskeleton. Liu *et al.*[8] used a rabbit animal model of ischemia and reperfusion and found that pretreatment with colchicine, a drug that depolymerizes microtubules,[9] locked the protective effect of ischemic preconditioning but had no effect on IS in a control group that did not receive preconditioning.[8] The precise role played by microtubules in this ischemia-preconditioned

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model is unknown, but several possible roles have been postulated. Considering their normal physiologic role, it is probable that disruption of microtubules would inhibit intracellular transport and steady-state distribution of enzymes (*e.g.*, PKC) that may be critical to the preconditioning process.

Despite the many similarities that have been demonstrated between ischemia-induced preconditioning and volatile anesthetic-induced preconditioning, it is not known whether cytoskeletal microtubules play a role in the preconditioning induced by volatile anesthetics. To determine whether an intact cytoskeleton is critically important in the process of volatile anesthetic-induced preconditioning, we used a rabbit model of isoflurane-induced myocardial preconditioning, with myocardial IS as the primary outcome variable. In this model, we tested whether colchicine would inhibit isoflurane-induced myocardial preconditioning.

Materials and Methods

Anesthesia and Surgical Preparation

The study was conducted according to the standards of the American Physiologic Society and with approval of our hospital's Animal Welfare Committee. New Zealand white rabbits (weight, 3.2–3.5 kg) were sedated with 70 mg/kg intramuscular ketamine. Surgical anesthesia was maintained by continuous intravenous infusion of 10 mg/ml propofol (Diprivan; Zeneca Pharmaceuticals, Wilmington, DE) started at 0.5–1.0 mg · kg^{-1} · min^{-1}.[10,11] During administration of 100% oxygen by mask, a tracheostomy was performed, and the trachea was intubated. Ventilation was controlled using a positive-pressure respirator (model 309-0612; Ohio Medical Products, Madison, WI) and inspiratory fraction of oxygen of 1.0. A carotid artery was isolated and cannulated with a 22-gauge catheter to measure blood pressure and to sample arterial blood. The ventilation rate was adjusted periodically to achieve normocapnia, and small doses of $NaHCO_3$ (44 mEq/50 ml) were administered when necessary to maintain physiologic blood *p*H (at 7.35–7.45). A three-lead surface electrocardiographic lead system was attached to the anterior chest wall, and the electrocardiogram was monitored using a Grass Instruments model 7D recorder (Quincy, MA). Intermittent recording of the electrocardiogram was used to calculate heart rate and to confirm ST-segment changes during ischemia. Arterial blood gases were measured using a Radiometer ABL 2 Acid-Base Laboratory (Copenhagen, Denmark). The end-tidal carbon dioxide tension was monitored using a calibrated Puritan-Bennet anesthetic agent monitor (Wilmington, MA). A warming blanket maintained core body temperature between 38°C and 39°C.

Each animal was administered a single intravenous dose of pancuronium (0.4 mg) just before median sternotomy to prevent muscle retractions during electrocautery and therefore to minimize bleeding. After exposing the heart by performing a median sternotomy, the pericardium was opened and a 2-0 polyester suture was passed quickly around the anterolateral coronary artery at approximately the midpoint of its epicardial course. The ends of the suture were passed through a 6-cm piece of plastic tubing to form a snare. The position of the suture varied somewhat among animals as a result of variations in coronary anatomy; therefore, a range of areas at risk (ARs) was obtained in each experimental group. The coronary artery was occluded when necessary by tightening the snare and then clamping the tube with a hemostat. Regional cyanosis and ST-segment elevation confirmed myocardial ischemia. Reperfusion was achieved by releasing the snare and was confirmed by visual observation of reactive hyperemia.

Experimental Protocols

After placement of the snare around the anterolateral coronary artery, we randomly assigned the rabbits to one of four groups in which the pretreatment was varied. All rabbits in all groups underwent the same 30 min of coronary artery occlusion, followed by 3 h of reperfusion (fig.1). The first group (control) received no pretreatment. The second group, an isoflurane-pretreated group, received isoflurane (15 min end-tidal isoflurane, 1.1%, or 0.5–0.6 minimum alveolar concentration in rabbits)[12] followed by a 15-min washout period. In the third group, an isoflurane-plus-colchicine group, rabbits were administered 2 mg/kg intravenous colchicine[13] 25 min before the isoflurane pretreatment. In the fourth group, a colchicine-control group, 2 mg/kg colchicine was administered 55 min before the start of the 30-min period of ischemia.

A stabilization period of 20–25 min was allowed after the surgical preparation was complete, before the experimental protocols were begun. The duration of the experiments was standardized across all four groups, as depicted in figure 1. Anticoagulation was achieved in each rabbit using 1,500 U beef lung heparin before the initial coronary artery occlusion. Ventricular fibrillation, if it occurred, was reversed using direct mechanical

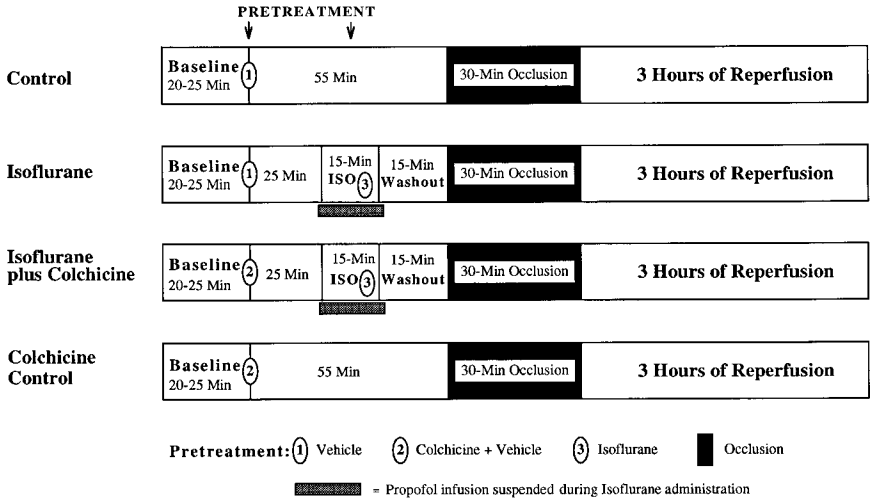

Fig. 1. Experimental protocols for the study. Baseline indicates a period of no experimental intervention. All rabbits were subjected to 30 min of coronary occlusion followed by 3 h of reperfusion. The four groups differed in the pretreatment regimen used. No pretreatment was given in the control group. In the isoflurane group, 15 min of isoflurane pretreatment at 1.1% end-tidal concentration was followed by a 15-min washout period. In the isoflurane-plus-colchicine group, 2 mg/kg colchicine was administered intravenously 55 min before the prolonged occlusion (and 15 min before isoflurane pretreatment). In the colchicine-control group, rabbits were administered 2 mg/kg colchicine but no isoflurane. At the end of the 3-h reperfusion period, infarct size and area at risk were measured.

stimulation: an index finger was flicked directly against the right ventricular side of the fibrillating heart one to three times to achieve defibrillation. No antiarrhythmic drugs were used. Failure to convert to an organized rhythm after three attempts was defined as intractable fibrillation. In all four groups, 3 h of reperfusion followed the 30-min coronary artery occlusion.

In the two isoflurane-pretreated groups, propofol infusion was interrupted briefly during isoflurane administration to maintain a nearly constant level of anesthesia and to avoid hypotension and possible hypotension-induced myocardial ischemia that could theoretically lead to inadvertent myocardial ischemic preconditioning. In general, a total of 7 to 8 min was necessary to achieve the target end-tidal isoflurane concentration of 1.1% isoflurane. After the end-tidal level of 1.1% was reached, the isoflurane concentration was held constant for 15 min, after which the propofol infusion was restarted, and isoflurane administration was discontinued. No measurable end-tidal isoflurane could be detected 4–6 min after the isoflurane was discontinued.

Mean arterial pressure was maintained at > 50 mmHg during ischemia and reperfusion by optimizing the ventricular preload. This was achieved by tilting the surgery table or by infusing 0.9% saline. In addition, the rate of propofol infusion was varied between 0.89 and 1.4 mg $\cdot$ kg^{-1} $\cdot$ min^{-1} during reperfusion to minimize hypotension in rabbits with large infarctions.

Hemodynamics

Hemodynamics were measured at end expiration at various times during the experimental protocol. Blood pressure was monitored continuously using a calibrated strain-gauge transducer (Abbot Critical Care Systems, North Chicago, IL) and a Grass Instruments model 7D polygraph. Heart rate was calculated from electrocardiography and averaged over several heartbeats during each measurement period. The rate–pressure product was calculated as mean arterial pressure times the heart rate.

Myocardial IS and AR

At the end of the 3-h reperfusion, the heart was stopped by anesthetic overdose and excised for measurement of IS and AR. The AR and area of infarction were identified by staining and measured by computerized planimetry, methods previously described in detail.[2,4,14] In brief, the AR was defined by reoccluding the coronary artery and perfusing the aortic trunk with fluorescent microspheres (Duke Scientific, Palo Alto, CA) suspended in a dextran solution. Each heart was then frozen in liquid nitrogen, sliced along the transverse axis into 2-mm sections, and stained with triphenyltetrazolium chloride to define the areas of infarction. AR, area of infarct, and total myocardial area were then digitized and measured. The averages for area of infarct and AR for each slice were converted to percentages, which were converted to grams of myocardium by multiplying by the total weight of each slice. IS for each rabbit is expressed as the sum of the grams of myocardium infarcted on all slices divided by the total grams at risk.

Drugs and Chemicals

Colchicine (Sigma Chemical Company, St. Louis, MO) was prepared as an aqueous solution with final concentration of 2 mg/ml. The fluorescent microspheres were

Table 1. Area at Risk, Infarct Size, Fraction of Risk Zone Infarcted, and Ventricular (LV + RV) Weight in Rabbit Hearts after Four Different Pretreatments and Subsequent Exposure to 30 min of LAD Coronary Occlusion and 3 h of Reperfusion

Treatment	Area at Risk (g)	Infarct Size (g)	IS:AR (%)	Ventricular (LV + RV) Weight (g)
Control (n = 8)	1.43 ± 0.38	0.48 ± 0.19	33.6 ± 8.8	5.2 ± 0.2
Isoflurane (n = 9)	1.39 ± 0.42	0.17 ± 0.14*	11.8 ± 9.1†	5.4 ± 0.5
Isoflurane plus colchicine (n = 8)	1.39 ± 0.34	0.46 ± 0.19	32.6 ± 8.7	5.0 ± 0.2
Colchicine control (n = 8)	1.23 ± 0.53	0.34 ± 0.19	27.6 ± 7.1	5.1 ± 0.6

Values are mean ± SD.

IS:AR = infarct size/area at risk; LV + RV = left plus right ventricle.

* Significantly different ($P < 0.05$) from control group by ANCOVA.

† Significantly different ($P < 0.05$) from control group by ANOVA.

prepared by adding 120 mg ZnS microspheres, 1 to 20 μm (Duke Scientific), to 30 ml dextran 40, 10%, containing 0.5 ml Tween 80 (Sigma). Triphenyltetrazolium chloride (Sigma) was prepared by dissolving 2 g 2,3,5-triphenyltetrazolium chloride in 200 ml phosphate buffer, 90 mM, (*p*H 8.5–8.6) at 37°C.

Data Analysis and Statistics

Values in tables, figures, and the text are expressed as the mean ± SD. The effect of pretreatment regimen on IS was tested by analysis of covariance using the AR as the covariate. AR, the fraction of the risk zone infarcted (IS:AR), and hemodynamic data were compared in all groups using one-way analysis of variance followed by the Student-Newman-Keuls test for comparison *versus* control. Differences in hemodynamic measurements were tested among groups and within each group. The frequency of ventricular fibrillation was compared among groups using chi-square analysis. Statistical analyses were performed using Systat 5.0 (Systat, Evanston, IL) and Statview 4.0 (Abacus Concepts, Berkeley, CA) software. For all statistical analyses, the fiducial limit of significance was chosen as 5%.

Results

Experimental Animals

Experiments were performed on 37 rabbits: 8 in the control group, 10 in the isoflurane-pretreated group, 8 in the isoflurane-plus-colchicine group, and 11 in the colchicine-control group. Three rabbits died in the colchicine-control group of ventricular fibrillation shortly after coronary occlusion. One rabbit in the isoflurane-pretreated group was excluded because of intractable hypotension that began during coronary occlusion.

IS

Areas at risk did not differ significantly among the four treatment groups (table 1). The effect of AR on IS size was similar in all four groups (fig. 2). However, pretreatment with isoflurane significantly reduced myocardial IS, independent of AR. Myocardial IS, expressed as a percentage of AR (IS:AR), was 33.6% ± 8.8% in the control group and was reduced significantly to 11.8% ± 9.1% by preexposure to 15 min of isoflurane (1.1 minimum alveolar concentration; fig. 3). Administration of colchicine before isoflurane pretreatment completely abolished the myocardial protective effect of isoflurane and returned IS to values comparable to those in the control group (IS:AR = 32.6% ± 8.7%; P = not significant). Colchicine

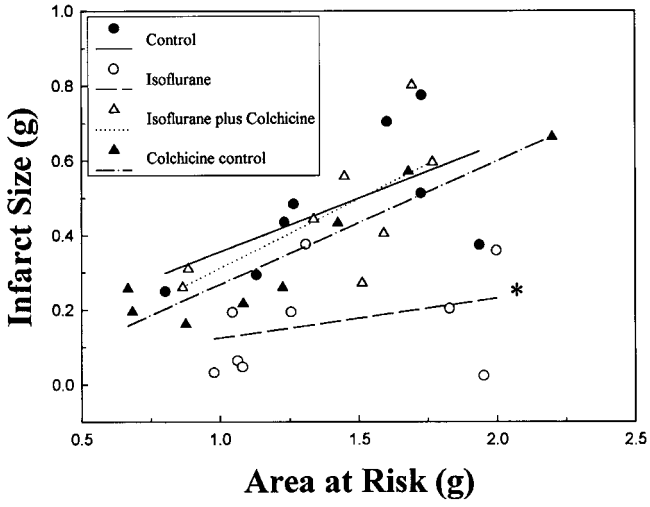

Fig. 2. The relation between myocardial area at risk and myocardial infarct size for each experimental group. The slopes of the four regression lines are not different. However, the elevation of the isoflurane line (asterisk) is lower than the elevation of the other regressions ($P < 0.05$ by analysis of covariance), indicating that isoflurane decreased infarct size, independent of area at risk.

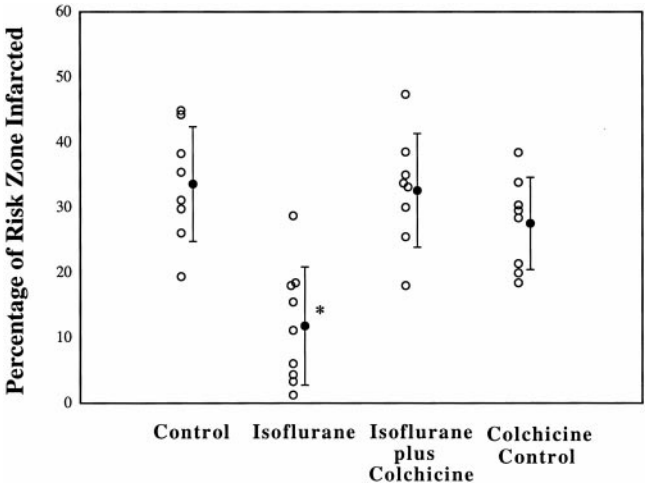

Fig 3. The area of infarcted myocardium is expressed as a percentage of the risk zone in each of four groups. Isoflurane pretreatment reduced infarct size significantly. The asterisk indicates a difference from control ($P < 0.05$ by analysis of variance and Newman-Keuls *post hoc* test). The preconditioning-like effect of isoflurane was eliminated by pretreatment with colchicine. Meanwhile, colchicine did not increase infarct size, expressed as a percentage of the area at risk, in control groups given 30-min coronary occlusions but no isoflurane pretreatment. Error bars represent mean ± SD.

alone did not alter IS (IS:AR = 27.6% ± 7.1%; P = not significant).

Hemodynamics

As previously noted, in the two isoflurane-pretreated groups, propofol infusion was interrupted briefly during administration of isoflurane to maintain a nearly constant level of anesthesia and to avoid hypotension. There were no significant differences in heart rate, mean arterial pressure, or rate–pressure product among the four treatment groups at baseline or immediately before coronary occlusion (table 2), which might account for the group differences in IS.

In both colchicine-treated groups, heart rate decreased significantly, by 10–20%, over the time of study (table 2). Mean arterial pressure, by experimental design, was held as constant as possible and did not change significantly over the course of the experiment. Because of the effect noted before on heart rate, the rate–pressure product decreased toward the end of reperfusion in both colchicine-treated groups.

Incidence of Transient Ventricular Fibrillation

As previously noted, three rabbits died of intractable ventricular fibrillation (all three in the colchicine-control group). In addition, short periods (< 90 s) of transient, nonlethal ventricular fibrillation were recorded in two rabbits (one in the colchicine-control group and one in the control group). The incidence of ventricular fibrillation (lethal plus nonlethal) was 36.4% in the control-plus-colchicine group, 12.5% in the control group, and 0% in the isoflurane-treated groups. The overall incidence of ventricular arrhythmia was significantly different among the four study groups by chi-square analysis ($P < 0.05$).

Discussion

This study confirms the "preconditioning" effect of isoflurane. When administered before prolonged myocardial ischemia and reperfusion, isoflurane exerts a potent cardioprotective effect, reducing myocardial IS by ≥ 50%.[1,4] In the current study, we found isoflurane pretreatment, compared with the controls that did not receive pretreatment, reduced IS substantially (11.8% ± 9.1% *vs.* 33.6% ± 8.8%), respectively. In addition, colchicine, a drug that depolymerizes microtubules,[9,15] completely abolished the isoflurane-induced preconditioning effect (IS:AR = 32.6% ± 8.7%) but had no effect on IS in our control group (IS:AR = 27.6% ± 7.1%; P = not significant).

The most important finding of the current study, that colchicine inhibits isoflurane-induced myocardial preconditioning, suggests that cytoskeletal microtubules play a role in the mechanism of isoflurane's cardioprotective effects. This result is similar to findings by Liu *et al.* in a study of classic ischemic preconditioning.[8] Using an acutely instrumented rabbit model, Liu *et al.* found that pretreatment with colchicine 30 min before ischemic preconditioning blocked the protective effect of preconditioning but had no effect on IS in a group that did not receive preconditioning.[8]

Role of Microtubules

The cellular functions served by microtubules are many and diverse, including cellular motility, cell extension and growth, intracellular transport, and basic structural support functions.[13,16–23] In view of these many functions, we cannot assign a specific role for microtubules or the cytoskeleton in isoflurane-induced preconditioning, nor does current evidence allow us to say definitely whether microtubular integrity is universally necessary for cardioprotection. With regard to currently known pathways mediating preconditioning, which may also be dependent on microtubular function, at least one possible mechanism deserves special mention: the proposed PKC pathway.[8,24]

Table 2. Hemodynamic Measurements in Rabbit Hearts after Four Different Pretreatments and Subsequent Exposure to 30 min of LAD Coronary Occlusion and 3 h of Reperfusion

	Control (n = 8)	Isoflurane (n = 9)	Isoflurane Plus Colchicine (n = 8)	Colchicine Control (n = 8)
Heart rate (beats/min)				
Baseline	252 ± 29	249 ± 20	247 ± 24	249 ± 20
Preocclusion	253 ± 22	237 ± 39	233 ± 23	214 ± 12
15 min into occlusion	247 ± 30	234 ± 45	232 ± 24	217 ± 14
30 min reperfusion	263 ± 32	238 ± 46	228 ± 23	216 ± 24*
1 h reperfusion	263 ± 32	232 ± 34	218 ± 23*	215 ± 27*
3 h reperfusion	248 ± 44	231 ± 36	218 ± 26	197 ± 37*†
Mean arterial pressure (mmHg)				
Baseline	69 ± 8	64 ± 5	64 ± 5	68 ± 8
Preocclusion	78 ± 7	69 ± 4	75 ± 13	78 ± 9
15 min into occlusion	70 ± 11	63 ± 4	72 ± 8	78 ± 11
30 min reperfusion	69 ± 12	65 ± 7	70 ± 7	70 ± 14
1 h reperfusion	69 ± 7	63 ± 7	66 ± 8	68 ± 10
3 h reperfusion	62 ± 8	64 ± 8	59 ± 7	59 ± 5
Rate–pressure product (beats/min × mmHg × 10^3)				
Baseline	17.6 ± 3.9	16.0 ± 1.5	15.9 ± 1.8	16.9 ± 2.8
Preocclusion	19.9 ± 2.7	16.5 ± 2.5	17.8 ± 4.3	16.8 ± 2.2
15 min into occlusion	17.5 ± 4.0	15.0 ± 3.7	16.9 ± 3.5	17.1 ± 2.5
30 min reperfusion	18.5 ± 4.8	15.7 ± 4.0	16.0 ± 2.0	15.3 ± 4.2
1 h reperfusion	18.4 ± 3.3	15.3 ± 2.4	14.3 ± 1.6*	14.7 ± 3.2*
3 h reperfusion	15.6 ± 3.5	14.7 ± 3.0	12.9 ± 2.3	11.6 ± 2.1*†

Hemodynamic variables were measured serially in all groups.

* Significantly different ($P < 0.05$) from the same respective value in control group by ANOVA.

† Significantly different ($P < 0.05$) from baseline by ANOVA.

On activation in cardiomyocytes, specific enzymatic isoforms of PKC are translocated to cytoskeletal elements and various subcellular locations.[25,26] Although the downstream phosphorylation targets of PKC in preconditioning are unknown at present, the process of PKC isoform translocation/activation is a key component of the process in many models. Pharmacologic inhibition of PKC also inhibits myocardial preconditioning.[24,27] Although we did not study the role of PKC in this model of isoflurane-induced preconditioning, our results would be consistent with the speculation that intracellular translocation of PKC is a key element in anesthetic-induced preconditioning. Disruption of intracellular transport functions of tubulin would be predicted to disrupt the transport of PKC.

One constraint of the current study is that we did not measure microtubular dissolution directly. Therefore, we cannot say to what degree the microtubular cytoskeleton was disrupted in this anesthetized rabbit model. However, colchicine is known to inhibit microtubular growth and induce microtubular disassembly,[9] and the doses used (2 mg/kg) are known to be effective, based on previous experimental work.[13] We cannot completely exclude the possibility that colchicine has other pharmacologic effects, unrelated to its effects on microtubules, that would influence the effectiveness of preconditioning.

Limitations

A possible limitation of this study is that propofol was not administered in the same doses in all groups; it was discontinued briefly during isoflurane administration to avoid anesthetic-induced hypotension. As has been noted previously,[4] it is unlikely that this experimental design led to any significant changes in our findings. Because propofol, which is reported to have a mild cardioprotective effect,[28] was discontinued during isoflurane administration, our study may have been slightly biased against finding a cardioprotective effect of isoflurane. To the contrary, we found a potent cardioprotective effect.

In summary, the microtubular depolymerizing agent colchicine inhibits the myocardial preconditioning effect of isoflurane. This implies that an intact microtubular cytoskeleton is required for the isoflurane preconditioning process.

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