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Isoflurane-enhanced Recovery of Canine Stunned Myocardium

Role for Protein Kinase C?

Wolfgang G. Toller, M.D., D.E.A.A., Matthew W. Montgomery, B.S.,† Paul S. Pagel, M.D., Ph.D.,‡ Douglas A. Hettrick, Ph.D.,§ David C. Warltier, M.D., Ph.D.,‖ Judy R. Kersten, M.D.‡*

Background: **Isoflurane enhances the functional recovery of postischemic, reperfused myocardium by activating adenosine A_1 receptors and adenosine triphosphate–regulated potassium channels. Whether protein kinase C is involved in this process is unknown. The authors tested the hypothesis that inhibition of protein kinase C, using the selective antagonist bisindolylmaleimide, attenuates isoflurane-enhanced recovery of stunned myocardium in dogs.**

Methods: **Fifty dogs were randomly assigned to receive intracoronary vehicle or bisindolylmaleimide (2 or 8 μg/min) in the presence or absence of isoflurane (1 minimum alveolar concentration). Five brief (5 min) coronary artery occlusions interspersed with 5-min reperfusion periods followed by 180 min of final reperfusion were used to produce myocardial stunning. Hemodynamics, regional segment shortening, and myocardial blood flow (radioactive microspheres) were measured at selected intervals.**

Results: **There were no differences in baseline hemodynamics, segment shortening, or coronary collateral blood flow between groups. Isoflurane significantly ($P < 0.05$) decreased heart rate, mean arterial pressure, rate pressure product, and the maximum rate of increase of left ventricular pressure ($+dP/dt_{max}$) in the presence or absence of bisindolylmaleimide. Sustained contractile dysfunction was observed in dogs that received vehicle (recovery of segment shortening to 12 ± 8% of baseline), in contrast to those that received isoflurane (75 ± 7% recovery). Bisindolylmaleimide at a dose of 2 μg/min alone enhanced recovery of segment shortening (50 ± 7% of baseline) compared with vehicle-pretreated dogs, and isoflurane in the presence of 2 μg/min bisindolylmaleimide further enhanced recovery of contractile function (79 ± 8% of baseline). In contrast, 8 μg/min bisindolylmaleimide alone (32 ± 12%) or combined with isoflurane (37 ± 17%) did not enhance recovery of segment shortening compared with vehicle-pretreated dogs.**

Conclusions: **The results indicate that protein kinase C inhibition using low doses of bisindolylmaleimide alone produces cardioprotection, and isoflurane further enhances this protection. In contrast, high doses of bisindolylmaleimide are not cardioprotective in the presence or absence of isoflurane. A role for protein kinase C during isoflurane-induced recovery of the stunned myocardium cannot be excluded. (Key words: Bisindolylmaleimide; myocardial ischemia; volatile anesthetics.)**

BRIEF, repetitive episodes of myocardial ischemia and reperfusion cause myocardial stunning, which is prolonged reversible contractile dysfunction without tissue necrosis.[1,2] Isoflurane enhances the recovery of stunned myocardium when it is administered before or during myocardial ischemia,[3–9] but the signal transduction cascade responsible for this myocardial protection has not been completely defined. Previously, we showed that isoflurane-enhanced recovery of postischemic, reperfused myocardium is mediated by activation of adenosine type 1 (A_1) receptors[9] and adenosine triphosphate–regulated potassium channels.[4,8,10] Recent evidence indicates that protein kinase C (PKC) activation may occur as a result of adenosine A_1 receptor stimulation and may directly modulate adenosine triphosphate–regulated potassium channel activity *in vitro*.[11–14] These actions may account for the cardioprotective effects of PKC activation during ischemic preconditioning *in vivo*.[15,16] Increased PKC activity has been implicated in anesthetic-induced reductions of myocardial

* Research Fellow in Anesthesiology.

† Medical Student.

‡ Associate Professor of Anesthesiology.

§ Assistant Professor of Anesthesiology.

‖ Professor of Anesthesiology, Pharmacology and Toxicology, and Medicine (Division of Cardiovascular Diseases), and Vice Chairman for Research of Anesthesiology.

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Address reprint requests to Dr. Kersten: Department of Anesthesiology, MEB 462C, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226. Address electronic mail to: jkersten@mcw.edu

infarct size,[17] but the role of PKC in enhanced functional recovery of the stunned myocardium produced by isoflurane or other volatile agents is unknown. We tested the hypothesis that inhibition of PKC, using the selective antagonist bisindolylmaleimide,[18] attenuates isoflurane-enhanced recovery of stunned myocardium in barbiturate-anesthetized, acutely instrumented dogs.

Materials and Methods

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal Care and Use Committee of the Medical College of Wisconsin. All procedures were in conformity with the *Guiding Principles in the Care and Use of Animals* of the American Physiologic Society and were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (Washington, DC, National Academy Press, 1996).

General Preparation

The experimental methods used in the current investigation have been described in detail previously.[4] Briefly, mongrel dogs of either sex (weight, 26 ± 1 kg [mean ± SEM]) were fasted overnight, anesthetized with sodium barbital (200 mg/kg) and sodium pentobarbital (15 mg/kg), and ventilated through an air-and-oxygen mixture (fraction of inspired oxygen = 0.25) after endotracheal intubation. Tidal volume and respiratory rate were adjusted to maintain arterial blood gas tensions within a physiological range (*p*H = 7.36–7.43; carbon dioxide tension = 28–34 mmHg). A double pressure transducer-tipped catheter was inserted into the left ventricle and aorta through the left carotid artery to measure left ventricular (LV) and aortic pressures, respectively. The maximum rate of increase of LV pressure ($+dP/dt_{max}$) was obtained by differentiating the LV pressure waveform. A thoracotomy was performed in the left fifth intercostal space, and a transit-time ultrasonic flow probe was placed around the ascending aorta to measure relative (less coronary blood flow) cardiac output. A 1.5- to 2-cm portion of the left anterior descending coronary artery (LAD) immediately distal to the first diagonal branch was isolated, and a precalibrated Doppler ultrasonic flow transducer and a silk ligature were positioned around the vessel to measure coronary blood flow velocity and to produce coronary artery occlusion and reperfusion, respectively. Heparin-filled catheters were inserted into the LAD (*via* a small catheter [PE-20] that was inserted over a needle [24 gauge; Angiocath; Becton Dickinson, Sandy, UT]), the left atrial appendage, and a femoral vein for intracoronary drug, radioactive microsphere, and fluid administration, respectively. The right femoral artery was cannulated for the withdrawal of reference blood flow samples. A pair of ultrasonic segment-length transducers was implanted in the LAD region to measure regional contractile function using the formula: %regional segment shortening = $(EDL - ESL) \cdot 100 \cdot EDL^{-1}$, where %segment shortening = the percentage of segment shortening, EDL = end-diastolic segment length, and ESL = end-systolic segment length. Regional myocardial perfusion was measured in the ischemic (LAD) and normal (left circumflex coronary artery) regions using the radioactive microsphere technique.[4] Relative diastolic coronary vascular resistance was calculated as the ratio of end-diastolic arterial pressure to peak diastolic coronary blood flow velocity. Hemodynamic data were monitored continuously during the experiment, recorded with a polygraph, and digitized *via* a computer interfaced with an analog-to-digital converter.

Experimental Protocol

Figure 1 shows the experimental protocol. After the surgical preparation was completed, dogs were assigned randomly to receive intracoronary vehicle (0.5 ml dimethylsulfoxide diluted in 8 ml saline, 0.9%) or bisindolylmaleimide (dissolved in 0.5 ml dimethylsulfoxide and diluted with saline, 0.9%, to a volume of 8 ml) in the presence or absence of isoflurane (1 minimum alveolar end-tidal concentration) in six separate groups of experiments (fig. 1). All dogs were subjected to five brief (5 min) coronary occlusions interspersed with 5-min periods of reperfusion, followed by a final 180-min period of reperfusion to produce stunned myocardium. In all groups, intracoronary drug infusion (0.1 ml/min) was started 30 min before the first LAD occlusion, maintained throughout all five occlusions, and discontinued at the end of the fifth occlusion (total infusion time = 75 min). Two groups of dogs received 2 μg/min bisindolylmaleimide during a period of 75 min (total dose = 150 μg; calculated intracoronary concentration of 100 nM, assuming an LAD blood flow of approximately 40 ml/min). Another two groups of dogs received 8 μg/min bisindolylmaleimide during a period of 75 min (total dose = 600 μg; calculated intracoronary concentration of 400 nM).

Statistical Analyses

Statistical analysis of data within and between groups was performed with multivariate analyses of variance for

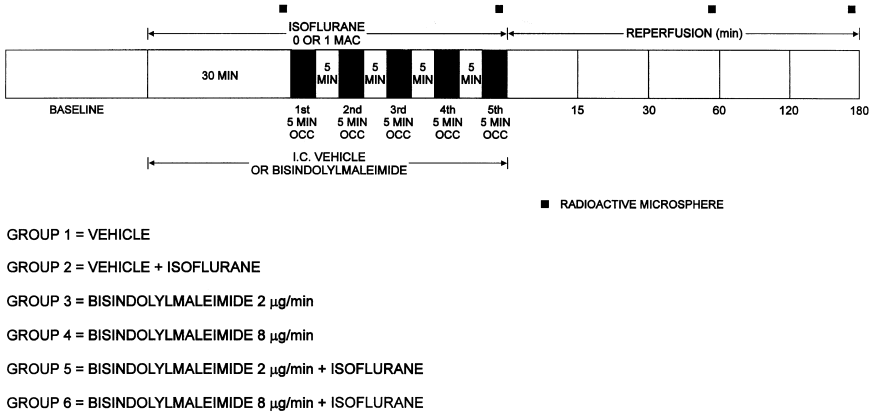

Fig. 1. The experimental protocol used to study the role of protein kinase C as a mediator of isoflurane-enhanced recovery of stunned myocardium using two doses of the selective protein kinase C inhibitor, bisindolylmaleimide. Dogs were subjected to five brief (5-min) periods of left anterior descending coronary artery (LAD) occlusion (OCC) interspersed with 5-min periods of reperfusion and followed by a final 18-min period of reperfusion. Systemic and coronary hemodynamics, regional contractile function (the percentage of segment shortening), and regional myocardial blood flow (radioactive microspheres) were measured at selected intervals.

repeated measures followed by application of the Student *t* test with Bonferroni correction for multiplicity. Changes in segment shortening were analyzed using the Wilcoxon signed rank sum test. Changes within and between groups were considered significant when $P < 0.05$. All data are expressed as the mean ± SEM.

Results

Sixty-four dogs were instrumented to provide 50 successful experiments. Ten dogs were excluded from data analysis because transmural coronary collateral blood flow exceeded 0.3 $ml \cdot min^{-1} \cdot g^{-1}$ (two vehicle, three vehicle and isoflurane, two low-dose bisindolylmaleimide, one low-dose bisindolylmaleimide and isoflurane, one high-dose bisindolylmaleimide, and one high-dose bisindolylmaleimide and isoflurane). Four additional dogs were excluded because of myocardial ischemia during the surgical preparation (one vehicle, two vehicle and isoflurane, and one high-dose bisindolylmaleimide).

Hemodynamic Effects of Myocardial Stunning

Systemic and coronary hemodynamics were similar among the groups under baseline conditions. Left anterior descending coronary artery occlusion increased LV end-diastolic pressure and decreased $+dP/dt_{max}$ and cardiac output in vehicle-treated dogs (table 1). Decreases in contractile performance were sustained in vehicle-treated dogs and were accompanied by increases in systemic and diastolic coronary vascular resistance after 180 min of reperfusion. In contrast to findings during vehicle alone, isoflurane caused significant ($P < 0.05$) decreases in heart rate, mean arterial and LV systolic pressures, rate pressure product, $+dP/dt_{max}$, and systemic vascular resistance (table 2). Compared with baseline values, LAD occlusion caused similar increases in LV end-diastolic pressure and decreases in $+dP/dt_{max}$ and cardiac output in the presence or absence of isoflurane. However, hemodynamics returned to baseline values after 180 min of reperfusion in dogs receiving isoflurane, in contrast to findings during drug vehicle alone.

Bisindolylmaleimide produced no hemodynamic effects (tables 3 and 4), and LAD occlusion caused similar hemodynamic effects in dogs that received bisindolylmaleimide or drug vehicle. In addition, bisindolylmaleimide did not alter the systemic or coronary hemodynamic effects of isoflurane (tables 5 and 6), except that sustained reductions in $+dP/dt_{max}$ and cardiac output were observed in all bisindolylmaleimide-treated dogs after 180 min of reperfusion.

Contractile Function during Myocardial Stunning

There were no differences in segment shortening among the experimental groups under baseline conditions or during brief LAD occlusions and reperfusions. Sustained contractile dysfunction was observed in vehicle-pretreated dogs after 180 min of reperfusion (12 ± 8% of baseline; fig. 2). In contrast, dogs that received isoflurane had enhanced recovery of segment shortening after 60 min (table 2) and achieved recovery to 75 ± 7% of baseline after 180 min of reperfusion. Bisindolylmaleimide alone enhanced recovery of contractile function in the low-dose but not the high-dose groups. However, no differences in recovery were observed between the low-and high-dose bisindolylmaleimide groups alone. Isoflurane in the presence of low-dose bisindolylmaleimide enhanced the recovery of contractile function as early as 60 min after reperfusion (table 5) and to a similar extent compared with isoflurane alone, but it caused signifi-

Table 1. Hemodynamic Effects of Myocardial Stunning in Dogs Receiving Drug Vehicle

				Reperfusion (min)				
	Baseline	Vehicle	5th Occlusion	15	30	60	120	180
HR (bpm)	133 ± 5	132 ± 5	127 ± 6	126 ± 8	125 ± 8	123 ± 9	125 ± 11	129 ± 12
MBP (mmHg)	91 ± 4	92 ± 4	88 ± 6	95 ± 8	95 ± 8	94 ± 9	96 ± 6	90 ± 6
RPP (mmHg · bpm · 10^3)	13.6 ± 0.7	13.7 ± 0.7	12.4 ± 1.0	13.5 ± 1.2	13.4 ± 1.4	13.0 ± 1.5	13.9 ± 1.5	13.4 ± 1.8
LVSP (mmHg)	100 ± 4	101 ± 4	97 ± 5	106 ± 7	107 ± 8	106 ± 8	109 ± 6	104 ± 7
LVEDP (mmHg)	5 ± 1	6 ± 1	13 ± 2*	9 ± 1*	8 ± 1	9 ± 1*	10 ± 2*	9 ± 1*
$+dP/dt_{max}$ (mmHg/s)	1,640 ± 160	1,620 ± 160	1,310 ± 120*	1,220 ± 100*	1,230 ± 120*	1,220 ± 130*	1,230 ± 120*	1,190 ± 140*
DCBFV (Hz · 10^2)	41 ± 4	38 ± 4	—	38 ± 4	35 ± 4	33 ± 4*	35 ± 4	31 ± 3*
DCVR (mmHg · Hz^{-1} · 10^{-2})	2.3 ± 0.3	2.4 ± 0.3	—	2.6 ± 0.4	2.8 ± 0.4	2.9 ± 0.4*	2.9 ± 0.3*	2.9 ± 0.4*
CO (l/min)	2.8 ± 0.2	2.6 ± 0.2	2.2 ± 0.2*	2.3 ± 0.2*	2.3 ± 0.2*	2.2 ± 0.2*	2.2 ± 0.2*	2.1 ± 0.3*
SVR (dyne · s · cm^{-5})	2,660 ± 160	2,910 ± 190	3,240 ± 310	3,350 ± 310	3,430 ± 370	3,570 ± 480	3,740 ± 390*	3,760 ± 510*
SV (ml)	21 ± 2	20 ± 2	18 ± 2	19 ± 2	19 ± 2	19 ± 3	19 ± 3	18 ± 3
SS (%)	19 ± 2	17 ± 1	−3 ± 1*	4 ± 1*	5 ± 1*	7 ± 2*	5 ± 1*	2 ± 2*

Data are mean ± SEM; n = 9.

HR = heart rate; MBP = mean aortic blood pressure; RPP = rate pressure product; LVSP and LVEDP = left ventricular systolic and end diastolic pressure, respectively; DCBFV = diastolic coronary blood flow velocity; DCVR = diastolic coronary vascular resistance; CO = cardiac output; SVR = systemic vascular resistance; SV = stroke volume; SS = segment shortening.

* Significantly ($P < 0.05$) different from baseline.

cantly greater recovery than did low-dose bisindolylmaleimide alone. No recovery of segment shortening was observed in experiments using high-dose bisindolylmaleimide, regardless of previous exposure to isoflurane (+ isoflurane: 37 ± 17%; − isoflurane: 32 ± 12% of baseline). However, no differences in recovery of segment shortening could be shown in dogs receiving isoflurane in the presence or absence of high-dose bisindolylmaleimide ($P = 0.066$).

Regional Myocardial Perfusion

Table 7 summarizes the transmural myocardial perfusion to the ischemic (LAD) and normal (left circumflex coronary artery) regions. There were no differences in

Table 2. Hemodynamic Effects of Myocardial Stunning in Dogs Receiving Drug Vehicle and Isoflurane

				Reperfusion (min)				
	Baseline	Vehicle + Isoflurane	5th Occlusion	15	30	60	120	180
HR (bpm)	130 ± 7	107 ± 6*†	105 ± 5*	104 ± 6*	107 ± 6*	117 ± 10	118 ± 10	118 ± 9
MBP (mmHg)	101 ± 3	73 ± 4*	63 ± 3*†	93 ± 6	101 ± 8	103 ± 7	109 ± 8	110 ± 7
RPP (mmHg · bpm · 10^3)	14.3 ± 1.1	8.8 ± 0.8*†	7.1 ± 0.7*†	10.6 ± 1.0*	11.9 ± 1.3	13.0 ± 1.2	13.8 ± 1.4	13.9 ± 1.2
LVSP (mmHg)	107 ± 4	77 ± 4*†	64 ± 4*†	99 ± 7	107 ± 9	109 ± 7	114 ± 8	119 ± 8
LVEDP (mmHg)	4 ± 1	7 ± 1	12 ± 1*	9 ± 1*	8 ± 1*	6 ± 1	5 ± 1	5 ± 1
$+dP/dt_{max}$ (mmHg/s)	1,700 ± 120	990 ± 50*†	840 ± 50*†	1,190 ± 60*	1,290 ± 90*	1,440 ± 90	1,600 ± 130	1,520 ± 110
DCBFV (Hz · 10^2)	36 ± 6	34 ± 4	—	51 ± 9*	45 ± 7	44 ± 8	47 ± 9	44 ± 9
DCVR (mmHg · Hz^{-1} · 10^{-2})	3.5 ± 1	2.5 ± 0.7	—	2.0 ± 0.3	2.3 ± 0.3	2.7 ± 0.5	2.8 ± 0.6	2.9 ± 0.5
CO (l/min)	2.2 ± 0.2	1.7 ± 0.2	1.5 ± 0.2*	2.1 ± 0.2	2.0 ± 0.2	1.9 ± 0.3	2.1 ± 0.2	1.9 ± 0.2
SVR (dyne · s · cm^{-5})	3,810 ± 280	3,680 ± 370*	3,750 ± 430	3,930 ± 460	4,360 ± 530	4,960 ± 650	4,660 ± 590	4,960 ± 530
SV (ml)	17 ± 2	17 ± 3	15 ± 3*	21 ± 3	20 ± 3	18 ± 4	19 ± 4	18 ± 3
SS (%)	14 ± 2	12 ± 2†	−4 ± 1*	8 ± 1*	8 ± 2*	10 ± 2	10 ± 2	10 ± 1†

Data are mean ± SEM; n = 8.

HR = heart rate; MBP = mean aortic blood pressure; RPP = rate pressure product; LVSP and LVEDP = left ventricular systolic and end diastolic pressure, respectively; DCBFV = diastolic coronary blood flow velocity; DCVR = diastolic coronary vascular resistance; CO = cardiac output; SVR = systemic vascular resistance; SV = stroke volume; SS = segment shortening.

* Significantly ($P < 0.05$) different from baseline.

† Significantly ($P < 0.05$) different from vehicle (table 1).

Table 3. Hemodynamic Effects of Myocardial Stunning in Dogs Receiving 2 μg/min Bisindolylmaleimide

				Reperfusion (min)				
	Baseline	BIS	5th Occlusion	15	30	60	120	180
HR (bpm)	131 ± 7	130 ± 7	127 ± 6	125 ± 6	125 ± 5	122 ± 5	122 ± 6	120 ± 7*
MBP (mmHg)	101 ± 6	98 ± 6	88 ± 6	94 ± 5	96 ± 5	98 ± 5	101 ± 4	99 ± 6
RPP (mmHg · bpm · 10^3)	14.5 ± 1.1	14.0 ± 0.9	12.1 ± 1.0	12.9 ± 0.7	13.0 ± 0.6	13.2 ± 0.7	13.3 ± 0.8	13.1 ± 1.1
LVSP (mmHg)	110 ± 6	105 ± 7	93 ± 6*	104 ± 6	106 ± 5	109 ± 6	108 ± 4	107 ± 6
LVEDP (mmHg)	5 ± 1	5 ± 1	7 ± 2	5 ± 1	5 ± 1	5 ± 1	5 ± 1	5 ± 1
$+dP/dt_{max}$ (mmHg/s)	1,860 ± 140	1,830 ± 80	1,450 ± 90*	1,470 ± 60*	1,510 ± 50*	1,570 ± 70*	1,530 ± 80*	1,460 ± 110*
DCBFV (Hz · 10^2)	40 ± 3	41 ± 4	—	47 ± 6	45 ± 5	45 ± 6	46 ± 4	45 ± 4
DCVR (mmHg · $Hz^{-1} \cdot 10^{-2}$)	2.5 ± 0.3	2.4 ± 0.3	—	2.1 ± 0.3	2.2 ± 0.3	2.4 ± 0.4	2.2 ± 0.2	2.2 ± 0.2
CO (l/min)	2.5 ± 0.4	2.4 ± 0.4	1.9 ± 0.4*	2.0 ± 0.4	2.0 ± 0.4	2.0 ± 0.3	1.7 ± 0.3*	1.6 ± 0.3*
SVR (dyne · s · cm^{-5})	3,900 ± 610	4,050 ± 710	4,390 ± 540	4,290 ± 500	4,560 ± 620	4,850 ± 930	5,980 ± 1120	5,840 ± 1010
SV (ml)	19 ± 3	19 ± 3	15 ± 3*	16 ± 3	16 ± 3	17 ± 3	14 ± 2*	14 ± 2*
SS (%)	17 ± 1	17 ± 1	−5 ± 1*	6 ± 2*	8 ± 2*	11 ± 2	11 ± 2†	8 ± 1*†

Data are mean ± SEM; n = 9.

BIS = bisindolylmaleimide; HR = heart rate; MBP = mean aortic blood pressure; RPP = rate pressure product; LVSP and LVEDP = left ventricular systolic and end diastolic pressure, respectively; DCBFV = diastolic coronary blood flow velocity; DCVR = diastolic coronary vascular resistance; CO = cardiac output; SVR = systemic vascular resistance; SV = stroke volume; SS = segment shortening.

* Significantly ($P < 0.05$) different from baseline.

† Significantly ($P < 0.05$) different from vehicle (table 1).

transmural myocardial perfusion or coronary collateral blood flow among the groups.

Discussion

Increased activity of an important family of serine-threonine kinase enzymes known collectively as PKC has been implicated in the intracellular signal transduction pathways responsible for myocardial protection during ischemic[15,19] and anesthetic-induced preconditioning.[17] Bisindolylmaleimide inhibits the activity of all known PKC isoforms, including calcium-dependent and -independent species in conventional, novel, and atypical classes.[18,20–22] The results of the present investigation indicate that high but not low doses of bisindolylmaleimide attenuate isoflurane-enhanced functional recovery of stunned myocardium independent of alterations in coronary collateral blood flow or systemic hemodynamics. These results are similar to those of Cope *et al.*,[17] who found that reductions in experimental myo-

Table 4. Hemodynamic Effects of Myocardial Stunning in Dogs Receiving 8 μg/min Bisindolylmaleimide

				Reperfusion (min)				
	Baseline	BIS	5th Occlusion	15	30	60	120	180
HR (bpm)	123 ± 4	119 ± 4	112 ± 4	114 ± 5	111 ± 4	108 ± 4	110 ± 6	112 ± 8
MBP (mmHg)	88 ± 6	89 ± 6	83 ± 9	95 ± 5	90 ± 7	94 ± 5	100 ± 5	99 ± 5
RPP (mmHg · bpm · 10^3)	12.0 ± 0.7	11.6 ± 0.6	10.2 ± 1.1	11.7 ± 0.6	11.0 ± 0.9	11.2 ± 0.9	11.8 ± 0.9	12.0 ± 1.1
LVSP (mmHg)	98 ± 6	96 ± 6	89 ± 8	100 ± 5	98 ± 6	103 ± 4	103 ± 6	103 ± 6
LVEDP (mmHg)	7 ± 1	6 ± 1	10 ± 3	7 ± 2	7 ± 2	8 ± 2	8 ± 2	8 ± 2
$+dP/dt_{max}$ (mmHg/s)	1,760 ± 140	1,610 ± 100	1,280 ± 120*	1,260 ± 60*	1,200 ± 70*	1,210 ± 70*	1,300 ± 100*	1,250 ± 100*
DCBFV (Hz · 10^2)	51 ± 7	47 ± 7	—	60 ± 11	54 ± 10	49 ± 8	52 ± 8	54 ± 9
DCVR (mmHg · $Hz^{-1} \cdot 10^{-2}$)	1.9 ± 0.4	2.2 ± 0.5	—	2.1 ± 0.7	2.2 ± 0.7	2.4 ± 0.7	2.4 ± 0.7	2.2 ± 0.5
CO (l/min)	2.9 ± 0.3	2.4 ± 0.3	1.9 ± 0.3*	1.9 ± 0.2*	1.9 ± 0.2*	1.7 ± 0.2*	1.6 ± 0.2*	1.6 ± 0.2*
SVR (dyne · s · cm^{-5})	2,490 ± 290	3,130 ± 410	3,640 ± 420*	4,010 ± 320*	3,880 ± 480*	4,420 ± 350*	5,160 ± 610*	5,120 ± 490*
SV (ml)	24 ± 3	21 ± 3	17 ± 3*	17 ± 2*	18 ± 2*	16 ± 2*	15 ± 1*	15 ± 1*
SS (%)	19 ± 1	18 ± 1	−4 ± 2*	5 ± 2*	6 ± 2*	7 ± 3*	8 ± 3*	7 ± 3*

Data are mean ± SEM; n = 7.

BIS = bisindolylmaleimide; HR = heart rate; MBP = mean aortic blood pressure; RPP = rate pressure product; LVSP and LVEDP = left ventricular systolic and end diastolic pressure, respectively; DCBFV = diastolic coronary blood flow velocity; DCVR = diastolic coronary vascular resistance; CO = cardiac output; SVR = systemic vascular resistance; SV = stroke volume; SS = segment shortening.

* Significantly ($P < 0.05$) different from baseline.

Table 5. Hemodynamic Effects of Myocardial Stunning in Dogs Receiving 2 μg/min Bisindolylmaleimide and Isoflurane

				Reperfusion (min)				
	Baseline	BIS + Isoflurane	5th Occlusion	15	30	60	120	180
HR (bpm)	123 ± 5	102 ± 4*†‡	99 ± 4*†‡	103 ± 4*	106 ± 5*	106 ± 5*	106 ± 7*	103 ± 8*
MBP (mmHg)	96 ± 4	62 ± 4*†‡	58 ± 5*†‡	93 ± 4	96 ± 3	105 ± 4	106 ± 6	103 ± 6
RPP (mmHg · bpm · 10^3)	13.0 ± 0.8	7.5 ± 0.7*†‡	6.7 ± 0.8*†‡	10.8 ± 0.8	11.5 ± 0.7	12.4 ± 0.8	12.4 ± 1.1	11.8 ± 1.1
LVSP (mmHg)	104 ± 4	74 ± 5*†‡	65 ± 4*†‡	102 ± 4	107 ± 4	115 ± 5	114 ± 7	115 ± 6
LVEDP (mmHg)	6 ± 1	8 ± 1	12 ± 2*	9 ± 2	8 ± 2	8 ± 2	7 ± 2	8 ± 2
$+dP/dt_{max}$ (mmHg/s)	1,790 ± 100	960 ± 40*†‡	760 ± 60*†‡	1,240 ± 60*	1,380 ± 60*	1,470 ± 60*	1,480 ± 60*	1,450 ± 110*
DCBFV (Hz · 10^2)	35 ± 2	38 ± 4	—	51 ± 7*	43 ± 4	41 ± 5	38 ± 4	39 ± 5
DCVR (mmHg · Hz^{-1} · 10^{-2})	2.5 ± 0.2	1.6 ± 0.3*	—	1.9 ± 0.4	2.2 ± 0.4	2.5 ± 0.4	2.8 ± 0.4	2.5 ± 0.4
CO (l/min)	2.7 ± 0.2	2.1 ± 0.1*	1.5 ± 0.1*	2.3 ± 0.2	2.4 ± 0.2	2.2 ± 0.2	1.9 ± 0.2*	1.9 ± 0.2*
SVR (dyne · s · cm^{-5})	3,000 ± 330	2,400 ± 210	3,190 ± 450	3,330 ± 300	3,400 ± 320	4,020 ± 460	4,790 ± 540*	4,570 ± 420*
SV (ml)	22 ± 2	21 ± 2	16 ± 1*	23 ± 2	23 ± 3	22 ± 3	19 ± 2	19 ± 2
SS (%)	18 ± 2	15 ± 2	−4 ± 1*	10 ± 1*†	13 ± 1*†	15 ± 2†	15 ± 2†	14 ± 2†‡

Data are mean ± SEM; n = 8.

BIS = bisindolylmaleimide; HR = heart rate; MBP = mean aortic blood pressure; RPP = rate pressure product; LVSP and LVEDP = left ventricular systolic and end diastolic pressure, respectively; DCBFV = diastolic coronary blood flow velocity; DCVR = diastolic coronary vascular resistance; CO = cardiac output; SVR = systemic vascular resistance; SV = stroke volume; SS = segment shortening.

* Significantly ($P < 0.05$) different from baseline.

† Significantly ($P < 0.05$) different from vehicle (table 1).

‡ Significantly ($P < 0.05$) different from BIS 2 μg/min (table 3).

cardial infarct size produced by halothane in rabbits depended on PKC activation. However, we also found that PKC inhibition alone may be cardioprotective, and isoflurane further enhances the protection afforded by low doses of bisindolylmaleimide.

Low doses of bisindolylmaleimide (2 μg/min) specific for PKC inhibition do not block the protective effects of isoflurane and may independently exert cardioprotective effects. The present results are similar to the results Tosaki *et al.*[23] found in experiments that evaluated the

Table 6. Hemodynamic Effects of Myocardial Stunning in Dogs Receiving 8 μg/min Bisindolylmaleimide and Isoflurane

				Reperfusion (min)				
	Baseline	BIS + Isoflurane	5th Occlusion	15	30	60	120	180
HR (bpm)	123 ± 3	107 ± 4*†	107 ± 5*	107 ± 5*	108 ± 5*	111 ± 6*	116 ± 6	120 ± 8
MBP (mmHg)	92 ± 4	66 ± 5*†‡	66 ± 4*†‡	93 ± 4	96 ± 3	102 ± 3	102 ± 3	98 ± 3
RPP (mmHg · bpm · 10^3)	12.5 ± 0.6	8.2 ± 0.8*†‡	8.1 ± 0.8*†	11.3 ± 0.8	11.7 ± 0.7	12.5 ± 0.9	13.0 ± 0.9	13.0 ± 1.1
LVSP (mmHg)	101 ± 3	75 ± 5*†	75 ± 4*	105 ± 4	108 ± 3	112 ± 3	110 ± 4	106 ± 4
LVEDP (mmHg)	5 ± 1	6 ± 1	12 ± 1*	8 ± 1	8 ± 1	6 ± 1	8 ± 2	6 ± 2
$+dP/dt_{max}$ (mmHg/s)	1,820 ± 130	1,070 ± 80*†‡	950 ± 90*	1,300 ± 70*	1,390 ± 70*	1,550 ± 120	1,470 ± 110*	1,410 ± 120*
DCBFV (Hz · 10^2)	43 ± 7	43 ± 7	—	48 ± 7	44 ± 7	48 ± 7	46 ± 7	40 ± 6
DCVR (mmHg · Hz^{-1} · 10^{-2})	2.7 ± 0.7	2.0 ± 0.5	—	2.6 ± 0.8	3.0 ± 0.8	2.9 ± 0.8	3.1 ± 0.9	3.2 ± 0.9
CO (l/min)	2.5 ± 0.1	2.0 ± 0.2*	1.7 ± 0.2*	2.1 ± 0.2	2.2 ± 0.2	2.1 ± 0.2	1.8 ± 0.2*	1.7 ± 0.2*
SVR (dyne · s · cm^{-5})	2,990 ± 230	2,820 ± 330	3,310 ± 310	3,850 ± 460	3,730 ± 370	4,220 ± 460*	4,960 ± 620*	4,960 ± 490*
SV (ml)	21 ± 1	19 ± 2	16 ± 2	20 ± 2	21 ± 2	20 ± 2	16 ± 2	15 ± 2*
SS (%)	16 ± 1	13 ± 1	−4 ± 1*	7 ± 2*	8 ± 2*	10 ± 2	8 ± 3*	7 ± 3*

Data are mean ± SEM; n = 9.

BIS = bisindolylmaleimide; HR = heart rate; MBP = mean aortic blood pressure; RPP = rate pressure product; LVSP and LVEDP = left ventricular systolic and end diastolic pressure, respectively; DCBFV = diastolic coronary blood flow velocity; DCVR = diastolic coronary vascular resistance; CO = cardiac output; SVR = systemic vascular resistance; SV = stroke volume; SS = segment shortening.

* Significantly ($P < 0.05$) different from baseline.

† Significantly ($P < 0.05$) different from vehicle (table 1).

‡ Significantly ($P < 0.05$) different from BIS 8 μg/min (table 4).

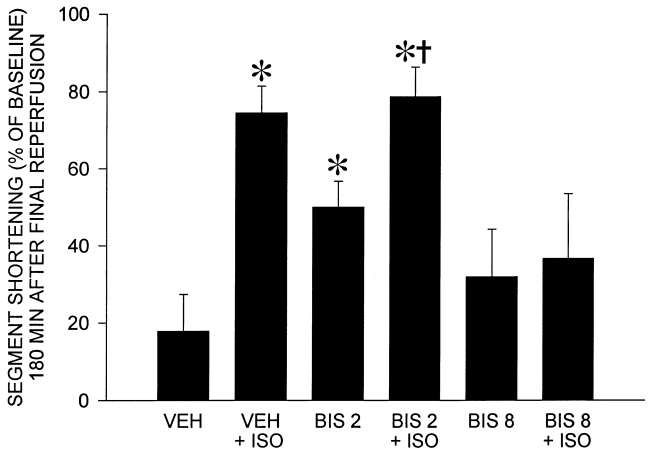

Fig. 2. Recovery of contractile function in the left anterior descending coronary artery perfused region 180 min after final reperfusion (expressed as a percentage of the baseline value) in dogs pretreated with vehicle (VEH), or bisindolylmaleimide (2 or 8 μg/min [BIS 2 and BIS 8, respectively]) and in the presence or absence of 1 minimum alveolar concentration isoflurane (ISO). *Significantly ($P < 0.05$) different from vehicle-pretreated dogs. †Significantly ($P < 0.05$) different from BIS 2.

role of PKC during ischemic preconditioning. The PKC antagonist, calphostin C, did not alter the protection afforded by ischemic preconditioning when administered at low concentrations (100 nM), enhanced protection at moderate concentrations (200 nM), and blocked the cardioprotective effects of ischemic preconditioning on arrhythmogenesis at high concentrations. Thus, the response to PKC inhibition using calphostin C was shown to be biphasic. Blockade of some PKC isoforms at low concentrations may have caused cardioprotective effects, whereas high concentrations may have differentially blocked the "cardioprotective" isoforms of PKC.[23]

In the current investigation, the estimated coronary concentrations of bisindolylmaleimide used were 100 and 400 nM (low and high doses, respectively). Because the half-maximal inhibitory concentration for PKC inhibition of calphostin C and bisindolylmaleimide are similar (40 nM and 10 nM, respectively), the results of our study also may indicate that the response to PKC inhibition is biphasic. This contention is further supported by findings that PKC inhibition with bisindolylmaleimide[24,25] or chelerythrine[25] enhanced recovery of LV developed pressure and reduced infarct size in hearts subjected to global[25] or regional[24] ischemia and reperfusion, whereas high doses of chelerythrine abolished the cardioprotective effects of ischemic preconditioning.[25] These findings were interpreted to indicate that cardioprotection during PKC activation is isoform specific and may be variable according to the end point of cardioprotection used.[23]

The effects of isoflurane or other volatile anesthetic agents on the activity of specific PKC isoforms are unknown. Increases in calcium-activated force in skinned arterial smooth muscle strips produced by isoflurane were blocked by bisindolylmaleimide but not by a PKC inhibitor specific for calcium-dependent isoforms.[26] These data suggested that isoflurane may increase the activity of ϵ-PKC,[26] a prominent calcium-independent PKC isoform prevalent in canine myocardium[20] and one

Table 7. Transmural Myocardial Blood Flow ($ml \cdot min^{-1} \cdot g^{-1}$) in the Ischemic (LAD) and Normal (LCCA) Regions

			Reperfusion (min)	
	Baseline	5th Occlusion	60	180
LAD region				
Vehicle	1.06 ± 0.08	0.13 ± 0.02*	0.97 ± 0.11	0.91 ± 0.12
Vehicle + isoflurane	1.33 ± 0.23	0.13 ± 0.03*	1.30 ± 0.18	1.02 ± 0.08
BIS (2 μg/min)	1.16 ± 0.10	0.15 ± 0.03*	1.15 ± 0.18	1.23 ± 0.22
BIS (8 μg/min)	1.03 ± 0.07	0.13 ± 0.03*	1.04 ± 0.09	1.11 ± 0.07
BIS (2 μg/min) + isoflurane	1.21 ± 0.18	0.08 ± 0.01*	1.17 ± 0.24	1.07 ± 0.11
BIS (8 μg/min) + isoflurane	1.31 ± 0.15	0.16 ± 0.03*	1.32 ± 0.14	1.30 ± 0.17
LCCA region				
Vehicle	1.06 ± 0.07	1.11 ± 0.07	1.01 ± 0.09	0.94 ± 0.07
Vehicle + isoflurane	1.26 ± 0.19	1.18 ± 0.16	1.30 ± 0.18	1.31 ± 0.29
BIS (2 μg/min)	1.27 ± 0.11	1.14 ± 0.08	1.31 ± 0.19	1.40 ± 0.23
BIS (8 μg/min)	1.01 ± 0.07	1.26 ± 0.18	1.04 ± 0.06	1.21 ± 0.06
BIS (2 μg/min) + isoflurane	1.24 ± 0.18	1.09 ± 0.18	1.36 ± 0.18	1.24 ± 0.18
BIS (8 μg/min) + isoflurane	1.27 ± 0.15	1.18 ± 0.14	1.29 ± 0.14	1.24 ± 0.15

Data are mean ± SEM.

BIS = bisindolylmaleimide; LAD = left anterior descending coronary artery; LCCA = left circumflex coronary artery.

* Significantly ($P < 0.05$) different from baseline.

that has been implicated as a critical mediator of ischemic preconditioning.[27,28] Although such a hypothesis may be highly plausible, direct evidence linking the ϵ-PKC or any other specific PKC isoform to myocardial protection afforded by isoflurane has yet to be identified and represents an important goal of future research.

The myocardial signal transduction pathways responsible for endogenous protection from ischemic injury exhibit substantial biologic redundancy. Preconditioning-induced reductions of myocardial infarct size elicited through multiple brief periods of ischemia and reperfusion appears to be relatively resistant to attenuation by PKC inhibitors. In contrast, cardiac muscle preconditioned by a single brief period of ischemia may be more susceptible to pharmacologic inhibition of PKC activity.[29,30] Multiple signal transduction cascades may be recruited during repetitive episodes of ischemia and reperfusion, and pharmacologic strategies using specific receptor antagonists or enzyme inhibitors of known constituents of these pathways may reveal the relative contribution of unique or redundant signaling to myocardial protection.[30] Consistent with this hypothesis, we have shown that isoflurane-induced protection against reversible[4,8] and irreversible[10] ischemic injury is mediated by adenosine triphosphate–regulated potassium channel activation. This energy-dependent ion channel has been shown to be the putative end-effector of both ischemic and anesthetic-induced preconditioning *in vivo* and is activated by various signaling processes, including adenosine A_1 and α_1-adrenergic receptors, $G_{i/o}$ proteins, and PKC, to reduce ischemic damage.[13,14,31] Evidence suggests that PKC may provide an important mechanistic link between stimulation of adenosine A_1 receptors and increases in adenosine triphosphate–regulated potassium channel activity.[11,12] The current results with the PKC inhibitor, bisindolylmaleimide, lend further support to the hypothesis that myocardial protection produced by isoflurane occurs as a result of a complex, multifactorial process involving A_1 receptors and adenosine triphosphate–regulated potassium channels in which PKC and its intracellular translocation[32] may play a role.

Signal transduction pathways other than PKC may also have been involved in cardioprotection mediated by isoflurane. A role for tyrosine kinase in ischemic preconditioning[33] was implicated by recent findings indicating that beneficial reductions in infarct size after a brief ischemic stimulus were abolished by the combination of PKC and tyrosine kinase inhibitors but not by either drug alone.[34] These findings raise the interesting possibility that isoflurane and PKC may participate in parallel cardioprotective pathways, as previously suggested to occur during ischemic preconditioning.[33] Low-dose bisindolylmaleimide may have inhibited the activity of specific (detrimental) isoforms of PKC, whereas isoflurane may have directly stimulated a tyrosine kinase pathway. Accordingly, high-dose bisindolylmaleimide may have inhibited the activity of all PKC isoforms and tyrosine kinase in the current investigation. The results suggest that complex intracellular signal transduction pathways mediate cardioprotection during isoflurane, and a complete elucidation of the involved pathways will require further *in vitro* investigation into the role of specific protein kinase isoforms.

The current results must be interpreted within the constraints of several potential limitations. The relative lack of selectivity of other PKC inhibitors, including staurosporine and polymyxin B, often has been a limiting factor in previous studies that evaluated the role of PKC in intracellular signaling during myocardial ischemia, because these drugs may also inhibit cyclic adenosine monophosphate-dependent protein kinase, myosin light chain kinase, and tyrosine kinase.[19] Bisindolylmaleimide has been shown to be highly PKC selective,[18] but higher doses of this drug may also inhibit the activity of other protein kinases.[18] Bisindolylmaleimide was administered through an intracoronary route to attain plasma concentrations that previously were shown effectively to inhibit PKC *in vitro*,[18] but plasma bisindolylmaleimide concentrations in the coronary circulation were not specifically measured in the current investigation. Interpretation of the data may also have been influenced by the load-dependent nature of regional segment shortening. However, this potential source of bias was probably minimal, because no differences in loading conditions during reperfusion were observed among the groups. Differential alterations in myocardial oxygen consumption are unlikely to account solely for the cardioprotective effects of isoflurane or bisindolylmaleimide in the current investigation. Isoflurane, but not bisindolylmaleimide, decreased the rate pressure product, yet low- but not high-dose bisindolylmaleimide was cardioprotective, regardless of previous exposure to isoflurane. However, coronary sinus oxygen tension was not determined, and myocardial oxygen consumption was not measured directly.

In conclusion, the current results show that isoflurane enhances the functional recovery of canine stunned myocardium. Inhibition of PKC with bisindolylmaleimide causes biphasic, dose-dependent effects on recovery of stunned myocardium. Whereas cardioprotective ef-

fects of low-dose bisindolylmaleimide are enhanced by isoflurane, high-dose bisindolylmaleimide, alone or combined with isoflurane, produces no cardioprotective effects. The findings provide additional evidence that isoflurane-induced myocardial protection occurs by a signal transduction pathway similar to that observed in ischemic preconditioning, and a role for PKC during isoflurane-induced cardioprotection cannot be excluded.

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References

1. Braunwald E, Kloner RA: The stunned myocardium: Prolonged, postischemic ventricular dysfunction. Circulation 1982; 66:1146–9

2. Bolli R: Mechanism of myocardial 'stunning.' Circulation 1990; 82:723–38

3. Warltier DC, al-Wathiqui MH, Kampine JP, Schmeling WT: Recovery of contractile function of stunned myocardium in chronically instrumented dogs is enhanced by halothane or isoflurane. ANESTHESIOLOGY 1988; 69:552–65

4. Kersten JR, Schmeling TJ, Hettrick DA, Pagel PS, Gross GJ, Warltier DC: Mechanism of myocardial protection by isoflurane. Role of adenosine triphosphate-regulated potassium (K_{ATP}) channels. ANESTHESIOLOGY 1996; 85:794–807

5. Oguchi T, Kashimoto S, Yamaguchi T, Nakamura T, Kumazawa T: Comparative effects of halothane, enflurane, isoflurane and sevoflurane on function and metabolism in the ischaemic rat heart. Br J Anaesth 1995; 74:569–75

6. Kanaya N, Fujita S: The effects of isoflurane on regional myocardial contractility and metabolism in 'stunned' myocardium in acutely instrumented dogs. Anesth Analg 1994; 79:447–54

7. Kanaya N, Kobayashi I, Nakayama M, Fujita S, Namiki A: ATP sparing effect of isoflurane during ischaemia and reperfusion of the canine heart. Br J Anaesth 1995; 74:563–8

8. Kersten JR, Lowe D, Hettrick DA, Pagel PS, Gross GJ, Warltier DC: Glyburide, a K_{ATP} channel antagonist, attenuates the cardioprotective effects of isoflurane in stunned myocardium. Anesth Analg 1996; 83: 27–33

9. Kersten JR, Orth KG, Pagel PS, Mei DA, Gross GJ, Warltier DC: Role of adenosine in isoflurane-induced cardioprotection. ANESTHESIOLOGY 1997; 86:1128–39

10. Kersten JR, Schmeling TJ, Pagel PS, Gross GJ, Warltier DC: Isoflurane mimics ischemic preconditioning via activation of K_{ATP} channels: Reduction of myocardial infarct size with an acute memory phase. ANESTHESIOLOGY 1997; 87:361–70

11. Sato T, O'Rourke B, Marban E: Modulation of mitochondrial ATP-dependent K^+ channels by protein kinase C. Circ Res 1998; 83:110–4

12. Speechly-Dick ME, Grover GJ, Yellon DM: Does ischemic preconditioning in the human involve protein kinase C and the ATP-dependent K^+ channel? Studies of contractile function after simulated ischemia in an atrial in vitro model. Circ Res 1995; 77:1030–5

13. Light PE, Allen BG, Walsh MP, French RJ: Regulation of adenosine triphosphate-sensitive potassium channels from rabbit ventricular myocytes by protein kinase C and type 2A protein phosphatase. Biochemistry 1995; 34:7252–7

14. Light PE, Sabir AA, Allen BG, Walsh MP, French RJ: Protein kinase C-induced changes in the stoichiometry of ATP binding activate cardiac ATP-sensitive K+ channels. A possible mechanistic link to ischemic preconditioning. Circ Res 1996; 79:399–406

15. Ytrehus K, Liu Y, Downey JM: Preconditioning protects ischemic rabbit heart by protein kinase C activation. Am J Physiol 1994; 266:H1145–52

16. Kitakaze M, Node K, Minamino T, Komamura K, Funaya H, Shinozaki Y, Chujo M, Mori H, Inoue M, Hori M, Kamada T: Role of activation of protein kinase C in the infarct size-limiting effect of ischemic preconditioning through activation of ecto-5'-nucleotidase. Circulation 1996; 93:781–91

17. Cope DK, Impastato WK, Cohen MV, Downey JM: Volatile anesthetics protect the ischemic rabbit myocardium from infarction. ANESTHESIOLOGY 1997; 86:699–709

18. Toullec D, Pianetti P, Coste H, Bellevergue P, Grand-Perret T, Ajakane M, Baudet V, Boissin P, Boursier E, Loriolle F: The bisindolylmaleimide GF 109203X is a potent and selective inhibitor of protein kinase C. J Biol Chem 1991; 266:15771–81

19. MitchellM.B., Meng X, Ao L, Brown JM, Harken AH, Banerjee A: Preconditioning of isolated rat heart is mediated by protein kinase C. Circ Res 1995; 76:73–81

20. Steinberg SF, Goldberg M, Rybin VO: Protein kinase C isoform diversity in the heart. J Mol Cell Cardiol 1995; 27:141–53

21. Simkhovich BZ, Przyklenk K, Kloner RA: Role of protein kinase C as a cellular mediator of ischemic preconditioning: A critical review. Cardiovasc Res 1998; 40:9–22

22. Erdbrugger W, Keffel J, Knocks M, Otto T, Philipp T, Michel MC: Protein kinase C isoenzymes in rat and human cardiovascular tissues. Br J Pharmacol 1997; 120:177–86

23. Tosaki A, Maulik N, Engelman DT, Engelman RM, Das DK: The role of protein kinase in C ischemic/reperfused preconditioned isolated rat hearts. J Cardiovasc Pharmacol 1996; 28:723–31

24. Vogt AM, Htun P, Arras M, Podzuweit T, Schaper W: Intramyocardial infusion of tool drugs for the study of molecular mechanisms in ischemic preconditioning. Basic Res Cardiol 1996; 91:389–400

25. Lasley RD, Noble MA, Mentzer RM Jr: Effects of protein kinase C inhibitors in in situ and isolated ischemic rabbit myocardium. J Mol Cell Cardiol 1997; 29:3345–56

26. Toda H, Su JY: Mechanisms of isoflurane-increased submaximum Ca2+-activated force in rabbit skinned femoral arterial strips. ANESTHESIOLOGY 1998; 89:731–40

27. Ping P, Zhang J, Qiu Y, Tang XL, Manchikalapudi S, Cao X, Bolli R: Ischemic preconditioning induces selective translocation of protein kinase C isoforms epsilon and eta in the heart of conscious rabbits without subcellular redistribution of total protein kinase C activity. Circ Res 1997; 81:404–14

28. Gray MO, Karliner JS, Mochly-Rosen D: A selective epsilon-protein kinase C antagonist inhibits protection of cardiac myocytes from hypoxia-induced cell death. J Biol Chem 1997; 272:30945–51

29. Miura T, Miura T, Kawamura S, Goto M, Sakamoto J, Tsuchida A, Matsuzaki M, Shimamoto K: Effect of protein kinase C inhibitors on

cardioprotection by ischemic preconditioning depends on the number of preconditioning episodes. Cardiovasc Res 1998; 37:700–9

30. Sandhu R, Diaz RJ, Mao GD, Wilson GJ: Ischemic preconditioning: Differences in protection and susceptibility to blockade with single-cycle versus multicycle transient ischemia. Circulation 1997; 96:984–95

31. Kersten JR, Gross GJ, Pagel PS, Warltier DC: Activation of adenosine triphosphate-regulated potassium channels: Mediation of cellular and organ protection. ANESTHESIOLOGY 1998; 88:495–513

32. Ismaeil M, Tkachenko I, Hickey R, Cason BA: Colchicine inhibits isoflurane-induced myocardial preconditioning (abstract). ANESTHESIOLOGY 1998; 89:A595

33. Baines CP, Wang L, Cohen MV, Downey JM: Protein tyrosine kinase is downstream of protein kinase C for ischemic preconditioning's anti-infarct effect in the rabbit heart. J Mol Cell Cardiol 1998; 30:383–92

34. Vahlhaus C, Schulz R, Post H, Rose J, Heusch G: Prevention of ischemic preconditioning only by combined inhibition of protein kinase C and protein tyrosine kinase in pigs. J Mol Cell Cardiol 1998; 30:197–209