Anesthesiology 2004; 100:547–54

Cyclooxygenase-2 Mediates Ischemic, Anesthetic, and Pharmacologic Preconditioning In Vivo

Dunbar Alcindor, B.S., John G. Krolikowski, B.A.,† Paul S. Pagel, M.D., Ph.D.,‡ David C. Warltier, M.D., Ph.D.,§ Judy R. Kersten, M.D.‖*

Background: **Cyclooxygenase-2 (COX-2) mediates the late phase of ischemic preconditioning (IPC), but whether this enzyme modulates early IPC, anesthetic-induced preconditioning (APC), or other forms of pharmacologic preconditioning (PPC) is unknown. The authors tested the hypothesis that COX-2 is an essential mediator of IPC, APC, and PPC *in vivo*.**

Methods: **Barbiturate-anesthetized dogs (n = 91) were instrumented for measurement of hemodynamics and randomly assigned to receive IPC (four 5-min coronary occlusions interspersed with 5-min reperfusions), APC (1.0 minimum alveolar concentration of isoflurane for 30 min), or PPC (selective mitochondrial K_{ATP} channel opener diazoxide, 2.5 mg/kg intravenous) in the presence or absence of pretreatment with oral aspirin (650 mg), the selective COX-2 inhibitor celecoxib (200 mg), or acetaminophen (500 mg) administered 24, 12, and 2 h before experimentation in 12 separate experimental groups. All dogs were subjected to a 60-min coronary artery occlusion followed by 3 h of reperfusion. Myocardial infarct size and coronary collateral blood flow were quantified with triphenyltetrazolium staining and radioactive microspheres, respectively. Myocardial 6-keto-prostaglandin $F_{1\alpha}$, a stable metabolite of prostacyclin, was measured (enzyme immunoassay) in separate experiments (n = 8) before and after isoflurane administration, in the presence or absence of celecoxib.**

Results: **No significant differences in baseline hemodynamics or the left ventricular area at risk for infarction were observed between groups. IPC, isoflurane, and diazoxide all decreased myocardial infarct size (9 ± 1, 12 ± 2, and 11 ± 1%, respectively) as compared with control (30 ± 1%). Celecoxib alone had no effect on infarct size (26 ± 3%) but abolished IPC (30 ± 3%), APC (30 ± 3%), and PPC (26 ± 1%). Aspirin (24 ± 3%) and acetaminophen alone (29 ± 2%) did not alter infarct size or abolish APC-induced protection (18 ± 1 and 19 ± 1%, respectively). Isoflurane increased myocardial 6-keto-prostaglandin $F_{1\alpha}$ to 463 ± 267% of baseline in the absence but not in the presence (94 ± 13%) of celecoxib.**

Conclusions: **The results indicate that COX-2 is a critical mediator of IPC, APC, and PPC in dogs. The role of cyclooxygenase enzymes as obligatory mediators of myocardial protection produced by diverse preconditioning stimuli may have implications for the clinical use of COX-2 inhibitors.**

CYCLOOXYGENASE-2 (COX-2) inhibitors are widely prescribed because these drugs produce antiinflammatory and analgesic effects. These drugs are also being used with increasing frequency as premedicants in surgical patients. Despite the widespread clinical use of these drugs, COX-2 inhibitors may cause adverse cardiovascular effects that potentially limit their therapeutic utility. A meta-analysis that included the results of two major randomized trials of the selective COX-2 inhibitors celecoxib and rofecoxib compared the relative risk of a cardiovascular event in patients treated with selective COX-2 as compared with nonselective cyclooxygenase inhibitors or placebo.[1] The risk of sustaining a thrombotic cardiovascular episode was twice as great in patients treated with rofecoxib as compared with naproxen.[1] The rate of myocardial infarction was also greater in celecoxib- or rofecoxib-treated patients as compared with those receiving placebo.[1] The mechanisms responsible for this apparent increase in cardiovascular risk in patients treated with COX-2 inhibitors have yet to be defined. Recent evidence suggests that COX-2 serves an essential protective role because this enzyme has been shown to mediate delayed preconditioning against myocardial infarction and stunning.[2] Volatile anesthetics also precondition myocardium against ischemic injury in experimental animals[3] and humans,[4] but the role of COX-2 in this anesthetic-induced preconditioning (APC) phenomenon has not been examined. Whether COX-2 is also involved in acute ischemic preconditioning (IPC) or other forms of pharmacologic preconditioning (PPC), such as those produced by direct openers of the mitochondrial adenosine triphosphate–regulated potassium (K_{ATP}) channel, is also unclear based on previously published data. We tested the hypothesis that the protective effects of isoflurane, IPC, and the selective mitochondrial K_{ATP} channel agonist diazoxide are abolished by celecoxib but not the cyclooxygenase-1 (COX-1) inhibitor aspirin or the cyclooxygenase-3 (COX-3) inhibitor acetaminophen in dogs.

Materials and Methods

All experimental procedures and protocols used in this investigation were reviewed and approved by the Institutional Animal Care and Use Committee of the Medical

* Medical Student, † Research Technologist, ‖ Professor of Anesthesiology, Pharmacology and Toxicology, Medical College of Wisconsin. ‡ Professor of Anesthesiology and Biomedical Engineering and Director of Cardiac Anesthesia, Medical College of Wisconsin; the Clement J. Zablocki Veterans Affairs Medical Center, Milwaukee, Wisconsin; and Department of Biomedical Engineering, Marquette University, Milwaukee, Wisconsin. § Professor of Anesthesiology, Biomedical Engineering, Pharmacology and Toxicology, and Medicine (Division of Cardiovascular Diseases) and Senior Vice Chairman of Anesthesiology, Medical College of Wisconsin; the Clement J. Zablocki Veterans Affairs Medical Center, Milwaukee, Wisconsin; and Department of Biomedical Engineering, Marquette University, Milwaukee, Wisconsin.

Received from the Departments of Anesthesiology, Pharmacology and Toxicology, and Medicine (Division of Cardiovascular Diseases), the Medical College of Wisconsin, Milwaukee, Wisconsin; the Clement J. Zablocki Veterans Affairs Medical Center, Milwaukee, Wisconsin; and the Department of Biomedical Engineering, Marquette University; Milwaukee, Wisconsin. Submitted for publication September 12, 2003. Accepted for publication November 24, 2003. Supported in part by National Institutes of Health grants HL 63705 (to Dr. Kersten), HL 54820 (to Dr. Warltier), GM 066730 (to Dr. Warltier), and GM 08377 (to Dr. Warltier) from the United States Public Health Service, Bethesda, Maryland.

Address reprint requests to Dr. Kersten: Medical College of Wisconsin, MEB-M4280, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226. Address electronic mail to: jkersten@mcw.edu. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

College of Wisconsin (Milwaukee, Wisconsin). Furthermore, all conformed to the *Guiding Principles in the Care and Use of Animals*[5] of the American Physiologic Society and were in accordance with the *Guide for the Care and Use of Laboratory Animals*.[6]

General Preparation

Instrumentation of dogs has been previously described in detail.[7] Briefly, mongrel dogs (weight, 20–24 kg) of either sex were anesthetized with sodium barbital (200 mg/kg) and sodium pentobarbital (15 mg/kg) and ventilated using positive pressure with an air-and-oxygen mixture after tracheal intubation. A dual micromanometer-tipped catheter was inserted into the aorta and left ventricle for measurement of aortic and left ventricular (LV) pressures and the maximum rate of increase of LV pressure ($+dP/dt_{max}$). Heparin-filled catheters were inserted into the left atrial appendage, the right femoral artery, and the right femoral vein for administration of radioactive microspheres, withdrawal of reference blood flow samples, and fluid or drug administration, respectively. The left anterior descending coronary artery (LAD) was isolated, and a silk ligature was placed around this vessel immediately distal to the first diagonal branch for production of coronary artery occlusion and reperfusion. Hemodynamic data were continuously monitored on a polygraph and digitized using a computer interfaced with an analog-to-digital converter.

Experimental Protocol

Baseline systemic hemodynamic data were recorded 90 min after instrumentation was completed (fig. 1). All dogs were subjected to a 60-min LAD occlusion followed by 3 h of reperfusion. Dogs were randomly assigned to receive oral celecoxib (200 mg), aspirin (650 mg), or acetaminophen (500 mg) three times (24, 12, and 2 h) before surgical instrumentation in the presence or absence of IPC, APC, or PPC in 12 separate experimental groups. IPC was produced with four 5-min periods of LAD occlusion interspersed with 5-min periods of reperfusion. APC was produced by administration of isoflurane (1.0 minimum alveolar concentration) for 30 min and discontinued 30 min before LAD occlusion. End-tidal concentrations of isoflurane were measured at the tip of the endotracheal tube by an infrared anesthetic analyzer that was calibrated with known standards before and during experimentation. Last, PPC was produced by administration of intravenous diazoxide (2.5 mg/kg over 15 min) immediately before coronary occlusion. Transmural myocardial blood flow was measured under baseline conditions, during LAD occlusion, and after 1 h of reperfusion. Dogs that developed intractable ventricular fibrillation or those with subendocardial blood flow greater than 0.15 ml $\cdot$ min^{-1} $\cdot$ g^{-1} were excluded from subsequent analysis.

Determination of Myocardial Infarct Size

Myocardial infarct size was measured at the end of each experiment as previously described.[8] Briefly, the LV area at risk (AAR) for infarction was separated from the normal zone, and the two regions were incubated at 37°C for 20–30 min in 1% 2,3,5-triphenyltetrazolium chloride in 0.1 M phosphate buffer adjusted to a pH of 7.4. After overnight storage in 10% formaldehyde, infarcted and noninfarcted myocardial areas within the AAR were carefully separated and weighed. Infarct size was expressed as a percentage of the AAR.

Determination of Regional Myocardial Blood Flow

Carbonized plastic microspheres (15 $\pm$ 2 μm [SD] in diameter) labeled with ^{141}Ce, ^{103}Ru, or ^{95}Nb were used to measure regional myocardial perfusion as previously described.[9] Transmural tissue samples were selected from the ischemic region (distal to the LAD occlusion) and subdivided into subepicardial, midmyocardial, and subendocardial layers of approximately equal thickness. Samples were weighed and placed in scintillation vials for isotope activity determination. Similarly, the activity of each isotope in the reference blood flow sample was assessed. Tissue blood flow was calculated as $Q_r \cdot C_m \cdot C_r^{-1}$, where Q_r is the rate of withdrawal of the reference blood flow sample (ml/min), C_m is the activity (counts $\cdot$ min^{-1} $\cdot$ g^{-1}) of the myocardial tissue sample, and C_r is the activity (counts/min) of the reference blood flow sample. Transmural blood flow was considered to be the average of subepicardial, midmyocardial, and subendocardial blood flows.

Determination of Myocardial 6-Keto-$PGF_{1\alpha}$

In separate experiments (n = 8), dogs were anesthetized and instrumented as described above. LV myocardium was biopsied at baseline and after 30 min of isoflurane in the absence or presence of pretreatment with celecoxib (200 mg orally at 24, 12, and 2 h before instrumentation). Myocardial 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) was measured with enzyme immunoassay (Cayman Chemical, Ann Arbor, MI).

Statistical Analysis

Statistical analysis of data within and between groups was performed with analysis of variance for repeated measures followed by the Student-Newman-Keuls test. The Mann–Whitney rank sum test was used to compare myocardial prostaglandin concentrations. Changes within and between groups were considered significant when the probability (*P*) value was less than 0.05. Data are expressed as mean $\pm$ SEM.

Results

Ninety-one dogs were instrumented to obtain 86 successful experiments. Two dogs were excluded because

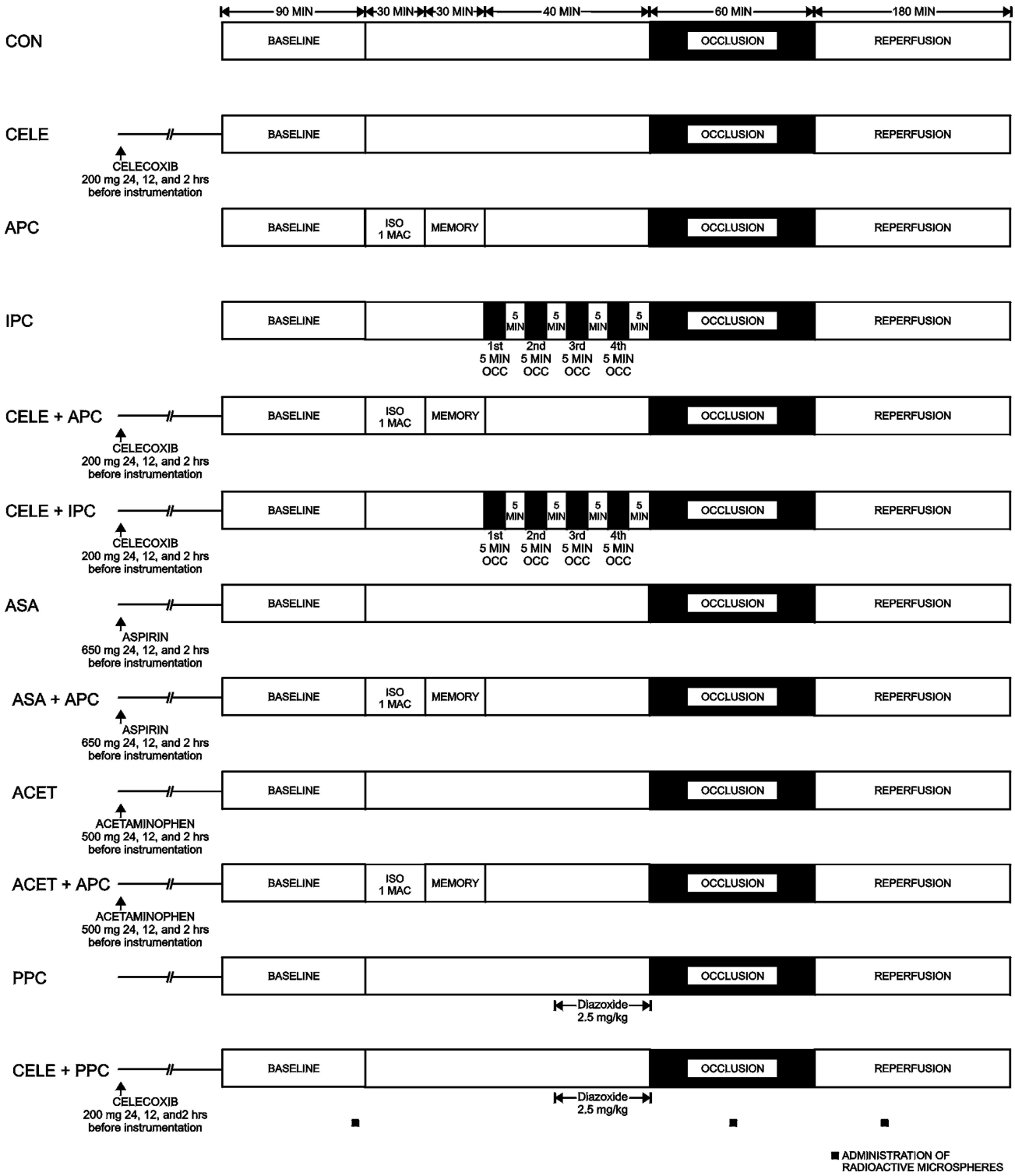

Fig. 1. Schematic illustration of the experimental protocols. ACET = acetaminophen; APC = anesthetic preconditioning with 1 minimum alveolar concentration (MAC) of isoflurane; ASA = aspirin; CELE = celecoxib; CON = control; IPC = ischemic preconditioning; ISO = isoflurane; OCC = coronary artery occlusion; PPC = pharmacologic preconditioning with diazoxide.

of intractable ventricular fibrillation (1 control; 1 diazoxide alone). Three dogs were excluded because subendocardial blood flow exceeded 0.15 ml · min^{-1} · g^{-1} (1 isoflurane alone, 1 aspirin plus isoflurane, and 1 celecoxib alone).

Hemodynamics and Coronary Collateral Blood Flow

There were no significant differences in systemic hemodynamics between experimental groups under baseline conditions, during LAD occlusion, or during reper-

fusion (table 1). Celecoxib, aspirin, and acetaminophen did not alter hemodynamics. Isoflurane caused significant ($P < 0.05$) decreases in heart rate, mean arterial pressure, LV systolic pressure, and LV $+dP/dt_{max}$ in the presence or absence of celecoxib, aspirin, or acetaminophen pretreatment. There were no differences in coronary collateral blood flow among groups (table 2).

Myocardial Infarct Size

The LV AARs were similar among groups (control, 37 ± 2%; celecoxib, 33 ± 4%; APC, 37 ± 2%; IPC, 39 ± 2%; celecoxib plus APC, 36 ± 2%; celecoxib plus IPC, 31 ± 3%; aspirin, 33 ± 2%; aspirin plus APC, 41 ± 3%; acetaminophen, 37 ± 3%; acetaminophen plus APC, 41 ± 4%; PPC, 44 ± 2%; celecoxib plus PPC, 36 ± 4% of left ventricle). IPC, APC, and PPC decreased myocardial infarct size (9 ± 1, 12 ± 2, and 11 ± 1% of the LV AAR, respectively) as compared with control (30 ± 1%; fig. 2). Celecoxib alone had no effect on infarct size (26 ± 3%) but abolished IPC (30 ± 3%), APC (30 ± 3%), and PPC (26 ± 1%). Aspirin and acetaminophen alone did not alter the extent of myocardial infarction (24 ± 3 and 29 ± 2%, respectively), nor did these drugs abolish the protection produced by APC (18 ± 1 and 19 ± 1%, respectively).

Myocardial 6-Keto-$PGF_{1\alpha}$

Isoflurane increased myocardial 6-keto-$PGF_{1\alpha}$ to 463 ± 267% of baseline (n = 4) in untreated dogs. In contrast, 6-keto-$PGF_{1\alpha}$ was unchanged by isoflurane in dogs (n = 4) pretreated with celecoxib (94 ± 13% of baseline).

Discussion

A variety of preconditioning stimuli are capable of eliciting protection in ischemic myocardium by activating common signal transduction pathways. Reactive oxygen species, membrane-bound receptors (*e.g.*, adenosine subtype 1, δ opioid), intracellular kinases (*e.g.*, protein kinase C, protein tyrosine kinase), and ion channels (*e.g.*, K_{ATP} channels) have been identified that play critical roles in triggering or mediating IPC[10] and APC.[11] Two proteins, including inducible nitric oxide synthase and COX-2, have been the focus of recent studies characterizing the more prolonged transition of myocardium from a vulnerable to a protected state.[12] This transition has been termed the *delayed* or *late phase* of IPC. Protection caused by acute or early IPC typically lasts for approximately 2 h after a brief ischemic episode; however, myocardium again becomes resistant to infarction 24 h later. This late phase of preconditioning is dependent on inducible nitric oxide synthase–derived nitric oxide and increased COX-2 expression and activity.[13] Many drugs, including volatile anesthetics,[14] may mimic the salutary action of both acute[3] and delayed IPC by activating similar signaling pathways.[11] The mitochondrial K_{ATP} channel has been strongly implicated during IPC[15] and APC[3] and has also been shown to mediate delayed preconditioning phenomena.[16] Direct activation of mitochondrial K_{ATP} channels with diazoxide produces both early and delayed protection, and these beneficial actions are blocked by a nitric oxide synthase inhibitor.[16] Prostaglandins also influence the activity of K_{ATP} channels. Intravenous administration of prostaglandins E_1 or E_0 decreased myocardial infarct size, and this beneficial effect was blocked by the selective mitochondrial K_{ATP} channel antagonist 5-hydroxydecanoate.[17] These provocative findings strongly suggested that interventions that alter prostanoid concentrations may favorably influence K_{ATP} channel activity and reduce the degree of myocardial injury in response to a subsequent ischemic event. Therefore, it has become increasingly apparent that endogenous molecules including nitric oxide[18] and COX-generated metabolites of arachidonic acid may potentiate K_{ATP} channel activity[17] and modulate the response of myocardium to ischemia.

The current results indicate that COX-2 is a critical mediator of IPC, APC, and PPC *in vivo*. The findings further suggest that COX-derived prostanoids may modulate K_{ATP} channel activity elicited by IPC or pharmacologic agonists, including isoflurane and diazoxide. The role of COX-2 and its prostanoid products in myocardial protection has been extensively studied and validated in models of delayed preconditioning against infarction and stunning.[19,20] In contrast, whether prostanoids are capable of favorably affecting acute ischemic injury remains controversial, as does the specific role of COX-2 in IPC and other forms of acute preconditioning. However, genetic and pharmacologic evidence suggests that cyclooxygenase or cyclooxygenase-derived metabolites may contribute to protection against ischemic injury. Recovery of LV pressure after global ischemia and reperfusion was significantly attenuated in mice with targeted deletions of the COX-1 or -2 gene.[21] Findings that myocardial ischemic injury is increased in COX-2 null mice implies that endogenous production of COX-2–derived prostanoids produces cardioprotective effects even though constitutive expression of protein is low or undetectable. Furthermore, prostaglandin E_2 (PGE_2) and 6-keto-$PGF_{1\alpha}$, a stable metabolite of prostacyclin (PGI_2), are present in myocardium of COX-1 null mice, indicating that constitutive COX-2 expression is sufficient to produce measurable quantities of cardioprotective eicosanoids.[2] Pharmacologic inhibition of COX-2 with celecoxib caused dose-dependent decreases in functional recovery of myocardium after global ischemia and reperfusion in isolated rabbit hearts.[22] The nonselective cyclooxygenase inhibitor indomethacin abolished reductions of infarct size after IPC in enflurane-anesthetized pigs.[23] Another nonselective cyclooxygenase inhibitor (meclofenamate) also abrogated the beneficial effects of

Table 1. Systemic Hemodynamics

				Reperfusion		
	Baseline	Intervention	30 min CAO	1 h	2 h	3 h
HR, min^{-1}						
Control	130 ± 6	130 ± 6	124 ± 7	123 ± 6	130 ± 4	130 ± 5
Celecoxib	137 ± 4	133 ± 4	128 ± 5	115 ± 4*	115 ± 5*	114 ± 5*
APC	130 ± 7	105 ± 4*†	117 ± 6*	111 ± 7*	113 ± 6*	117 ± 6*
IPC	132 ± 3	127 ± 3	127 ± 4	121 ± 4*	119 ± 3*	119 ± 5*
Celecoxib + APC	137 ± 4	117 ± 5*	123 ± 5*	118 ± 6*	115 ± 7*	118 ± 10*
Celecoxib + IPC	131 ± 6	130 ± 6	125 ± 7	115 ± 3*	116 ± 3*	119 ± 3*
Aspirin	142 ± 5	141 ± 5	131 ± 5	122 ± 6*	119 ± 8*	121 ± 9*
Aspirin + APC	145 ± 3	120 ± 2*	136 ± 4	119 ± 4*	125 ± 6*	125 ± 5*
Acetaminophen	130 ± 6	130 ± 6	131 ± 5	127 ± 6	120 ± 8	122 ± 9
Acetaminophen + APC	139 ± 7	113 ± 6*	128 ± 8*	118 ± 6*	117 ± 6*	121 ± 7*
PPC	135 ± 7	132 ± 6	132 ± 6	124 ± 6	125 ± 7	125 ± 8
Celecoxib + PPC	135 ± 3	131 ± 4	127 ± 3	123 ± 5*	115 ± 3*	114 ± 5*
MAP, mmHg						
Control	96 ± 4	98 ± 4	92 ± 4	91 ± 5	97 ± 5	97 ± 4
Celecoxib	103 ± 2	103 ± 4	93 ± 4	96 ± 6	106 ± 5	100 ± 6
APC	94 ± 5	61 ± 3*†	91 ± 3	95 ± 2	96 ± 2	96 ± 3
IPC	91 ± 2	85 ± 4	82 ± 3	95 ± 5	102 ± 5	96 ± 3
Celecoxib + APC	103 ± 3	69 ± 3*†	86 ± 2*	90 ± 3	90 ± 7	102 ± 2
Celecoxib + IPC	102 ± 3	99 ± 2	91 ± 4	97 ± 8	102 ± 7	108 ± 6
Aspirin	96 ± 6	104 ± 5	88 ± 5	93 ± 3	94 ± 5	95 ± 5
Aspirin + APC	100 ± 3	69 ± 3*†	90 ± 6	88 ± 5	97 ± 4	97 ± 5
Acetaminophen	91 ± 5	91 ± 5	75 ± 5*	84 ± 4	90 ± 6	88 ± 7
Acetaminophen + APC	96 ± 4	66 ± 2*	87 ± 4	102 ± 7	105 ± 5	104 ± 5
PPC	97 ± 4	85 ± 7	84 ± 6*	90 ± 5*	95 ± 5	96 ± 4
Celecoxib + PPC	101 ± 5	101 ± 5	87 ± 4	101 ± 5	106 ± 7	98 ± 4
LVSP, mmHg						
Control	104 ± 4	107 ± 5	95 ± 5	92 ± 5	99 ± 5	101 ± 5
Celecoxib	114 ± 2	114 ± 4	96 ± 5*	99 ± 6	110 ± 6	102 ± 6
APC	105 ± 4	72 ± 2*†	95 ± 3	97 ± 2	101 ± 2	101 ± 2
IPC	98 ± 3	91 ± 4†	86 ± 3	100 ± 6	105 ± 5	98 ± 3
Celecoxib + APC	113 ± 4	77 ± 3*†	91 ± 3*	91 ± 5*	94 ± 7*	107 ± 3
Celecoxib + IPC	114 ± 4	109 ± 3	95 ± 5	102 ± 9	106 ± 9	115 ± 9
Aspirin	105 ± 6	113 ± 6	92 ± 4	97 ± 4	98 ± 6	100 ± 6
Aspirin + APC	110 ± 3	78 ± 3*†	96 ± 6	89 ± 6*	100 ± 5	101 ± 5
Acetaminophen	100 ± 5	100 ± 5	81 ± 6*	89 ± 4	95 ± 6	94 ± 7
Acetaminophen + APC	105 ± 4	73 ± 3*†	92 ± 4	106 ± 8	110 ± 5	110 ± 6
PPC	104 ± 4	94 ± 6	84 ± 4*	94 ± 4	101 ± 4	102 ± 3
Celecoxib + PPC	108 ± 6	109 ± 6	92 ± 5*	108 ± 6	109 ± 8	104 ± 5
LVEDP, mmHg						
Control	4 ± 1	4 ± 1	17 ± 3*	21 ± 4*	17 ± 4*	14 ± 3*
Celecoxib	5 ± 1	4 ± 1	13 ± 2*	18 ± 2*	15 ± 3*	13 ± 1*
APC	7 ± 2	10 ± 2†	13 ± 2*	13 ± 2*	11 ± 2*	11 ± 1
IPC	7 ± 1	8 ± 1	10 ± 2†	10 ± 2	11 ± 2	11 ± 2
Celecoxib + APC	6 ± 1	9 ± 2	15 ± 1*	17 ± 2*	16 ± 3*	16 ± 3*
Celecoxib + IPC	8 ± 2	8 ± 1	14 ± 2*	18 ± 2*	15 ± 2*	16 ± 2*
Aspirin	7 ± 1	8 ± 1	13 ± 1	14 ± 3*	11 ± 3	11 ± 3
Aspirin + APC	6 ± 1	7 ± 1	12 ± 2*	14 ± 1*	14 ± 2*	13 ± 2*
Acetaminophen	5 ± 1	5 ± 1	14 ± 2*	15 ± 1*	13 ± 1*	14 ± 2*
Acetaminophen + APC	5 ± 1	7 ± 1	11 ± 1*	15 ± 2*	14 ± 2*	12 ± 1*
PPC	5 ± 1	5 ± 1	11 ± 1*	11 ± 2*	10 ± 2*	10 ± 2*
Celecoxib + PPC	6 ± 1	7 ± 1	12 ± 1*	13 ± 2*	13 ± 1*	15 ± 1*
LV + dP/dt_{max}, mmHg/s						
Control	1,750 ± 50	1,820 ± 60	1,560 ± 160	1,300 ± 80*	1,370 ± 80*	1,340 ± 80*
Celecoxib	1,860 ± 90	1,900 ± 90	1,600 ± 140*	1,350 ± 70*	1,360 ± 80*	1,280 ± 100*
APC	1,760 ± 170	960 ± 50*†	1,480 ± 110*	1,440 ± 80*	1,430 ± 90*	1,390 ± 100*
IPC	1,710 ± 130	1,580 ± 120	1,570 ± 140	1,480 ± 130	1,350 ± 80*	1,210 ± 90*
Celecoxib + APC	1,760 ± 70	1,070 ± 60*†	1,460 ± 80*	1,470 ± 110*	1,320 ± 110*	1,440 ± 100*
Celecoxib + IPC	1,910 ± 100	1,800 ± 60	1,760 ± 100	1,510 ± 130*	1,500 ± 60*	1,540 ± 60*
Aspirin	1,740 ± 100	1,800 ± 100	1,450 ± 50*	1,570 ± 80	1,480 ± 90*	1,430 ± 90*
Aspirin + APC	1,840 ± 60	1,130 ± 30*†	1,480 ± 170*	1,250 ± 20*	1,240 ± 50*	1,330 ± 80*
Acetaminophen	1,790 ± 100	1,790 ± 100	1,410 ± 150*	1,540 ± 90*	1,390 ± 80*	1,300 ± 100*
Acetaminophen + APC	1,760 ± 50	1,030 ± 50*†	1,550 ± 60	1,530 ± 130	1,350 ± 50*	1,300 ± 60*
PPC	1,930 ± 190	2,050 ± 200	1,600 ± 150*	1,510 ± 90*	1,490 ± 100*	1,460 ± 100*
Celecoxib + PPC	1,850 ± 90	1,820 ± 90	1,450 ± 70*	1,610 ± 80*	1,480 ± 80*	1,310 ± 70*

Data are presented as mean ± SEM. Intervention = preconditioning stimuli with ischemia, isoflurane, or diazoxide (see text). n = 7 in each group except pharmacologic preconditioning with diazoxide (PPC); n = 9.

* Significantly ($P < 0.05$) different from baseline. † Significantly ($P < 0.05$) different from the respective value in control experiments.

APC = anesthetic preconditioning with isoflurane; CAO = coronary artery occlusion; HR = heart rate; IPC = ischemic preconditioning; LVEDP = left ventricular end-diastolic pressure; LVSP = left ventricular systolic pressure; MAP = mean arterial blood pressure.

Table 2. Transmural Myocardial Perfusion in the Ischemic (LAD) Region

	Baseline	30 min CAO	1 h Reperfusion
Control	1.38 ± 0.13	0.07 ± 0.02*	1.68 ± 0.09
Celecoxib	0.98 ± 0.26	0.05 ± 0.01*	1.61 ± 0.19*
APC	0.79 ± 0.10	0.07 ± 0.01*	1.76 ± 0.33*
IPC	0.88 ± 0.06	0.08 ± 0.02*	1.51 ± 0.32*
Celecoxib + APC	1.06 ± 0.16	0.04 ± 0.01*	1.51 ± 0.15
Celecoxib + IPC	0.88 ± 0.13	0.07 ± 0.01*	1.68 ± 0.17*
Aspirin	0.89 ± 0.09	0.06 ± 0.02*	1.64 ± 0.23*
Aspirin + APC	0.93 ± 0.06	0.06 ± 0.01*	1.67 ± 0.18*
Acetaminophen	0.92 ± 0.12	0.10 ± 0.02*	1.54 ± 0.30*
Acetaminophen + APC	0.94 ± 0.11	0.06 ± 0.01*	2.06 ± 0.17*
PPC	0.86 ± 0.07	0.05 ± 0.01*	1.99 ± 0.31*
Celecoxib + PPC	0.94 ± 0.10	0.07 ± 0.01*	1.88 ± 0.28*

Data are presented as mean ± SEM. (ml · min^{-1} · g^{-1}). n = 7 in each group, except pharmacologic preconditioning with diazoxide (PPC), n = 9.

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

APC = anesthetic preconditioning with isoflurane; CAO = coronary artery occlusion; IPC = ischemic preconditioning; LAD = left anterior descending coronary artery.

IPC on ischemia and reperfusion-induced arrhythmias in dogs.[24] Conversely, administration of indomethacin[25] or aspirin[26] failed to block IPC in rat myocardium *in vitro* or *in vivo*, respectively. IPC-induced protection against contractile dysfunction after ischemia and reperfusion also remained intact in COX-1 and -2 knockout mice.[21] These findings suggested that important species differences (rodents *vs.* larger mammals) may exist that complicate interpretation of studies designed to evaluate the role of cyclooxygenase in protection against ischemic injury. In addition, the previous data also suggested that differences in experimental design (isolated hearts *vs. in vivo* preparations), variability in drug selectivity for cyclooxygenase isoforms, or shunting of arachidonic acid through alternate metabolic pathways (*e.g.*, leukotrienes, hydroxyeicosatetraenoic acids) may also be important in determining the outcome of studies in which cyclooxygenase activity is manipulated.

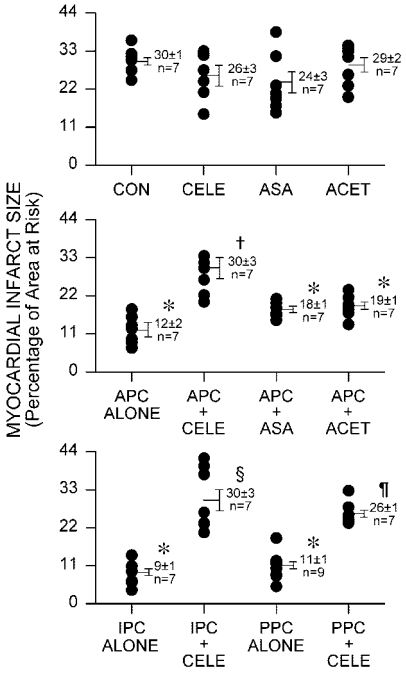

Fig. 2. Myocardial infarct size expressed as a percentage of left ventricular area at risk in dogs receiving no preconditioning stimuli (CON), celecoxib (CELE), aspirin (ASA), or acetaminophen (ACET) in the presence or absence of anesthetic preconditioning (APC), ischemic preconditioning (IPC), or pharmacologic preconditioning (PPC). * Significantly ($P < 0.05$) different from CON. † Significantly ($P < 0.05$) different from APC alone. § Significantly ($P < 0.05$) different from IPC alone. ¶ Significantly ($P < 0.05$) different from PPC alone.

A wide variety of physical, chemical, inflammatory, and mitogenic stimuli liberate arachidonic acid from membrane phospholipids by activating phospholipase A_2, thereby providing the substrate for cytosolic cyclooxygenase.[27] Two major forms of cyclooxygenase have been identified. COX-1 is often described as the constitutively expressed isoform, whereas COX-2 is classically identified as an inducible form of the enzyme whose production may be stimulated by cytokines, growth factors, phorbol esters, and bacterial lipopolysaccaride.[28] Despite the relative simplicity of these definitions, COX-2 may also be constitutively expressed in renal parenchyma,[29] neural tissue,[30] and myocardium.[20] Prostaglandins synthesized by COX-1 have been strongly implicated in the maintenance of gastrointestinal mucosal function and vascular and cellular homeostasis.[27,28] COX-1 is also the only enzyme isoform expressed in platelets, and activation of this isoform is directly responsible for thromboxane A_2 (TXA_2)–dependent platelet aggregation.[27,28] Nonsteroidal antiinflammatory drugs (*e.g.*, ibuprofen) inhibit the activity of both cyclooxygenase isoforms at the cyclooxygenase active site[31] that initiates the committed step in the biosynthesis of prostaglandins. Aspirin inhibits cyclooxygenase activity by irreversibly acetylating the enzyme and is 100 times more selective for COX-1 than -2.[32] In contrast, celecoxib is 375 times more selective for COX-2 than -1.[28]

Both COX-1 and -2 are relatively unaffected by acetaminophen,[32] but acetaminophen has recently been shown to inhibit a newly identified isoform variant of COX-1, identified as COX-3.[32] COX-2 has been shown to be a major source of PGE_2 and PGI_2 biosynthesis in humans.[28] Production of these eicosanoids is significantly decreased by celecoxib. Aspirin and other COX-1 antagonists significantly decrease TXA_2 concentrations and TXA_2-dependent platelet aggregation.[28] These drugs also reduce PGE_2 and PGI_2 concentrations, but decreases in these vasodilatory prostaglandins are less pronounced than concomitant declines in thromboxanes.[28,33] Therefore, aspirin seems to favorably modify the ratio of PGI_2 to TXA_2, whereas COX-2 selective antagonists may theoretically produce adverse cardiovascular consequences by selectively reducing PGI_2 concentrations and allowing the relatively unopposed action of TXA_2.[1]

In the current investigation, celecoxib alone did not affect infarct size but abolished the protective effects of preconditioning by repetitive brief ischemia, exposure to isoflurane, and administration of diazoxide in a canine model of myocardial infarction independent of alterations in systemic hemodynamics or coronary collateral blood flow. In contrast, aspirin or acetaminophen did not block reductions of myocardial infarct size in response to preconditioning with isoflurane. In addition, the current results are the first to show that isoflurane increases myocardial concentrations of 6-keto-$PGF_{1\alpha}$. Pretreatment with celecoxib abolished the formation of this cardioprotective prostanoid in response to isoflurane. In view of previous results obtained in large mammals, the current findings strongly suggest that COX-2–derived prostanoids, such as PGI_2, may enhance mitochondrial K_{ATP} channel activation in response to ischemic and pharmacologic stimuli, including isoflurane. The current results further suggest that COX-1 selective antagonists seem to be less likely to impair signal transduction during APC by maintaining a favorable balance between PGI_2 and TXA_2, but further investigation will clearly be needed to verify this hypothesis. The actions of cyclooxygenase antagonists have been shown to be dose dependent.[27] For example, small quantities of aspirin did not block late preconditioning against myocardial stunning, but higher doses abolished IPC-enhanced recovery of contractile function and prevented increases in myocardial 6-keto-$PGF_{1\alpha}$ and PGE_2 after late preconditioning.[33] Taken together, the current and previous findings suggest that prostanoids derived from COX-2 are important endogenous mediators of preconditioning that seem to be recruited in response to ischemic or pharmacologic stimuli. Inhibition of COX-2 with selective antagonists or higher doses of nonselective blockers may interfere with this important adaptive response during ischemia–reperfusion injury.

The current investigation should be interpreted within the constraints of several potential limitations. The dose of cyclooxygenase inhibitors was based on a comparable dose in humans and on previous reports in the literature. Dose–response relations to cyclooxygenase inhibitors were not specifically examined in the current investigation. However, the dose of aspirin used in the current investigation that did not block APC was similar to the dose previously used in rabbits to block delayed preconditioning. Therefore, the relative dose-dependent selectivity of cyclooxygenase inhibitors may be species related, and similar results may not be observed in humans. The area of the left ventricle at risk for infarction and coronary collateral blood flow are important determinants of the extent of myocardial infarction. No differences in these variables were observed between experimental groups that would account for the current findings. Isoflurane caused similar alterations in the hemodynamic determinants of myocardial oxygen consumption in the presence or absence of cyclooxygenase inhibitors. Therefore, it seems highly unlikely that the current findings are related to differences in myocardial oxygen consumption among groups. However, coronary venous oxygen tension was not measured and myocardial oxygen consumption was not directly quantified in the current investigation.

In summary, the current results indicate that reductions in myocardial infarct size produced by repetitive brief ischemia, exposure to isoflurane, and administration of diazoxide were abolished by selective inhibition of COX-2 independent of alterations in systemic hemodynamics or coronary collateral blood flow in dogs. In contrast, aspirin and acetaminophen failed to block isoflurane-induced protection against ischemic injury. The current results are consistent with the hypothesis that inhibition of COX-2 decreases PGI_2 and PGE_2 concentrations and alters signaling responsible for myocardial protection, including activation of K_{ATP} channels, during IPC, APC, and PPC. The findings confirm and extend recent evidence suggesting that COX-2 is an essential cardioprotective protein.

The authors thank David A. Schwabe, B.S.E.E. (Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, Wisconsin), for technical assistance and Mary Lorence-Hanke, A.A. (Department of Anesthesiology, Medical College of Wisconsin), for assistance in preparation of the manuscript.

References

1. Mukherjee D, Nissen SE, Topol EJ: Risk of cardiovascular events associated with selective COX-2 inhibitors. JAMA 2001; 286:954–9

2. Bolli R, Shinmura K, Tang XL, Kodani E, Xuan YT, Guo Y, Dawn B: Discovery of a new function of cyclooxygenase (COX)-2: COX-2 is a cardioprotective protein that alleviates ischemia/reperfusion injury and mediates the late phase of preconditioning. Cardiovasc Res 2002; 55:506–19

3. 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

4. De Hert SG, ten Broecke PW, Mertens E, Van Sommeren EW, De Blier IG, Stockman BA, Rodrigus IE: Sevoflurane but not propofol preserves myocardial function in coronary surgery patients. ANESTHESIOLOGY 2002; 97:42–9

5. World Medical Association, American Physiological Society: Guiding prin-

ciples for research involving animals and human beings. Am J Physiol Regul Integr Comp Physiol 2002; 283:R281–3

6. Institute of Laboratory Animal Resources: Guide for the Care and Use of Laboratory Animals, 7th edition. Washington, D.C., National Academy Press, 1996

7. 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

8. Warltier DC, Zyvoloski MG, Gross GJ, Hardman HF, Brooks HL: Determination of experimental myocardial infarct size. J Pharmacol Methods 1981; 6:199–210

9. Domenech RJ, Hoffman JI, Noble MI, Saunders KB, Henson JR, Subijanto S: Total and regional coronary blood flow measured by radioactive microspheres in conscious and anesthetized dogs. Circ Res 1969; 25:581–96

10. Gross GJ, Peart JN: KATP channels and myocardial preconditioning: An update. Am J Physiol Heart Circ Physiol 2003; 285:H921–30

11. Tanaka K, Ludwig LM, Kersten JR, Pagel PS, Warltier DC: Mechanisms of cardioprotection by volatile anesthetics. ANESTHESIOLOGY 2004; 100:707–21

12. Li Q, Guo Y, Xuan YT, Lowenstein CJ, Stevenson SC, Prabhu SD, Wu WJ, Zhu Y, Bolli R: Gene therapy with inducible nitric oxide synthase protects against myocardial infarction via a cyclooxygenase-2-dependent mechanism. Circ Res 2003; 92:741–8

13. Shinmura K, Xuan YT, Tang XL, Kodani E, Han H, Zhu Y, Bolli R: Inducible nitric oxide synthase modulates cyclooxygenase-2 activity in the heart of conscious rabbits during the late phase of ischemic preconditioning. Circ Res 2002; 90:602–8

14. Tanaka K, Ludwig LM, Krolikowski JG, Alcindor D, Pratt PF, Kersten JR, Pagel PS, Warltier DC: Isoflurane produces delayed preconditioning against myocardial ischemia and reperfusion injury: Role of cycloxygenase-2. ANESTHESIOLOGY 2004; 100:525–31

15. Gross GJ, Auchampach JA: Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. Circ Res 1992; 70:223–33

16. Ockaili R, Emani VR, Okubo S, Brown M, Krottapalli K, Kukreja RC: Opening of mitochondrial KATP channel induces early and delayed cardioprotective effect: Role of nitric oxide. Am J Physiol Heart Circ Physiol 1999; 277:H2425–34

17. Hide EJ, Ney P, Piper J, Thiemermann C, Vane JR: Reduction by prostaglandin E1 or prostaglandin E0 of myocardial infarct size in the rabbit by activation of ATP-sensitive potassium channels. Br J Pharmacol 1995; 116:2435–40

18. Shinbo A, Iijima T: Potentiation by nitric oxide of the ATP-sensitive K+ current induced by K+ channel openers in guinea-pig ventricular cells. Br J Pharmacol 1997; 120:1568–74

19. Guo Y, Bao W, Wu WJ, Shinmura K, Tang XL, Bolli R: Evidence for an essential role of cyclooxygenase-2 as a mediator of the late phase of ischemic preconditioning in mice. Basic Res Cardiol 2000; 95:479–84

20. Shinmura K, Tang XL, Wang Y, Xuan YT, Liu SQ, Takano H, Bhatnagar A, Bolli R: Cyclooxygenase-2 mediates the cardioprotective effects of the late phase of ischemic preconditioning in conscious rabbits. Proc Natl Acad Sci U S A 2000; 97:10197–202

21. Camitta MG, Gabel SA, Chulada P, Bradbury JA, Langenbach R, Zeldin DC, Murphy E: Cyclooxygenase-1 and -2 knockout mice demonstrate increased cardiac ischemia/reperfusion injury but are protected by acute preconditioning. Circulation 2001; 104:2453–8

22. Rossoni G, Muscara MN, Cirino G, Wallace JL: Inhibition of cyclo-oxygenase-2 exacerbates ischaemia-induced acute myocardial dysfunction in the rabbit. Br J Pharmacol 2002; 135:1540–6

23. Gres P, Schulz R, Jansen J, Umschlag C, Heusch G: Involvement of endogenous prostaglandins in ischemic preconditioning in pigs. Cardiovasc Res 2002; 55:626–32

24. Vegh A, Szekeres L, Parratt JR: Protective effects of preconditioning of the ischaemic myocardium involve cyclo-oxygenase products. Cardiovasc Res 1990; 24:1020–3

25. Murphy E, Glasgow W, Fralix T, Steenbergen C: Role of lipoxygenase metabolites in ischemic preconditioning. Circ Res 1995; 76:457–67

26. Li Y, Kloner RA: Cardioprotective effects of ischaemic preconditioning are not mediated by prostanoids. Cardiovasc Res 1992; 26:226–31

27. FitzGerald GA, Patrono C: The coxibs, selective inhibitors of cyclooxygenase-2. N Engl J Med 2001; 345:433–42

28. McAdam BF, Catella-Lawson F, Mardini IA, Kapoor S, Lawson JA, FitzGerald GA: Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: The human pharmacology of a selective inhibitor of COX-2. Proc Natl Acad Sci U S A 1999; 96:272–7

29. Khan KN, Paulson SK, Verburg KM, Lefkowith JB, Maziasz TJ: Pharmacology of cyclooxygenase-2 inhibition in the kidney. Kidney Int 2002; 61:1210–9

30. Tocco G, Freire-Moar J, Schreiber SS, Sakhi SH, Aisen PS, Pasinetti GM: Maturational regulation and regional induction of cyclooxygenase-2 in rat brain: Implications for Alzheimer's disease. Exp Neurol 1997; 144:339–49

31. Kalgutkar AS, Crews BC, Rowlinson SW, Garner C, Seibert K, Marnett LJ: Aspirin-like molecules that covalently inactivate cyclooxygenase-2. Science 1998; 280:1268–70

32. Chandrasekharan NV, Dai H, Roos KL, Evanson NK, Tomsik J, Elton TS, Simmons DL: COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: Cloning, structure, and expression. Proc Natl Acad Sci U S A 2002; 99:13926–31

33. Shinmura K, Kodani E, Xuan YT, Dawn B, Tang XL, Bolli R: Effect of aspirin on late preconditioning against myocardial stunning in conscious rabbits. J Am Coll Cardiol 2003; 41:1183–94