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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

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Abstract

More than 10 years after its discovery, the function of cyclooxygenase-2 (COX-2) in the cardiovascular system remains largely an enigma. Many scholars have assumed that the allegedly detrimental effects of COX-2 in other systems (e.g. proinflammatory actions and tumorigenesis) signify a detrimental role of this protein in cardiovascular homeostasis as well. This view, however, is ill-founded. Recent studies have demonstrated that ischemic preconditioning (PC) upregulates the expression and activity of COX-2 in the heart, and that this increase in COX-2 activity mediates the protective effects of the late phase of PC against both myocardial stunning and myocardial infarction. An obligatory role of COX-2 has been observed in the setting of late PC induced not only by ischemia but also by δ-opioid agonists and physical exercise, supporting the view that the recruitment of this protein is a central mechanism whereby the heart protects itself from ischemia. The beneficial actions of COX-2 appear to be mediated by the synthesis of PGE_2 and/ or PGI_2. Since inhibition of iNOS in preconditioned myocardium blocks COX-2 activity whereas inhibition of COX-2 does not affect iNOS activity, COX-2 appears to be downstream of iNOS in the protective pathway of late PC. The results of these studies challenge the widely accepted paradigm that views COX-2 activity as detrimental. The discovery that COX-2 plays an indispensable role in the anti-stunning and anti-infarct effects of late PC demonstrates that the recruitment of this protein is a fundamental mechanism whereby the heart adapts to stress, thereby revealing a novel, hitherto unappreciated cardioprotective function of COX-2. From a practical standpoint, the recognition that COX-2 is an obligatory co-mediator (together with iNOS) of the protection afforded by late PC has implications for the clinical use of COX-2 selective inhibitors as well as nonselective COX inhibitors. For example, the possibility that inhibition of COX-2 activity may augment myocardial cell death by obliterating the innate defensive response of the heart against ischemia/reperfusion injury needs to be considered and is the object of much current debate. Furthermore, the concept that the COX-2 byproducts, PGE_2 and/ or PGI_2, play a necessary role in late PC provides a basis for novel therapeutic strategies designed to enhance the biosynthesis of these cytoprotective prostanoids in the ischemic myocardium. From a conceptual standpoint, the COX-2 hypothesis of late PC expands our understanding of the function of this enzyme in the cardiovascular system and impels a critical reassessment of current thinking regarding the biologic significance of COX-2.

Keywords

Ischemia; Nitric oxide; Preconditioning; Reperfusion

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1. Introduction

The phenomenon of ischemic preconditioning (PC), whereby brief episodes of sublethal ischemia render the myocardium resistant to subsequent ischemic stress, occurs in two phases: an early phase that starts within a few minutes after the initial ischemic stimulus and lasts for 2–3 h, and a late phase, which begins 12–24 h later and lasts for 3–4 days [1,2]. The late phase of ischemic PC is caused by the simultaneous activation of multiple stress-responsive signaling pathways, resulting in the shift of the heart to a phenotype that confers sustained protection against both reversible (stunning) and irreversible (infarction) myocardial ischemia / reperfusion injury [2]. During the past decade, late PC has become the focus of intense investigative efforts aimed at identifying the molecular mechanisms that underlie this powerful defensive adaptation of the heart. The clinical implications of these studies are potentially vast, since elucidation of the molecules that confer the preconditioned phenotype could conceivably enable therapeutic exploitation of this endogenous protective mechanism in patients with coronary artery disease who are at risk of acute myocardial infarction or other acute coronary events.

2. Mechanism of late PC

The shift of the heart to a late preconditioned (defensive) phenotype is the result of a complex cascade of molecular events that begins with an effective PC stimulus (such as reversible ischemia or pharmacologic and physical stimuli) and culminates in the synthesis of new proteins that render the heart tolerant to subsequent ischemic injury [2] (Fig. 1). The elements that constitute this molecular cascade can be conceptually subdivided into three major components: (i) ‘triggers’ or initiators of late PC, (ii) ‘mediators’ or effectors of late PC, and (iii) signaling pathways that connect these two groups of molecules [2] (Fig. 1). Several chemical species, including nitric oxide (NO), reactive oxygen species, adenosine, and opioids, have been shown to serve as triggers of the late PC response [2]. These triggers activate a series of kinases and transcription factors that include the ε isoform of PKC, the Src and/ or Lck isoform of the Src family of protein tyrosine kinases (PTKs), Janus kinases (JAK) 1 and 2, nuclear factor-κB (NF-κB), signal transducers and activators of transcription (STAT) 1 and 3, and probably other as-yet-unknown kinases and transcription factors [2] (Fig. 1). Recruitment of these pathways eventually culminates in transcriptional activation of cardioprotective genes and increased expression of proteins that confer resistance to ischemic injury (mediators of late PC). The first mediator of late PC to be identified was the inducible isoform of nitric oxide synthase (iNOS) [3–6]. However, given the complexity of the signaling pathways activated during late PC, it seemed implausible to us that iNOS would be the only cardio-protective protein involved. We postulated that late PC is a polygenic response that requires the coordinated upregulation of multiple proteins.

3. Rationale for the COX-2 hypothesis of late PC

Cyclooxygenase (COX)-2 is the rate-limiting enzyme in prostaglandin (PG) synthesis, catalyzing the conversion of arachidonic acid to PGH_2 [7,8]. Two distinct COX isoforms have been characterized so far: COX-1, which is present in most cells and is responsible for constitutive prostanoid formation, and COX-2, which is induced in response to stress but is also constitutively expressed in some tissues (e.g. kidney, brain, endothelial cells) [7,8]. Our hypothesis that COX-2 is a co-mediator of the protection afforded by late PC (together with iNOS) was predicated on several considerations. First, COX-2 is known to be co-induced together with iNOS in various cell types, including cardiac myocytes, in response to stresses such as cytokines, hypoxia, and ischemia [9–21]. Second, the signaling elements that control the expression of COX-2 during stress appear to be similar to those that control the

induction of iNOS, because they include reactive oxygen species [9,22], protein kinase C (PKC) [23,24], protein entyrosine kinases (PTKs) [25,26], and nuclear factor-kappa B (NF-κB) [10,22,27]. Third, an impressive body of evidence indicates that prostanoids (and their mimetics) exert salutary actions during myocardial ischemia / reperfusion, including attenuation of stunning and reduction in infarct size [28–45]. Despite these facts, however, virtually nothing was known regarding the role of COX-2 either in ischemic PC or in myocardial ischemia / reperfusion in general.

Accordingly, 4 years ago we formulated the COX-2 hypothesis of late PC and began a series of investigations to test it. The results of these studies have demonstrated that COX-2 activity is essential for late PC to occur, supporting a cytoprotective role of this protein and challenging the common paradigm that COX-2 activity is detrimental. The purpose of the present essay is to review the evidence supporting the recent recognition that COX-2 is a cardioprotective enzyme. We will first review the role of COX-2 in the late phase of ischemic and pharmacologic PC and then its role in the early phase of ischemic PC and in nonpreconditioned myocardium.

4. Role of COX-2 in ischemia-induced late PC

4.1. Ischemic PC upregulates COX-2

For this and other studies, we utilized a well-established conscious rabbit model of late PC [3,4,46–48]. The first question we addressed was: Is COX-2 induced by the brief episodes of ischemia that elicit late PC? Rabbits were preconditioned with a sequence of six 4-min coronary occlusion /4-min reperfusion cycles (a protocol that has been shown to induce late PC against both myocardial stunning and myocardial infarction [3,4,46–48]) and were euthanized at selected times thereafter. RNase protection assays were used to detect and quantitate COX-2 mRNA in myocardial tissue samples [46]. Low but detectable COX-2 mRNA levels were present in control rabbits. COX-2 mRNA levels were significantly increased in the ischemic-reperfused region at 1 h after ischemic PC (+231 ± 64%), remained elevated at 3 h, and returned to near control values by 24 h (Fig. 2). We then analyzed COX-2 protein expression in samples harvested 24 h after ischemic PC using standard Western immunoblotting. In control rabbits, over 99% of total COX-2 protein was found in the membranous fraction. Fig. 3 illustrates a representative Western immunoblotting analysis of COX-2. A weak COX-2 signal was detected in control hearts. When rabbits were preconditioned 24 h earlier, the expression of COX-2 in the ischemic-reperfused region increased significantly (+216 ± 79%) [10]. No COX-2 immunoreactivity was detectable in the cytosolic fractions of preconditioned hearts [46].

To determine whether the increased COX-2 protein expression was associated with increased COX-2 enzymatic activity, the myocardial content of the major arachidonic acid metabolites (PGD_2, PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ [stable metabolite of PGI_2], and TXB_2 [stable metabolite of TXA_2]) was measured by enzyme immunoassay. Ischemic PC resulted in a robust increase in PGE_2 and 6-keto-$PGF_{1\alpha}$ (+250±85 and +259±107%, respectively, vs. controls) in the ischemic–reperfused region 24 h later (Fig. 4) [46]. $PGF_{2\alpha}$ levels were also elevated but to a much lesser extent (Fig. 4). The increase in PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$ was completely abrogated when rabbits preconditioned 24 h earlier were given the selective COX-2 inhibitors NS-398 or celecoxib 40 min before euthanasia (Fig. 4) [46]. Thus, the doses of NS-398 and celecoxib used in this study were effective in blocking the increase in COX activity associated with ischemic PC. Importantly, neither NS-398 nor celecoxib lowered prostanoid levels in the nonischemic region below control values (Fig. 4), indicating that constitutive COX activity (COX-1) was not suppressed by these drugs. There was no significant difference in the myocardial content of PGD_2 or TXB_2 between control

and preconditioned hearts [46]. Taken together, these results (Figs. 2–4) demonstrate that ischemic PC upregulates the myocardial expression and activity of COX-2.

4.2. COX-2 activity is necessary for the protective effects of ischemia-induced late PC against myocardial stunning

The next logical question was: Does the upregulation of COX-2 following ischemic PC play a necessary role in the manifestation of the preconditioned phenotype (i.e. of cardiac tolerance to ischemia) or is it merely an epi-phenomenon? This question is critical, as the number of proteins that are upregulated by ischemia is very large but only few of them are likely to be causally involved in late PC. As indicated above, the doses of NS-398 and celecoxib used in our study [46] effectively ablated the increased COX-2 activity associated with late PC (Fig. 4), thereby providing a pharmacologic tool to interrogate the functional significance of COX-2 in this phenomenon. Accordingly, we carried out studies to determine whether these same doses of NS-398 and celecoxib interfere with the cardioprotective effects of late PC. Because myocardial stunning and myocardial infarction are two different phenomena [49], we studied late PC against stunning and late PC against infarction separately.

To examine late PC against stunning, conscious rabbits were subjected to a sequence of six 4-min coronary occlusions /4-min reperfusion cycles for three consecutive days (days 1, 2, and 3) [46]. The recovery of regional myocardial function was assessed as left ventricular (LV) systolic thickening fraction by using the pulsed Doppler probe [47]. The total deficit of systolic wall thickening (WTh) after reperfusion (an integrative assessment of the overall severity of myocardial stunning) [47] was calculated by measuring the area comprised between the systolic WTh-vs.-time line and the baseline (100% line) during the 5-h recovery phase after the sixth reperfusion. In all groups, thickening fraction on day 1 remained significantly depressed for 4 h after the sixth reperfusion and returned to values not significantly different from preocclusion values by 5 h. Thus, as previously observed in this model [3,46– 48], the sequence of six 4-min coronary occlusion /4-min reperfusion cycles resulted in severe stunning that lasted, on average, for 4 h. As expected [3,46–48], in control rabbits the recovery of WTh was improved on days 2 and 3 compared with day 1, resulting in a significant decrease in the total deficit of WTh on both days, compared with day 1 (Fig. 5). This indicates the development of late PC against stunning [3,46–48]. Similar results were obtained in rabbits given DMSO (the vehicle used for both NS-398 and celecoxib) on day 2 (Fig. 5).When rabbits were given NS-398 or celecoxib prior to the six occlusion / reperfusion cycles on day 2, however, the recovery of WTh during the 5-h reperfusion period was not improved on day 2 compared with day 1, so that the total deficit of WTh did not differ significantly between day 1 and day 2 (Fig. 5). Thus, the protective effects of late PC against stunning on day 2 were completely abrogated by the administration of either NS-398 or celecoxib. Administration of NS-398 or celecoxib on day 1 had no effect on the deficit of WTh on the same day (Fig. 5), indicating that these drugs, in themselves, do not affect the severity of myocardial stunning in nonpreconditioned myocardium.

4.3. COX-2 activity is necessary for the protective effects of ischemia-induced late PC against myocardial infarction

To examine late PC against infarction, conscious rabbits were preconditioned with a sequence of six 4-min coronary occlusion /4-min reperfusion cycles and then subjected, 24 underlyh later, to a 30-min coronary occlusion followed by 3 days of reperfusion. As expected [4,46,48,50], infarct size was significantly smaller in rabbits subjected to six occlusion / reperfusion cycles 24 h earlier (PC group) compared with controls, indicating a late PC effect against myocardial infarction (Fig. 6) [46]. In contrast, in rabbits treated with either NS-398 or celecoxib prior to the 30-min occlusion, infarct size was similar to that

measured in controls (Fig. 6), indicating that both drugs abrogated late PC. When NS-398 or celecoxib were given in the absence of ischemic PC, infarct size did not differ from that observed in controls (Fig. 6), indicating that these drugs did not affect the extent of cell death in nonpreconditioned myocardium [46]. This finding suggests that COX-2 activity does not play an important role in modulating infarct size in naïve (nonpreconditioned) myocardium.

To determine whether the obligatory role of COX-2 in late PC is species-specific, we then examined the role of COX-2 in the mouse utilizing a well-established model of myocardial infarction [51] produced by a 30-min coronary occlusion followed by 24 h of reperfusion. As expected [51], ischemic PC (six 4-min coronary occlusion /4-min reperfusion cycles) resulted, 24 h later, in a significant reduction in infarct size, indicating a late PC effect analogous to that observed in the rabbit (Fig. 7) [52]. As we had found in rabbits, this cardioprotective effect was ablated when mice were given NS-398 prior to the 30-min coronary occlusion, indicating that COX-2 activity is essential for the infarct-sparing effects of late PC in mice (Fig. 7) [52]. Again, administration of NS-398 had no effect on infarct size in the absence of ischemic PC (Fig. 7).

In summary, these two studies [46,52] demonstrate that COX-2 plays an obligatory role in the cardioprotective effects of ischemia-induced late PC in two different species (rabbits and mice), supporting the notion that the recruitment of this protein is a general mechanism underlying cardiac adaptation to stress. Furthermore, COX-2 is essential both for late PC against stunning and for late PC against infarction.

5. Role of COX-2 in pharmacologic PC

5.1. COX-2 activity is necessary for the protective effects of δ-opioid receptor- and NO donor-induced late PC

Having identified an obligatory role of COX-2 in ischemia-induced late PC, we sought to determine whether pharmacologic and other stimuli known to induce a late PC response also act via upregulation of COX-2 activity.

Activation of δ-opioid receptors has been shown to elicit a delayed cardioprotective effect against myocardial infarction that mimics that induced by ischemia [53,54]. In recent studies in conscious rabbits, we have found that administration of the δ-opioid receptor agonist BW373U86 alleviates the myocardial stunning induced 24 h later by a sequence of six 4-min occlusion / reperfusion cycles (δ-opioid-induced late PC against stunning). We used this model to test the role of COX-2. When rabbits were given the COX-2 inhibitors NS-398 or celecoxib prior to the consequence of six occlusion / reperfusion cycles 24 h after BW373U86, the recovery of WTh was indistinguishable from control rabbits, indicating that the late PC effect against stunning was ablated. In addition, BW373U86 upregulated the myocardial expression of COX-2 protein and the content of PGE_2 and 6-keto-$PGF_{1\alpha}$ 24 h later. Taken together, these data demonstrate that activation of δ-opioid receptors upregulates COX-2 activity in the myocardium and that this phenomenon plays an obligatory role in δ-opioid receptor-induced late PC against myocardial stunning.

Furthermore, in recent studies in mice subjected to a 30-min coronary occlusion followed by 24 h of reperfusion, we have found that administration of COX-2 inhibitors shortly before the 30-min occlusion blocks the late PC effect induced by pretreatment with NO donors (DETA/NO) as well as that induced by prior physical exercise, indicating that COX-2 is also a mediator of NO donor-induced and exercise-induced late PC.

5.2. COX-2 activity is not necessary for adenosine A_1 or A_3 receptor-induced late PC against myocardial infarction

The adenosine A_1 receptor agonist 2-chloro-N^6-cyclopentyladenosine (CCPA) and the adenosine A_3 receptor agonist N^6-3-iodobenzyladenosine-5′-*N*-methylcar-boxamide (IB-MECA) have been shown to elicit a delayed phase of protection against infarction similar to the late phase of ischemia-induced PC [55–57]. Using our conscious rabbit model, we found that neither CCPA nor IB-MECA upregulated COX-2 protein expression 24 h after their administration [50], despite the fact that both of these agonists were given in doses that elicit delayed cardioprotection [57]. Nevertheless, since COX-2 is constitutively expressed in the rabbit heart [46,50], it remains possible that this enzyme might be activated 24 h after CCPA or IB-MECA treatment and thus contribute to cardioprotection even though its protein expression is unchanged. To test this possibility, infarct size was measured in rabbits subjected to a 30-min coronary occlusion followed by 72 h of reperfusion [50]. Both CCPA and IB-MECA, given 24 h prior to the 30-min occlusion, resulted in a significant reduction in infarct size indicative of a late PC effect, consistent with prior observations [57]. Administration of NS-398 at the same dose that was previously shown to effectively abolish the enhanced COX-2 activity elicited by ischemia and the concomitant cardioprotection in the same conscious rabbit model [46], failed to block the infarct-sparing effects of either CCPA or IB-MECA, indicating that COX-2 activity is not necessary for these effects to occur [50]. Thus, unlike δ-opioid receptor-induced late PC against stunning, the mechanism of adenosine A_1 and A_3 receptor-induced late PC against infarction is independent of COX-2 activity.

6. Mechanism of upregulation of COX-2 by ischemia

Although COX-2 has been found to be upregulated by phorbol ester and oxidative stress in isolated neonatal myocytes [9], no information is available regarding the modulation of COX-2 in adult myocardium. Specifically, nothing is known regarding the signaling pathways where- by a sublethal ischemic stress leads to increased expression of COX-2 in the heart. Since the development of late PC is triggered by the formation of NO and reactive oxygen species during the initial PC stimulus and by the subsequent early activation of a cascade that involves the sequential recruitment of PKC, Src /Lck PTKs, and NF-κB [2], it seemed logical to hypothesize that these elements participate in the induction of COX-2 in response to sublethal myocardial ischemia.

Using our conscious rabbit model, we found [58] that the increase in COX-2 protein expression observed 24 h after ischemic PC was completely prevented when the animals were pretreated (before the PC protocol) with the PKC inhibitor chelerythrine, the tyrosine kinase inhibitor lavendustin-A, or the NF-κB inhibitor diethyldithiocarbamate (DDTC), given at doses which completely inhibit activation of PKCε [59], Src and Lck PTKs [60], and NF-κB [61], respectively, in this rabbit model. Thus, induction of COX-2 protein expression in preconditioned myocardium requires PKC-, Src /Lck PTK-, and NF-κB-dependent signaling (Fig. 1). In contrast, administration of the antioxidant *N*-2-mercaptopropionyl glycine (MPG) or the NOS inhibitor N^{ω}-nitro-L-arginine (L-NA) prior to ischemic PC did not block the upregulation of COX-2 24 h later, indicating that the generation of NO and reactive oxygen species during the PC ischemia is not necessary for the increase in COX-2 protein expression to occur [58]. Since both L-NA and MPG were given in doses that completely block the development of late PC in this conscious rabbit model [3,47,48,62], the failure of these agents to block COX-2 induction [58] implies that NO and reactive oxygen species participate in the late phase of ischemic PC by upregulating proteins other than COX-2 (e.g. iNOS) [3–6], thereby supporting the notion [2] that the switch of the heart to a preconditioned phenotype is a polygenic response (i.e. COX-2 induction is necessary but not sufficient). As the activation of *COX-2* gene transcription

usually requires the combinatorial actions of various transcription factors [7,63], it seems very likely that the mechanism responsible for upregulating this enzyme in the heart is much more complex. Further studies will be needed to decipher the intricate network of kinases and transcription factors that underlie the recruitment of this cardioprotective protein.

7. COX-2 activity in preconditioned myocardium is modulated by iNOS

As indicated above, iNOS plays a necessary role in mediating the cardioprotective effects of late PC [3–6]. Since both iNOS and COX-2 are obligatory co-mediators of late PC, the question naturally arises to whether these two proteins act in series or are independent (i.e. parallel) effectors of cardioprotection. A possible 'cross-talk' between NOS and COX has been extensively examined in noncardiac tissues, with conflicting results (reviewed in Ref. [64]). While some studies suggest that NO enhances COX-2 activity [16,65–68], others have concluded that NO inhibits it [69–73] or has no effect [74–76]. The relationship between iNOS and COX-2 in the heart has not been evaluated.

In a recent study [58], we have examined this issue in the same conscious rabbit model in which the role of COX-2 was previously demonstrated [46]. We found that the increase in myocardial prostanoids (PGE_2 and 6-keto-$PGF_{1\alpha}$) observed 24 h after ischemic PC (on day 2) was ablated when two selective iNOS inhibitors (SMT and 1400W) were given on day 2, 30–40 min prior to harvesting of tissue samples [58]. On the other hand, administration of NS-398 or celecoxib on day 2 did not have any appreciable effect on iNOS activity. These data indicate that the enhanced prostanoid biosynthesis associated with late PC is dependent upon iNOS-derived NO whereas the enhanced iNOS activity is independent on COX-2-derived prostanoids [58]. Thus, COX-2 is located downstream of iNOS in the protective pathway of late PC, implying that iNOS protects, at least in part, by recruiting COX-2. We propose that stimulation of COX-2 activity and production of cytoprotective prostanoids, such as PGE_2 and PGI_2, may be a previously unrecognized mechanism by which NO exerts its salubrious effects on the ischemic myocardium (Fig. 1).

Interestingly, the increase in prostanoid levels 24 h after ischemic PC was not affected by the administration on day 2 of the soluble guanylyl cyclase inhibitor ODQ (given at doses that block the increase in cGMP levels associated with late PC [77]), indicating that iNOS-derived NO activates cardiac COX-2 via cGMP-independent mechanisms [58]. This supports the hypothesis of a direct interaction between NO and the COX-2 molecule [65].

8. Does aspirin abrogate COX-2-dependent late PC?

Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit COX activity and are widely used clinically. Acetylsalicylic acid (aspirin) is the most commonly used NSAID for relieving pain, inflammatory symptoms, and fever [78]. In addition, aspirin has established efficacy for preventing cardiovascular events [78–80]. Aspirin inhibits COX-1 activity by acetylating serine 530, which is located close to the active site (tyrosine 385) of COX-1; acetylation of this serine residue hinders the access of arachidonic acid to the catalytic site [81,82]. Aspirin also inhibits COX-2 by a similar mechanism but with less potency [83]. Although the ability of low doses of aspirin to inhibit COX-1 activity is well established [78,84], it is unknown whether these doses can also interfere with the cardio-protective effects of late PC by inhibiting COX-2 as well. Mounting evidence indicates that both ischemic and pharmacologic PC occur in patients with coronary artery disease [85,86]. Since aspirin at low doses is currently recommended for the prophylaxis of cardiac and cerebral ischemic events and is given to almost all patients with coronary artery disease [78], it is important to determine whether the use of aspirin affects the development of late PC.

We have recently examined this issue in conscious rabbits in which late PC against stunning was induced with a sequence of six 4-min coronary occlusion /4-min reperfusion cycles on three consecutive days [87]. We found that administration on day 2 of 5 mg/kg of aspirin, which was sufficient to inhibit platelet aggregation, did not inhibit either the increase in myocardial levels of PGE_2 and 6-keto-$PGF_{1\alpha}$ or the late PC protection against myocardial stunning; in contrast, administration on day 2 of 25 mg /kg of aspirin completely abrogated both the increase in myocardial prostanoids and the cardioprotective effects of late PC [87]. Thus, low doses of aspirin, which are widely used to prevent cardiovascular events in patients, do not interfere with the cardioprotective effects of late PC against myocardial stunning. In contrast, higher doses of aspirin, which are used for analgesic / antipyretic purposes, completely abrogate late PC, suggesting that they should be used with caution in patients with atherosclerotic cardiovascular disease, since they may deprive the heart of its innate defensive response. That low-dose aspirin inhibits platelet aggregation (a COX-1-dependent phenomenon) but not late PC (a COX-2-dependent phenomenon) probably results from the fact that aspirin exhibits less potency for COX-2 than for COX-1 inhibition [83] because the substrate channel of COX-2 is larger and more flexible than that of COX-1 [88]. For example, in intact cells aspirin is 166 times more active against COX-1 (IC_{50}=0.3 μg/ml) than against COX-2 (IC_{50}=50 μg/ml) [83]. The results summarized above [87] were obtained in the context of late PC against stunning; whether similar conclusions apply to late PC against infarction is not known.

9. Role of COX-2 in early PC

Studies of the effects of nonselective COX inhibitors on early PC have yielded conflicting results [89–91]. Perhaps the most conclusive study is that of Camitta et al. [92], who found that deletion of either COX-1 or COX-2 by gene targeting had no effect on early PC in isolated mouse hearts.

10. Effect of COX-2 on ischemia / reperfusion injury in nonpreconditioned myocardium

Our finding that COX-2 is an obligatory co-mediator of protection during late PC is consistent with an increasing number of reports in other systems suggesting a beneficial role of this enzyme in ischemia and other settings. Studies in isolated neonatal cardiomyocytes have shown that oxidative stress upregulates COX-2 and the addition of a COX-2-specific inhibitor enhances oxidative stress-induced injury and apoptosis, indicating that COX-2 is protective in this system [9]. A pro-apoptotic effect of inhibiting COX-2 has also been reported in other cell types [93–95]. In a recent study in isolated mouse hearts [92], Camitta et al. [92] found that the postischemic recovery of LV function was impaired in COX-2 null mice compared with controls, implying that constitutively-expressed COX-2 is cardioprotective. Interestingly, COX-2 protein was not detectable by Western immunoblotting in wild-type hearts; however, PGE_2 and 6-keto-$PGF_{1\alpha}$ were still present in COX-1 null hearts (albeit at lower levels than in controls), suggesting that sufficient COX-2 was present under baseline conditions to generate these prostanoids [92]. This concept is corroborated by the finding that loss of COX-2 aggravated ischemia–reperfusion injury [92], which implies that constitutively-expressed COX-2 is sufficient to produce cardioprotective prostanoids even though its protein expression may not be detectable. The fact that ablation of COX-2 exacerbated ischemia–reperfusion injury also implies that COX-1 cannot substitute for the loss of COX-2.

Taken together, the observation of Camitta et al. [92] support a cardioprotective role of constitutively-expressed COX-2 in the heart, a novel concept that warrants further investigation. Interestingly, acute inhibition of COX-2 with indomethacin failed to

exacerbate ischemia–reperfusion injury while supplementation with prostaglandins failed to reverse the detrimental effects of COX-2 deletion, suggesting that in that study COX-2 protected the heart by indirect actions [e.g. altered expression of other gene(s)] rather than by the direct effects of its metabolites [92].

11. Mechanism of COX-2-mediated cardioprotection

COX-2 catalyzes the conversion of arachidonic acid to PGH_2, which can be metabolized to various eicosanoids, including PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and TXA_2 [7,8]. The precise range of prostanoids generated by COX-2 depends on the cell types and their inherent prostanoid synthetic pathways [7,8]. Our published data in rabbits [46] as well as unpublished data in mice indicate that the two main products of COX-2 activity in preconditioned myocardium are PGE_2 and PGI_2 (Fig. 3), suggesting that the cardioprotective effects of COX-2 are related to the biological actions of these two prostanoids. A large number of studies have shown that PGE_2 and PGI_2 (and their analogs) exert salutary actions during myocardial ischemia / reperfusion, resulting in attenuation of stunning [96,97] and reduction in infarct size [29–31,35– 41,44,45,98]. The cytoprotective actions of PGE_2 and PGI_2 have been attributed to antagonism of adenylyl cyclase [28,34–36,42,99,100], activation of ATP-sensitive potassium channels [36,40,41,99], inhibition of Ca^{2+} influx [99], and attenuation of neutrophil infiltration [38,39,44,45] (Table 1). Interestingly, these actions are reminiscent of those of NO [101], suggesting that prostanoids and NO might possibly have additive or synergistic mechanisms of cytoprotection. Since activation of ATP-sensitive potassium channels is necessary for late PC against infarction [53,102,103] but not for late PC against stunning [103], this mechanism could account for the antiinfarct but not for the antistunning actions of COX-2; the latter must involve other mechanisms.

12. COX-2 activity: friend or Foe?

The conclusion that COX-2 mediates the beneficial effects of late PC may seem surprising or even paradoxical, because the activity of COX-2 is generally thought to be detrimental [8,21]. Specifically, induction of COX-2 is believed to play a role in inflammation, toxic shock, cancer, and apoptosis [8,21,71,104–111]. A recent study has reported the expression of COX-2 in ischemic human myocardium and in dilated cardiomyopathy, but not in normal cardiomyocytes [112], a finding which has been interpreted (without proof) to indicate a role of COX-2 in cardiac disease. Evidence has been reported suggesting that COX-2 contributes to ischemia / reperfusion injury in the brain [113,114].

However, there is also evidence suggesting physiologically important or salutary actions of COX-2 in other situations [9,93–95,104,107–111,115–120]. For example, COX-2 protects cardiomyocytes against oxidative stress [9], exerts anti-apoptotic actions in various cell types [9,93–95,104,107–111], and has recently been identified as a major source of systemic PGI_2 biosynthesis in humans [115]. Indeed, sheer stress induces COX-2 expression in endothelial cells and a substantial amount of eicosanoid production by endothelial cells results from the action of COX-2 [121]. COX-2-dependent production of PGI_2 in endothelial cells may exert antithrombotic effects by counteracting COX-1-dependent production of TXA_2 in platelets [119,122,123]. The finding that genetic disruption of COX-2 results in cardiac fibrosis [116] also suggests that COX-2 expression may be protective. Furthermore, it is now recognized that COX-2 is constitutively expressed in the kidney [120,124] and in the brain [125–127] and plays an important role in maintaining renal function [119,120] and in modulating neural responses [119]. Whether COX-2 exerts proinflammatory actions in reperfused myocardium remains unknown; even if it does, these actions would not

necessarily be deleterious because inflammation is likely to be a consequence rather than a cause of myocardial ischemia / reperfusion injury.

We propose that the pathophysiological role of COX-2 is much more complex than hitherto appreciated, and that this enzyme may exert either beneficial or deleterious effects depending on the intensity of its induction, the pathophysiological setting, and the ability of specific cells to metabolize PGH_2 produced by COX-2 into cytoprotective prostanoids. The experimental studies reviewed in this essay document an essential role of COX-2 as a mediator of cardioprotection during the late phase of ischemic or pharmacologic PC. Interestingly, the clinical experience accumulated with COX-2 inhibitors in the past three years suggests that COX-2 exert protective effects in patients with cardiovascular disease [123]. In the VIGOR trial [128], the relative risk of developing cardiovascular events with rofecoxib vs. naproxen was 2.38 (95% confidence interval, 1.39–4.00; $P = 0.002$). A recent meta-analysis of all randomized clinical trials of COX-2 antagonists (primarily VIGOR [128] and CLASS [129]) has concluded that these agents significantly increase the risk of myocardial infarction [123]. One explanation put forth for these differences was that COX-2 inhibitors may have a prothrombotic action, since they suppress endothelial production of PGI_2 (which is mostly derived from COX-2 [115,121,122]) while leaving platelet production of TBA_2 (which is exclusively due to COX-1 [8,119,122]) unaltered, thereby causing an imbalance between antithrombotic and prothrombotic prostanoids [122,123]. Another possibility, however, is that COX-2 antagonists may abrogate late PC, thereby increasing the severity of myocardial ischemia / reperfusion injury. Regardless of the mechanism involved, a prospective randomized trial of the effect of COX-2 inhibitors on cardiovascular events seems warranted [123].

13. Conclusions

More than 10 years after its discovery [130,131], the function of COX-2 in the cardiovascular system remains largely an enigma. Many scholars have assumed that the allegedly detrimental effects of COX-2 in other systems (e.g. proinflammatory actions, pain, tumorigenesis, among others) predict a detrimental role of this protein in cardiovascular homeostasis as well. This view, however, is ill-founded. Asides from the fact that a causative role of COX-2 activity in many of the aforementioned processes has not been proven but rather has been suspected on the basis of correlative data, tumorigenic and proinflammatory actions in other organs cannot be extrapolated to signify detrimental cardiovascular actions without data.

The evidence reviewed herein expands our understanding of this protein. Specifically, the discovery that COX-2 activity plays an indispensable role in the antiischemic effects of late PC has revealed a novel, heretofore-un-appreciated cardioprotective function of COX-2, thereby impelling a critical reassessment of current assumptions regarding the significance of this enzyme in the cardiovascular system. From a conceptual standpoint, the COX-2 hypothesis of late PC challenges the widely accepted view that this protein is detrimental to the heart. From a practical standpoint, the recognition that COX-2 is an obligatory co-mediator (together with iNOS) of the protection afforded by late PC has implications for the clinical use of COX-2 selective inhibitors as well as nonselective COX inhibitors. For example, the possibility that inhibition of COX-2 activity may augment myocardial cell death and dysfunction by obliterating the innate defensive response of the heart against ischemia / reperfusion injury (late PC) needs to be considered, particularly in light of recent clinical data suggesting an increase in cardiovascular events among patients treated with COX-2 inhibitors. Finally, the concept that the arachidonic acid metabolites, PGE_2 and/or PGI_2, play a necessary role in late PC provides a basis for novel therapeutic strategies aimed

at enhancing the biosynthesis of these cytoprotective eicosanoids in the ischemic myocardium.

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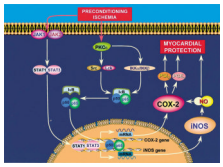

Fig. 1.

Schematic representation of our current understanding of the cellular mechanisms whereby COX-2 is upregulated by ischemic PC and participates in cardioprotection. A sublethal ischemic stress (ischemic PC) activates a complex signal transduction cascade that includes PKC (specifically, the ε isoform), PTKs (specifically, Src and/ or Lck kinases), and probably other as-yet-unknown kinases, leading to phosphorylation of IκBα and mobilization of the transcription factor NF-κB. In addition, ischemic PC activates the non-receptor tyrosine kinases JAK1 and JAK2 with subsequent tyrosine phosphorylation and activation of the transcription factors STAT1 and STAT3. Other, as yet unknown, transcription factors are most likely involved as well. The promoter of both the *iNOS* and the *COX-2* genes contains cognate sequences for NF-κB and STAT1/STAT3. Binding of NF-κB and STAT1/STAT3 to these promoters results in a coordinated transcriptional activation of the *iNOS* and *COX-2* genes with synthesis of new iNOS and COX-2 proteins. The activity of newly-synthetized COX-2 protein requires iNOS-dependent NO generation whereas the activity of iNOS does not require COX-2-dependent prostanoid generation. Thus, COX-2 is downstream of iNOS in the pathophysiological cascade of late PC. iNOS-derived NO can protect the myocardium from recurrent ischemia both via direct actions and via activation of COX-2-dependent synthesis of cardioprotective prostanoids. Among the products of COX-2, PGE_2 and/ or PGI_2 appear to be the most likely effectors of cytoprotection. A similar upregulation of COX-2 can be elicited pharmacologically by δ-opioid receptor agonists but not by adenosine A_1 or A_3 receptor agonists.

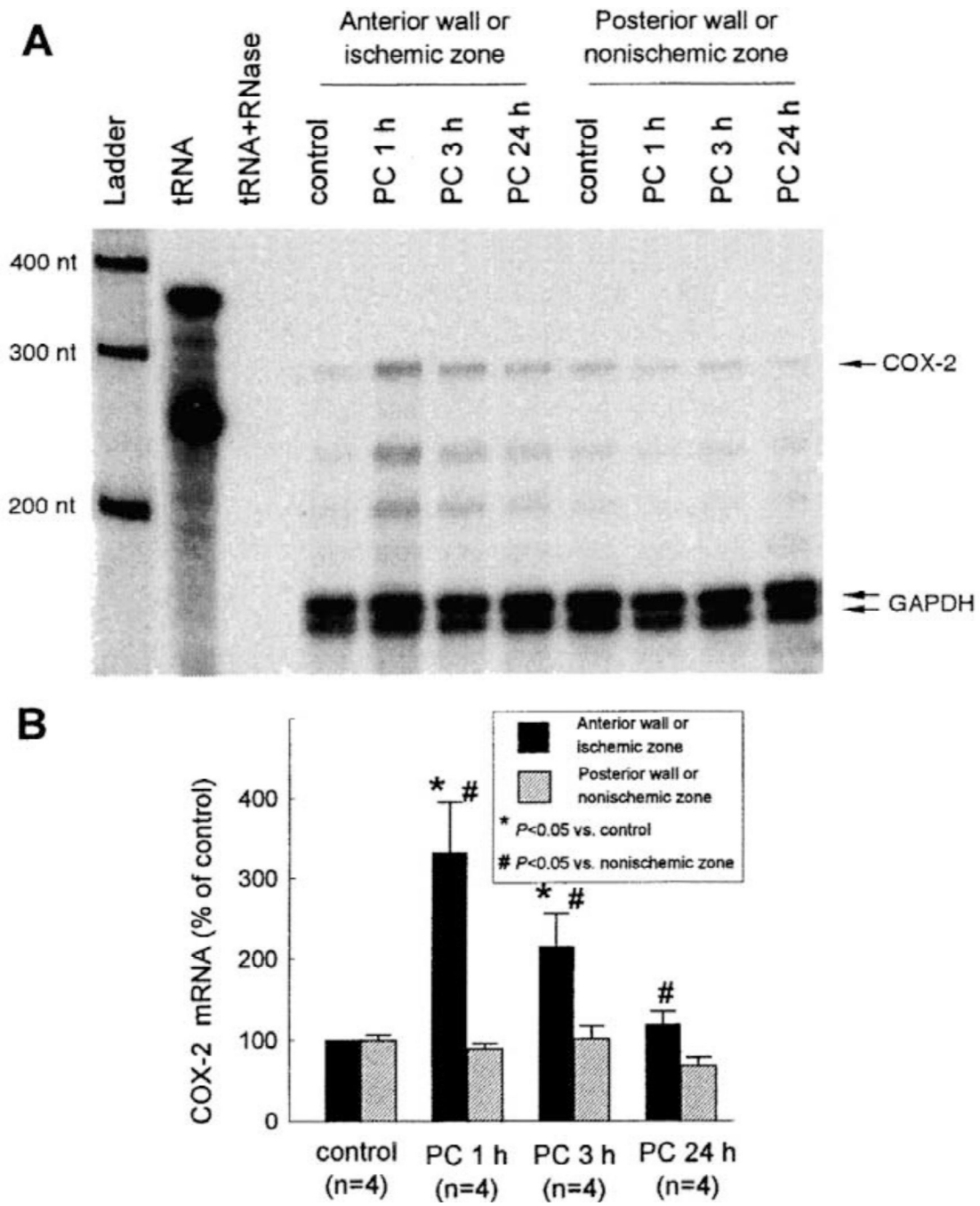

Fig. 2.

Effect of ischemic PC on COX-2 mRNA expression in rabbit myocardium. Tissue samples were obtained from the anterior and posterior LV wall of control rabbits and from the ischemic-reperfused and nonischemic regions of rabbits that underwent ischemic PC with six 4-min coronary occlusion /4-min reperfusion cycles and were euthanized 1 h, 3 h, or 24 h later. (A) Representative RPA gel; (B) densitometric analysis of COX-2 mRNA signals. Each COX-2 signal was normalized to the GAPDH signal from the same sample to control for RNA loading. The normalized values are expressed as percentage of the signal in the anterior LV wall of control hearts. Data are means±S.E.M. (Reproduced with permission of the National Academy of Sciences from Shinmura et al. [46]).

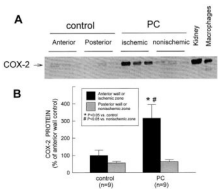

Fig. 3.

Effect of ischemic PC on the expression of COX-2 protein in rabbit myocardium. Tissue samples were obtained as described in the legend to in Fig. 2 from control rabbits and from rabbits that underwent ischemic PC 24 h earlier. (A) COX-2 immunoreactivity in the membranous fraction increased markedly in the ischemic-reperfused region 24 h after ischemic PC. Robust expression of COX-2 was observed in rabbit kidney and in murine macrophages stimulated with interferon-γ and lipopolysaccharide (positive controls). (B) Densitometric analysis of COX-2 signals in the membranous fraction. In all samples, the densitometric measurements of COX-2 immunoreactivity were expressed as a percentage of the average value measured in the anterior LV wall of control rabbits. Data are means ±S.E.M. (Reproduced with permission of the National Academy of Sciences from Shinmura et al. [46]).

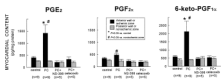

Fig. 4.

Effect of ischemic PC on the myocardial content of PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$ (measured by EIA). In rabbits that underwent ischemic PC 24 h earlier, the levels of PGE_2 and 6-keto-$PGF_{1\alpha}$ in the ischemic-reperfused region increased markedly vs. control rabbits; the levels of $PGF_{2\alpha}$ were higher than in the nonischemic region of the same group but did not differ significantly from controls. The increase in PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$ was completely abrogated when rabbits were given NS-398 or celecoxib 24 h after ischemic PC (40 min before euthanasia). The myocardial content of PGE_2 and 6-keto-$PGF_{1\alpha}$ in the nonischemic region was similar in all groups, indicating that the COX-2 inhibitors did not affect constitutive production of these eicosanoids by COX-1. Data are means±S.E.M. (Reproduced with permission of the National Academy of Sciences from Shinmura et al. [46]).

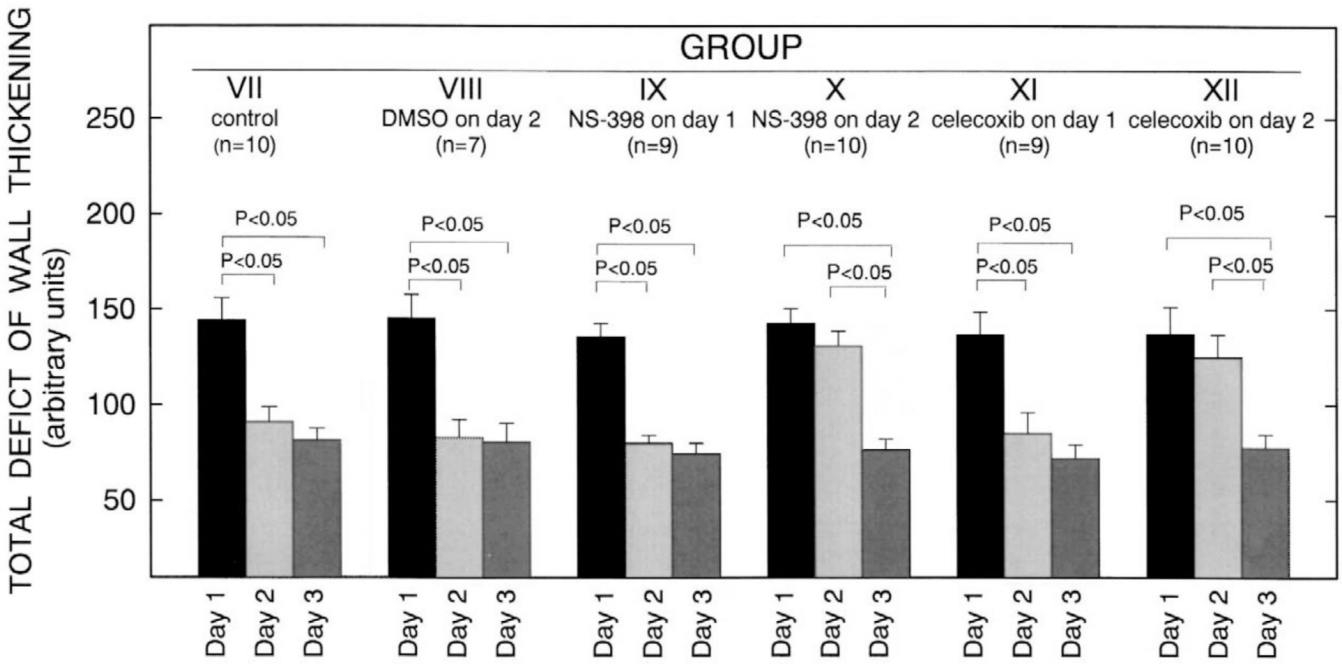

Fig. 5.

Total deficit of WTh after the sixth reperfusion (a measure of the severity of myocardial stunning) on days 1, 2, and 3 in groups VII (control, *n* = 10), VIII (DMSO on day 2, *n* = 7), IX (NS-398 on day 1, *n* = 9), X (NS-398 on day 2, *n* = 10), XI (celecoxib on day 1, *n* = 9) and XII (celecoxib on day 2, *n* = 10). The total deficit of WTh was measured in arbitrary units, as described in the text. Data are means±S.E.M. (Reproduced with permission of the National Academy of Sciences from Shinmura et al. [46]).

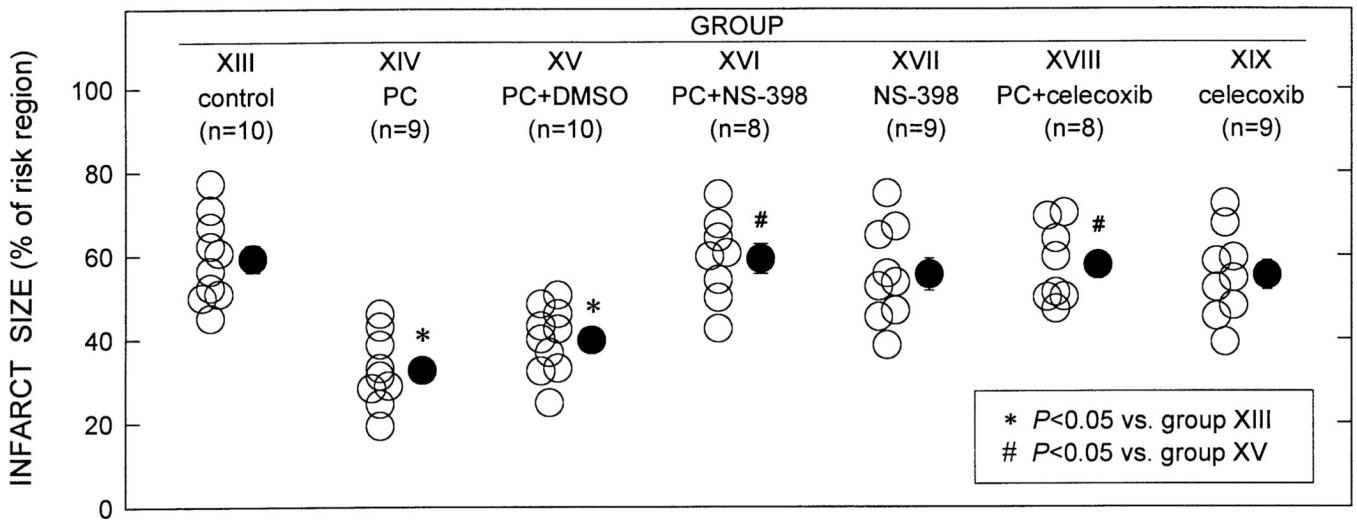

Fig. 6.

Myocardial infarct size in groups XIII (control, *n* = 10), XIV (PC, *n* = 9), XV(PC+DMSO, *n* = 10), XVI (PC+NS-398, *n* = 8), XVII (NS-398, *n* = 9), XVIII (PC+celecoxib, *n* = 8), and XIX (celecoxib, *n* = 9). Infarct size is expressed as a percentage of the region at risk of infarction. Solid circles represent means±S.E.M. (Reproduced with permission of the National Academy of Sciences from Shinmura et al. [46]).

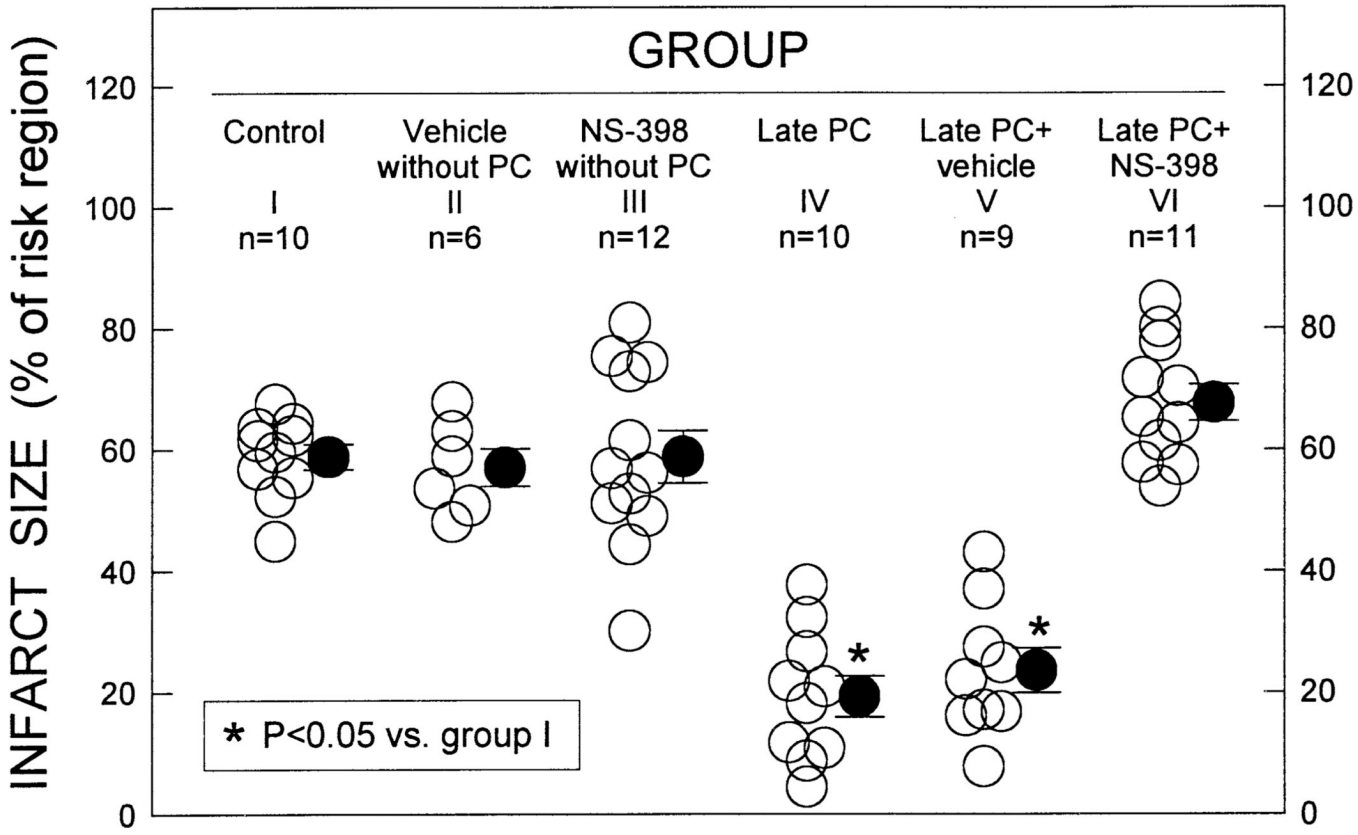

Fig. 7.

Infarct size, expressed as a percent of the risk region, in the six groups of mice. Open circles indicate individual measurements, solid circles represent means±S.E.M. (Reproduced with permission of Steinkopff Verlag from Guo et al. [52]).

Table 1

Mechanism for the cardioprotective effects of prostanoids

Effect	Mechanism
More likely	
	Inhibition of Ca^{2+} influx
	Antagonism of adenylyl cyclase
	Opening of K_{ATP} channels
Less likely	
	Attenuation of neutrophil infiltration