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Mechanisms of Protection Afforded by Cyclooxygenase Inhibitors to Endothelial Function Against Ischemic Injury in Rat Isolated Hearts

[Articles]

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Summary:

The aim of this study was to assess whether cyclooxygenase (COX) inhibitors protect the endothelial function against the deleterious effect of ischemia and reperfusion. Isolated rat hearts perfused under constant-flow conditions were exposed to 30 min of partial ischemia (flow, 1 ml/min) followed by 20 min of reperfusion, after which coronaries were precontracted with U-46619, and the response to the endothelium-dependent vasodilator, serotonin (5-HT), was compared with that of the endothelium-independent vasodilator, sodium nitroprusside (SNP). In untreated hearts, ischemia diminished selectively 5-HT-induced vasodilation, compared with sham hearts (without ischemia). The vasodilation to SNP was unaffected in all groups. Pretreatment with 6-MNA, 30 μM, a COX-2 inhibitor with some activity on COX 1, diclofenac, 1 μM (COX-1 and -2), or 1-(7-carboxyheptyl) imidazole, 10 μM [thromboxane (TX) synthase inhibitor] but not indomethacin, 10 μM (COX-1 inhibitor) preserved the vasodilation induced by 5-HT after ischemia. Enzyme immunoassays indicated that all COX inhibitors decreased the concentration of TXB_2 and 6-keto-$PGF_{1[alpha]}$ [stable metabolites of TXA_2 and prostacyclin (PGI_2), respectively] in coronary effluent during ischemia. Furthermore, indomethacin was the only one to abolish the concentration of PGE_2 during ischemia and early reperfusion. No clear trend on ventricular postischemic recovery could be observed between treated and untreated groups under our experimental protocols. These data suggest that, under our conditions, 6-MNA, diclofenac, and 1-7-CHI, but not indomethacin, protect the endothelial function via a reduction in TX concentration. Disparities between COX inhibitors may be due to the complete abolition of PGE_2 concentration during ischemia and reperfusion in the indomethacin group.

An increasing number of experimental studies attribute an important role to eicosanoids in myocardial ischemia and reperfusion injury (1). Phospholipase activation with the subsequent cleavage of membrane phospholipids and fatty acid accumulation within the ischemic tissue are initial events in myocardial ischemia. A large increase in arachidonic acid in ischemic myocardium has been reported (2). Cardiac tissue has the capacity to synthesize all the major types of eicosanoids (3). Although the coronary vasculature and endothelium were once considered to be the only important cardiac sites of prostaglandin synthesis, PGE_2 and $PGF_{2[alpha]}$ also are produced in cardiac myocytes (4).

In ischemia induced by coronary ligation, enhanced production of eicosanoids is known to occur, but their exact role in the development of ischemic injury remains to be clarified (5-8). Their involvement in ischemia-and-reperfusion injury is extremely complex, primarily because both beneficial and deleterious effects of these compounds have been proposed (7,9). Prostacyclin (PGI_2) is the major prostaglandin produced by the heart during ischemia and reperfusion, and its release is well documented in the literature (10,11). Some authors also reported enhanced release of $PGF_{2[alpha]}$ and PGE_2 from cardiac tissue after coronary ligation (5,12). As vasodilators, PGE_2 and PGI_2 (13) may have protective effects during ischemia and reperfusion.

Furthermore, endogenous release of thromboxanes (TXA_2) during ischemia and reperfusion has been reported to contribute to the progression of myocardial infarction (14,15) and the increased occurrence of arrhythmias after coronary artery occlusion (10). TXA_2 is a potent vasoconstrictor and has enhanced membrane-permeability action (16).

Beneficial effects of TX-synthase inhibitors (17,18) and TX-receptor antagonists (19) have been reported in models of ischemia and reperfusion. Thus TXA_2 could be potentially deleterious in myocardial ischemia, extending ischemic damage in the heart and leading to increased cell death (18).

Moreover, in isolated rat hearts, structurally dissimilar nonsteroidal antiinflammatory drugs (NSAIDs) including aspirin, ibuprofen, indomethacin (20), naproxen (21), and flurbiprofen (22), as well as sulfinpyrazone (23), all of which inhibit formation of PGs and TX, were shown to enhance post-ischemic ventricular recovery (20,23) and reduce myocardial infarct size (21,22,24) after reperfusion. The protective role of indomethacin is controversial. Another study reported that a pretreatment with indomethacin exacerbates cardiac ischemia (25). For the moment, little is known about the effect of COX or TX synthase inhibitors on endothelial coronary function during ischemia.

The first aim of this study was therefore to evaluate whether COX inhibitors afford protection against ischemic injury to the endothelium of coronary vessels in the isolated rat heart. The second aim was to identify the mechanisms whereby these agents provide this protection. One mechanism postulated to explain this protective effect of COX was through inhibition of TX formation.

METHODS

Preparation of hearts

The investigation was performed in accordance with the Canadian Council on Animal Care. Male Sprague-Dawley rats (300-350 g) were narcotized with CO_2 until complete loss of consciousness and promptly decapitated. The thorax was rapidly opened, and the heart excised and immersed in ice-cold heparinized buffer (10 IU/ml). It was immediately mounted on the experimental setup and perfused at constant flow by means of a digital roller pump. A 20-ml compliance chamber along the perfusion line ensured a continuous flow. The flow rate was adjusted during the first part of the experiment to obtain a coronary perfusion pressure of ~75 mm Hg. The flow rate was held constant, and the perfusion pressure was monitored to calculate coronary resistance. Flow rate was measured during all the experiments with an in-line ultrasonic flow probe and meter (model T106; Transonic Systems Inc., Ithaca, NY, U.S.A.). The normal perfusion solution consisted of a modified Krebs-Henseleit buffer containing (in mM): NaCl, 118; KCl, 4; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1; $NaHCO_3$, 24; D-glucose, 5; and pyruvate, 2. The perfusate was gassed with 95% O_2/5% CO_2 (pH 7.4) and kept at a constant temperature of 37°C. All drugs were administered through a Y-connector in the aortic cannula with syringe pumps (model 11; Harvard Apparatus, South Natick, MA, U.S.A.) at 1/100 of the coronary flow rate. Adequate mixing of the drugs was ensured by the turbulent flow created in the reverse drop-shaped aortic cannula. All concentrations mentioned in the text and figures refer to the final concentration after mixing. Coronary perfusion pressure was measured with a pressure transducer connected to a side arm of the aortic perfusion cannula. Isovolumetric left ventricular pressure and its first derivative (dP/dt) was measured with a fluid-filled latex balloon inserted into the left ventricle and connected to a second pressure transducer. The volume of the balloon was adjusted to obtain a diastolic pressure of between 5 and 10 mm Hg. Heart rate was derived from the left ventricular pressure trace by a tachograph. Data were recorded on a polygraph system (Grass model 79 polygraph; Astra-Med Inc., West Warwick, R.I.).

Experimental protocols

The animals were randomized into 10 groups, which were exposed to two different experimental protocols (Fig. 1). The hearts in all groups were subjected to a 20-min stabilization period. Drug or vehicle infusion was then started, followed by an additional 30-min perfusion period. The ischemic groups were subjected to a partial ischemia (flow rate, 1 ml/min) before a 20-min reperfusion period. The sham groups were not exposed to ischemia/reperfusion, but to a time-matched normal perfusion. After these periods, coronary arteries were precontracted with 0.1 μM U-46619 administered throughout the end of the experiment. Fifteen minutes after the beginning of U-46619 infusion, the endothelial function was evaluated by the vasodilation produced by 10 μM serotonin (5-HT), whereas coronary smooth muscle function was evaluated with 3 μM sodium nitroprusside (SNP). These infusions were maintained for 10 min, which was long enough to reach a steady state. A washout period of 10 min was allowed between each infusion. Vasodilation was evaluated by computing percentage changes in coronary resistance (coronary perfusion pressure divided by coronary flow), measured immediately before each drug infusion, and after a new steady state.

Graphic

FIG. 1. Diagram showing the different experimental protocols. Each experiment started with a 20-min stabilization period, followed by infusion of either diclofenac (diclo, 1 μM), indomethacin (indo, 10 μM), 6-methoxy-2-naphthyl acetic acid (6-MNA, 30 μM), or vehicle. Hearts in the ischemia protocol underwent 30 min of low-flow (1 ml/min) ischemia and 20 min of reperfusion. For each group, endothelial and smooth muscle function was tested after the 20-min reperfusion period. Hearts in the sham protocol were used as time-matched controls. Coronary arteries were precontracted by a continuous infusion of 0.1 μM U-46619. After 15 min, infusion of 5-HT (10 μM) was started for 10 min. A washout

period of 10 min was allowed between 5-HT and SNP (3 μM, 10 min) infusions. Effluent samples were collected after stabilization period (T-30), before ischemia (T0), at the end of ischemia (T29), and 30 s after the beginning of reperfusion (T30.5). The horizontal rule at the bottom of the figure represents time in minutes. Numbers between parenthesis indicate the numbers of rats in each group.

Sham and ischemic hearts were treated with either 1 μM diclofenac, a COX-1 and COX-2 inhibitor (26); 10 μM indomethacin, a COX-1 inhibitor (26); 30 μM 6-methoxy-2-naphthyl acetic acid (6-MNA), a somewhat more selective inhibitor for COX-2 than for COX-1 (27); 10 μM 1-(7-carboxyheptyl)imidazole (1-7-CHI), a TX synthase inhibitor (28); or vehicles, starting after the 20-min stabilization period and lasting throughout the ischemic period.

TXB_2, 6-keto $PGF_{1[alpha]}$, and PGE_2 determination in coronary effluent

Coronary effluent samples were collected 4 times for each protocol (after the 20-min stabilization period, just before ischemic period, 29 min after the beginning of ischemic period, and 30 s after the beginning of reperfusion (Fig. 1).

TXB_2, 6-keto $PGF_{1[alpha]}$ (stable hydrolysis products of TXA_2 and PGI_2, respectively), and PGE_2 in coronary effluent samples were measured by enzyme immunoassays (EIAs) (Biotrak; Amersham, Life Science, Buckinghamshire, U.K.).

Statistical analysis

Values represent the mean ± SEM. Statistical significance of differences between means was evaluated by a two-way analysis of variance with Scheffé post hoc test. In the presence of an interaction between the different groups, one-way analyses of variance were used for each group. A commercially available software program (Systat for Windows) was used. Only probability values (p) <0.05 were considered to be statistically significant.

Drugs

6-MNA was a kind gift of SmithKline Beecham Pharma. 1-7-CHI was bought from Tocris (Ballwin, MO, U.S.A.). All other drugs were obtained from Sigma-Aldrich (Mississauga, Ontario, Canada). Indomethacin (1 mM) was prepared in 600 μl 100% dimethylsulfoxide (DMSO) and 30 ml of Krebs-Henseleit buffer. 6-MNA, 3 mM, was dissolved in 300 μl 10 mM NaOH, this solution being added directly to 30 ml H_2O. U-46619 (28.5 mM) was dissolved in 100% ethanol and diluted with Krebs-Henseleit buffer to obtain the desired final concentration. DMSO (0.02%), and NaOH (0.1 mM), at the concentrations obtained in the final dilutions, had no effect on any of the hemodynamic variables studied and on the dilator responses to 5-HT and SNP. All the other drugs were dissolved in Krebs-Henseleit buffer.

RESULTS

Vascular function

Untreated groups. Coronary resistance measured just before 0.1 μM U-46619 perfusion (n = 18) was 5.94 ± 0.29 mm Hg · min/ml, for a coronary flow rate of 6.72 ± 0.22 ml/min/g (mean heart weight of 1.90 ± 0.05 g). Infusion of U-46619 induced a significant ($p < 0.05$) vasoconstriction in all groups of hearts (coronary resistance of 10.10 ± 0.82 and 11.53 ± 0.75 mm Hg · min/ml in sham and ischemia groups, respectively). Perfusion of 10 μM 5-HT produced a diminution in coronary resistance of -25.2 ± 4.8% in the sham group, and 30 min of partial ischemia significantly diminished the 5-HT-induced vasodilation by more than half (Fig. 2A). Endothelium-independent vasodilation to 3 μM SNP was not affected by ischemia and was found to be comparable in the two groups of hearts (sham and ischemic; Fig. 2B).

Graphic

FIG. 2. Change in coronary resistance ([DELTA]%) induced by 10 μM serotonin (5-HT; **A, C, E,** and **G**) and 3 μM sodium nitroprusside (SNP; **B, D, F,** and **H**) in untreated hearts **(A, B)**, in diclofenac-pretreated hearts **(C, D)**, in indomethacin-pretreated hearts **(E, F)**, and in 6-MNA-pretreated hearts **(G, H).** Open and solid columns represent sham and ischemic hearts. *p < 0.05, compared with sham hearts.

Diclofenac-treated groups. Inhibition of COX-2 and COX-1 with diclofenac (1 μM) was accompanied by a small but nonsignificant increase in coronary resistance when measured just before 0.1 μM U-46619 perfusion (6.77 ± 0.29 vs. 5.94 ± 0.29 mm Hg · min/ml in untreated hearts; $p > 0.05$). The perfusion rate was 5.72 ± 0.24 ml/min/g (mean heart weight, 1.98 ± 0.03 g). Infusion of U-46619 (0.1 μM, n = 17) induced a significant ($p < 0.05$) vasoconstriction in all diclofenac-treated groups (coronary resistance, 11.73 ± 0.08 and 11.96 ± 0.64 mm Hg · min/ml in sham (n = 8) and ischemic (n = 9) groups, respectively). Vasodilation produced by 10 μM 5-HT (-30.2 ± 6.2% in sham hearts, n = 8) was almost totally preserved in the ischemic group (n = 9; Fig. 2C). Vasodilation to 3 μM SNP was comparable in the two diclofenac-treated groups (sham and ischemic; Fig. 2D).

Indomethacin-treated groups. Inhibition of COX-1 with indomethacin (10 μM) produced no significant increase in coronary resistance when measured just before 0.1 μM U-46619 perfusion (6.04 ± 0.36 mm Hg · min/ml, n = 7 vs. 5.94 ± 0.29 mm Hg · min/ml, n = 8 in untreated hearts; $p > 0.05$). The perfusion rate was 5.39 ± 0.21 ml/min/g (mean heart weight, 2.28 ± 0.07 g). Infusion of U-46619 (0.1 μM, n = 15) induced a significant ($p < 0.05$) vasoconstriction in all indomethacin-treated groups (coronary resistance, 10.27 ± 0.67 and 10.75 ± 0.85 mm Hg · min/ml in sham (n = 7) and ischemic (n = 8) groups, respectively). Vasodilation produced by 10 μM 5-HT (-33.7 ± 2.7% in sham hearts, n = 7) was reduced by more than half in the ischemic group (Fig. 2E). Vasodilation to 3 μM SNP was comparable in the two indomethacin-treated groups (sham and ischemic; Fig. 2F).

6-MNA-treated groups. The somewhat more selective inhibition of COX-2 than COX-1 with 6-MNA (30 μM) was accompanied by a small but nonsignificant increase in coronary resistance when measured just before 0.1 μM U-46619 perfusion (7.00 ± 0.52 vs. 5.94 ± 0.29 mm Hg · min/ml in untreated hearts; $p > 0.05$). The perfusion rate was 5.52 ± 0.29 ml/min/g (mean heart weight, 2.03 ± 0.10 g). Infusion of U-46619 (0.1 μM, n = 10) induced a significant ($p < 0.05$) vasoconstriction in all 6-MNA-treated groups (coronary resistance, 11.11 ± 1.14 and 13.03 ± 1.02 mm Hg · min/ml in sham (n = 4) and ischemic (n = 6) groups, respectively). Vasodilation produced by 10 μM 5-HT (-24.1 ± 8.2% in sham hearts, n = 4) was almost totally preserved in the ischemic group (n = 6; Fig. 2G). Vasodilation to 3 μM SNP was comparable in the two 6-MNA-treated groups (sham and ischemic; Fig. 2H).

1-7-CHI-treated groups. Inhibition of thromboxane synthase with 1-7-CHI (10 μM) produced a significant increase in coronary resistance when measured just before 0.1 μM U-46619 perfusion (8.05 ± 0.81 mm Hg · min/ml, n = 6 vs. 5.94 ± 0.29 mm Hg · min/ml in untreated hearts; $p < 0.05$). The perfusion rate was 5.64 ± 0.21 ml/min/g (mean heart weight, 2.10 ± 0.14 g). Infusion of U-46619 (0.1 μM, n = 15) induced a significant ($p < 0.05$) vasoconstriction in all 1-7-CHI-treated groups (coronary resistance, 13.04 ± 1.56 and 11.39 ± 0.88 mm Hg · min/ml in sham (n = 6) and ischemic (n = 6) groups, respectively). Vasodilation produced by 10 μM 5-HT (-35.9 ± 4.9% in sham hearts, n = 6) was preserved in the ischemic group (-32.7 ± 4.1%, n = 6). Vasodilation to 3 μM SNP was comparable in the two 1-7-CHI-treated groups (-38.7 ± 6.3%, n = 6 and -39.7 ± 3.8, n = 6 in sham and ischemic groups, respectively).

Myocardial function

The inotropic characteristics of hearts pretreated with diclofenac, indomethacin, 6-MNA, and 1-7-CHI were comparable to those of untreated hearts: dP/dt_{max} values measured before the 30-min low-flow ischemia were 1,980 ± 449 (n = 9), 1,808 ± 190 (n = 9), 1,775 ± 119 (n = 8), 1,508 ± 47 (n = 6), and 1,625 ± 73 mm Hg/s (n = 6) for untreated, and hearts pretreated with diclofenac, indomethacin, 6-MNA, and 1-7-CHI, respectively. Low-flow ischemia was accompanied by a severe reduction in dP/dt_{max} (Fig. 3) in all groups. Indomethacin and 6-MNA pretreatment improved early postischemic dP/dt_{max} (Fig. 3B and C), but the other treatments had no effect (Fig. 3A).

Graphic

FIG. 3. Change in dP/dt_{max} ([DELTA]%) observed during 30-min low-flow ischemia (1 ml/min) and 20-min reperfusion in diclofenac **(top),** indomethacin **(middle),** and in 6-MNA-pretreated hearts **(bottom).** Solid circles and open triangles represent untreated ischemic and pretreated ischemic hearts, respectively. *p < 0.05 compared with untreated ischemic hearts.

Arachidonic acid cascade products in coronary effluent

Thromboxane B_2. Before COX or TX synthase inhibitor pretreatment (T-30), TXB_2 in the coronary effluent amounted to 74.7 ± 6.4 pM, n = 21 (Fig. 4). Treatment with diclofenac, 1-7-CHI, or indomethacin produced a nonsignificant decrease in TXB_2 just before the ischemic period (T0). At the end of the low-flow ischemia (T29), levels of TXB_2 were significantly increased in the ischemic nontreated group (n = 3) versus the sham untreated group (n = 3; $p < 0.05$). This increase in TXB_2 levels was partially but significantly blocked by all COX (n = 4, 3, 3) and TX (n = 3) inhibitors ($p < 0.05$). After 30 s of reperfusion (T30.5) no statistically significant difference between treatments was found.

Graphic

FIG. 4. Coronary effluent concentration of TXB_2 after stabilization period (T-30), before ischemia (T0), at the end of ischemia (T29), and 30 s after the beginning of reperfusion (T30.5). *p < 0.05 compared with the indicated group(s).

6-Keto PGF$_{1[alpha]}$. Before COX or TX synthase inhibitor pretreatment (T-30), 6-keto $PGF_{1[alpha]}$ measured in the coronary effluent was 654 ± 56 p*M*, n = 21 (Fig. 5). Treatment with 6-MNA, diclofenac, and indomethacin, but not 1-7-CHI, produced a significant decrease in 6-keto $PGF_{1[alpha]}$ levels just before the ischemic period (T0) ($p < 0.05$). At the end of the low-flow ischemia (T29), levels of 6-keto $PGF_{1[alpha]}$ were significantly increased in the ischemic nontreated group (n = 3) versus the sham untreated group (n = 3; $p < 0.05$). This increase in 6-keto $PGF_{1[alpha]}$ levels was partially but significantly blocked by all COX (n = 4, 3, 3) inhibitors at times T29 and T30.5 ($p < 0.05$).

Graphic

FIG. 5. Coronary effluent concentration of 6-keto $PGF_{1[alpha]}$ after stabilization period (T-30), before ischemia (T0), at the end of ischemia (T29), and 30 s after the beginning of reperfusion (T30.5). *p < 0.05 compared with the indicated group(s).

PGE$_2$. Before COX or TX synthase inhibitor pretreatment (T-30), PGE_2 in the coronary effluent amounted to 255 ± 31 p*M*, n = 21 (Fig. 6). Treatment with indomethacin abolished significantly ($p < 0.05$) the PGE_2 concentration just before the ischemic period (T0). At the end of the low-flow ischemia (T29), levels of PGE_2 were significantly increased in the ischemic nontreated group (n = 3) versus the sham untreated group (n = 3; $p < 0.05$). This increase in PGE_2 levels was partially but significantly blocked by all COX (n = 4, 3, 3) and TX synthase (n = 3) inhibitors ($p < 0.05$). The levels of PGE_2 measured in indomethacin-pretreated group (n = 3) were significantly lower than those observed in the other treated groups ($p < 0.05$). After 30 s of reperfusion (T30.5), once again, levels of PGE_2 measured in indomethacin-pretreated group (n = 3) were significantly lower than those observed in the other treated groups ($p < 0.05$).

Graphic

FIG. 6. Coronary effluent concentration of PGE_2 after stabilization period (T-30), before ischemia (T0), at the end of ischemia (T29), and 30 s after the beginning of reperfusion (T30.5). *p < 0.05 compared with the indicated group(s).

DISCUSSION

In this study, we evaluated whether COX inhibitors, in addition to their well-known protective effect on myocardial function, could also prevent endothelial cell dysfunction induced by ischemia/reperfusion injury in resistance coronary arteries. The contribution of TXs in the deleterious effect of ischemia and reperfusion also was evaluated. The major findings of this study were (a) pretreatment with COX inhibitors like 6-MNA and diclofenac, but not indomethacin, and with a TX synthase inhibitor like 1-7-CHI perfused before ischemia prevented endothelial dysfunction produced by ischemia/reperfusion; (b) an increased concentration of TXs was observed during ischemia; and (c) inhibition of this increase in TX concentration by COX and TX synthase inhibitors could explain partially that protection.

The concentrations of the different inhibitors used in this study were selected according to the current literature. Meade et al. (27) reported that indomethacin at 10 μ*M* and 6-MNA at 30 μ*M* were completely selective for COX-1 and COX-2, respectively, and inhibited 80% of the activity of their respective target enzyme in COS-1 cells. Likewise, diclofenac at 1 μ*M* inhibits ~80% of COX-1 and COX-2 activities (26). In bovine platelets, 1-7-CHI at 10 μ*M* inhibits 92% of TX synthase (29). Therefore all inhibitors, at the concentrations used in our study, were, according to the literature, equipotent regarding their respective target enzymes. However, indomethacin was more potent in reducing PGE_2 concentration than were the other COX inhibitors. Furthermore, 1-7-CHI reduced PGE_2 concentration on reperfusion. Therefore additional effects of these inhibitors, as on PGE_2 isomerase, cannot be ruled out.

In this study, the vasodilation to 5-HT was used as an index of endothelial function. 5-HT has been shown to be an endothelium-dependent vasodilator in several isolated vessel preparations (30,31) as well as in isolated rat hearts (32). In isolated hearts, the coronary vasodilation to 5-HT is blocked after treatment with NO-synthase inhibitors (31,32). Thus the vasodilatory response to 5-HT is indicative of the ability of endothelial cells to generate and release NO. Our data show that endothelium-dependent vasodilation of resistance coronary arteries to 5-HT is drastically decreased after ischemia/reperfusion injury. However, the same vessels retained the ability to dilate to SNP, an endothelium-independent vasodilator. This indicates that, under these conditions, ischemia/reperfusion altered selectively the functionality of the endothelium without affecting that of smooth muscle cells. Pretreatment with COX inhibitors, 6-MNA and diclofenac, but not indomethacin, prevented the reduction in the vasodilation to 5-HT, suggesting that these agents can protect endothelial function in resistance coronary arteries against the deleterious effect of ischemia/reperfusion. A protective effect similar to that of COX inhibitors was observed with the TX synthase inhibitor, 1-7-CHI. To our best knowledge, this is the first report of a protective effect of COX inhibitors against endothelial dysfunction in resistance coronary arteries.

Role of TXs in deleterious effect of ischemia/reperfusion

In the untreated groups, ischemia was accompanied by an important increase in TXB_2 levels measured in the coronary effluent. This increase in TXB_2 was correlated with impairment of endothelial function, as reflected by the reduction of endothelium-dependent vasodilation to 5-HT. The unaltered dilator response to SNP in all untreated groups implies that the decreased vasodilation to 5-HT in untreated groups was not the result of a nonspecific smooth muscle dysfunction. Pretreatment with COX and TX synthase inhibitors, 30 min before and during ischemia, blocked the increase in TXB_2 concentration and preserved the endothelial function against ischemia, with the notable exception of indomethacin. These data suggest that elevated TX concentration is one of the mechanisms involved in the deleterious effect of ischemia on endothelial function observed with ischemia/reperfusion.

TXA_2 is known to be an important mediator in acute myocardial ischemia due to its well-recognized and marked vasoconstrictor, platelet-aggregatory, and membrane-lytic effects (16,33). Complete inhibition of TXs synthesis reduced infarct size (17,34) and prevented ischemia-induced arrhythmias (35). It is now clear that TXA_2 is important in myocardial injury, but not directly to the endothelium. The beneficial effect of TX synthase inhibitors on myocardium might be attributed to decreased formation and inhibition of action of thromboxanes, as suggested by several studies (17,36). Platelets and mast cells are the major source of TXs (36). Under our experimental conditions, there are no platelets and few or no tissue-trapped mast cells. In this study with isolated hearts, TXs may be produced by the endothelium, smooth muscle, or cardiac myocytes, with 1-7-CHI acting directly on these tissues.

PGs and TXs release, observed during either anoxia or ischemia and reperfusion, can be abolished by pretreatment with COX inhibitors. Several groups reported similar observations with indomethacin, aspirin, and meclofenate (10,20). This reduction in TXs should mimic the protective role of the TX synthase inhibitor. However, in our study, this holds true only for two of the three COX inhibitors tested (6-MNA and diclofenac). This apparent contradiction has been reported in the past by other research groups: one group reported that indomethacin may increase infarct size in dogs (25), with another group demonstrating that ibubrofen reduces infarct size, whereas aspirin tends to increase it (37). Opposite effects of indomethacin and ibuprofen on infarct size have also been reported experimentally (38) and clinically (39). To explain the opposite effect of indomethacin and ibuprofen, it was postulated that ibuprofen was a weaker inhibitor of COX than indomethacin (40). Based on these data, it was assumed that ibuprofen would inhibit PGI_2 formation less extensively than indomethacin (41). However, this was not the case in our study.

In our experimental model, measurement of PGE_2 in the coronary effluent could partially explain the differences observed between COX inhibitors. Indomethacin, but not 6-MNA and diclofenac, reduced the concentration of PGE_2 to near-undetectable levels in the isolated rat heart, and this could in part explain the absence of protective effect of indomethacin on endothelial function during ischemia. In favor of this hypothesis, some authors postulated that endogenous prostaglandins (most probably PGI_2 and PGE_2) exert a "braking effect" on the adrenergic responsiveness of coronary arterial smooth muscle and thereby result in a vasodilation (42) with a redistribution of coronary flow during ischemia. Furthermore, we recently observed that a perfusion with 3 n*M* PGE_2 for 15 min before ischemia protects the endothelial function in a similar experimental model (J-F. Bouchard and D. Lamontagne, unpublished observation).

The mechanism by which PGE_2 could protect the endothelial function is still largely unknown. We reported earlier that PGE_2 can activate adenosine triphosphate (ATP)-sensitive potassium channels (43). These channels have been involved in the endothelial protective effect of ischemic preconditioning (44). However, we recently observed that the endothelial protective effect of exogenous PGE_2 in the isolated rat heart was not antagonized by the K_{ATP} channel blocker, glibenclamide (J-F. Bouchard and D. Lamontagne, unpublished data).

Alternatively, EP_1 receptors activate phospholipase C to release inositol 1,4,5-trisphosphate and 1,2-diacylglycerol (45). The latter compound in combination with intracellular calcium then causes the translocation and activation of protein kinase C (PKC) (46). Activated PKC may phosphorylate secondary effectors, which would be responsible for the protective effects of PGE_2. In support of that, treatment of isolated rat hearts with chelerythrine prevents the endothelial protective effect of exogenous PGE_2 (J-F. Bouchard and D. Lamontagne, unpublished data). PGE_2 can also act on EP_2 receptors, stimulating the production of cyclic adenosine 3',5'-monophosphate (AMP_c) and inducing vasodilation (45).

Effect of COX and TX synthase inhibitors on myocardial function

In our study, dP/dt_{max} and dP/dt_{min}, which represent the capacity of the ventricle to contract during systole and its ability to relax during diastole, were used to evaluate the contractile function of the hearts. These variables recovered rapidly and completely within the 20-min reperfusion period. Furthermore, indomethacin and 6-MNA improved the dP/dt_{max} only during early reperfusion, whereas all the others failed to improve ischemic or postischemic ventricular recovery. The inability of these COX and TX synthase inhibitors to improve ventricular recovery is most probably due to the fact that our ischemic conditions are too mild to depress the contractile function severely, leaving little room for improvement.

The advent of therapeutic capability for restoring blood flow to ischemic myocardium has stimulated clinical and experimental interest regarding cardiac effects of ischemia and reperfusion. Preservation of myocardial and endothelial function during ischemia and reperfusion is one of the major priorities of modern medicine. The future of the described drugs promises to be fruitful, not only to unlock the mechanism of important humoral mediators such as PGs, but also to provide the care giver with new therapeutic interventions in acute, life-threatening disease states, such as myocardial infarction, reperfusion injury, coronary spasm, and angina pectoris.

In conclusion, these data suggest that 6-MNA, diclofenac, but not indomethacin perfusions, before and during ischemia, afford protection to the endothelial function against ischemia in resistance coronary arteries. The similar protective effect of 1-7-CHI, a specific inhibitor of TX synthase, and the decreased concentration of TXs during perfusion of COX and TX synthase inhibitors, suggest the implication of TXs in the deleterious effect of ischemia. In our conditions, the lack of protective effect of indomethacin may be due not to an incapacity to inhibit the synthesis of TXs, but to its important reduction in PGE_2 concentration during ischemia and early reperfusion.

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REFERENCES

1. Mullane KM, Read N, Salmon JA, Moncada S. Role of leukocytes in acute myocardial infarction in anesthetized dogs: relationship to myocardial salvage by anti-inflammatory drugs. *J Pharmacol Exp Ther* 1984;228:510-22. **Bibliographic Links** | [Context Link]

2. Prinzen FW, van der Vusse GJ, Arts T, Roemen TH, Coumans WA, Reneman RS. Accumulation of nonesterified fatty acids in ischemic canine myocardium. *Am J Physiol* 1984;247:H264-72. **Bibliographic Links** | [Context Link]

3. van Bilsen M, Engels W, van der Vusse GJ, Reneman RS. Significance of myocardial eicosanoid production. *Mol Cell Biochem* 1989;88:113-21. **Bibliographic Links** | [Context Link]

4. Ahumada GG, Sobel BE, Needleman P. Synthesis of prostaglandins by cultured rat hearts myocytes and cardiac mesenchymal cells. *J Mol Cell Cardiol* 1980;12:685-700. **Bibliographic Links** | [Context Link]

5. Alexander RW, Kent KM, Pisano JJ, Keiser HR, Cooper T. Regulation of postocclusive hyperemia by endogenously synthesized prostaglandins in the dog heart. *J Clin Invest* 1975;55:1174-81. **Bibliographic Links** | [Context Link]

6. Coker SJ, Marshall RJ, Parratt JR, Zeitlin IJ. Does the local myocardial release of prostaglandin E_2 or F_{2alpha} contribute to the early consequences of acute myocardial ischaemia? *J Mol Cell Cardiol* 1981;13:425-34. **Bibliographic Links** | [Context Link]

7. Karmazyn M. Synthesis and relevance of cardiac eicosanoids with particular emphasis on ischemia and reperfusion. *Can J Physiol Pharmacol* 1989;67:912-21. **Bibliographic Links** | [Context Link]

8. Bend JR, Karmazyn M. Role of eicosanoids in the ischemic and reperfused myocardium. *Experientia Suppl* 1996;76:243-62. **Bibliographic Links** | [Context Link]

9. Karmazyn M. The 1990 Merck Frosst Award: ischemic and reperfusion injury in the heart: cellular mechanisms and pharmacological interventions. *Can J Physiol Pharmacol* 1991;69:719-30. **Bibliographic Links** | [Context Link]

10. Coker SJ, Parratt JR, Ledingham IM, Zeitlin IJ. Thromboxane and prostacyclin release from ischaemic myocardium in relation to arrhythmias. *Nature* 1981;291:323-4. **Bibliographic Links** | [Context Link]

11. Engels W, van Bilsen M, De Groot MJ, et al. Ischemia and reperfusion induced formation of eicosanoids in isolated rat hearts. *Am J Physiol* 1990;258:H1865-71. **Bibliographic Links** | [Context Link]

12. Rabinowitz B, Arad M, Shotan A, Klein R, Har ZY, Elazar E. Effects of brief and prolonged ischemia on eicosanoid synthesis in dog and rat hearts. *J Basic Clin Physiol Pharmacol* 1995;6:39-52. **Bibliographic Links** | [Context Link]

13. Woditsch I, Schror K. Prostacyclin rather than endogenous nitric oxide is a tissue protective factor in myocardial ischemia. *Am J Physiol* 1992;263:H1390-6. **Bibliographic Links** | [Context Link]

14. Schmitz JM, Apprill PG, Buja LM, Willerson JT, Campbell WB. Vascular prostaglandin and thromboxane production in a canine model of myocardial ischemia. *Circ Res* 1985;57:223-31. **Bibliographic Links** | [Context Link]

15. Grover GJ, Parham CS. Role of thromboxane A_2 in the control of myocardial O_2 supply/consumption balance and severity of ischemia during pacing-induced ischemia. *Circ Res* 1989;64:575-82. **Bibliographic Links** | [Context Link]

16. Lefer AM, Darius H. A pharmacological approach to thromboxane receptor antagonism. *Fed Proc* 1987;46:144-8. **Bibliographic Links** | [Context Link]

17. Burke SE, Lefer AM, Smith GM, Smith JB. Prevention of extension of ischaemic damage following acute myocardial ischaemia by dazoxiben, a new thromboxane synthetase inhibitor. *Br J Clin Pharmacol* 1983;15(suppl 1):97S-101S. **Bibliographic Links** | [Context Link]

18. Sato N, Endo T, Kiuchi K, Hayakawa H. Effects of a thromboxane synthetase inhibitor, Y-20811, on infarct size, neutrophil accumulation, and arrhythmias after coronary artery occlusion and reperfusion in dogs. *J Cardiovasc Pharmacol* 1993;21:353-61. **Bibliographic Links** | [Context Link]

19. Singh J, Seth SD, Manchanda SC, Seth S. Protective actions of a thromboxane receptor antagonist, SQ 29548 on the ischemic myocardium: morphologic and hemodynamic effects. *Prostaglandins Leukot Essent Fatty Acids* 1997;56:105-10. **Bibliographic Links** | [Context Link]

20. Karmazyn M. Contribution of prostaglandins to reperfusion-induced ventricular failure in isolated rat hearts. *Am J Physiol* 1986;251:H133-40. **Bibliographic Links** | [Context Link]

21. Smith EF, Lefer AM. Stabilization of cardiac lysosomal and cellular membranes in protection of ischemic myocardium due to coronary occlusion: efficacy of the nonsteroidal anti-inflammatory agent, naproxen. *Am Heart J* 1981;101:394-402. **Bibliographic Links** | [Context Link]

22. Fiedler VB. Role of arachidonic acid metabolites in cardiac ischemia and reperfusion injury. *Pharmacotherapy* 1988;8:158-68. **Bibliographic Links** | [Context Link]

23. Karmazyn M. A direct protective effect of sulphinpyrazone on ischaemic and reperfused rat hearts. *Br J Pharmacol* 1984;83:221-6. **Bibliographic Links** | [Context Link]

24. Jugdutt BI, Hutchins GM, Bulkley BH, Becker LC. Salvage of ischemic myocardium by ibuprofen during infarction in the conscious dog. *Am J Cardiol* 1980;46:74-82. **Bibliographic Links** | [Context Link]

25. Jugdutt BI, Hutchins GM, Bulkley BH, Pitt B, Becker LC. Effect of indomethacin on collateral blood flow and infarct size in the conscious dog. *Circulation* 1979;59:734-43. **Ovid Full Text** | **Bibliographic Links** | [Context Link]

26. Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ, Vane JR. Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. *Proc Natl Acad Sci U S A* 1993;90:11693-7. **Bibliographic Links** | [Context Link]

27. Meade EA, Smith WL, DeWitt DL. Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. *J Biol Chem* 1993;268:6610-4. **Bibliographic Links** | [Context Link]

28. Kayama N, Sakaguchi K, Kaneko S, et al. Inhibition of platelet aggregation by 1-alkylimidazole derivatives, thromboxane A synthetase inhibitors. *Prostaglandins* 1981;21:543-54. **Bibliographic Links** | [Context Link]

29. Yoshimoto T, Yamamoto S, Hayaishi O. Selective inhibition of prostaglandin endoperoxide thromboxane isomerase by 1-carboxyalkylimidazoles. *Prostaglandins* 1978;16:529-40. **Bibliographic Links** | [Context Link]

30. Hoyer D, Clarke DE, Fozard JR, et al. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol Rev* 1994;46:157-93. **Bibliographic Links** | [Context Link]

31. Lamontagne D, Pohl U, Busse R. N^G-Nitro-L-arginine antagonizes endothelium-dependent dilator responses by inhibiting endothelium-derived relaxing factor release in the isolated rabbit heart. *Pflugers Arch* 1991;418:266-70. **Bibliographic Links** | [Context Link]

32. Mankad PS, Chester AH, Yacoub MH. 5-Hydroxytryptamine mediates endothelium dependent coronary vasodilatation in the isolated rat heart by the release of nitric oxide. *Cardiovasc Res* 1991;25:244-8. **Bibliographic Links** | [Context Link]

33. Ellis EF, Oelz O, Roberts LJ, et al. Coronary arterial smooth muscle contraction by a substance released from platelets: evidence that it is thromboxane A_2. *Science* 1976;193:1135-7. **Bibliographic Links** | [Context Link]

34. Wargovich TJ, Mehta J, Nichols WW, et al. Reduction in myocardial neutrophil accumulation and infarct size following administration of thromboxane inhibitor U-63,557A. *Am Heart J* 1987;114:1078-85. **Bibliographic Links** | [Context Link]

35. O'Connor KM, Friehling TD, Kowey PR. The effect of thromboxane inhibition on vulnerability to ventricular fibrillation in the acute and chronic feline infarction models. *Am Heart J* 1989;117:848-53. **Bibliographic Links** | [Context Link]

36. Shekher A, Singh M. Role of eicosanoid inhibition of ischemia reperfusion injury: intact and isolated rat heart studies. *Methods Find Exp Clin Pharmacol* 1997;19:223-9. **Bibliographic Links** | [Context Link]

37. Flynn PJ, Becker WK, Vercellotti GM, et al. Ibuprofen inhibits granulocyte responses to inflammatory mediators: a proposed mechanism for reduction of experimental myocardial infarct size. *Inflammation* 1984;8:33-44. **Bibliographic Links** | [Context Link]

38. Jugdutt BI, Becker LC. Prostaglandin inhibition and myocardial infarct size. *Clin Cardiol* 1981;4:117-24. **Bibliographic Links** | [Context Link]

39. Jugdutt BI, Basualdo CA. Myocardial infarct expansion during indomethacin or ibuprofen therapy for symptomatic post infarction pericarditis. influence of other pharmacologic agents during early remodelling. *Can J Cardiol* 1989;5:211-21. **Bibliographic Links** | [Context Link]

40. Kaplan HB, Edelson HS, Korchak HM, Given WP, Abramson S, Weissman G. Effects of non-steroidal anti-inflammatory agents on human neutrophil functions in vitro and in vivo. *Biochem Pharmacol* 1984;33:371-8. **Bibliographic Links** | [Context Link]

41. Hohlfeld T, Strobach H, Schror K. Stimulation of endogenous prostacyclin protects the reperfused pig myocardium from ischemic injury. *J Pharmacol Exp Ther* 1993;264:397-405. **Bibliographic Links** | [Context Link]

42. Rubanyi G, Vanhoutte PM. Inhibitors of prostaglandin synthesis augment beta-adrenergic responsiveness in canine coronary arteries. *Circ Res* 1985;56:117-25. **Ovid Full Text** | **Bibliographic Links** | [Context Link]

43. Bouchard J-F, Dumont E, Lamontagne D. Evidence that prostaglandins I_2, E_2, and D_2 may activate ATP sensitive potassium channels in the isolated rat heart. *Cardiovasc Res* 1994;28:901-5. **Bibliographic Links** | [Context Link]

44. Bouchard J-F, Lamontagne D. Mechanisms of protection afforded by preconditioning to endothelial function against ischemic injury. *Am J Physiol* 1996;271:H1801-6. [Context Link]

45. Campbell WB, Halushka PV. Lipid-derived autacoids: eicosanoids and platelet-activating factor. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Goodman Gilman A, eds. *Goodman and Gilman's the pharmacological basis of therapeutics*. 9th ed. New York: McGraw-Hill, 1996:601-16. [Context Link]

46. Ren J, Karpinski E, Benishin CG. The actions of prostaglandin E_2 on potassium currents in rat tail artery vascular smooth muscle cells: regulation by protein kinase A and protein kinase C. *J Pharmacol Exp Ther* 1996;277:394-402. **Bibliographic Links** | [Context Link]

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