Effect of COX inhibitors and NO on renal hemodynamics following ischemia-reperfusion injury in normotensive and hypertensive rats

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Knight, Sarah, and Edward J. Johns. Effect of COX inhibitors and NO on renal hemodynamics following ischemia-reperfusion injury in normotensive and hypertensive rats. Am J Physiol Renal Physiol 289: F1072–F1077, 2005. First published June 14, 2005; doi:10.1152/ajprenal.00430.2004.—The processes involved in the renal damage resulting from ischemia-reperfusion injury are poorly understood. This study examined the contribution of prostaglandins and nitric oxide (NO) in the vascular responses to ischemia-reperfusion injury in the kidneys of normotensive and hypertensive rats. Groups of Wistar and stroke-prone spontaneously hypertensive rats (SHRSP) were dosed with polyethylene glycol vehicle, aspirin (53.5 mg·kg⁻¹·day⁻¹), NO-aspirin (100 mg·kg⁻¹·day⁻¹), or celecoxib (10 mg·kg⁻¹·day⁻¹) for 7 days. On day 7, rats were anesthetized with chloralose/urethane and the left kidney was exposed to a 30-min period of ischemia followed by 90-min reperfusion. Renal cortical and medullary perfusions were monitored throughout using laser-Doppler flowmetry. In the vehicle- and celecoxib-treated Wistar rats, cortical and medullary postischemic perfusion was reduced to 66 and 62% and 53 and 62%, respectively (all P < 0.05), of baseline. The ischemia-induced reductions in cortical and medullary flux were ameliorated in the aspirin and NO-aspirin groups where flux fell to 96 and 78% and 105 and 83%, respectively (P < 0.05). There was a fall in cortical and medullary flux in the postischemic period in the vehicle-treated SHRSP to 82 and 77% (P < 0.05). These findings show that nonselective cyclooxygenase (COX) inhibition, and to an even greater extent NO donation, provided protection to the renal vasculature from ischemic injury in the Wistar rat but not in the SHRSP. This would suggest that prostaglandins are less important in the development of renal ischemia-reperfusion injury during hypertension and both COX isoforms must be inhibited to offset the decrease in renal hemodynamics.

NO-NSAIDs; hypertension

RENAIISCHEMICINJURYISAMAJORCAUSEOFACUTERENALFAILURE (ARF) which carries a high mortality rate in humans (1). ARF is associated with renal vascular and tubular damage and decreased excretory function and involves the action of a number of autocrine and paracrine factors that may contribute to ischemic cell damage. During renal ischemia, there is an increase in the production of inflammatory mediators and vasoactive molecules such as ANG II (28), an increased production of free radicals (9) as well as an increase in renal prostaglandin production (26) while NO appears to play a smaller role in tubular reabsorption and vascular diameter than in normotensive strains. Preischemic endothelial function is also proposed to influence the degree of ischemic renal injury; therefore, the possibility arises that the stroke-prone SHR (SHRSP) will be less able to respond and will undergo enhanced renal damage in response to the ischemia (13).

The aim of the study was to investigate the involvement of the individual COX enzymes in renal ischemia-reperfusion injury and to elucidate the potential benefit of COX inhibition together with an NO donor. A balance will exist between the COX enzymes and NO availability which will be important in the vascular response of the kidney to ischemia raising the possibility that nonselective COX inhibition will ameliorate ischemic injury. Increased NO availability using NO-aspirin may provide additional benefit by further counteracting vasoconstriction. A second objective was to evaluate whether COX inhibition and NO had a similar impact on the renal responses to ischemia in the SHRSP.

METHODS

All procedures were performed in accordance with National guidelines and the European Community Directive 86/609/EC. The studies described herein conformed with the guidelines and practices of the University College Cork Animal Experimentation Ethical Committee. Four groups of male Wistar rats, 250–350 g, were obtained from Charles River Laboratories (Marston, Kent, UK), and four groups of male SHRSP, 250–350 g, were bred in the Biomedical Services Unit.

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Each group was dosed orally with either vehicle 0.2 ml•100 g body wt⁻¹•day⁻¹ polyethylene glycol (Sigma, Poole, Dorset, UK), aspirin (NicOx, Sophia Antipolis, France) at 53.5 mg•kg body wt⁻¹•day⁻¹, NO-aspirin (NCX-4016, NicOx) at 100 mg•kg body wt⁻¹•day⁻¹, or celecoxib (NicOx) at 10 mg•kg body wt⁻¹•day⁻¹, for 7 days before the acute experiment. Previous reports showed that the doses used were sufficient for celecoxib to block COX-2 activity by 75%, and NO-aspirin to effectively inhibit COX-1 and -2 enzymes and generate NO (21, 22). The dose of aspirin used was equimolar to that of NO-aspirin. Rats were given free access to standard chow and water until 12 h before surgery when food was restricted. On day 7, the rat was initially anesthetized with 1 ml of chloralose/urethane (1.65/25 mg/ml ip, respectively) which was maintained with supplemental doses of 0.05 ml every 30 min or as required. The left femoral vein was cannulated for the infusion of saline (150 mM NaCl at 3 ml/h) and anesthetic. The left femoral artery was cannulated to monitor blood pressure and heart rate. The left kidney was exposed via a flank incision and placed in a custom-made cup. The renal capsule was removed and one 1.5-mm diameter fiber optic probe was placed 2 mm into the renal cortex and another 5 mm into the renal medulla. The probes were connected to a calibrated laser-Doppler flowmeter using a standard colloid solution representing 100 perfusion units (PU; Perimed PF3, Stockholm, Sweden) such that 1 V was equivalent to 100 PU. The rat was allowed to stabilize for 60–120 min and measurements were started when blood pressure was stable for a minimum of 20 min. Mean blood pressure was measured over 2 min, during each perfusion measurement, at each time point. Baseline perfusion measurements were taken for 2 min in the cortex and medulla and thereafter a nontraumatic clamp was placed on the renal artery for 30 min as previously described (9). On removal of the clamp, 2-min cortical and medullary measurements were taken after 2, 10, and every 10 min for 90 min. The animal was killed with an overdose of anesthetic, background laser-Doppler measurements were taken 20 min later, and results were corrected for background. The kidney was sectioned to confirm the location of the probes.

Values are expressed as means ± SE. The difference between the experimental groups was evaluated by two-way ANOVA followed by Bonferroni and Dunn post hoc analysis; statistical significance was taken at P < 0.05.

RESULTS

Baseline variables. Body weight was similar in all groups and there was no difference between drug treatment groups or rat strain (Table 1). There was no difference in baseline mean arterial pressure (MAP) between the vehicle-, aspirin-, and NCX-4016-treated Wistar groups, but the celecoxib group had a significantly lower baseline MAP than all Wistar groups (Table 1; P < 0.05). All drug treatments appeared to reduce baseline MAP in the SHRSP, when compared with the vehicle-treated group (P < 0.05). The vehicle-, aspirin-, and celecoxib-treated SHRSP had a higher baseline MAP than the similarly drug-treated Wistar rats (Table 1; all P < 0.05). Baseline cortical perfusion was similar in all Wistar groups, except the celecoxib-treated group which was lower than the vehicle (P < 0.05). Cortical perfusion was higher than medullary perfusion in all Wistar groups (P < 0.05). Medullary perfusion under basal conditions in the aspirin- and celecoxib-treated Wistar groups were lower than that recorded in the vehicle group (P < 0.05).

Blood pressure responses to renal ischemia. The vehicle-treated SHRSP had a higher MAP than the vehicle-treated Wistar rats (Fig. 1) at baseline and throughout the reperfusion period (P < 0.05). MAP increased 29 mmHg in the vehicle-treated Wistar group during renal artery clamping, whereas there was an increase of just 5 mmHg in the vehicle-dosed SHRSP (P < 0.05). During the ischemic period and 2 min postischemia, the MAP increased from baseline in all Wistar groups (Fig. 2A). MAP was significantly lower pre- and postischemia in the celecoxib-treated Wistar group compared with the vehicle group (P < 0.05; Fig. 2A). The celecoxib-treated SHRSP also displayed a reduced MAP compared with the vehicle group, as did the NCX-4016-treated SHRSP group (P < 0.05; Fig. 2B). There was a mean increase in MAP, as a result of the clamping of the renal artery, of 15 ± 5 mmHg in the Wistar groups, whereas there was just a 7 ± 2-mmHg increase in MAP in the SHRSP groups (Fig. 2B).

Renal hemodynamic responses to ischemia in Wistar rats. The percentage changes from baseline cortical and medullary perfusions following ischemia for all groups of Wistar rats are shown in Fig. 3, A and B, respectively. In the vehicle-treated group, 2 min after clamp removal, cortical perfusion was 70% of baseline and remained depressed throughout the 90-min study period (Fig. 3A). The aspirin- and NO-aspirin-treated groups displayed a significantly greater recovery in cortical

Table 1. Baseline medullary and cortical flux in all groups

<table>
<thead>
<tr>
<th></th>
<th>Wistar</th>
<th>SHRSP</th>
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<tr>
<td></td>
<td>Vehicle</td>
<td>Aspirin</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Weight</td>
<td>315±14</td>
<td>303±9</td>
</tr>
<tr>
<td>Baseline MAP, mmHg</td>
<td>102±2</td>
<td>103±2</td>
</tr>
<tr>
<td>Baseline cortical, PU</td>
<td>174±30</td>
<td>135±22</td>
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<tr>
<td>Baseline medullary, PU</td>
<td>86±11†</td>
<td>34±6†</td>
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Data are means ± SE. MAP, mean arterial pressure; PU, perfusion units; SHRSP, stroke-prone spontaneously hypertensive rats. *P < 0.05 comparing drug treatments. †P < 0.05 comparing kidney location.
perfusion than that observed in the vehicle-treated group, reaching 100% of basal levels at 90 min ($P < 0.05$). In the aspirin-treated group, 2 min after the ischemic challenge cortical perfusion was reduced to 63% of baseline but climbed to reach basal values 30 min after removal of the clamp, and thereafter levels plateaued until 90 min when PU fell by 7%. The NO-aspirin-treated group also displayed an initial decrease in mean cortical perfusion 2 min after the ischemic period to 88% of baseline. Perfusion gradually rose to reach baseline levels 30 min after the clamp was removed, whereupon it continued to rise and exceeded basal levels and those of the aspirin group by some 10% for the remainder of the 90 min study ($P < 0.05$). The celecoxib-treated group displayed a similar pattern to the vehicle group, that is of decreased cortical perfusion as a result of the ischemic challenge, which was a greater reduction compared with that observed in the aspirin and NO-aspirin groups (both $P < 0.05$). There was a fall in cortical perfusion in the celecoxib-treated group from 2 min after the ischemic period, which rose to exceed baseline levels 30 min postischemia, reaching a peak at 60 min of 140%, finishing at 126% of baseline.

The medullary perfusion in the celecoxib- and NO-aspirin-treated groups displayed a different pattern to the cortical flux period of ischemia by some 22%. However, the depressed medullary perfusion in the postischemic period was not as marked as the vehicle- and celecoxib- and aspirin-treated groups and displayed a greater recovery during reperfusion ($P < 0.05$). Postischemic red cell flux in the vehicle group was similar in both the cortex and medulla, remaining at between 62 and 66% of baseline. By contrast, in the aspirin-treated group the medullary flux remained depressed at 78% of baseline whereas the cortical flux returned to 96% of basal flux. The NO-aspirin-treated group displayed a similar pattern to the aspirin-treated group, and the medullary flux was depressed at 83% of baseline, with cortical flux reaching 106%. There was a slightly altered pattern in the celecoxib-treated group where the mean medullary flux during reperfusion reached 62% of baseline and exceeded that of the cortex, which reached only 53%.

**Renal hemodynamic responses to ischemia in SHRSP.** There was a fall in cortical perfusion to 53–65% of baseline in all SHRSP groups 2 min after the removal of the arterial clamp (Fig. 4A). Postischemic flux increased from 2 min to reach a maximum of 90% of baseline in the aspirin- and vehicle-treated groups. By contrast, in the NO-aspirin-treated group, after the initial fall, the postischemic flux remained at ~90% of baseline until 60 min after the ischemic challenge when flux increased to reach a peak of 126% at 90 min postischemia. The celecoxib-treated group displayed an initial decrease in cortical flux, which rose to exceed baseline levels 30 min postischemia, reaching a peak at 60 min of 140%, finishing at 126% of baseline.

All groups displayed a fall in medullary red cell flux as a result of the 30-min period of renal ischemia (Fig. 3B). In the vehicle-treated group, medullary perfusion did not recover immediately following the ischemic challenge and flux remained below 70% of baseline throughout the remaining 90 min of study. The aspirin-treated group also showed a depressed red cell flux in the postischemia period, but perfusion was significantly higher than that observed in the vehicle- and celecoxib-treated groups ($P < 0.05$). Medullary perfusion in the NO-aspirin-treated group was lower than baseline after the
Levels fell to between 57 and 64% of baseline 2 min after the ischemic insult and recovered to a maximum of 67 to 71%. The vehicle and aspirin groups displayed a smaller initial fall in flux after the ischemic insult but flux fell to 83 and 62%, respectively, 90 min postischemia.

**DISCUSSION**

It was evident from the vehicle-treated Wistar rats that following the period of ischemia, there was a large reduction in perfusion in both the cortex and medulla, which persisted throughout the 90 min following the ischemic challenge. These reductions in red cell flux most likely reflected a vasoconstriction of the renal resistance vessels, namely, the interlobular arteries and the afferent and efferent arterioles. The net vasoconstriction could have resulted from an increased release of vasoactive substances such as arachidonic acid metabolites (34), endothelins, and reactive oxygen species, which have been reported to occur in response to similar ischemic challenges (9, 33). Another mechanism by which blood flow may have been lowered is by COX-1-mediated release of thromboxane from trapped, activated thrombocytes within the renal vessels (10).

Selective COX-2 inhibition caused a reduction in baseline MAP in the Wistar rats, which continued after ischemia, indicating a basal vasoconstrictor role of the COX-1 metabolites usually counteracted by COX-2 metabolites (Table 1 and Fig. 2A). Selective COX-2 inhibition also resulted in a reduced basal MAP in the SHRSP, as did nonselective inhibition with aspirin and NCX-4016, suggesting the COX metabolites play a role in the increased blood pressure of the SHRSP strain. As expected, the SHRSP had a higher basal MAP than the similarly treated Wistar rats, but this difference was abolished by NCX-4016 treatment, suggesting NO donation resulted in a vasodilatation and fall in MAP in the SHRSP (Table 1). Selective COX-2 inhibition had a deleterious effect on both cortical and medullary baseline perfusion in the Wistar rat, as did aspirin treatment on medullary perfusion, suggesting COX metabolites, particularly arising from COX-2 activity, are important in maintaining perfusion in the Wistar rat (Table 1).

In the Wistar rats, the nonselective inhibition of COX with aspirin resulted in improved cortical and medullary perfusion after the ischemic challenge, compared with that observed in the vehicle group (Fig. 3). Interestingly, NSAID treatment has been reported to have a detrimental effect on renal blood flow in renal disease states (10) but the present data suggested that

**Fig. 4.** Response of cortical (A) and medullary (B) perfusions to renal ischemia in SHRSP given either vehicle, aspirin, NO-aspirin, or celecoxib. The celecoxib-treated SHRSP had a higher postischemic flux than the other groups (*P < 0.05).

**Fig. 5.** Mean postischemic cortical (A) and medullary (B) flux, comparing Wistar and SHRSP. There was a significantly higher postischemic cortical flux in the celecoxib-treated SHRSP than the Wistar group (*P < 0.05), whereas the vehicle-treated SHRSP had a lower flux and the aspirin- and NO-aspirin-treated SHRSP had a higher medullary flux than the Wistar rats (*P < 0.05).
in this acute model, the COX metabolites play a deleterious role, as inhibition of these enzymes ameliorated the ischemic injury. The normal balance between vasoconstrictor prostaglandins, such as thromboxane (TXA), and vasodilatory prostaglandins, such as prostacyclin (PGI₂), may have been disrupted during ischemia reperfusion injury resulting in a net vasoconstriction. This view is supported by Lelcuk et al. (14), who have previously shown that a specific thromboxane synthase inhibitor had beneficial morphological effects following renal ischemia-reperfusion injury. Pretreatment with the selective COX-2 inhibitor celecoxib resulted in no net change in the regional hemodynamic responses to the ischemic challenge compared with the vehicle group; that, is both cortical and medullary perfusions were depressed. COX-2 is constitutively expressed in the cortical thick ascending and the medullary interstitial cells (7) and inflammation is associated with increased levels of renal COX-2 activity (3), but whether COX-2 activity is increased within the time frame used in the present study is unclear, but COX-2 metabolites may have contributed little to the hemodynamic effects of the acute renal ischemic challenge. The results of the present study indicated there may have been an increased production of COX-1 metabolites that contributed to the reduction in perfusion in response to renal ischemia, as it was partially prevented by aspirin pretreatment but not by celecoxib.

NO has been reported to play a role in the renal response to ischemia (33). The results from the NO-aspirin-treated group were striking in that there was a rapid return in cortical perfusion to basal levels and above and during the postischemic period. There was a similar but less pronounced effect in the medulla. The improved cortical and medullary perfusion exceeded that of the aspirin-treated group, which suggested that the donation of NO combined with nonselective COX inhibition had a greater beneficial effect on ischemia-reperfusion injury than COX inhibition alone. During the renal response to ischemia, increased reactive oxygen species can cause a breakdown in endogenous NO production and thereby remove the buffering action of NO to the increased vasoconstrictor factors such as endothelins (4) and ANG II (24, 25). It has also been proposed that endogenous generation of endothelial-derived NO is reduced in the postischemic kidney, due in part to a reduced wall shear stress (18). Therefore, the possibility exists that the increased availability of NO arising from the NO-aspirin offset the decreased production during the postischemic period and compensated for the reduced endogenous NO. The mechanisms underlying the protective effect of NO-aspirin are unclear, but the important finding from this study was that administration of NO donor NSAID largely abolished the depressed perfusion of blood through both the cortical and medullary regions of the kidney in the postischemic kidney.

Perfusion in the medulla was reduced in the vehicle- and celecoxib-treated groups and was 62% of baseline over the course of the 90-min postischemic period. This reduction in perfusion was similar to that observed in the cortex, indicating that ischemia had a comparable vasoconstrictor effect on the renal vasculature in both regions of the kidney. Pretreatment with aspirin and NO-aspirin caused a better postischemia recovery in medullary perfusion compared with the celecoxib and vehicle groups, although the magnitude of the recovery was less than that observed in the cortex. One explanation for this disparity is that the medulla may be more sensitive to ischemic damage than the cortex (15). The renal medulla contains high levels of prostaglandins, and COX inhibition has been reported to result in redistribution of blood flow from the juxtamedullary area to the cortex (23), which may account for the differences in recovery in perfusion from the ischemic insult in the aspirin-, celecoxib-, and NO-aspirin-treated groups. Together, these findings show that as a consequence of the renal artery clamping during the ischemia-reperfusion period, blood perfusion was reduced in both the cortex and medulla. Treatment with a selective COX-2 had no effect on the hemodynamic response to ischemia in either the cortex or medulla. Nonselective COX inhibition to a large extent prevented the postischemia-related reduction in perfusion, which was further improved when combined with a NO moiety. The reasons for this remain uncertain but may be as a result of interactions between COX products, endothelins, and reactive oxygen species.

There was a postischemic fall in cortical red cell flux in the vehicle- and aspirin-treated SHRSP, indicating that nonselective COX inhibition afforded little protection to the vasculature from the ischemic insult (Fig. 4). NO donation combined with COX enzyme inhibition produced a postischemic increase in flux from basal levels. One possibility is that the NO supplied by the NO-aspirin counteracted the effect of NO depletion caused by the damaged endothelium following the ischemic insult (18). Increased NO availability may also have caused a reduction in ROS concentrations, resulting in reducing vasoconstriction. COX-2 is present in small quantities in the normal kidney, but expression is upregulated during ischemia (27). Selective COX-2 inhibition counteracted the postischemic reduction in cortical flux in the SHRSP, an effect that was not apparent with the nonselective inhibition of COX. This suggests that the balance between vasoactive prostaglandins may have been altered, resulting in an overall vasodilation. All SHRSP groups experienced a reduction in postischemic medullary flux. The vasodilation that was observed in the cortical circulation in the celecoxib- and NO-aspirin-treated groups was not apparent in the medullary vasculature (Fig. 4). This data indicated that the inhibition of COX-2 and NO donation was not sufficient to prevent the medullary ischemic injury.

There were similarities in the vascular responses of the two strains to renal ischemia-reperfusion injury, but the effect of COX inhibition and NO donation yielded different responses. COX-2 inhibition had a greater beneficial effect on cortical flux in the SHRSP than the Wistar rats, suggesting that COX-2 played a greater role in the development of cortical ischemic injury in the hypertensive animals (Fig. 4). Aspirin and NO-aspirin pretreatment provided greater medullary vascular protection to ischemia in the Wistar rats than in the SHRSP (Fig. 5). One interpretation of these findings is that prostaglandins play a more important role in the development of ischemic injury in the Wistar than the SHRSP. The postischemic medullary vasculature of the Wistar rat may have greater depletion of endogenous NO or be more sensitive to NO than the SHRSP. It is well established that SHRSP blood vessels are less responsive to vasodilator substances than those of Wistar rats (2), but there is evidence that endothelial responsiveness to NO is unaltered in SHRSP (17). These data suggest that nonselective COX inhibition and NO donation have a beneficial action on the renal vasculature during ischemia-reper-
fusion injury, but this effect was not apparent in the hypertensive state.

GRANTS

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REFERENCES


