Physiol. Res. 49: 129-133, 2000

Effect of Indomethacin and Deendothelisation on Vascular Responses in the Renal Artery

V. KRISTOVÁ, M. KRIŠKA, R. VOJTKO, A. KURTANSKÝ[1]

Department of Pharmacology and [1]Department of Physiology, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic

Received July 30, 1999
Accepted September 21, 1999

Summary

Vasodilator prostaglandins (PGE_2, PGI_2) play an important role in the regulation of renal blood flow. Hence, inhibition of their production with nonsteroidal anti-inflammatory drugs increases renal vascular resistance and exerts adverse renal effects. It has been reported that besides endothelium-derived prostaglandin products, nitric oxide (NO) may be mainly involved in regulation of renal functions. The aim of our study was to evaluate the effect of cyclooxygenase inhibition with indomethacin and endothelium removal on vascular responses of the renal artery as a model vessel. Isolated segments of rabbit renal arteries were perfused at constant flow. Indomethacin administration (10^{-5}mol.l^{-1}) significantly increased the responses to single doses (0.1, 1, 10 μg) of noradrenaline (NA) as compared with the controls. In indomethacin-pretreated vessels, subsequent deendothelisation by air bubbles enhanced the constrictor responses to NA. In reversed order, when deendothelisation was followed by indomethacin administration, the responses to NA were similar in character. A comparison of renal artery responses to NA in both experimental situations did not reveal any significant differences. It can be supposed that endothelial and non-endothelial factors may be involved in local regulation of renal vascular tone.

Key words

Renal artery • Vasoconstrictor responses • Indomethacin • Deendothelisation

Introduction

Vasodilator prostaglandins (PG) play an important role in the regulation of renal blood flow. Thus the inhibition of their formation from arachidonic acid with nonsteroidal anti-inflammatory drugs (NSAIDs) can result in adverse renal effects, e.g. acute renal failure or renal damage (Carmichael and Shankel 1985, Palmer 1995, McLaughlin *et al.* 1998). The main mechanism of action of NSAIDs concerns the inhibition of cyclooxygenase (COX) as described originally by Vane (1971). Recently, the existence of two isoforms of COX has been demonstrated: COX-1, a constitutive form and COX-2, an inducible form of the enzyme which are differently inhibited by NSAIDs (Battistini *et al.* 1994). Indomethacin represents a mixed type of COX-1/COX-2 inhibitor, so that a blockade of COX-1 impairs some physiological functions including regulation of renal blood flow.

Besides vasodilator prostaglandins (PGE_2, PGI_2), there is increasing evidence that endothelial products such as nitric oxide (NO) are involved in the control of

© 2000 Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic
Fax +420 2 293 894
E-mail physres@biomed.cas.cz
http://www.lf2.cuni.cz/physiolres/physiolres.htm

renal function (Gonzáles *et al.* 1998). The results obtained in this study suggest that the renal hemodynamic and excretory functions may be more sensitive to the prolonged cyclooxygenase inhibition, when NO production is reduced. Inhibition of NO synthesis has been found to potentiate the effect of several vasoconstrictors, such as 5-hydroxytryptamine, endothelin-1, thromboxane A_2-analogue U46619 (Gude *et al.* 1998), noradrenaline (Kristová *et al.* 1999) in different vascular beds, leading to general vasoconstriction and blood pressure increase (Rees *et al.* 1989, Gardiner *et al.* 1990). Therefore, the interaction of both arachidonate and NO pathways may play an important physiological role in the regulation of vascular tone (Di Rosa *et al.* 1996). From this point of view it is important to determine whether vascular responses are endothelium-dependent and mediated by either prostaglandins or other endothelium-derived products. The aim of our study was to evaluate the effect of cyclooxygenase inhibition with indomethacin and endothelium removal on vasoconstrictor responses to noradrenaline in the rabbit renal artery as a model vessel.

Methods

Rabbits (Cincilla) of both sexes weighing 2.5-3 kg were used in our experiments. The animals were sacrificed by cervical dislocation.

Experimental procedure

Both renal arteries were excised together with the kidney and placed in Tyrode's solution. After isolation and cannulation, vessel segments were transferred into a vessel chamber and perfused in parallel at a constant flow with Tyrode's solution (in $mmol.l^{-1}$): NaCl 137, KCl 2.7, $MgCl_2$ 1.1, NaH_2PO_4 0.32, $CaCl_2$ 0.9, $NaHCO_3$ 11.9, glucose 5.5. The perfusion solution was saturated with 95 % O_2/5 % CO_2, maintained at 37 °C and at pH 7.25-7.35. The changes of perfusion pressure were measured by means of a tensometric transducer LDP 102 and registered on a recorder TZ 4200, Tesla. After an equilibration period of 30 min, the basal perfusion pressure was adjusted to 18-20 mm Hg at a flow rate of 22 ml/min.

Experimental protocol

Both renal arteries were handled identically. After equilibration, standard doses of noradrenaline (0.1, 1, 10 μg) were injected directly into the cannula (controls) in a volume of 0.1 ml at approximately 5-min intervals. The vessels were than continuously perfused for 15 min with indomethacin in a concentration of 10^{-5} $mol.l^{-1}$. After this time, deendothelisation by air bubbles lasting 2 min was performed and the same doses of noradrenaline were used to evaluate the vasoconstrictor responses of vessel segments (Fig. 1). In another group of preparations, the effect of noradrenaline was evaluated in a reverse arrangement when deendothelisation was preceded by indomethacin administration.

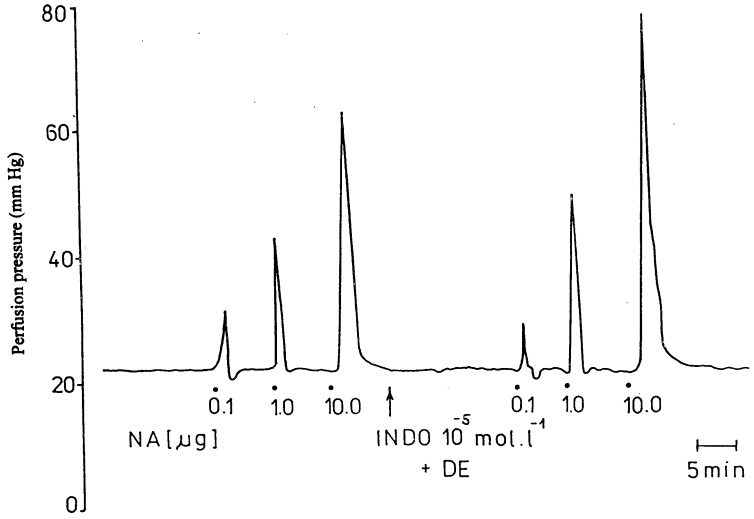

Fig. 1. *Rabbit renal artery responses to noradrenaline (NA) before and after deendothelisation (DE) in indomethacin-pretreated preparation (INDO). The characteristic recording from a single experiment.*

Drugs

Noradrenaline hydrogentartarate (Léčiva, Czech Republic) and indomethacin (Sigma) were used for all experiments. Indomethacin was dissolved in ethanol. For dilution of all drugs Tyrode's solution was used. All solutions were freshly prepared before each experiment.

Statistical evaluation of results

The data from renal arteries (expressed in mm Hg) were evaluated using the Wilcoxon test for pairwise comparison. For the comparison of two independent samples, the Mann-Whitney U-test was used. All values are expressed as median and confidence limits of the median.

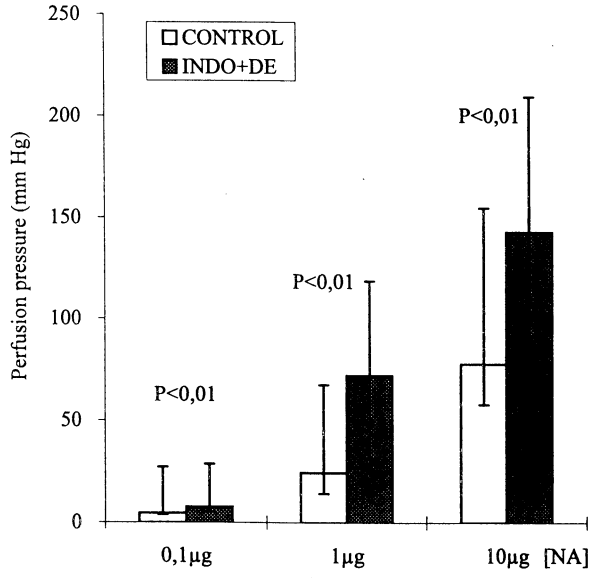

Fig. 2. *Comparison of constrictor responses of rabbit renal artery to noradrenaline (NA) before (CONTROL) and after indomethacin-pretreatment and deendothelisation (INDO + DE). All values are expressed as medians and their confidence limits.*

Results

Isolated segments of rabbit renal arteries contracted in a dose-dependent manner by noradrenaline (NA) under the conditions of *in vitro* perfusion. In indomethacin-pretreated vessels subsequent deendothelisation (DE) by air bubbles enhanced the contrictor responses to single doses of noradrenaline (controls versus indomethacin + DE expressed as median and its confidence limits in mm Hg): 0.1 μg: 4.5 (3.8; 27) vs. 7.5 (5.2; 28.5), 1 μg: 24.4 (14.2; 67.5) vs. 72 (31.5; 118.5), 10 μg: 77.65 (57.8; 154.5) vs. 142.5 (91.5; 209.2), $p<0.01$, n=8 (Fig. 2). In reversed order, when deendothelisation was followed by indomethacin administration, similar changes of responses to NA were found (controls vs. DE + indomethacin): 0.1 μg: 6 (2.2; 18) vs. 9.35 (4.5; 18), 1 μg: 15.75 (6; 48) vs. 36 (15; 73.5), 10 μg: 38.6 (11.2; 69) vs. 55.5 (21.8; 105.8), $p<0.01$, n=12 (Fig. 3). A comparison of vasoconstrictor responses to NA (values relative to controls) in the renal arteries affected either by indomethacin + deendothelisation or deendothelisation + indomethacin (Fig. 4) did not reveal any significant differences between these groups of preparations: 0.1 μg: 1.62 (0.97; 2.76) vs. 1.68 (1.13; 3), 1 μg: 2.68 (1.48; 4.46) vs. 2.2 (1.76; 3.88), 10 μg: 1.65 (1.35; 1.91) vs. 1.93 (1.1; 2.43).

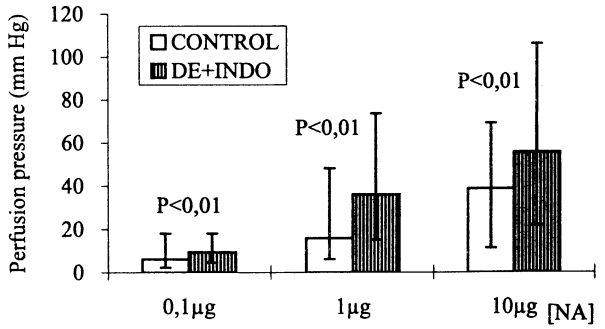

Fig. 3. *Comparison of constrictor responses of rabbit renal artery to noradrenaline (NA) before (CONTROL) and after deendothelisation followed by indomethacin administration (DE + INDO). All values are expressed as medians and their confidence limits.*

Discussion

The present results indicated that vasoconstrictive responses to noradrenaline were significantly potentiated in both experimental situations: in indomethacin-pretreated renal arteries with subsequent deendothelisation and in deendothelised preparations treated with indomethacin. No significant differences were found in responses to noradrenaline between these groups.

It is generally accepted that NSAIDs act by inhibiting prostaglandin production by a blockade of cyclooxygenase. The adverse effects of these drugs, including renal damage, are also believed to result from the inhibition of COX. The discovery of two isoforms of cyclooxygenase COX-1 and COX-2 has provided an explanation of some remarkable discrepancies in NSAID adverse effects (Pairet and Engelhardt 1996).

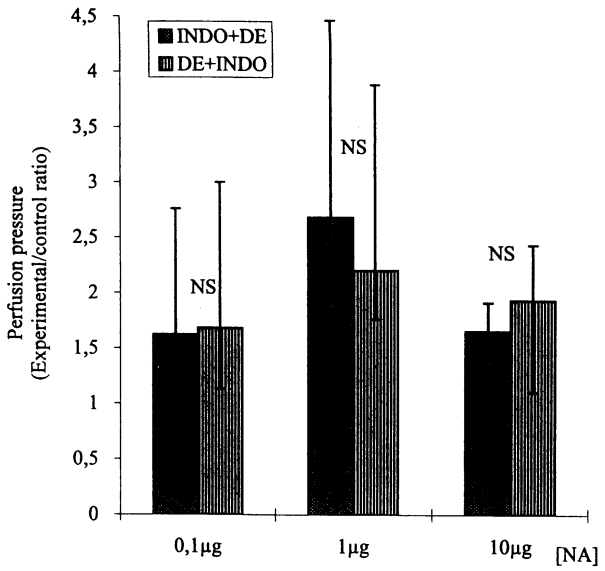

Fig. 4. *Relative constrictor response to noradrenaline (NA) in rabbit renal artery subjected to two different procedures: indomethacin-pretreatment with subsequent deendothelisation (INDO + DE) and deendothelisation followed by indomethacin administration (DE + INDO). The values (given as medians and their confidence limits) are expressed as the ratio of responses found in experimental and control arteries.*

Indomethacin, a relatively non-selectively acting drug, inhibits COX-1 and COX-2 over a similar concentration range. It is thus a more effective inhibitor of COX-1 than ibuprofen or diclofenac, suggesting an increased risk of renal problems with indomethacin. It was demonstrated in numerous studies that indomethacin enhances vasoconstrictor responses to various agents such as noradrenaline (Michibayashi 1984) and phenylephrine (Malomvolgyi *et al.* 1996) by preventing vasodilator PG formation. We assessed the effect of indomethacin on vascular responses to noradrenaline in previous studies (Djibril *et al.* in press) in which significant potentiation of these responses after indomethacin administration was found not only in renal but also in femoral arteries. The effect of indomethacin on vascular contractions *in vitro* may result from inhibition of COX-1 present in the vascular endothelium. Therefore, elimination of the endothelium effect on these responses may disclose mechanisms which are not endothelium-dependent. Our results suggest that the enhanced vasoconstrictor responses to noradrenaline in deendothelised renal arteries pretreated with indomethacin involve both endothelial and non-endothelial mechanisms. When deendothelisation preceded indomethacin administration, the potentiation of constrictor responses to NA indicate endothelium-independent mechanisms. We can only speculate which factors in vascular smooth muscle participate in this effect.

To examine the possibility that vascular smooth muscle is modulated by COX-2 after endothelium removal will require to evaluate the effects of selective COX-2 inhibitors such as nimesulid or nabumeton. Increased responses to vasoconstrictor stimuli after deendothelisation have been reported by Carrier and White (1985) and Tesfamariam *et al.* (1992). This is in agreement with our findings in perfused rabbit femoral arteries (Kriška *et al.* 1989). It is likely that the decreased production of vasodilator factors such as NO and PGI_2 enhances the vascular responses to vasoconstrictors. It is still possible that non-endothelial constrictor agents, e.g. endothelin or thromboxane, may be involved in the potentiation of these responses. We can not also rule out an additional effect of indomethacin on membrane ion transport. In conclusion, increased responses to noradrenaline after cyclooxygenase inhibition and deendothelisation suggest that both endothelial and non-endothelial factors are involved in the regulation of renal vascular tone.

Acknowledgements

This study was supported by grant VEGA N°1/4120/97 of the Slovak Grant Agency of Sciences.

This work was presented at the International Symposium "*Nitric Oxide: From Molecular Level to Clinical Application*" held in Bratislava, June 27-29, 1999 and published as an abstract in *Physiol Res* **48:** 33P, 1999.

References

BATTISTINI B, BOTTING R, BAKHLE YS: COX-1 and COX-2: toward the development of more selective NSAIDs. *Drug News Perspect* **7:** 501-512, 1994.

CARMICHAEL J, SHANKEL SW: Effects of NSAIDs on prostaglandins and renal functions. *Am J Med* **78:** 992-1000, 1985.

CARRIER GO, WHITE RE: Enhancement of alpha-1 and alpha-2 adrenergic agonists induced vasoconstriction by removal of endothelium in rat aorta. *J Pharmacol Exp Ther* **232:** 682-687, 1985.

DI ROSA M, IALENTI A, IANARO A, SAUTEBIN L: Interactions between nitric oxide and cyclooxygenase pathways. *Prostagland Leukot Essent Fatty Acids* **54:** 229-238, 1996.

DJIBRIL MN, KRISTOVÁ V, FAČKOVCOVÁ D, KRIŠKA M: Comparison of vascular responses to selected NSAIDs in rabbit femoral and renal arteries. *Acta Physiol Hung* (in press).

GARDINER SM, COMPTON AM, BENNETT T, PALMER RMJ, MONCADA S: Control of regional blood flow by endothelium-derived nitric oxide. *Hypertension* **15:** 486-492, 1990.

GONZÁLES JD, LLINÁS MT, NAVA E, GHIADONI L, SALAZAR FJ: Role of nitric oxide and prostaglandins in the long-term control of renal function. *Hypertension* **32:** 33-38, 1998.

GUDE NM, KING RG, BRENNECKE SP: Autacoid interactions in the regulation of blood flow in the human placenta. *Clin Exp Pharmacol Physiol* **25:** 706-711, 1998.

KRIŠKA M, BABÁL P, KRISTOVÁ V, CAŇOVÁ R: The evaluation of perfused vessel reactivity in relation to the endothelium (in Slovak). *Čs Fysiol* **38:** 153, 1989.

KRIŠKA M, KRISTOVÁ V, VOJTKO R, KURTANSKÝ A: Effect of indomethacin and deendothelisation on vascular responses in the renal artery. *Physiol Res* **48:** 33P, 1999.

KRISTOVÁ V, DJIBRIL MN, VOJTKO R, KRIŠKA M, KURTANSKÝ A: Effect of L-NNA and indomethacin interactions on noradrenaline-induced vasoconstrictions in renal and femoral arteries. *Pflügers Arch* **437** (Suppl): R161 1999.

MALOMVOLGYI B, HADHAZY P, TEKES K, KOLTAI MZ, POGATSA G: Comparison of the vasodilatory effects of bradykinin in isolated dog renal arteries and in buffer-perfused dog kidneys. *Acta Physiol Hung* **84:** 9-18, 1996.

MCLAUGHLIN JK, LIPWORTH L, CHOW WH, BLOT WJ: Analgesic use and chronic renal failure: a critical review of the epidemiologic literature. *Kidney Int* **54**: 679-686, 1998.

MICHIBAYASHI T: Effect of endogenous prostaglandin E on the vasoconstrictor response to noradrenaline. *Jpn J Pharmacol* **36:** 365-370, 1984.

PALMER BIFF F: Renal complications associated with use of NSAIDs. *Invest Med* **43:** 516-533, 1995.

PAIRET M, ENGELHARDT G: Differential inhibition of COX-1 and COX-2 in vitro and pharmacological profile in vivo of NSAIDs. In: *Improved Non-steroid Anti-inflammatory Drugs. COX-2 Enzyme Inhibitors.* J VANE, J BOTTING, R BOTTING (eds), Kluwer Academic Publishers, Dortmund, William Harvey Press, New York, 1996, pp 103-119.

REES D, PALMER RMJ, MONCADA S: Role of endothelium-derived nitric oxide in regulation of blood pressure. *Proc Natl Acad Sci USA* **86**: 3375-3378, 1989.

TESFAMARIAM B, WEISBROD RM, COHEN RA: Cyclic GMP modulators of vascular adrenergic neurotransmission. *J Vasc Res* **29**: 396-404, 1992.

VANE JR: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature* **231**: 232-235, 1971.

Reprint requests

V. Kristová, M.D., Ph.D., Department of Pharmacology, Faculty of Medicine, Comenius University, Sasinkova 4, 813 72 Bratislava, Slovak Republic, e-mail: farmakologia@fmed.uniba.sk.