EXPERIMENTAL STUDY

Comparison of vasoconstrictor responses to selected NSAIDs in rabbit renal and femoral arteries

Kristova V, Djibril NM, Fackovcova D, Kriska M, Kurtansky A

*Department of Pharmacology, Faculty of Medicine, Comenius University, Bratislava, Slovakia.***bll@fmed.uniba.sk**

Abstract

Background: **Nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to induce adverse renal effects, which are closely related to physiological inhibition of renal prostaglandin synthesis.**
Aim: **This study was aimed to evaluate the effect of drugs inhibiting both cyclooxygenase (COX) isoforms COX-1 and COX-2 on vasoconstrictor responses to noradrenaline in the rabbit renal artery and to compare these responses with femoral artery as a systemic vessel.**
Methods: **Rabbit femoral and renal arteries were perfused with a constant flow. Vascular responses to drugs were measured and registered as changes in perfusion pressure.**
Results: **It was found that the vasoconstrictor responses to noradrenaline were significantly enhanced after administration of all NSAIDs in both the renal and femoral arteries. The effect of indomethacin on renal vasoconstrictor responses was more pronounced compared to ibuprofen or phenacetin. Comparison of NSAIDs effects on renal and femoral arteries did not show significant differences.**
Conclusions: **These results demonstrate an increase of vasoconstrictor activity after NSAIDs administration without significant differences between the renal and femoral arteries. The strongest potentiation of the vasoconstrictor responses in the renal artery was found with indomethacin.** ***(Tab. 1, Fig. 4, Ref. 20.)***
Key words: renal and femoral arteries, nonsteroidal anti-inflammatory drugs, vasoconstrictor responses.

Vasodilator prostaglandins (PGs), prostacyclin, and prostaglandin E2 are produced predominantly in the endothelium under physiological conditions. These compounds are important in the control of renal hemodynamic and excretory function (Gonzáles et al, 1998), maintenance of a low pulmonary vascular resistance (Barnard et al, 1992), regulation of blood flow in foetal placental vessels (Gude et al, 1998) and other vascular beds. Alterations in the production of vasodilator prostaglandins and other autacoids (nitric oxide) may be associated with disturbances of blood flow in some vascular beds (Nies, 1986; Gude et al, 1998). Besides pathological situations, this alteration can also result from pharmacologically induced inhibition of prostaglandin synthesis by nonsteroidal anti-inflammatory drugs (NSAIDs).

NSAIDs are among the most widely used pharmacological agents and are often accompanied by adverse effects. In addition to frequent gastrointestinal disturbances, NSAIDs may have serious nephrotoxic effects (Carmichael and Shankel, 1985; Palmer, 1995). Most NSAIDs are characterised by a common mechanism of action - inhibition of the key enzyme in prostaglandin biosynthesis, cyclooxygenase (COX), as described originally by Vane (1971). In spite of similar mechanisms of action of individual drugs there are differences in their ability to block two distinct isoforms of cyclooxygenase. Cyclooxygenase 1 (COX-1) — the constitutive form of the enzyme, regulates basic physiological functions mediated by prostaglandins (e.g. peripheral vascular resistance, renal blood flow and glomerular filtration rate, renal sodium excretion). Cyclooxygenase 2 (COX-2) is an inducible enzyme, primarily up-regulated in response to

Department of Pharmacology, Faculty of Medicine, Comenius University, Bratislava, and Department of Physiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Address for correspondence: V. Kristova, MD, PhD, Dept of Pharmacology, Faculty of Medicine, Comenius University, Sasinkova 4, SK-813 72 Bratislava A, Slovakia.
Phone: +421.2.59357227 Fax: +421.2.59357508

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tissue damage during inflammation (Pairet and Engelhardt, 1996). These facts provide insight into the mechanisms of action and adverse effects of NSAIDs.

Most of the available data are on the effects of indomethacin on vascular reactivity. Less information is found about the effects of other NSAIDs, especially considering data comparing effects of selected drugs on different types of vessels. The aim of this study was: 1) To evaluate effects of selected NSAIDs (indomethacin, ibuprofen, phenacetin) on vasoconstrictor responses to noradrenaline in rabbit renal and femoral arteries in vitro. 2) To compare these effects between both arteries.

Materials and methods

Rabbits (Cincilla) of both sexes weighing 2.5—3.0 kg were used in the experiments. The animals were sacrificed by cervical dislocation. The following experimental procedures were used:

Isolated femoral artery: Both femoral arteries were carefully removed up to the knee level using a procedure described previously (Kristová et al, 1993). Polyethylene cannules were introduced into each artery, fixed with a cotton thread and the vessel segment was cut up into constant lengthes of 5 mm. Two paired vessel segments from the same animal were placed into the perfusion system and perfused parallelly at constant flow with Tyrode's solution (the composition in mM: NaCl 137.0, 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. Changes of pressure in the perfusion system were detected by means of tensometric transducer (LDP 102) and registered on recorder (TZ 4200, Tesla). After an equilibration period of 30 minutes, the basal perfusion pressure was adjusted at 20 mm Hg with a flow rate of 25 ml/min.

Isolated renal artery: Renal arteries were excised together with the kidneys and placed in Tyrode's solution. After isolation and cannulation the vessel segments were transferred into a vessel chamber and perfused at similar conditions as described above (the basal perfusion pressure: 14—18 mmHg, flow rate: 22 ml/min).

Experimental protocol: Both the femoral and renal arteries were handled identically. After equilibration, the standard doses of noradrenaline (0.1; 1; 10 g) were injected directly into the cannula in a volume of 0.1 ml at intervals of approximately 5 minutes. Then the vessel segments were continuously perfused during a period of 15 minutes with single NSAIDs (indomethacin, ibuprofen, phenacetin) in concentration of 10^{-5} mol.l^{-1} followed by the same doses of noradrenaline. The experimental protocol is illustrated in Fig. 1.

Drugs: Noradrenaline hydrogentartarate, indomethacin, ibuprofen, and phenacetin. NSAIDs were dissolved in ethanol. For dilution of drugs Tyrode's solution was used. All solutions were prepared fresh before each experiment.

Statistical evaluation of results: The data (expressed in mmHg) were evaluated using tests for pairwise comparisons, the Wilcoxon test for dependent samples and the Wilcoxon—Mann—Whitney test for independent samples, respectively. Multiple comparisons of differences of effects between tested drugs (expressed in the percentage of controls) were calculated using the test of Kruskal—Wallis.

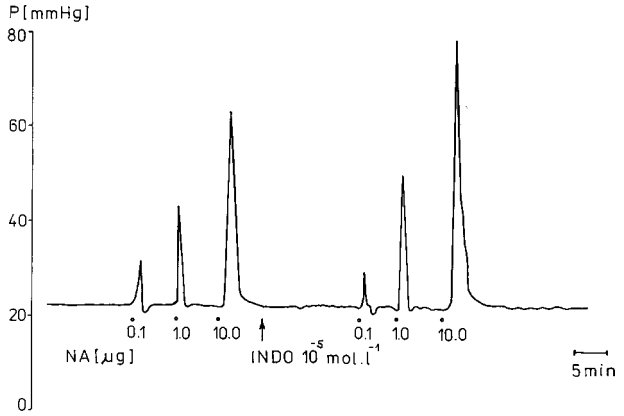

Fig. 1. Responses of a isolated renal artery to noradrenaline before and after indomethacin administration. Record from the experiment. P — perfusion pressure, NA — noradrenaline, INDO — indomethacin.

Results

Both renal and femoral arteries contracted in a dose-dependent manner to noradrenaline under in vitro perfusion conditions. When exposed to single NSAID (concentration 10^{-5} mol.l^{-1}) noradrenaline-induced contractions were significantly enhanced in both arterial preparations. The vasoconstrictor responses of rabbit renal and femoral arteries to noradrenaline in the presence of indomethacin, ibuprofen and phenacetin are shown in Figs 2, 3 and 4.

In the renal artery, responses to noradrenaline increased after indomethacin administration (controls versus indomethacin, all values expressed as medians in mmHg): at dose 0.1 µg, 6.5

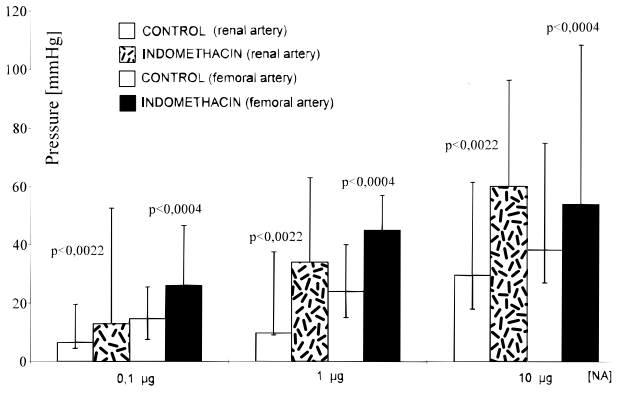

Fig. 2. Effect of indomethacin on vasoconstrictor responses to noradrenaline in the rabbit femoral artery (n=16) and renal artery (n=12). All values expressed as medians and their confidence limits. NA — noradrenaline.

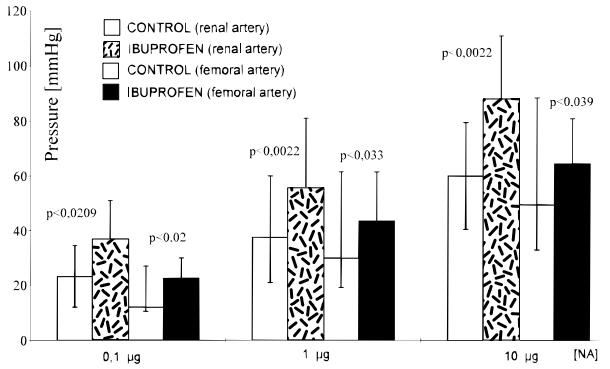

Fig. 3. Effect of ibuprofen on vasoconstrictor responses to noradrenaline in the rabbit femoral artery (n=13) and renal artery (n=12). All values expressed as medians and their confidence limits. NA — noradrenaline.

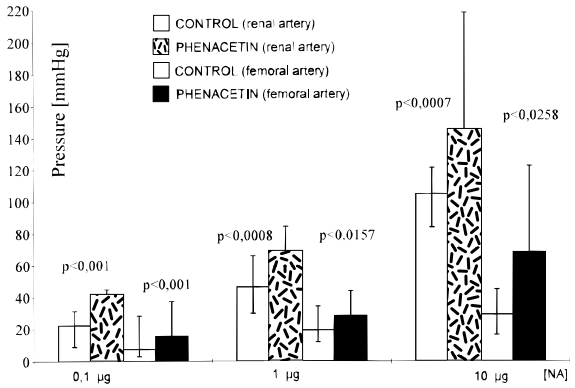

Fig. 4. Effect of phenacetin on vasoconstrictor responses to noradrenaline in the rabbit femoral artery (n=14) and renal artery (n=15). All values expressed as medians and their confidence limits. NA — noradrenaline.

vs 12.75; p<0.002, at dose 1.0 µg, 9.75 vs 33.75; p<0.002, at dose 10.0 µg, 29.6 vs 60.0; p<0.002 (Fig. 2). Results obtained in the femoral artery were the following (controls vs indomethacin): at dose 0.1 µg, 14.63 vs 26.0; p<0.004, at dose 1.0 µg, 24.0 vs 45.0; p<0.004 at dose 10.0 µg, 38.25 vs 54.0; p<0.004 (Fig. 2).

Comparison of responses to noradrenaline after administration of ibuprofen revealed increased responsiveness in the renal arteries at all doses when compared with controls: at dose 0.1 µg, 3.25 vs 36.75; p<0.029, at dose 1.0 µg, 37.5 vs 55.5; p<0022, at dose 10.0 µg, 60.0 vs 88.0; p<0.0022 (Fig. 3). In the femoral arteries similarly potentiated responses to noradrenaline were found (controls vs ibuprofen): at dose 0.1 µg, 12.0 vs 22.5; p<0.02, at dose 1.0 µg, 30.0 vs 43.5; p<0.033, at dose 10.0 µg, 49.5 vs 64.5; p<0.03 (Fig. 3).

Significantly enhanced responses to noradrenaline after phenacetin administration in the renal artery were as follows (controls vs phenacetin): at dose 0.1 µg, 22.5 vs 42.0;3 p<0.001, at dose 1.0 µg, 46.5 vs 69.0; p<0.0008, and at dose 10.0 µg, 105.0 vs 145.5; p<0.0007 (Fig. 4). Similar potentiation of vasoconstrictor responses was demonstrated in the femoral artery (controls vs phenacetin): at dose 0.1 µg, 7.5 vs 15.75; p<0.001, at dose 1.0 µg, 19.5 vs 28.5; p<0.0157, and at dose 10.0 µg, 29.5 vs 68.25, p<0.0258 (Fig. 4).

Vasoconstrictor responses to noradrenaline after indomethacin, ibuprofen and phenacetin administration in both the renal and femoral arteries were compared as the percentages of controls. The effect of indomethacin on renal vasoconstrictor responses was relatively more pronounced compared with ibuprofen and phenacetin effects. Comparison of NSAIDs effects did not reveal significant differences between the responses of the renal and femoral arteries (Tab. 1).

Discussion

The results of this study showed that the vasoconstrictor responses to noradrenaline were significantly potentiated by all selected nonsteroidal anti-inflammatory agents in both rabbit renal and femoral arteries. Similar results were obtained in the renal artery rings (unpublished results). However, no significant differences in the responses to noradrenaline between these groups of vessels were found. These findings support our assumption

Tab. 1. Effects of indometacin, ibuprofen and phenacetin on vascular responses to noradrenaline (NA) in rabbit renal and femoral artery. Data are expressed as medians with their confidence limits in the percentage of control pressures.

NA [µg]	Artery	Indometacin	$P_{Indo\ vs\ Ibu}$	Ibuprofen	$P_{Ibu\ vs\ Phen}$	Phenacetin	$P_{Indo\ vs\ Phen}$
	renal	207 (162; 269)	NS	153 (122; 277)	NS	144 (130; 208)	<0,05
0,1	p	NS		NS		NS	
	femoral	193 (136; 256)	NS	163 (117; 233)	NS	155 (125; 222)	NS
	renal	232 (185; 283)	<0,01	155 (121; 195)	NS	133 (124; 154)	<0,00025
1,0	p	<0,0062		NS		<0,0275	
	femoral	150 (125; 188)	NS	138 (97; 278)	NS	168 (142; 200)	NS
	renal	203 (157; 227)	<0,01	144 (120; 217)	NS	136 (121; 180)	<0,01
10	p	NS		NS		NS	
	femoral	157 (130; 172)	NS	131 (83; 171)	NS	163 (115; 85)	NS

that increased vasoconstrictor activity in both types of vessels as a result of vasodilator prostaglandins inhibition by NSAIDs may be associated with increased vascular resistance and diminished blood flow through these vascular beds. The potentiation of vasoconstrictor responses by NSAIDs found in two different types of vessels suggests a nonselective effect of these drugs on vascular beds.

Although potentiation of vasoconstrictor responses to noradrenaline with all examined drugs was found in both the renal and femoral arteries the comparison of the drug effects in the renal artery revealed a most pronounced effect to indomethacin when compared with the effects of ibuprofen or phenacetin. Indomethacin is a widely used nonsteroidal antiflogistic used among others for the treatment of rheumatoid arthritis in humans. It is a relatively non-selective drug, and inhibits COX-1 and COX-2 over a similar concentration range. Indomethacin is a more potent inhibitor of COX-1 than ibuprofen (Laneuville et al, 1994) suggesting an increased risk of renal side effects. Previous studies have demonstrated that indomethacin increases vasoconstrictor responses to some vasoconstrictor agents such as noradrenaline (Michibayshi, 1984) and phenylephrine (Malomvolgyi et al, 1996) by preventing vasodilator PG formation. Indomethacin leads to disturbances in the villous microcirculation in rat ileum (Ruh et al, 1999). Experimental and clinical studies indicate a relationship between indomethacin usage during pregnancy and persistent fetal circulation (Stevens and Schreiner, 1982). Indomethacin was successfully used for "pharmacological" closure of the ductus arteriosus in premature infants with patency of this fetal shunt (Heymann et al, 1976; Thalji et al, 1980). This is in agreement with our previous findings that indomethacin enhanced contractibility of the ductus arteriosus and pulmonary artery from guinea pig and rabbit fetuses (Kriška et al, 1984). We believe that the effect of indomethacin on vascular wall involves decreased production of vasodilator prostaglandins but a direct effect on vascular smooth muscle cannot be excluded.

In our experiments the vasoconstrictor activity of ibuprofen was weaker then that of indomethacin, which may explain the less pronounced renal adverse effects seen with ibuprofen. This is supported by findings that ibuprofen inhibits COX-2 relative to COX-1 more effectively than indomethacin (Laneuville et al, 1994).

An other type of drug, phenacetin, used as an analgesic and antipyretic agent similarly enhanced vessel reactivity. This effect cannot be explained unambiguously by peripheral blockade of cyclooxygenase because phenacetin lacks anti-inflammatory properties. The major phenacetin metabolite, paracetamol, is without effect on COX-2 and inhibits COX-1 very weakly (Mitchell et al, 1994). It is well known that phenacetin induces renal damage, so-called „classic" analgesic nephropathy, and this could result from a direct effect of phenacetin on renal tissues (Palmer, 1995; Fačkovcová et al, 2000).

The present findings may also help to explain NSAID's interaction with antihypertensive drugs on a pharmacodynamic level. Based on clinical studies, the effect of angiotensin enzyme inhibitors is diminished if they are combined with anti-inflammatory drugs such as indomethacin. The consequence of this interaction is a failure of antihypertensive treatment (Polónia, 1997).

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