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Interactions between nitric oxide and prostanoids in isolated perfused kidneys of the rat

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1 The present study was aimed to assess the interaction between nitric oxide (NO) and thromboxane (Tx) A_2-prostaglandin (PG) H_2 in single-pass perfused isolated kidneys of the rat.

2 Noradrenaline (NA, 63 and 110 nM) dose-dependently elevated the renal vascular resistance (RVR), the glomerular filtration rate (GFR) and the urinary excretion of sodium ($U_{Na}V$). Infusion of N^{ω}-nitro-L-arginine methyl ester (L-NAME, 100 μM), an inhibitor of NO synthesis, enhanced the effects of NA on RVR and on $U_{Na}V$, but decreased those on GFR. The TxA_2-PGH_2 (TP) receptor blockade by GR32191B (10 μM) attenuated this potentiating effect of L-NAME.

3 When renal perfusion pressure was stepwise increased from 90 to 150 mmHg, L-NAME similarly decreased renal perfusion flow rate and GFR.

4 The venous excretion of TxB_2 and 6-keto-$PGF_{1\alpha}$ was increased by L-NAME in baseline conditions as well as after NA or increasing renal perfusion pressure (RPP).

5 These results suggest that: (1) TxA_2 and PGH_2 play an important role in the overall effect of the renal prostanoids, (2) NO strongly interacts with the cyclo-oxygenase pathway and reduces the prostanoid synthesis in the kidney, and (3) the pressor effect of L-NAME partly relies upon the vasoconstrictor effect of TxA_2 and PGH_2.

Keywords: Prostanoids; TP receptor; nitric oxide; isolated perfused kidney

Introduction

In a previous study with isolated perfused kidneys of the rat, we have shown that inhibition of the nitric oxide (NO) synthase by N^{ω}-nitro-L-arginine methyl ester (L-NAME) potentiates the contractile responses to noradrenaline (NA) and that this effect is markedly attenuated after inhibition of the cyclo-oxygenase pathway by indomethacin (Zhang & Sassard, 1993). Since we observed that the NA-induced vasoconstriction partly depends upon the activation of the thromboxane (Tx) A_2-prostaglandin (PG) H_2 receptors (Zhang & Sassard, 1993), it can be hypothesized that either TxA_2-PGH_2 (TP) receptors are involved in the release of NO or conversely, that NO inhibits the prostanoid synthesis as suggested by Bordet & Largarde (1988) and Jameson *et al.* (1993).

Therefore, in the present work we aimed to assess the mechanisms of the interactions between NO and the prostanoids acting on TP receptors. Hence, we determined (1) the influence of TP receptor blockade on the effects of L-NAME after NA administration, and (2) the influence of L-NAME on the effects of TP receptor stimulation on smooth muscle cell contraction as well as on prostanoid receptors.

Methods

Animals

Eight-week old male Sprague-Dawley rats (Iffa-Credo, les Oncins, France) weighing 294 ± 11 g ($n=24$) were used. Animals were housed under constant conditions of temperature (21 ± 1°C), lighting (8 h00 min to 20 h00 min) and humidity (60 ± 10%). They were fed a standard diet (Elevage UAR A03, Villemoisson sur Orge, France) and had free access to tap water.

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Isolated kidney preparation

After the rats were anaesthetized with sodium pentobarbitone (45 mg kg^{-1}, i.p.), their right kidney was isolated as previously described (Liu *et al.*, 1990). Briefly, after a midline abdominal incision, the right adrenal artery and small lombar arteries were tied off. The right kidney was removed from peripheral fat pads and transferred without interruption of the renal blood flow into a small metallic cup maintained at 37°C. After an injection of heparin (1000 iu i.v.), four polyethylene catheters were respectively inserted into (1) the superior mesenteric artery, facing the origin of the right renal artery, to ensure perfusion of the kidney; (2) the infrarenal aorta to allow the measurement of the renal perfusion pressure (RPP, mmHg); (3) the suprarenal vena cava to collect the renal venous effluent and (4) the ureter to collect urine samples. The left renal artery was then ligated and, immediately after the beginning of the perfusion, the suprarenal aorta and the infrarenal vena cava were tied off. The right kidney was then excised, trimmed of adhering tissue and completely isolated. Finally, the left kidney was removed and weighed.

Perfusion medium

Kidneys were perfused with a blood free modified Krebs-Henseleit solution containing 35 g l^{-1} of a gelatin derivative (Haemaccel, Behring, Marburg, Germany) used as an oncotic agent. The final electrolyte composition of the perfusate was (in mM): NaCl 100, KCl 3.8, $CaCl_2$ 1.1, $MgCl_2$ 0.6, KH_2PO_4 1.2 and $NaHCO_3$ 25. In addition, the medium contained (mM) D-glucose 10.0, sodium pyruvate 2.0, oxaloacetic acid 1.0, sodium DL-lactate 5.0 and urea 6.0. Just before use, the perfusate was filtered through a millipore filter (0.8 μm) and added with 0.5 g l^{-1} polyfructosan (Inutest, Leavosan, Linz, Austria) for determination of the glomerular filtration rate (GFR, ml min^{-1} g^{-1}). The solution was continuously bubbled with a 95% O_2 and 5% CO_2 mixture and single-pass perfused in an open thermostatically controlled circuit with a peristaltic pump (Minipuls 2, Gilson, Paris, France). Experiments 1 and 2 were

realized at a constant flow rate, providing a RPP of 87.5±0.9 mmHg ($n=17$) at the end of the period of stabilization. Conversely, in experiment 3, kidneys ($n=7$) were perfused at different RPP levels fixed by a specially designed device servocontrolling the perfusion flow rate.

Renal function parameters

The RPP was continuously recorded (Model BS 273, Gould Inc. Cleveland, OH, U.S.A.) at the aorto-renal artery junction through a pressure transducer (model P23 ID, Statham Instrument Division, Gould Inc., Oxnard, CA, U.S.A.). Renal perfusion flow rate (RPF, ml min^{-1} g^{-1}) was measured by weighing the venous effluent. Renal vascular resistance (RVR, mmHg ml^{-1} min^{-1} g^{-1}) was calculated as the ratio of RPP to RPF. GFR (ml min^{-1} g^{-1}) was determined by polyfructosan clearance (Technicon autoanalyzer). Sodium concentration was measured by flame photometry (IL meter model 243, Lexington, MA, U.S.A.) and used to calculate the sodium urinary excretion ($U_{Na}V$, μmol min^{-1} g^{-1}) and reabsorption rate (R_{Na}, %). All these parameters were corrected for the weight of the unperfused left kidney, since the weight of the right kidney increased during the perfusion. The venous release of TxB_2 and 6-keto-$PGF_{1\alpha}$ (the stable derivatives of TxA_2 and prostacyclin, respectively) were measured by specific immunoassays after separation by high performance liquid chromatography (Geoffroy *et al.*, 1985).

Protocols

Experiment 1: TP receptor blockade and effects of L-NAME on the NA responses The experiment was performed in 7 kidneys. After a stabilization period of 30 min, urine samples were collected for 10 min and the venous effluent during the last minute of this baseline period. Then two concentrations of NA (63 and 110 nM) were infused for 3 min each, separated by a 17 min interval. The two NA infusions were repeated for 20 min after the beginning of an infusion of 100 μM L-NAME which was maintained until the end of the experiment, and then 20 min after the beginning of an infusion of 10 μM GR32191B, a specific TP receptor antagonist (Lumley *et al.*, 1989). This concentration inhibited by 91±2.4% ($n=6$) the contractile effect of 270 nM of U46619, a specific TP agonist (Coleman *et al.*, 1980). Urine samples were collected for 7 min before (control) and 6 min after the start of NA infusions. At the end of each experiment, TP receptor blockade was verified with an infusion of 270 nM of U46619.

Experiment 2: L-NAME and prostanoid synthesis In 10 kidneys, after a 30 min stabilization period, NA (63 nM) was infused for 3 min before and 20 min after the beginning of an infusion of L-NAME (100 μM). The venous effluent was collected for 1 min before (control) and during the last minute of the NA infusion.

Experiment 3: RPP and prostanoids synthesis In order to evaluate if the modifications of the prostanoid release induced by L-NAME could be due to the inhibition of NO *per se* or were only a consequence of the resulting increase in RPP, the venous release of TxB_2 was followed in 7 kidneys at three different RPP levels (90, 120 and 150 mmHg for 6 min) before and 20 min after beginning an infusion of 100 μM L-NAME. The venous effluent was collected during the last 2 min of each RPP level.

Statistics

Data are presented as means±s.e.mean. Statistical evaluations were performed by the non parametric test of Wilcoxon for paired data.

Drugs

NA and L-NAME were purchased from Sigma Chemical Co. (St. Louis, MI, U.S.A.). GR32191B ([1R-[1α(Z),2β,3β,5α]]-(+)-7-[5-[[(1,1'-biphenyl)-4-yl]methoxy]-3-hydroxy-2-(1-piperidinyl)cyclopentyl]-4-heptenoic acid, hydrochloride) was a generous gift from Glaxo Group Research Limited (Ware, Hertfordshire, U.K.).

Results

As shown in Figure 1, NA dose-dependently elevated RVR and GFR. Since the kidneys were perfused at a constant flow

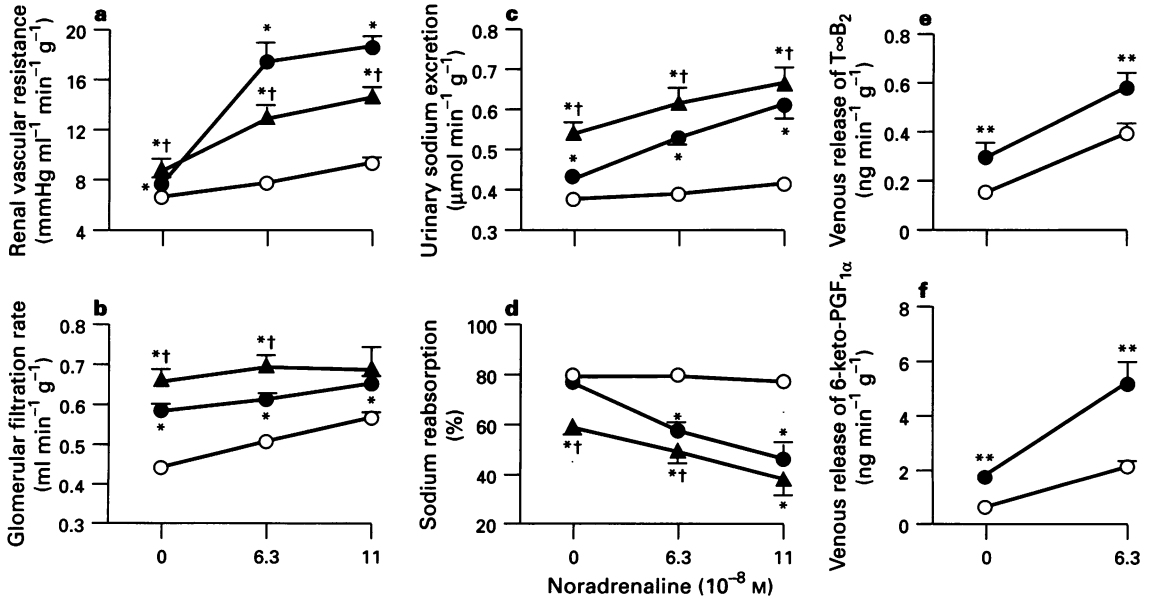

Figure 1 Effects of N^{ω}-nitro-L-arginine methyl ester (L-NAME, 100 μM) on the responses to noradrenaline of (a) the renal vascular resistance, (b) glomerular filtration rate, (c) urinary excretion of sodium, (d) sodium reabsorption ($n=7$) and of the venous excretion of (e) thromboxane B_2 (TxB_2) and (d) 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) ($n=10$) before and after thromboxane A_2-prostaglandin H_2 receptor blockade by GR32191B (10 μM). Symbols are: control, (○) L-NAME (●), L-NAME+GR32191B (▲). $^{*}P<0.05$ and $^{**}P<0.01$ vs control, $^{\dagger}P<0.01$ vs L-NAME.

rate, the filtration fraction varied in a similar manner to the GFR and thus was also increased. These effects were associated with a concentration-related increase in $U_{Na}V$ without any significant change in R_{Na}. L-NAME significantly ($P<0.05$) increased baseline RVR (from 6.3 ± 0.1 to 7.3 ± 0.2 mmHg ml^{-1} min^{-1}) and more markedly GFR (from 0.44 ± 0.01 to 0.59 ± 0.02 ml min^{-1} g^{-1}); $U_{Na}V$ increased from 12.5 ± 0.8 to 19.1 ± 0.9 μEq min^{-1} g^{-1} ($P<0.05$) while R_{Na} remained unchanged (from 80.4 ± 0.9 to $77.4\pm0.6\%$). L-NAME markedly potentiated the effects of NA on RVR but tended to decrease those on GFR. This effect was associated with an increase in $U_{Na}V$ and a decrease in R_{Na}. GR32191B markedly attenuated the potentiating effect of L-NAME on the NA-induced increases in RVR and enhanced those on GFR and $U_{Na}V$ suggesting a production of TxA_2 or PGH_2 in the glomerular arterioles. L-NAME significantly ($P<0.01$) increased the venous excretion of TxB_2 and 6-keto-$PGF_{1\alpha}$ under baseline conditions as well as after stimulation by NA. This effect did not correlate with the simultaneous changes in RPP, except for the effect on TxB_2 and that only after NA administration ($r=0.63$, $P<0.05$) but not after L-NAME or NA together with L-NAME.

As shown in Figure 2, stepwise increases in RPP from 90 to 150 mmHg were followed by significant ($P<0.001$) elevations of RPF, GFR and $U_{Na}V$ and by a decrease in R_{Na}. At the same time, the filtration fraction increased between 90 and 120 mmHg and remained stable thereafter. Under baseline conditions (RPP = 90 mmHg), L-NAME significantly ($P<0.001$) decreased RPF but did not affect the other parameters. When RPP was increased to 150 mmHg, RPF, GFR and $U_{Na}V$ were significantly ($P<0.01$) reduced by L-NAME whereas R_{Na} and the filtration fraction were not altered.

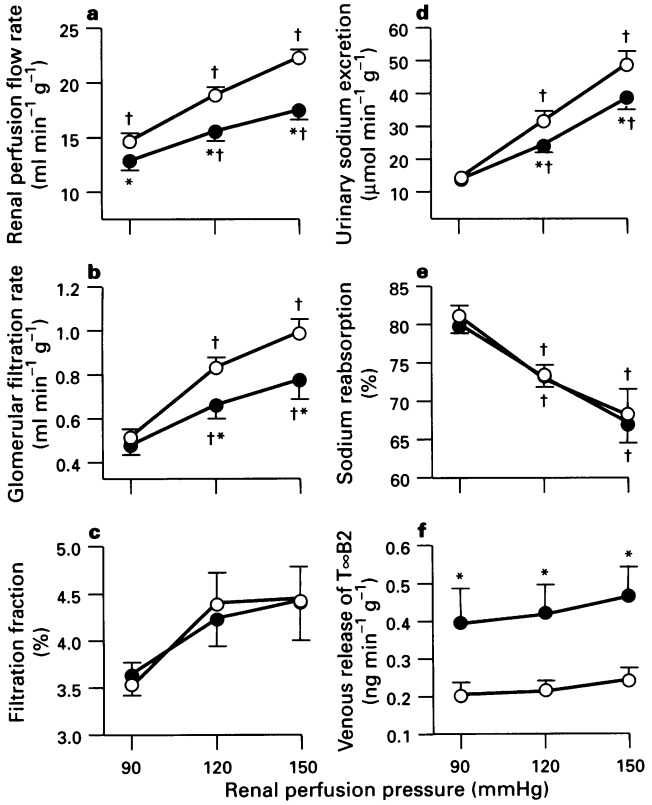

Figure 2 Effect of L-NAME 100 μM (●) on (a) renal perfusion flow rate, (b) glomerular filtration rate, (c) filtration fraction, (d) urinary sodium excretion, (e) sodium reabsorption and (f) on the venous release of TxB_2 in the baseline conditions and after stepwise increases in the renal perfusion pressure. $^*P<0.01$ vs control (○); $^\dagger P<0.05$ vs baseline (90 mmHg).

Stepwise increases in RPP did not significantly modify the venous release of TxB_2 while L-NAME infusion markedly increased it by 100% over the range of RPP studied.

Discussion

The present study was aimed to assess the relationships between NO and prostanoids acting on TP receptors in the isolated perfused kidney of the rat. The main finding was that NO and TxA_2- PGH_2-like metabolites strongly interact in the kidney, since inhibition of NO synthesis enhanced the release of the prostanoids which suggests that NO may inhibit the cyclo-oxygenase pathway.

Our previous experiments have demonstrated that the NA-induced release of NO and vasoconstriction are prostanoid-dependent in the preglomerular vessels of the rat isolated perfused kidney. Indeed, cyclo-oxygenase inhibition by indomethacin decreased the pressor effect of NA as well its potentiation by L-NAME (Zhang & Sassard, 1993). Since, among the prostanoids, TxA_2 and PGH_2 are the most potent vasoconstrictors and since they act predominantly at the preglomerular level (Ferrario *et al.*, 1989), the first objective of this study was to confirm that the interactions between NO and the prostanoids mainly rely upon these two cyclo-oxygenase-dependent products. For that purpose, we evaluated the effect of L-NAME on the NA response before and after administration of GR32191B, a specific TP receptor antagonist, using an experimental model fully validated for study of renal haemodynamics and prostanoid synthesis (Liu *et al.*, 1990).

As expected, L-NAME administration increased the effects of NA on RVR and decreased those on GFR indicating that the pressor effect of NA is partly masked by the concomitant release of NO. When RPP was increased stepwise from 90 to 150 mmHg, inhibition of the NO synthase by L-NAME did not modify the filtration fraction since the induced changes in both RPF and GFR were of similar magnitude. Such a result suggests that pressure and/or shear stress result in the preglomerular release of NO which is consistent with data previously published by Imig & Roman (1992) in the rat juxtamedullary microvascular preparation and by Ito & Ren (1993) in rabbit afferent arterioles. However, a simultaneous constriction of the postglomerular vessels is also induced by L-NAME, otherwise the decrease of GFR might be greater than that of the RPF. This has been demonstrated both *ex vivo* by Gabbai *et al.* (1992) in the same experimental model and *in vivo* by use of micropuncture techniques in the rat (Zatz & De Nucci, 1991) and rabbit (Denton & Anderson, 1994). The precise respective importance of pre- and post-glomerular effects of L-NAME cannot be evaluated from the present study and requires a more detailed analysis of intrarenal haemodynamics.

TP receptor blockade by GR32191B markedly attenuated the potentiating effect of L-NAME on the NA-induced vasoconstriction which indicates that the effect of L-NAME largely depends on TP receptor stimulation. Although it has been suggested that these receptors are also activated by $PGF_{2\alpha}$ (Kennedy *et al.*, 1982) and by TxA_2-like metabolites of the 20-hydroxyeicosatetraenoic (HETE) acid (Escalante *et al.*, 1989), the involvement of these eicosanoids in the effects of GR32191B appears to be unlikely. Indeed, $PGF_{2\alpha}$ is a weak vasoconstrictor compared to TxA_2 or PGH_2 (Kennedy *et al.*, 1982) and it was recently shown that the vasconstrictor response to 20-HETE acid was not affected by SQ29548, a specific TP receptor antagonist (Ma *et al.*, 1993). Therefore, since TP receptor blockade results in the same effect as cyclo-oxygenase inhibition, the present results strongly suggest that the previously obtained attenuation by indomethacin of the pressor effect of L-NAME mainly relies upon inhibition of the synthesis of PGH_2/TxA_2 themselves.

Although the mechanisms underlying this interaction remain unclear, one possibility could be that NO inhibits prostanoid synthesis. Interestingly, L-NAME increased the venous

release of TxB_2 and 6-keto-$PGF_{1\alpha}$ in baseline conditions as well as in response to NA. This effect is unlikely to rely upon the associated increase in RPP and shear stress which may have activated the phospholipase A_2 via an elevation of the cytosolic Ca^{2+} concentration. Indeed, stepwise increases in RPP did not significantly modify the venous output of TxB_2 which confirms the results of Wang & Voelkel (1989) that prostanoid synthesis was not tightly coupled with the magnitude of the contraction. Therefore, the most likely explanation is that NO directly modifies the phospholipase A_2 or more, probably, the cyclo-oxygenase activity (Gryglewski, 1993) and inhibits the release of TxA_2 and PGH_2. This hypothesis is supported by the finding that L-NAME markedly activated the synthesis of TxB_2 at any RPP level studied. This suggests that the potentiating action of L-NAME on the NA-induced vasoconstriction is partly mediated by the vasoconstrictor effect of PGH_2/TxB_2. Nafrialdi *et al.* (1994) recently found the same result in anaesthetized Wistar rats; they showed that the acute pressor effect of L-NAME is partly mediated by cyclo-oxygenase products, mainly TxA_2. As discussed by Jameson *et al.* (1993), NO can convert the ferric form of the cyclo-oxygenase into an inactive ferrous form. Such an inhibition has been recently demonstrated in rat Kupffer cells by Stadler *et al.* (1993). Therefore, the renal haemodynamic effects of acute NO synthesis inhibition is likely to rely upon both the removal of the direct vasodilator effect of NO and an increase of the vasoconstrictor prostanoid synthesis, in addition to the potentiation of other endogenous vasoconstrictor such as angiotensin II (Sigmon & Beierwattes, 1993) and endothelin (Qiu *et al.*, 1995).

However, Franchi *et al.* (1994) have recently hypothesized that NO could activate rather than inhibit the cyclo-oxygenase, since they observed that NO synthesis inhibition by N_G-monomethyl-L-arginine decreased the basal production of PGE_2 in the oestrogen-treated rat uterine tissue. However, they failed to demonstrate that the synthesis of the other prostanoids was also depressed which argues against the direct activation of cyclo-oxygenase. Similarly, Salvemini *et al.* (1995) reported that endotoxin *E. coli* lipopolysaccharide (LPS) treatment, which is known to increase the expression of the inducible isoform of the NO synthase markedly elevated plasma 6-keto-$PGF_{1\alpha}$ concentrations and the urinary excretion of both PGE_2 and 6-keto-$PGF_{1\alpha}$ in unrestrained conscious rats. However, it is noteworthy that LPS is proinflammatory and therefore may directly stimulate phospholipase A_2 or cyclo-oxygenase. In addition the prostaglandin concentrations presented in this study were measured directly in unextracted samples. This presumably explains the fact that they were unusually elevated and therefore difficult to interpret safely.

In conclusion, the present study demonstrates that, in the rat isolated perfused kidney: (1) TxA_2 and PGH_2 play an important role in the overall effect of the renal prostanoids, (2) NO strongly interacts with the cyclo-oxygenase pathway and reduces the prostanoid synthesis, and (3) the pressor effect of L-NAME partly relies upon the vasoconstrictor effects of TxA_2 and PGH_2.

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