Eicosanoid-dependence of responses of pre- but not postglomerular vessels to noradrenaline in rat isolated kidneys

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1 We investigated the role of nitric oxide (NO) and of vasoactive eicosanoids in the control of renal vascular resistance (RVR) and glomerular filtration rate (GFR) and of their responses to noradrenaline (NA). This study was conducted in single-pass perfused, isolated kidney preparations of the rat.

2 NA (63, 110 and 160 nM) dose-dependently increased RVR and to a lesser degree GFR.

3 In baseline conditions, N^ω-nitro-L-arginine methylester (L-NAME, 100 μM) increased GFR more than RVR, thus demonstrating a basal release of NO which predominates in postglomerular vessels.

4 In kidneys stimulated with NA, L-NAME potentiated the increases in RVR but not in GFR. Indomethacin (1.5, 150 nM and 15 μM) did not alter GFR but markedly and dose-dependently reduced the NA-induced increase in RVR. Similar results were obtained with GR 32191B (10 and 100 μM), a prostaglandin H₂/thromboxane A₂ (PGH₂/TxA₂) receptor antagonist.

5 Indomethacin (15 μM) suppressed the enhancing effects of L-NAME on RVR responses to NA but did not affect those on GFR.

6 It is concluded that the mechanisms of the response to NA differ among pre- and postglomerular vessels. In preglomerular vessels the vasoconstrictor action and the NO release depend upon the activation of PGH₂/TxA₂ receptors, while both are eicosanoid-independent in the postglomerular vessels.

Keywords: Rat isolated kidney; nitric oxide; cyclo-oxygenase; prostaglandin H₂; thromboxane A₂

Introduction

It is well known that kidneys markedly influence the long-term blood pressure level through the pressure-natriuresis mechanism (Cowley, 1992). This mechanism which allows urinary sodium excretion to equilibrate sodium intake is partly dependent upon the preglomerular vascular resistance which limits the amount of filtered sodium. In that respect, recent experiments highlighted the possible role of nitric oxide (NO) and of prostanoids. NO decreases renal vascular resistance and enhances glomerular filtration rate and natriuresis both *in vitro* (Mattson *et al.*, 1992; Johnson & Freeman, 1992; Salazar *et al.*, 1992; Majid & Gabriel Navar, 1992) and in isolated kidney preparations (Radermacher *et al.*, 1990; Welch *et al.*, 1991; Gardes *et al.*, 1992). Concerning prostanoids, the vasoconstrictor effects of prostaglandin (PG)H₂ and of thromboxane (Tx)A₂ are well known and have been shown to participate in the control of renal vascular resistance in genetically hypertensive rats of the Japanese (Dai *et al.*, 1992) and of the Lyon strains (Liu *et al.*, 1991).

The present work aimed to assess the role of NO in the control of renal functions. In order to avoid the interference of circulating vasoactive substances, the study was conducted in the single-pass perfused isolated kidney preparation. In addition, to eliminate the possible influence of co-released eicosanoids by endothelial cells (Fulton *et al.*, 1992) the experiments were repeated in the presence of indomethacin which blocks cyclo-oxygenase.

Methods

Animals

Eight-week old male Sprague-Dawley rats (Iffa-Credo, Les Oncins, France) were used. Animals were housed under con-

stant conditions of temperature (21 ± 1°C), lighting (08 h 00 min to 20 h 00 min) and humidity (60 ± 10%). They were fed a standard diet (Elevage UAR, AO3 Villemoisson s/Orge, France) and had free access to tap water.

Isolated kidney preparations

After rats were anaesthetized with sodium pentobarbitone (45 mg kg⁻¹, i.p.), the right kidney was isolated according to Schmidt & Imbs (1980). Briefly, after a midline abdominal incision, the right adrenal artery and small lumbar arteries were tied off. The right kidney was removed from peripheral fat pads and transferred without interruption of the renal blood flow to a small metallic double-walled cup maintained at 37°C. After an injection of heparin (1,000 iu, i.v.), polyethylene catheters were 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 for collecting 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 whole right kidney was then excised, trimmed of adhering tissue and completely isolated. The left kidney was removed and weighed.

Perfusion medium

The perfusate was a blood-free modified Krebs-Henseleit solution containing 35 g l⁻¹ of a gelatin derivative (Haemacel, Behring, Marburg, Germany) as a colloid osmotic agent. The final electrolyte composition of the perfusate was as follows (mM): NaCl 100, KCl 3.8, CaCl₂ 1.1, MgCl₂ 0.6, KH₂PO₄ 1.2 and NaHCO₃ 25.0. In addition, the medium contained (mM) D-glucose 10.0, sodium pyruvate 2.0, oxaloacetic acid 1.0, sodium DL-lactate 5.0, L-glutamic acid 5.0 and urea 6.0. Just before use, the perfusate was filtered

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through a millipore filter (0.8 μm) and polyfructosan (Inutest, Laevosan, Linz, Austria) (0.5 g l^{-1}) added for determination of the glomerular filtration rate (GFR). The solution was continuously bubbled with 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) at a constant flow rate.

Renal function parameters

The RPP was continuously recorded (Model BS 273, Statham Instrument Division, Gould Inc. Cleveland, Ohio, U.S.A.) at the aorto-renal artery junction through a pressure transducer (model P231D, Statham Instrument Division, Gould Inc., Oxnard, Calif., U.S.A.). Renal perfusion flow rate (RPF, $\text{ml min}^{-1} \text{g}^{-1}$) was measured by weighing the venous effluent. Renal vascular resistance (RVR, $\text{mmHg ml}^{-1} \text{min}^{-1} \text{g}^{-1}$) was calculated as the ratio RPP/RPF. GFR ($\text{ml min}^{-1} \text{g}^{-1}$) was determined by polyfructosan clearance (Technicon autoanalyzer). All these parameters were corrected for the weight of the unperfused left kidney, since the weight of the right kidney increased after the perfusion. The venous release of PGE_2 and TxB_2 , the stable derivative of TxA_2 was measured by specific immunoassays after high performance liquid chromatography separation (Benzoni *et al.*, 1981).

Protocols

Experiment 1 Seven kidneys were perfused at a RPP of $88 \pm 1 \text{ mmHg}$. After a 30 min stabilization period, urines were collected during a baseline period of 10 min and venous effluent during the last min of this baseline period. Then three concentrations (63, 110, and 160 nM) of noradrenaline (NA) were infused for 3 min each, separated by a 17 min interval. These NA infusions were repeated 20 min after the beginning of an infusion of N^G -nitro-L-arginine methyl ester (L-NAME), a specific inhibitor of nitric oxide synthesis, at a concentration of $100 \mu\text{M}$ (Radermacher *et al.*, 1990). As previously described (Liu *et al.*, 1991), urines were collected for 7 min before (control) and 6 min after the start of each NA infusion. The venous effluent was collected for 1 min before (control) and during the last min of each NA infusion.

Experiment 2 In 7 kidneys, indomethacin, a cyclo-oxygenase inhibitor, was added to the perfusate at a final concentration of $15 \mu\text{M}$, and the preparations were studied according to the above protocol.

Experiment 3 In 9 kidneys, after a 30 min stabilization period, NA (160 nM) was infused for 3 min before (control) and 30 min after the beginning of an infusion of indomethacin (1.5 nM, 150 nM and $15 \mu\text{M}$). Each kidney received randomly in a cumulative way 2 of these 3 concentrations. Therefore each of them was studied in 6 kidneys. Urine and venous effluent were obtained according to experiment 1.

Experiment 4 In 6 kidneys, after a 30 min stabilization period, NA (160 nM) was administered for 3 min before (control) and 30 min after the infusion of two cumulative concentrations (10 and $100 \mu\text{M}$) of GR32191B, a prostaglandin H_2 /thromboxane A_2 ($\text{PGH}_2/\text{TxA}_2$) receptor antagonist. These two concentrations were chosen after pilot experiments which showed that they efficiently inhibited the contractile effects of U46619 (270 nM), a $\text{PGH}_2/\text{TxA}_2$ receptor agonist (ΔRPP of controls: $+14 \pm 1.1 \text{ mmHg}$; GR32191B $10 \mu\text{M}$: $+2 \pm 0.3 \text{ mmHg}$ and GR32191B $100 \mu\text{M}$: $0 \pm 0 \text{ mmHg}$). Urine and venous effluents were collected according to Experiment 1.

Statistics

Data are mean $\pm$ s.e.mean. The nonparametric Wilcoxon test was used to assess the effects of NA. One way analysis of variance was used to determine the differences among groups.

Figure 1 Effects of N^G -nitro-L-arginine methyl ester (L-NAME), ($100 \mu\text{M}$) on the responses to noradrenaline of rat isolated kidneys in the absence and in the presence of indomethacin (Ind, $15 \mu\text{M}$) on renal vascular resistance (RVR, a) and glomerular filtration rate (GFR, b). Controls (O, $n = 7$); L-NAME ($\square$, $n = 7$); Ind ($\bullet$, $n = 7$); L-NAME + Ind ($\blacksquare$, $n = 7$). * $P < 0.05$ L-NAME or Ind vs controls; † $P < 0.05$ L-NAME + Ind vs L-NAME.

Figure 2 Effects of indomethacin ($\square$, 1.5 nM; $\square$, 150 nM; $\blacksquare$, $15 \mu\text{M}$; $n = 6$ in each group) on renal vascular resistance (RVR, a), glomerular filtration rate (GFR, b) and the venous release of prostaglandin E_2 (PGE_2 , c) and thromboxane B_2 (TxB_2 , d) in rat isolated kidneys challenged with noradrenaline (160 nM). * $P < 0.05$ and ** $P < 0.01$ vs controls ($\square$, $n = 9$).

Drugs

NA, L-NAME, indomethacin and U46619 (9,11-dideoxy-11 α , 9 α -epoxymethano-prostaglandin F_{2 α}) were from Sigma chemical Co., St. Louis, MO, U.S.A. GR32191B ([1R-[1 α (Z),2 β ,3 β ,5 α]]-(+)-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, UK.

Results

As shown by Figure 1, L-NAME significantly ($P < 0.05$) increased baseline RVR from 7.3 ± 0.3 to 8.8 ± 0.4 mmHg ml⁻¹ min⁻¹ g⁻¹ and more markedly GFR. NA dose-dependently elevated both parameters. L-NAME enhanced only the NA-induced increase in RVR. Indomethacin did not change baseline RVR and GFR. It did not significantly alter the NA effects on GFR but blunted those on RVR. When given together with L-NAME, indomethacin almost completely suppressed the potentiation by L-NAME of NA-induced vasoconstrictor effects, leaving GFR values unchanged.

Figure 2 indicates that in kidneys infused with NA, indomethacin did not alter GFR but dose-dependently reduced RVR. This reduction was marked with indomethacin at a concentration of 15 μ M which profoundly inhibited

the venous release of PGE₂ ($-92 \pm 2\%$) and TxB₂ ($-62 \pm 15\%$).

Figure 3 shows that a PGH₂/TxA₂ receptor antagonist exerted similar effects to those of indomethacin as it did not change the baseline RVR and GFR, but markedly opposed the effects of NA on RVR leaving GFR unchanged.

Discussion

The present work aimed to assess the role of NO in the control of renal vascular resistance and glomerular filtration rate. In order to eliminate the influence of uncontrolled circulating vasoactive substances, it was carried out in single-pass perfused isolated kidneys. This preparation was shown to be valuable for the study of renal vessels responses. In that respect, comparison of the simultaneous changes in RVR and in GFR made it possible to differentiate between the effects which predominate on the pre- or on the postglomerular vasculature. Despite its numerous advantages, single-pass perfused isolated kidneys are not fully satisfactory for the study of tubular functions since the sodium reabsorption never exceeded 90%. In addition, since in baseline conditions the vessels of such a preparation are almost maximally vasodilated it is interesting to challenge them with known concentrations of a vasoconstrictor agent. In the present work, we used low concentrations of NA, infused for short period of times and separated by long intervals to avoid the development of desensitization (Liu *et al.*, 1991). As usual, NA dose-dependently enhanced RVR and to a lower degree GFR.

The functional role of NO release was assessed by use of L-NAME at a concentration (100 μ M) which fully blocks NO synthesis (Radermacher *et al.*, 1990). In baseline conditions, L-NAME increased RVR and to a greater extent GFR, thus demonstrating that a basal NO release exists which predominates in postglomerular vessels. Such a basal NO release is likely to be due to shear stress activation of endothelial cells in the kidney as already demonstrated in other vascular beds (Koller & Kaley, 1991; Lamontagne *et al.*, 1992). A more important release by post- than by preglomerular vessels is in accordance with the data of Tolins & Raij (1991) and of Majid & Gabriel Navar (1992) who reported that NO synthase blockade decreases renal blood flow more than GFR. After stimulation with NA, L-NAME produced dramatic increases in RVR leaving the response of GFR unchanged. This indicates that, unlike shear stress, NA induces a more important NO release in pre- than in postglomerular vessels.

To avoid the possible interferences of the vasoactive eicosanoids which are released by NA in the kidneys (Liu *et al.*, 1991) the experiments were repeated in the presence of indomethacin at a concentration (15 μ M) that we found to decrease the overall renal release of the vasoconstrictor PGE₂ and TxA₂ by more than 60%. It was observed that indomethacin did not affect the NA effects on GFR, but dose-dependently and markedly inhibited those on RVR. Therefore it appears that in pre- but not in postglomerular vessels, NA may provoke the release of vasoconstrictor eicosanoids which play an important part in the contractile response. There are few reports of the inhibitory effects of cyclo-oxygenase inhibitors on vasoconstriction. However, similar findings were made in isolated lung (Shaw *et al.*, 1992) in coronary vascular beds (Lee *et al.*, 1991) stimulated with 5-hydroxytryptamine, vasopressin or phenylephrine, as well as in rat isolated aortae constricted with endothelin (Taddei & Vanhoutte, 1993). Taken as whole, these data suggest that our observation is not specific for NA. In order to approach the nature of the eicosanoids which are important in the contractile response to NA, we used a specific PGH₂/TxA₂ receptor antagonist GR32191B, at two concentrations (10 and 100 μ M) that were chosen after the demonstration that they blocked the vasoconstrictor effect of

Figure 3 Effects of a prostaglandin H₂/thromboxane A₂ (PGH₂/TxA₂) receptor antagonist (GR32191B; ▨, 10 μ M; ▩, 100 μ M) on renal vascular resistance (RVR a,b), glomerular filtration rate (GFR c,d) in 6 isolated rat kidneys before (baseline) and after the infusion of noradrenaline (160 nm). ** $P < 0.01$ vs controls (□).

U46619, an agonist at these PGH₂/TxA₂ receptors. Interestingly, as well as indomethacin, GR32191B dose-dependently reduced the response on RVR to NA, leaving GFR values unchanged. Therefore it is possible to conclude that the eicosanoids released by NA acting on preglomerular vessels are PGH₂ and/or TxA₂. When considering preparations submitted to L-NAME, it was observed that indomethacin did not consistently affect GFR but blunted the potentiating action of L-NAME on the RVR responses to NA. These data suggest that the NA-induced release of PGH₂ and/or TxA₂ is an important intermediate step which leads to the release of NO. This indicates an interaction within the preglomerular vessels between eicosanoids and NO as has been described in macrophages (Marotta *et al.*, 1992).

In conclusion, the present work shows that, in rat isolated kidney preparations the mechanisms involved in the contractile effects of NA differ between the pre- and the post-glomerular vascular beds. In preglomerular vessels both vasoconstriction and the release of NO which partly opposes it, depend upon the release of PGH₂ and/or TxA₂. In post-glomerular vessels both are eicosanoid-independent. Although difficult to extrapolate to *in vivo* conditions, these data may be of interest for *in vitro* investigations.

We are grateful to J. Sacquet for prostanoid measurements, Professor D. Benzonni for stimulating comments, and Glaxo Research laboratories for their generous gift of the compound GR32191B.

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(Received April 21, 1993
Accepted May 7, 1993)