JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2006, 57, Suppl 11, 179–185
www.jpp.krakow.pl

M. KUCZERISZKA, B. BĄDZYŃSKA, E. KOMPANOWSKA-JEZIERSKA

CYTOCHROME P-450 MONOOXYGENASES IN CONTROL OF RENAL HAEMODYNAMICS AND ARTERIAL PRESSURE IN ANAESTHETIZED RATS

Laboratory of Renal and Body Fluid Physiology, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland

The renal regulatory role of cytochrome P450 dependent metabolites of arachidonic acid (AA), vasodilator epoxyeicosatrienoic acids (EETs) and vasoconstrictor 20-hydroxyeicosatetraenoic acid (20-HETE), was examined in anaesthetised rats. We measured renal artery flow (RBF), cortical (CBF) and medullary (MBF) perfusion (laser-Doppler) and medullary tissue nitric oxide (NO, selective electrode), after non-selective inhibition of CYP-450 pathway with 1-aminobenzotriazole (ABT, 10 mg/kg i.v.) or after selective inhibition of 20-HETE synthesis with HET0016 (Taisho Co, Yoshino-cho, Japan), infused into renal artery at 0.3 mg/kg/h or into renal medulla at rates increasing from 0.15 to 1.5 mg/kg/h. ABT caused significant (by 13.7%) decrease in RBF without changing MBF. Renal arterial HET0016 increased MBF (not RBF or CBF) from 152±12 to 174±12 perfusion units (+16%, $P<0.001$), while medullary tissue nitric oxide was significantly increased ($P<0.001$). After renal medullary HET0016, renal perfusion indices were significantly higher than after HET0016 solvent (β-cyclodextrin). Total renal blood flow seems to be under vasodilator control of EETs whereas renal medullary perfusion under tonic suppression by 20-HETE. The data document, for the first in the whole kidney studies, the functional antagonism of 20-HETE and NO.

Key words: *20-hydroxyeicosatetraenoic acid, nitric oxide, intrarenal circulation*

INTRODUCTION

The role of cytochrome P450 pathway of arachidonic acid (AA) metabolism in the control of renal circulation and excretion has not been clearly defined. CYP-450 dependent monooxygenases generate hydroxyeicosatetraenoic acids

(HETEs) and epoxyeicosatrienoic acids (EETs), which modulate vascular smooth muscle tone and ion channel activity. 20-HETE is a renal vasoconstrictor, an essential component of the tubuloglomerular feedback, and was also found to inhibit salt transport in the proximal tubule and thick ascending limb of the loop of Henle (1, 2); because of this wide spectrum of action it may have both pro- and antihypertensive properties. In general, CYP-450 pathway metabolites of AA may have opposed effects on renal excretion. For instance, 20-HETE dependent vasoconstriction would decrease renal haemodynamics and promote fluid retention while, on the other side, EET dependent vasodilatation and transport inhibition would promote excretion.

Recent development of a highly selective inhibitor of 20-HETE synthesis (3) has broadened our knowledge of the agent's role in physiology and pathophysiology, however, the inhibitor (*N*-hydroxy-N' -(4-butyl-2-methylphenyl) formamidine, HET0016) has been used so far in only one whole kidney study (4). In the present work the renal effects of non-selective inhibition of CYP-450 pathway were compared with effects of selective inhibition of 20-HETE synthesis in normal anaesthetised rats. In order to ensure a substantial degree of inhibition in the kidney while avoiding systemic effects, HET0016 was infused either into the renal artery, to deliver it to the bloodstream of the whole kidney, or directly to the tissue (interstitium) of the renal medulla. The latter route was thought promising as in the blood 20-HETE and its inhibitors may be avidly bound by plasma proteins (5, 6), which may limit their filtration in the glomeruli and final action. Considering the functional antagonism of vasoconstrictor 20-HETE and vasodilator nitric oxide, we determined also the effect of ABT and HET0016 on medullary tissue NO which was measured polarographically, using a selective electrode.

MATERIAL AND METHODS

The experimental procedures were approved by the extramural First Ethical Committee, Warsaw. Male Wistar rats fed a standard pellet diet were anaesthetized with sodium thiopental (Sandoz GmbH, Kundl, Austria), 100 mg/kg intraperitoneally. The left kidney was exposed from a subcostal flank incision; the femoral vein and artery were cannulated for fluid infusions and systemic blood pressure measurement, respectively. The total renal blood flow (RBF) was measured using a renal artery probe and Transonic T106 flowmeter (Transonic System Inc., Ithaca, N.Y., USA). The cortical laser-Doppler flux (CBF) was measured using laser-Doppler Periflux 4001 system (Perimed AB, Jarfalla, Sweden) and PF 407 probe placed on kidney surface. The inner medullary flux (MBF) was measured by a needle probe (PF 402) inserted into the kidney to the depth of 5 mm. A stainless steel cannula was used for infusion of fluids into the left renal artery. Other technical details of the experiments have been described previously (7).

For measurement of the medullary NO signal, a needle-shaped ISO-NO 200 sensor (0.2 mm in diameter), connected with Nitric Oxide Meter (ISO-NO MARK II, World Precision Instruments, Inc., USA), was inserted to the depth of 5 mm. To verify *in vitro* the responsiveness of the sensor, the curve relating the readings (nA) to known increasing concentrations of NO released from S-

Nitroso-N-acetyl-D, L penicillamine (SNAP) was established as described by Zhang and Broderick (8). The results of studies *in vivo* were expressed in pA. *In vivo* tests confirmed a dose-dependent decrease in tissue NO signal in response to intravenous administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME), and an increase in NO after renal artery infusion of SNAP (9).

In experiments involving administration of drugs into the renal medullary interstitium, two 26 G stainless steel cannulas were inserted into the kidney to the depth of 5.5 mm, close to the outer-inner medullary junction; the rate of infusion was 1 ml/h. In these experiments the renal tissue NO concentration was not determined. The following protocols were used:

Effects of intravenous infusion of ABT (n= 9)

After two 30-min control periods, 1-aminobenzotriazole (Fluka Chemie GmbH, Buchs, Netherlands), a non-selective inhibitor of cytochrome P450 monooxygenases, was applied as a short i.v. infusion, at the dose of 10 mg/kg. Then three 30-min measurement periods were performed. In time control experiments (n= 6), isotonic saline was given. In these experiments CBF was not determined.

Effects of renal artery infusion of HET0016 (n=9)

HET0016, a selective inhibitor of cytochrome P450 ω-hydroxylase, was synthesized by M. Sato (Taisho Pharmaceutical CO., LTD, Yoshino-cho, Japan). The drug was always dissolved in β-cyclodextrin hydrate (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Two 30-min control periods (isotonic saline i.a.) were followed by a 30-min period of β-cyclodextrin solvent infusion (1.42 mg/kg/h). Subsequently, HET0016 was infused at a rate of 0.3 mg/kg/h for the following three experimental periods. In additional three time control experiments, β-cyclodextrin solvent of HET0016 was infused.

Effects of intramedullary infusion of HET0016 (n=9)

Two 30-min control periods (isotonic saline i.v.) were followed by a 30-min period of solvent infusion (7.1 mg/kg/h of β-cyclodextrin hydrate). Subsequently, increasing doses of HET0016 were infused into the renal medulla. Each dose of drug: 0.15, 0.4, 0.75 or 1.5 mg/kg/h was infused for two 30-min measurement periods. In additional six time control experiments, β-cyclodextrin was infused into the medulla instead of HET0016.

For evaluation of the trends and differences over time, repeat measurement ANOVA and paired Student t test were used. $P<0.05$ was accepted as the significance level.

RESULTS

No significant changes in MAP, RBF, CBF or MBF were observed after infusion of ABT or HET0016 solvents. During intravenous ABT infusion MAP was stable at 114-118 mmHg. A significant decrease in RBF was seen, from 6.8 ± 0.6 to 5.8 ± 0.7 ml/min ($P<0.05$), without any change in MBF. Simultaneously, medullary tissue NO increased transiently (not significant) and reached the maximum 1 h after ABT administration (*Fig. 1*., left panel). Since ABT interferes *in vitro* with the NO signal, the data from the short infusion period, when the blood level was the highest, were discarded.

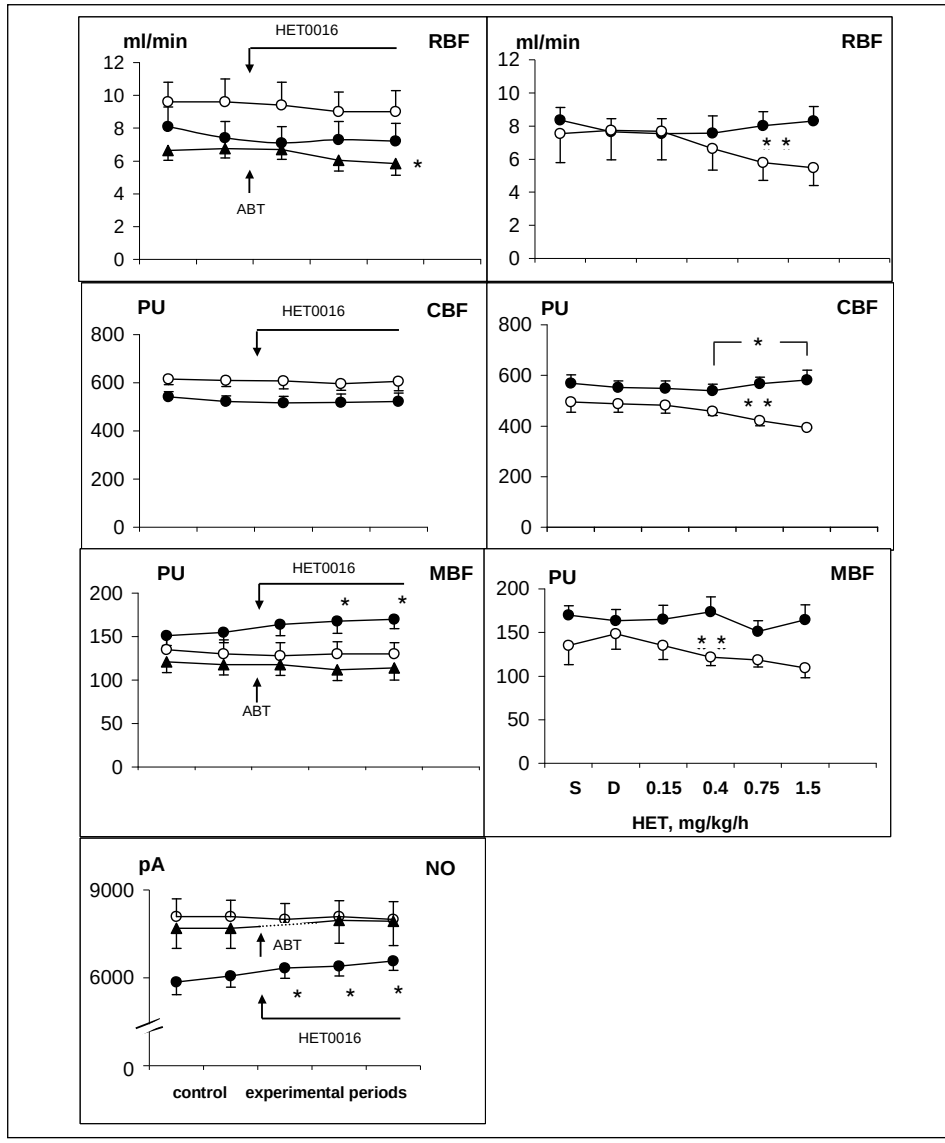

Fig. 1. Effects of non-selective inhibition of CYP-450 dependent monooxygenases (ABT) and selective inhibition of 20-HETE synthesis (HET0016) on renal haemodynamics and tissue NO. ***Left Panel***: Effects of i.v. administration of ABT (-▲-, 10 mg/kg) and renal artery infusion of HET0016 (-●-,0.3 mg/kg/h). ***Right panel***: Effects of intramedullary infusion of HET0016 (-●-. 0.15, 0.4, 0.75 or 1.5 mg/kg/h).

The profiles for cyclodextrin solvent of HET0016 (-○-) are also shown. RBF, CBF, MBF - total renal, cortical and medullary blood flow, respectively. NO - medullary tissue nitric oxide signal. PU - laser-Doppler perfusion units. S - isotonic saline, D - β-cyclodextrin solvent of HET0016. * significant increase by repeat measurement ANOVA; ** significant difference between the HET0016 and cyclodextrin profiles (P = 0.02 to 0.002 by unpaired Student t test).

During renal artery infusion of HET0016, MAP remained stable at 110-114 mmHg (data not shown). HET0016 induced a significant increase in MBF by 16% without changing RBF or CBF; also the medullary tissue NO increased significantly (*Fig. 1*, left panel).

During medullary interstitial HET0016 infusion at increasing rates, the MAP remained stable and the indices of cortical perfusion increased (CBF) or tended to increase (RBF) whereas MBF did not show consistent changes (*Fig. 1*., right panel). Intramedullary infusion of HET0016 solvent (β-cyclodextrin) tended to decrease progressively the renal haemodynamics while MAP did not change. A comparison of the pooled data for HET0016 and for cyclodextrin profiles by unpaired Student t test disclosed significant differences for RBF, CBF and MBF. Thus, HET0016 significantly increased the three parameters when compared with the effects of the solvent alone.

DISCUSSION

Inhibition of 20-HETE synthesis using a specific inhibitor of ω-hydroxylase infused into the renal artery selectively increased MBF, suggesting that 20-HETE suppressed perfusion of the medulla. This is in agreement with earlier evidence that nonselective inhibition of cytochrome P450 dependent pathways of AA metabolism decreased blood flow through the renal medulla (1, 2) but not with our present failure to decrease MBF using ABT.

The demonstration of a decrease in RBF after ABT suggests that perfusion of the renal cortex was under vasodilatator influence of EETs. This result would be compatible with the evidence indicating that EETs released by the afferent glomerular arteriole could affect the tone of the efferent arteriole (10).

Our results show that, at least in anaesthetized rats, elimination of CYP450 dependent compounds does not acutely alter arterial blood pressure. One explanation may be that, although EETs and HETEs have well-defined pro- and antihypertensive properties, their opposed effects may have been in equilibrium. Nor do the present findings argue against the role of these agents in long term control of arterial pressure. The renal production of 20-HETE and EETs is altered in many models of hypertension and blockade of this pathway alters blood pressure in several of these models. Moreover, ABT treatment or selective inhibition of renal expression of CYP450 enzymes by antisense oligonucleotides was found to reduce blood pressure in spontaneously hypertensive rats (SHR) (6).

When HET0016 was administered directly to the medullary tissue, RBF and CBF increased slightly while MBF did not change. These results are not easy to interpret because of the instability of the intramedullary solvent control. An inhibition of vasoconstrictor 20-HETE by renal medullary infusion of HET0016 appeared to limit the reduction of renal perfusion dependent on β-cyclodextrin,

however, it is impossible to say how this inhibition would affect the perfusion under normal circumstances.

In an early work an inhibition of all cytochrome P450 enzymes with 17-octadecynoic acid (17-ODYA) selectively increased renal papillary blood flow in anaesthetised rats; the increase being similar to those observed after infusions into the renal artery or into the interstitium of the renal cortex (11). These results are compatible with our demonstration of a selective increase in MBF in response to renal artery infusion of HET0016, and with the earlier evidence indicating that inhibition of 20-HETE synthesis is a potent mechanism of nitric oxide dependent vasodilatation in the kidney (1, 2).

The increase in MBF observed by us after renal artery infusion of HET0016 was associated with a definite increase in medullary tissue nitric oxide (NO). The observed mean increase in the signal of 330 pA was not trivial. Using the same methodology, an increase of about 500 pA above the basal NO level was seen after a large hypotensive dose of an NO donor (S-Nitroso-N-acetyl-D,L-penicillamine, SNAP) (9 and own unpublished data). Our results fit well with the data of others showing that inhibition of 20-HETE synthesis contributes up to 50-75% of the vasodilator response to NO donors in small renal arterioles *in vitro* and *in vivo* (10). Furthermore, our study provides the first direct demonstration in the whole kidney of the recognised functional antagonism of 20-HETE and NO. The finding suggests that, in the absence of 20-HETE, the NO that is normally consumed for inhibition of cytochrome P450 became available and could be detected in the tissue.

Aknowledgments: HET0016 was generously supplied by Dr. Mariko Sato, Taisho Pharmaceutical Co., Saitama, Japan. We are indebted to Professor Andrzej Lipkowski from the Department of Neuropeptides of our Institute, for his advice on preparation of HET0016 solutions for *in vivo* use.

REFERENCES

1. Imig JD. Eicosanoid regulation of the renal vasculature. *Am J Physiol* 2000; 279: F965-F981.
2. Roman RJ. P-450 Metabolites of arachidonic acid in the control of cardiovascular function. *Physiol Rev* 2002; 82: 131-185.
3. Miyata N, Taniguci K, Ishimoto T, et al. HET0016, a potent and selective inhibitor of 20-HETE synthetizing enzyme. *Br J Pharmacol* 2001; 133: 325-329.
4. Lopez B, Moreno C, Salom MG, Roman RJ, Fenoy FJ. Role of guanylyl cyclase and cytochrome P-450 on renal response to nitric oxide. *Am J Physiol* 2001; 281: F420-F427.
5. Widstrom RL, Norris AW, Spector AA. Binding of cytochrome P-450 monooxygenase and lipoxygenase pathway products by fatty acid-binding protein. *Biochemistry* 2001; 40: 1070-1076.
6. Sarkis A, Roman RJ. Role of Cytochrome P450 Metabolites of Arachidonic Acid in Hypertension. *Current Drug Metabolism* 2004; 5 (3): 1-12.

7. Kompanowska -Jezierska E, Walkowska A, Sadowski J. Role of prostaglandin cyclooxygenase and cytochrome P450 pathways in the mechanism of natriuresis which follows hypertonic saline infusion in the rat. *Acta Physiol Scan* 2003; 177: 93-99.
8. Zhang X, Broderick M. Amperometric detection of nitric oxide. *Mod Asp Immunobiol* 2000; 1: 160-165.
9. Bądzyńska B, Grzelec-Mojzesowicz M, Sadowski J. Effect of exogenous angiotensin II on renal tissue nitric oxide and intrarenal circulation in anaesthetized rats. *Acta Physiol Scand* 2004; 182: 313-318.
10. Sarkis A, Lopez B, Roman J. Role of 20-hydroxyeicosatetraenoic acid and epoxyeicosatrienoic acids in hypertension. *Curr Opin Nephrol Hypertens* 2004; 13: 205-214.
11. Zou AP, Ma YH, Sui ZH, et al. Effects of 17-Octadecynoic acid, a suicide-substrate inhibitor of cytochrome P450 fatty acid ω-hydroxylase, on renal function in rats. *J Pharmacol & Experiment Therapeutics* 1994; 268: 474-481.

Received: November 21, 2006
Accepted: November 24, 2006

Author's address: E. Kompanowska-Jezierska, Laboratory of Renal and Body Fluid Physiology, M. Mossakowski Medical Research Centre, Polish Academy of Sciences, Pawińskiego 5, 02-106 Warsaw, Poland; fax: +48 (22) 6685532; e-mail: renal@cmdik.pan.pl