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EFFECTS OF RENAL NERVES AND PLASMA EPOXYEICOSATRIENOIC ACIDS ON BLOOD PRESSURE, RENAL HEMODYNAMICS AND EXCRETION IN SPONTANEOUSLY HYPERTENSIVE RATS

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Spontaneously hypertensive rats (SHR) display deficiency of epoxyeicosatrienoic acids (EETs). Their possible interaction with renal sympathetic nerves remains unexplored; synthesis of EET-A [disodium (S)-2-(13-(3-pentyl)ureido)-tridec-8(Z)-enamido)succinate], a stable 14,15-EET analog, helps clarify the issue. In anesthetized SHR, untreated or pretreated with EET-A, we assessed early responses of blood pressure (MAP), renal hemodynamics and excretion, and indices of nitric oxide (NO) activity, to bilateral noninvasive renal denervation (DNX). DNX significantly decreased MAP, with or without EET-A pretreatment. Renal perfusion decreased in EET-A treated but not in control rats. After EET-A pretreatment DNX decreased renal excretion of sodium and total solutes, compared to increasing tendency in untreated rats. In EET-A treated but not in untreated SHR denervation reduced the excretion of NO metabolites. Antihypertensive action of EET-A in anesthetized SHR was not clearly dependent on renal nerve activity. On the other hand, DNX unmasked the unexpected effect of EET-A to lower renal perfusion. The mechanism of this novel finding is unclear, as is also the simultaneous post-denervation decrease in renal excretion, again, observed only under EET-A treatment. Possibly, the decrease was secondary to falling MAP and renal perfusion. Increased renal excretion of nitric oxide metabolites under EETs elevation strongly suggests facilitation of NO release; the effect that was observed only with intact renal nerve activity.

Key words: *blood pressure, hypertension, kidney denervation, disodium (S)-2-(13-(3-pentyl)ureido)-tridec-8(Z)-enamido)succinate, kidney circulation, nitric oxide, spontaneously hypertensive rats, epoxyeicosatrienoic acids, renal perfusion, sodium excretion*

INTRODUCTION

Hypertension is a major risk factor for heart disease, stroke or kidney failure, and new therapeutic strategies are still in high demand. The therapeutic potential of epoxyeicosatrienoic acids (EETs) in cardiovascular and renal diseases, including hypertension, has been repeatedly demonstrated (1-3). Among many designed and tested EET analogs, 14,15-EET-A [disodium (S)-2-(13-(3-pentyl)ureido)-tridec-8(Z)-enamido)succinate] (EET-A), a vasodilator and natriuretic agent, is one of the most promising. Experiments with various rat strains have shown its antihypertensive and renoprotective action (4, 5).

We confirmed recently such properties using intravenous EET-A administration in acute experiments with spontaneously hypertensive rats (SHR) (6). However, in chronic studies we found that orally administered EET-A failed to lower blood pressure, similarly in the phase of hypertension development (7) and in adult SHR with established hypertension (8). The reason for the failure is still unclear. The SHR is a genetic model of hypertension with many features similar with those of human primary hypertension. An increase in peripheral vascular resistance in SHR is first induced by neurogenic factors and later aggravated by structural vascular changes (9, 10).

There is growing evidence that impaired activity of EETs belongs in the complex mechanism responsible for blood pressure elevation in SHR. In fact, circulating (plasma) levels of EETs are lowered, largely to due to increased expression and activity of soluble epoxide hydrolase (sEH), an enzyme that metabolizes biologically unstable EETs to inactive dihydroxyeicosatrienoic acids (DHETs) (11). Specifically, the expression of the Ephx2 gene in the kidney which codes sEH was reported to be elevated. Similarly, increased sEH activity (leading to enhanced concentration of DHETs) has recently been suggested as a causal effect of the attenuation of EETs-dependent cardioprotection observed in end-stage heart failure in mice (12). Increased sEH activity in SHR is claimed to contribute to the development of hypertension (13), and its blockade effectively increased tissue EETs level (14). We have also provided evidence of impaired generation of 14,15-EET in the kidney (8).

It will be noticed, however, that in SHR the peripheral activity of EETs may be countered by their central effects and

a local increase in EETs may result in increased reactive oxygen species (ROS) production, leading to an increase of blood pressure (BP) (15). Since SHR display hyperpermeability of the blood-brain barrier (BBB) (16) EET-A supplementation would both enhance the peripheral vasodilator effects but, especially in the long term and with sustained drug's penetration, act in the opposite direction, *via* mechanisms operating in the brain. This may be the reason for the lack of pressure decrease after EET-A supplementation as observed in our chronic studies with SHR) (8).

It is known that, apart from the hormonal and paracrine status, the neural input to the kidney and the stimuli generated in the kidneys and ascending to the brain can contribute to the control of BP. Therefore, in this study we aimed to find out how the renal sympathetic nerve activity would influence blood pressure, possibly by altering renal hemodynamics and excretion and, in particular, if such influence would depend on the activity of EETs. Therefore we compared the effect of renal denervation in SHR that were untreated or pre-treated with EET-A. Our own method of non-invasive denervation was applied, one that does not interfere with determination of whole kidney and renal regional hemodynamics. In addition, we measured tissue medullary NO signal and urine excretion of nitric oxide metabolites.

MATERIALS AND METHODS

Subjects

Second Local Ethical Committee (Warsaw, Poland) approved all protocols and surgical preparations of animals, which are in accordance with the European Union Directive 63/2010 and the corresponding Polish regulations (15 Jan 2015) regarding the usage of laboratory animals.

Male spontaneously hypertensive rats (SHR) were purchased from the animal house of Mossakowski Medical Research Institute, Polish Academy of Sciences (MMRI PAS, Warsaw, Poland) and all the experiments were performed in the Department of Renal and Body Fluid Physiology of MMRI PAS. Rats were aged 16–17 weeks (established hypertension), weighing 289–343 g. They were housed in enriched environment (humidity 45–55%, 12 h light/12 h dark cycle, temperature 22–23°C) and were fed standard laboratory diet (SSNIFF GmbH, Soest, Germany) and given water *ad libitum*.

Surgical preparations

Rats were anesthetized intraperitoneally with sodium thiopental (100 mg/kg, Thipen, Samarth, Life Sciences PVT. Ltd., Mumbai, India) and were placed on a servo-controlled heated table to maintain body temperature at 37°C. Tracheotomy was performed to ensure free airways. For fluid infusion the femoral vein was cannulated and the solution of 3% albumin from bovine serum was infused (10 ml/kg/h) to equilibrate the plasma volume after the surgery. Thereafter this solution was replaced by either EET-A solution [14,15-EET analog; disodium (S)-2-(13-(3-pentyl)ureido)-tridec-8(Z)-enamido)succinate] or its isotonic saline solvent. The femoral artery was cannulated for mean arterial blood pressure (MAP) measurement (Stoelting blood pressure system, Wood Dale, Illinois, USA).

A cuff probe placed on the renal artery was used for measurement of total renal blood flow (RBF). Laser-Doppler Periflux 4001 system (Perimed AB, Jarfalla, Sweden) was used to measure blood perfusion of individual kidney zones. The first probe was placed on the kidney surface to measure cortical blood flow (CBF). The second (needle) probe was inserted into the depth of 4 mm in to the kidney to measure medullary blood flow (MBF).

The medullary level of nitric oxide (NO) was measured by ISO-NOP 200 sensor (0.2 mm in diameter) combined with Nitric Oxide Meter (ISO-NO MARK II, World Precision Instruments, Inc., Sarasota, FL, USA). The needle-shape sensor was inserted into the kidney to the depth of 5 mm.

Both kidneys were prepared to perform a variant of a relatively non-invasive local chemical (novocaine-induced) renal denervation (DNX). Unlike the standard surgical-plus-phenol application procedure (17), the technique enables reliable renal function assessment very soon after DNX. First, the right kidney was exposed *via* flank incision. A thin wire loop was placed around the renal nerve fibers (leading from the coeliac ganglion to the kidney) together with the connective tissue and fat located anteriorly cephalad to the right renal artery-aortic junction. Similarly, another wire loop was prepared for left kidney denervation. Thereafter, both ends were threaded through a thick-walled polyethylene tube (0.6 mm inner diameter) and left loose. The loop was adequately tightened and immediately a novocaine solution (2% in 0.15 ml, Polfa, Warsaw, Poland) was injected through the tube on the renal fibers. The left kidney was denervated immediately after the right one, so the result was bilateral renal denervation. To accomplish a transient rather than sustained denervation, the tissue within the wire loop was not electrocoagulated, as was done in the original procedure (18).

The left ureter was cannulated for timed urine collection, to measure urine flow (V) and to determine total solute and sodium excretion ($U_{osm}V$, $U_{Na}V$), and nitrate/nitrite.

Protocol

EET-A was administered throughout the experiment. The effective EET-A dose (5 mg/kg/h) was established in previous acute experiments (6). After stabilization of the parameters measured (about one hour), control recording was done during a 30-min urine collection. Thereafter, denervation of the right and left kidney was performed, and 30-minute experimental measurements during nerve blockade were done. A separate untreated group was also studied as time-control (bilateral renal denervation performed under infusion of EET-A's saline solvent).

Analytical procedures

Urine samples were collected in the presence of butylated hydroxytoluene to prevent oxidation (8), processed immediately or stored at –80°C until further analysis. Flame photometer (Jenway PFP7, Dunmow, Essex, UK) was used to measure the concentration of sodium in urine. Urine osmolality was evaluated by a cryoscopic osmometer (Osmomat 030; Gonotec GmbH, Berlin, Germany). Colorimetric assay was used to measure the urinary level of nitric oxide metabolites (Nitrate/Nitrite Colorimetric Assay Kit, Cat: 780001, Cayman Chemical, Michigan, USA). To assess the excretion of 8-isoprostane a solid phase enzyme-linked immunosorbent assay was used (516351, Cayman Chemical, Ann Arbor, MI, USA).

Statistical analysis

All the statistical analysis was performed using Graph-Pad Prism software (Graph Pad Software, San Diego, California, USA). Two-way analysis of variance (ANOVA) with Bonferroni's multiple comparisons test was used to compare the values between groups and to evaluate the changes within each group (before and after DNX). Unpaired t test was used to compare the changes (Δ) between groups in each parameter.

Values exceeding 95% probability limits ($p<0.05$) were considered statistically significant. All values are expressed as means ±SEM.

RESULTS

The baseline (pre-denervation) values for the EET-A treated and the control group receiving solvent (0.9% saline) are collected in *Table 1*. While all parameters were slightly higher in the EET-A group, the difference from the value measured in control rats was significant for CBF only and of border line significance ($p<0.058$) for $U_{Na}V$.

Fig. 1A-1D shows the effects of bilateral renal denervation (non-invasive procedure) on mean arterial pressure (MAP), renal total, cortical and medullary blood flow (RBF, CBF, and MBF respectively). In addition, the differences are shown in the responses to DNX between the rats treated with EET-A and controls (*Fig. 1E-1H*). In rats treated with EET-A, DNX caused a mean 13% decrease in MAP compared with a 9% decrease in the group receiving solvent; the between-group difference in the pressure decline was not significant. After denervation RBF decreased significantly in EET-A treated rats and tended, to increase in the control group; the between-group difference was significant ($p=0.03$). For CBF the pattern of response to DNX was similar as with RBF whereas for MBF no significant effects were seen.

Fig. 2 shows that in rats receiving EET-A, $U_{osm}V$ decreased significantly after bilateral renal denervation, whereas the decrease in V and $U_{Na}V$ did not reach statistical significance level. Nevertheless, the decreases in $U_{osm}V$ and $U_{Na}V$ significantly differed from the responses seen in control rats ($p=0.025$ and 0.016, respectively), however, DNX did not significantly affect renal excretion in solvent-infused rats.

Fig. 3A shows that without renal denervation the excretion of nitric oxide metabolites ($U_{NOx}V$) in the EET-A treated group was significantly higher than in the untreated animals: 0.26±0.06 vs. 0.05±0.02 nmol/min (two-way repeated measurement ANOVA with Bonferroni's multiple comparisons test, $F=10.42$, $p=0.0121$). DNX did not significantly alter NO_x excretion, similarly under EET-A treatment and in control rats (*Fig. 3A*). However, there was a significant difference between the change in this parameter in EET-A treated versus untreated rats (*Fig. 3C*). Remarkably, the difference in $U_{NOx}V$ was demonstrable only in the presence of renal nerve activity because it was clearly abolished by DNX. Another aspect of interrelation of NO, EETs and renal nerve activity is presented in *Fig. 3B* and *3D* which shows that renal denervation did not change the NO signal in renal medullary tissue, similarly in EET-A treated and untreated rats. This suggests that NO generated in the kidney did not substantially contribute to changes in overall body NO generation and its modification by EETs and renal nerve activity.

After denervation, urinary excretion of 8-isoprostane, a marker of oxidative stress, was tested in three rats pretreated with EET-A and showed a trend to decrease from 50.8±5.0 to 37.1±4.5 pg/min (NS), whereas in untreated rats, it remained virtually unchanged (46.7±6.8 versus 43.3±9.5 pg/min).

DISCUSSION

SHR are characterized by excessive activation of the sympathetic nervous system and a growing body of evidence supports the role of eicosanoids in the background of hypertension (19). Specifically, SHR show decreased levels of vasodilator and natriuretic EETs in plasma (20) and also reduced generation of 14,15-EET in the kidney (8). Our previous acute studies of anesthetized SHR with established hypertension showed that the analog of EETs [EET-A; (S)-2-(13-(3-pentyl)ureido)-tridec-8(Z)-enamido) disodium succinate], improved renal perfusion and lowered blood pressure (6).

Interestingly, a new experimental observation has shown that hypertension in SHR is associated with enhanced pro-contractile effect of perivascular adipose tissue on vasoconstriction dependent on endogenous noradrenaline released from arterial sympathetic nerves, however the nature of this phenomenon has not been fully elucidated (21).

We present here the first attempt to investigate whether the activity of the renal sympathetic nerves (RSNA) and its effects on systemic and renal circulation in SHR would be modified by EETs. We examined if the effects of bilateral renal denervation (DNX) depend on the actual plasma concentration of 14,15-EET, which was raised by infusion of EET-A, a stable synthetic analog.

Effect of EET-A on hemodynamic and excretory parameters (pre-DNX baseline values)

Even though in the rats receiving the EET analog only the renal cortical blood flow was significantly higher than in the control group, the results are compatible with the previously reported renal vasodilatory and natriuretic effects of EET-A in SHR (6, 22).

The present demonstration of lower blood pressure in the EET-A group accords with our previous acute studies with SHR receiving the analog intravenously (6). On the other hand, in our long-term studies in which EET-A was administered in drinking water, we found that, when administered to adult SHR, EET-A did not show any antihypertensive properties (8). This finding could be related to the central effect of the analog in the brain, considering its prolonged effective penetration of the damaged blood-brain barrier (BBB), since it has been shown that, in contrast to peripheral effects, increased level of EETs in the brain leads to a significant increase in BP in SHR, probably due to inhibition of the baroreceptor reflex and an increased ROS production (15). However, a direct measurement of EET-A concentration in the cerebrospinal fluid would be needed to confirm this hypothesis. Admittedly, different basic conditions in

Table 1. Basal values of mean arterial blood pressure (MABP), total renal blood flow (RBF), cortical flow (CBF), medullary flow (MBF) and renal excretion of water (V), total solutes ($U_{osm}V$) and sodium ($U_{Na}V$) in rats treated with EET-A or solvent (control).

	MABP (mmHg)	**RBF** (ml/min)	**CBF PU**	**MBF PU**	**V** (µl/min)	**$U_{osm}V$** (µosmol/min)	**$U_{Na}V$** (µmol/min)
EET-A	154±5	5.4±0.5	622±40*	240±34	3.6±0.5	4.5±1.3	0.30±0.07*
Control	160±5	4.8±0.5	468±38	200±50	2.7±0.5	2.3±0.8	0.10±0.03

Mean ±SEM. * Significantly different versus control group $p<0.05$ (unpaired t test); n=6–8.

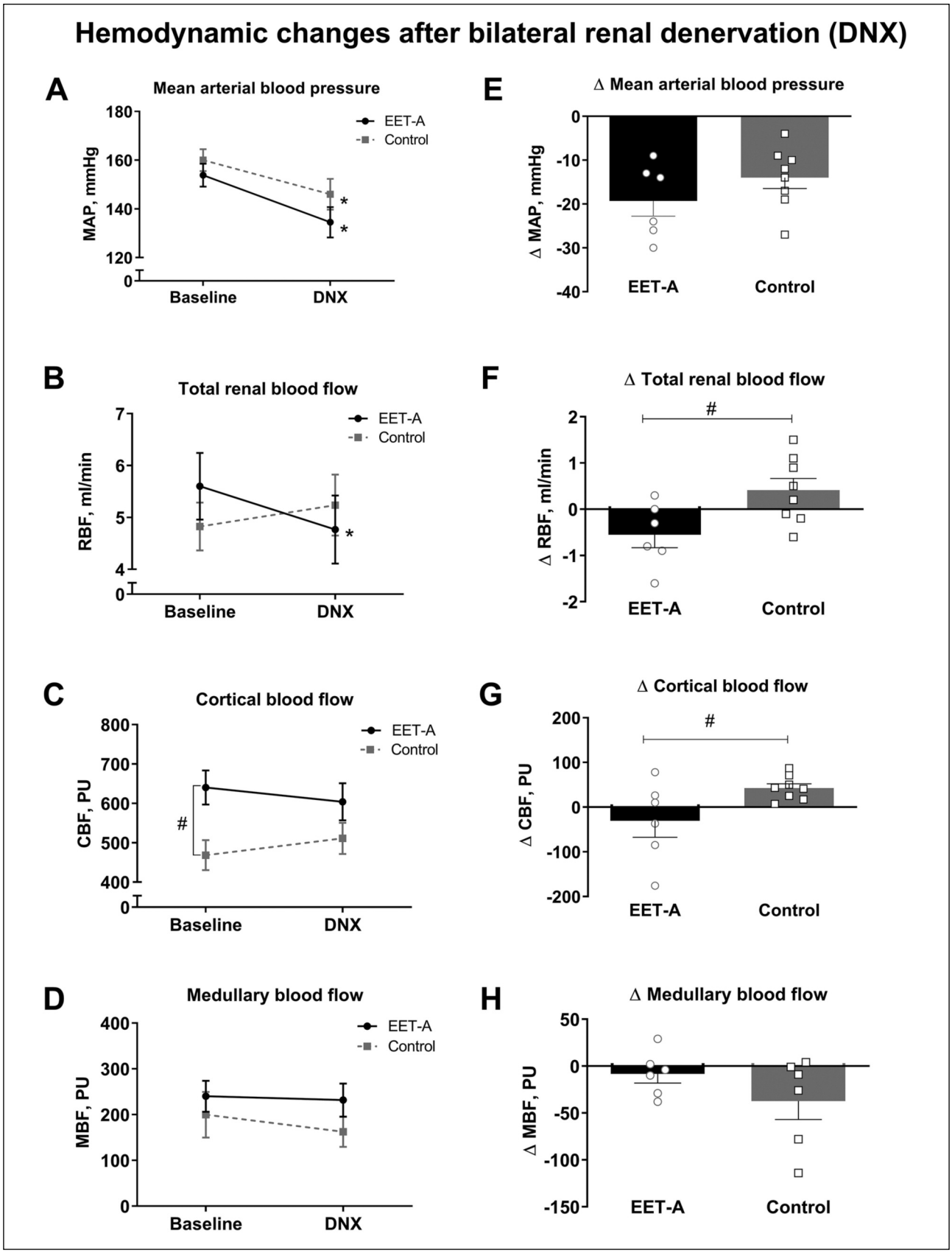

Fig. 1. A comparison of the effects of the bilateral renal denervation (0.15 ml of 2% novocaine solution) on mean arterial blood pressure (MAP) - panels A, E; total renal blood flow (RBF) - panels B, F; cortical blood flow (CBF) - panels C, G; and medullary blood flow (MBF) - panels D, H, in rats treated with EET-A or solvent (control). Right-hand panels (E, F, G, H) show the differences between post-DNX changes in the treated and control groups. Mean ±SEM. * significantly different from control period within the same group at $p<0.05$ (two-way ANOVA with Bonferroni's multiple comparisons test); # significantly different from control group at $p<0.05$ (unpaired t test); n=6–8.

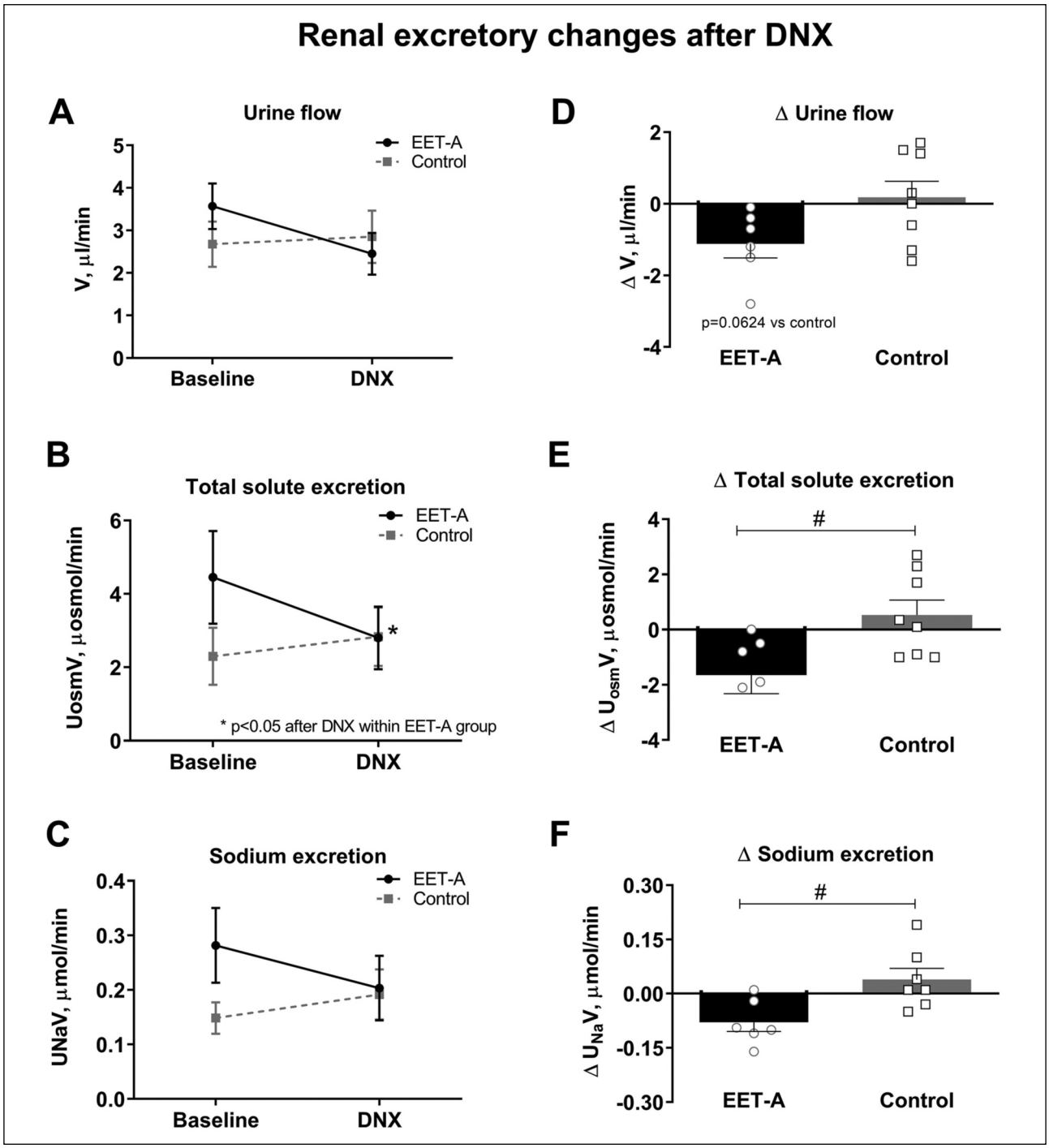

Fig. 2. A comparison of the effects of the bilateral renal denervation (0.15 ml of 2% novocaine solution) on urine flow (V) - panels A, D and total solute and sodium excretion (UosmV, UNaV - panels B, E and C, F, respectively) in rats treated with EET-A or solvent (control). Right-hand panels (D, E, F) show the differences between post-DNX changes in the treated and control groups. Mean ±SEM. * significantly different from control period within the same group at $p<0.05$ (two-way ANOVA with Bonferroni's multiple comparisons test); # significantly different from control group at $p<0.05$ (unpaired t test); n=6–8.

acute and long-term studies (anesthesia, drug administration route, follow-up time) might also account for the discrepant findings. Therefore, the present results refer only to relatively fast responses, without considering possible compensatory mechanisms.

To assess the changes in blood pressure, renal circulation and indices of NO activity directly after bilateral renal denervation, we modified our DNX technique which originally consisted of topical application of a local anesthetic (1% novocaine) and electrocoagulation of the tissue encompassing renal nerve fibres (18, 23) by refraining from the latter procedure component (electrocoagulation) but increasing novocaine concentration to 2%. We found earlier that application of 1% novocaine alone induced typical functional signs of renal denervation, quite

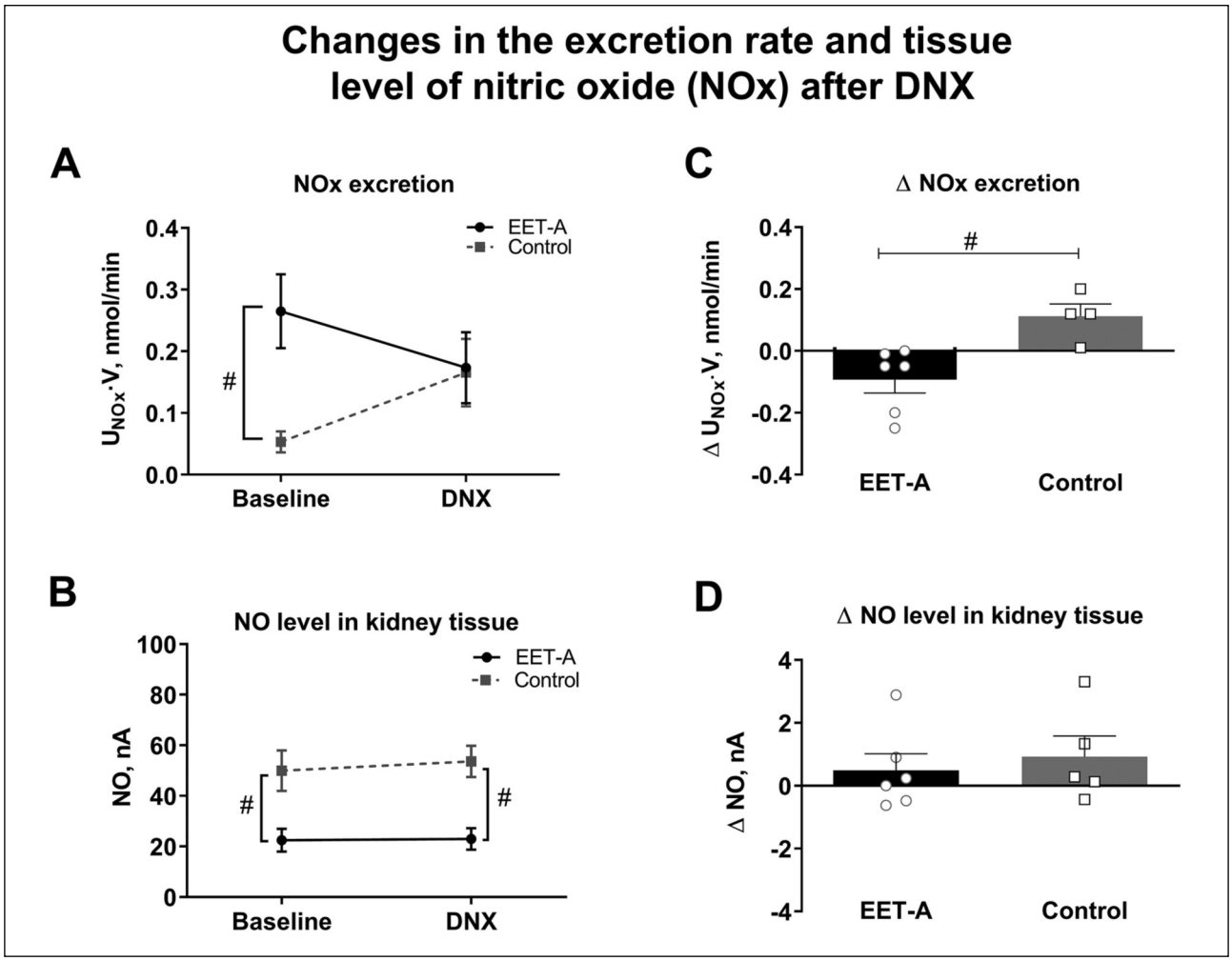

Fig. 3. A comparison of the effects of the bilateral renal denervation (0.15 ml of 2% novocaine solution) on the excretion of nitric oxide metabolites (UNOxV) in EET-A treated and in untreated group - panels A, C; and on DNX-induced changes in kidney tissue nitric oxide level (ΔNO) in EET-A pretreated and in untreated control group - panels B, D. Means ±SEM. * significant difference between the treated and untreated group at $p<0.05$ (two-way ANOVA with Bonferroni's multiple comparisons test); # significant difference between the post-denervation change in kidney NO in the EET-A treated and untreated group at $p<0.05$ (unpaired t test, n=4–6).

similar with those obtained with electrocoagulation (18). With this approach we were able to record renal hemodynamic parameters directly after DNX and avoided a disturbance by electrocoagulation of tissue NO recording or even a damage of NO electrodes. We have verified that total and cortical renal blood flow clearly increased (about 9%) and then remained stable for at least 30 minutes after novocaine application.

Given the vast evidence that renal sympathetic nerve activity exerts vasoconstrictior control of the renal vasculature (24), the observed changes in total and cortical renal blood flows after novocaine-DNX indicate interruption of sympathetic input to the kidney and prove the effectiveness of our renal denervation methodology.

Our main findings regarding the effects of bilateral acute renal denervation as related to EETs levels in SHR were: 1) Blood pressure was significantly lowered, both with and without EET-A pre-treatment; 2) EET-A pre-treatment resulted in a post-DNX decrease in total and renal cortical blood flow, as opposed to the increasing tendency seen in untreated SHR; 3) EET-A treated SHR showed a post-DNX decrease in the excretion of total osmoles and sodium; 4) EET-A pre-treatment reduced the post-DNX excretion of NO metabolites.

Hemodynamic changes after denervation

Acute bilateral renal denervation in anesthetized SHR significantly reduced MAP in untreated rats by about 9%; the decrease was sustained over 30 minutes of observation (*Fig. 1A*). In agreement with earlier reports, in SHR hypertension is caused, among other factors, by sympathetic hyperactivity, and renal denervation performed by various techniques can lower the pressure (25-27). The crucial finding of the present study was that in SHR the post-denervation decrease in blood pressure did not differ between the situation with usual and elevated plasma EETs level.

Although both efferent and afferent renal nerves may play an important role in the regulation of RBF, the effects of the former are better documented (28). The distinct tendency to a post-denervation increase in total renal and cortical blood flow (despite a concurrent decrease in blood pressure) observed after denervation in the untreated group (see *Fig. 1*) strongly suggests that the cortical circulation was under tonic vasoconstrictor influence of the sympathetic renal nerves.

On the other hand, it is not clear why the post-denervation decrease in blood pressure was associated with decreasing renal

perfusion in rats pre-treated with EET-A, in contrast to the increasing tendency in the control group which was an expected post-DNX response (29, 30).

Possibly, with somewhat greater post-DNX blood pressure decrease in rats pre-treated with EET-A the autoregulation range (which is shifted upwards in SHR (31)) was exceeded and renal perfusion was falling, as usual, with decreasing renal perfusion pressure. However, after the initial increase in RBF in response to EET-A (prior to DNX) and potential a simultaneous action of novocaine and EET-A on the K^+ channels of the kidney microvessels could occur (32, 33), which further complicates the interpretation of our results.

Interestingly, in the control group denervation tended to decrease MBF, despite an opposite trend observed for CBF (*Fig. 1D*). It is known that the control mechanisms of the medullary and cortical circulation can differ: for instance, renal nerve stimulation induced a significantly smaller blood flow decrease in the medulla than in the renal cortex (34). In general, MBF appears to be under strong local control which can offset neurogenic effects (35). In SHR medullary perfusion was reported to be lower than in normotensive controls whereas the cortical blood flow was similar (36).

Excretion changes after denervation

Maintenance of sodium and water balance by the kidneys is essential for long-term control of arterial pressure. One function of the sympathetic nerves innervating renal tubules is stimulation of sodium reabsorption leading to decreased urinary sodium excretion (37).

Impairment of sodium transport in the kidney is thought to be one of the genetic causes of hypertension in spontaneously hypertensive rats (38) which were early found to have a reduced ability to excrete sodium and water compared to WKY (Wistar Kyoto) rats (39).

Renal denervation results in decreased sodium reabsorption but in SHR the change is less pronounced than in WKY rats (40). In our study DNX did not alter renal excretion in the control group. This could be a consequence of a concurrent reduction of MAP (and renal perfusion pressure) and perhaps some decrease in MBF. It was proposed that even small changes in the perfusion of the medulla can have a profound effect on sodium and water excretion (41).

EETs have been demonstrated to reduce fluid transport in the proximal and distal tubules, mostly by inhibiting the epithelial sodium channel (ENaC) (2). While, as could be predicted, the pre-denervation renal excretion parameters at least tended to be higher in SHR receiving EET-A (*Table 1*, *Fig. 2A-2C*), the results of denervation were unexpected: in control SHR renal excretion tended to increase slightly whereas in EET-A pretreated rats $U_{osm}V$ clearly decreased and V and $U_{Na}V$ tended to decrease. The reason for such response might be simply related to blood pressure decrease below the level needed in SHR to ensure the excretion of usual amount of sodium. Alternatively, it must be considered that after EET-A treatment DNX caused a moderate significant decrease in RBF (as opposed to 8% elevation in control rats) as well as at least a tendency to a decrease in CBF and MBF. Very likely, the fall in CBF was associated with a fall in the glomerular filtration rate (GFR), which may have contributed to the observed decrease in excretion. Moreover, it should be mentioned that both EETs elevation and renal denervation would decrease tubular sodium reabsorption upstream from the macula densa region and increase tubular fluid delivery to this site. This would result in an enhancement of the TGF-dependent decrease in renal excretion. No such denervation effect was seen in the control group *i.e.* without EET-A elevation. The limitation of this study is the lack of measurement of GFR, which would facilitate interpretation of the results. Unfortunately, with our methodology, at low urine flow inulin precipitates, which would interfere with determination of the basic excretion and metabolic parameters.

Nitric oxide changes after denervation

The bioavailability of NO was reported to be reduced in SHR (42). Our finding that the excretion of nitric oxide metabolites ($U_{NOx}V$) in the EET-A treated group was significantly higher than in the untreated SHR (*Fig. 3A*) suggests that EET-A treatment facilitated global generation of NO. Indeed, EETs have been demonstrated to increase NO release in various tissues (43, 44). Remarkably, we showed that such action was demonstrable only in the presence of renal nerve activity: it was clearly abolished by DNX. The response to DNX differed in EET-A treated and untreated rats: in the former $U_{NOx}V$ tended to decrease, in contrast to an increasing tendency seen in the latter. The response observed in control rats agrees with the evidence that in SHR the bioavailability of NO increases after ablation of the renal nerves (45). On the other hand, it is not clear (i) why intact renal nerve activity is needed for EETs to stimulate NO generation, and (ii) why DNX causes a decrease instead of an increase in $U_{NOx}V$ under greater availability of EETs. The question can hardly be addressed here because our denervation method excluded both the neural input to the kidney (efferent nerves) and the afferent traffic to brain centers involved in the control of the overall sympathetic activity and of the peripheral vascular tone.

It is noteworthy that EET-A treatment did not affect the NO signal measured in the renal tissue after denervation, while the excretion of NO metabolites appeared to decrease (not significant). This suggests that kidney tissue was not an important source of NO during changes in its global pool that was at least roughly reflected by $U_{NOx}V$.

Limitations and conclusions

In this study we aimed to evaluate the role of EETs and renal denervation in the regulation of blood pressure, renal hemodynamics and excretion of spontaneously hypertensive rats. It is important to mention that we used only male rats, which is a clear limitation of our study. Differences in the renal activity of CYP have been demonstrated between male and female rats (46), therefore for future studies both sexes should be employed in future studies.

Application of the epoxyeicosatrienoic acid analog (EET-A) strengthened the evidence that EETs lower blood pressure in SHR, at least under anesthesia and in the early phase of treatment. This action was not clearly dependent on the renal nerve activity. On the other hand, with elevated EETs renal denervation unexpectedly lowered renal perfusion. This is a novel finding but the underlying mechanism is unclear. Also unexpectedly, in rats with elevated EETs (potent natriuretics!) renal denervation induced a decrease in renal excretion, the response opposite to the usual denervation natriuresis. Possibly, the decrease was due to a simultaneous reduction of blood pressure and renal hemodynamics. Unlike under basal conditions, under EETs elevation renal perfusion was seen to strictly follow blood pressure, without usual autoregulation. A novel finding was that renal denervation abolishes EETs-dependent facilitation of the release of NO, which points to the complexity of the interaction of EETs and renal nerve activity on NO bioavailability.

Abbreviations: BBB, blood-brain barrier; BP, blood pressure; CBF, cortical blood flow; DNX, bilateral renal denervation; EETs,

epoxyeicosatrienoic acids; EET-A, analog of 14,15-EET; ENaC, epithelial sodium channel; GFR, glomerular filtration rate; MAP, mean arterial blood pressure; MBF, medullary blood flow; NO, nitric oxide; RBF, total renal blood flow; ROS, reactive oxygen species; sEH, soluble epoxide hydrolase; SHR, spontaneously hypertensive rats; TGF, tubulo-glomerular feedback; $U_{osm}V$, total solute excretion; $U_{Na}V$, sodium excretion; $U_{NOx}V$, excretion of nitric oxide metabolites; V, urine flow; WKY, Wistar Kyoto rats.

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