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Eprosartan modulates the reflex activation of the sympathetic nervous system in sodium restricted healthy humans

Henrik Vase, Thomas G. Lauridsen, Jesper N. Bech & Erling B. Pedersen

Department of Medical Research, Holstebro Hospital and Aarhus University, Aarhus, Denmark

Correspondence
Dr Henrik Vase MD, Department of Medical Research, Holstebro Hospital, Laegaardvej 12, 7500 Holstebro, Denmark.
Tel.: + 45 9912 5427
Fax: + 45 9912 5422
E-mail: hevas@ringamt.dk

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- A sympatho-inhibitory effect of ACE-inhibitors and AT_1 receptor antagonists has been widely demonstrated in animal models, but in humans this effect tends only to be present during chronic treatment in conditions with pre-existing high levels of sympathetic activity.
- Sodium restriction increases renal sympathetic nerve activity and the activity of the renin-angiotensin system and may be a favourable condition to demonstrate sympatho-inhibition as a short-term effect of the AT_1 receptor antagonist eprosartan in healthy humans.

WHAT THIS STUDY ADDS

- Results from our study indicate that during sodium restriction eprosartan has a small inhibitory effect on nonbaroreflex mediated activation of the sympathetic nervous system.
- During arterial baroreflex mediated activation of the sympathetic nervous system this effect is, however, completely overruled by an increased sensitivity of the arterial baroreflex.

AIMS

To test the hypothesis that eprosartan inhibits both nonbaroreflex and arterial baroreflex mediated activation of the sympathetic nervous system, assessed by renal tubular function, systemic haemodynamics and vasoactive hormones, in sodium restricted healthy humans.

METHODS

The effect of eprosartan on urinary sodium, lithium and water excretion, heart rate (HR), blood pressure and vasoactive hormones was measured before, during and after a cold pressor test (CPT) and sodium nitroprusside (SNP) infusion in a randomized, placebo controlled, double-blind, crossover study in 17 healthy subjects. Glomerular filtration rate and renal tubular function were determined by a continuous infusion clearance technique and vasoactive hormones by radioimmunoassays.

RESULTS

Eprosartan attenuated the impact of the CPT on HR (mean difference from placebo (95% confidence interval) (3.9 (0.7, 7.0) min^{-1}) and mean arterial pressure (MAP) (4.7 (0.3, 9.2) mmHg), but no effect of eprosartan was observed on the impact of the CPT on renal tubular function. During a SNP induced reduction in MAP of 10 mmHg eprosartan decreased fractional excretions of sodium (0.46 (0.14, 0.76)%) and lithium (5.1 (2.5, 7.6)%) and tended to increase HR (4.1 (–0.26, 8.4) min^{-1}) and plasma concentrations of norepinephrine (33.8 (–5.8, 72.1) pg ml^{-1}).

CONCLUSIONS

These findings suggest that during mild sodium restriction eprosartan has a small inhibitory effect on nonbaroreflex mediated activation of the sympathetic nervous system. During arterial baroreflex mediated activation of the sympathetic nervous system this effect is, however, completely overruled by an increased sensitivity of the arterial baroreflex.

Introduction

There are extensive interactions between the sympathetic nervous system (SNS) and the renin-angiotensin system (RAS). The role of the SNS in the regulation of renin secretion from the juxta-glomerular apparatus is well established, while substantial evidence also exists for the fact that angiotensin II (Ang II) facilitates the activity of the SNS on several levels including stimulation of central nervous system sympathetic outflow and ganglionic transmission and facilitation of synaptic transmission through increased norepinephrine release and decreased norepinephrine reuptake [1, 2].

In various *in vitro* and animal experiments AT_1 receptor antagonists possess sympatho-inhibitory abilities both with and without the presence of exogenous Ang II [3–8]. In studies involving humans, this effect of the AT_1 receptor antagonists has been far more difficult to demonstrate. Studies evaluating the short-term effect of an AT_1 receptor antagonist show minimal [9] or absent [10–15] antiadrenergic effects or even increased sympathetic outflow [16]. In fact a sympatho-inhibitory effect of AT_1 receptor blockade seems only to be present after long-term treatment during conditions with high pre-existing levels of sympathetic activity such as heart failure [17], obesity related hypertension [18] and chronic kidney disease [19].

We therefore reasoned that to demonstrate a sympatho-inhibitory effect of short-term AT_1 receptor blockade a condition with increased levels of sympathetic would be necessary. Since dietary sodium restriction increases the activity of the SNS and the RAS [20] this could be a favourable experimental setting.

Our hypothesis was that short-term administration of the AT_1 receptor antagonist eprosartan inhibited both the nonbaroreflex mediated SNS activation and the arterial baroreflex mediated SNS activation during mild sodium restriction in healthy humans.

Renal sympathetic nerve activity plays an important role in regulating renin secretion, renal handling of sodium and water and ultimately blood pressure. To assess renal sympathetic nerve activity indirectly, renal tubular sodium handling, expressed as fractional sodium excretion, was chosen as the primary endpoint.

Thus, the purpose of the study was to investigate the effect of eprosartan on renal tubular function, systemic haemodynamics and vasoactive hormones during a cold pressor test and during a reduction in mean arterial pressure of 10 mmHg during sodium nitroprusside infusion in sodium restricted healthy humans.

Methods

Participants

Inclusion criteria Healthy male and female volunteers were recruited by advertisement in public and private institutions aged 18–65 years with a body mass index less or equal to 30 kg m^{-2}. Women were required to use oral hormonal contraceptives or an intrauterine anticonceptive device.

Exclusion criteria Subjects were excluded if there was a history or clinical signs of heart, lung, kidney or endocrine organ disease, clinically significant abnormal biochemical screening of the blood regarding haemoglobin, sodium, potassium, creatinine, albumin, bilirubin, alanine aminotransferase, alkaline phosphatase, cholesterol and glucose, clinically significant abnormal screening of the urine regarding albumin and glucose, arterial hypertension, defined as daytime ambulatory blood pressure above 135 mmHg systolic or above 85 mmHg diastolic, malignant disease, Alcohol abuse, current medical treatment, drug abuse, pregnancy or breast feeding, known intolerance or allergy to eprosartan or sodium nitroprusside or blood donation within 1 month of the start of the study.

Withdrawal criteria Subjects were withdrawn from the study if they developed of an exclusion criterion, clinically significant side-effects to the drugs used in the trial, lack of compliance or withdrawal of consent.

Ethics

The local Medical Ethics Committee (journal number 2006–1025) approved the study. All participants received written and oral information and gave their consent by signature.

Design

We performed a randomized, placebo-controlled, double blind, crossover study. Each subject was studied on two separate days at least 14 days apart. On each study day and on the day before the subjects received either 600 mg eprosartan or placebo in a randomized order with all other procedures being identical. The randomization and blinding of the study drug was conducted by the hospital pharmacy.

Effect variables

Primary effect variable was fractional excretion of sodium (FE_{Na}). Secondary effect variables were urinary sodium excretion rate (U_{Na}), lithium clearance (C_{Li}), fractional excretion of lithium (FE_{Li}), plasma concentrations of norepinephrine (NE), heart rate (HR), blood pressure, glomerular filtration rate (GFR), free water clearance (C_{H2O}), urine volume, urinary excretion of aquaporin-2 (u-AQP2) and plasma concentrations of vasopressin (AVP), renin (PRC), aldosterone (Aldo), atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and Ang II.

Number of participants

An increase in FE_{Na} of 0.004 was considered the relevant difference between eprosartan and placebo. The standard

deviation was estimated to be 0.005. With a level of significance of 5% and a power of 90%, 16 healthy subjects needed to be included in the trial.

Procedure

For 4 days prior to each study day the subjects consumed a standardized diet and fluid intake. If the individually estimated energy requirement was above or below 9500 kJ day^{-1} a diet containing 11 000 kJ day^{-1} or 8000 kJ day^{-1}, respectively, was supplied. Of the total energy content 55% was from carbohydrates, 15% from proteins and 30% from lipids. Fluid intake was 250 ml/1000 kJ day^{-1} and sodium content was 60–70 mmol day^{-1}.

Tablets of eprosartan or placebo were ingested at 07.00 h on the study day and on the day before. A 24 h urine collection was performed the day before the study and the subjects ingested 300 mg lithium carbonate at 22.00 h for the measurement of lithium clearance

On the study day the participants arrived at 08.00 h in the laboratory. Indwelling catheters were placed in the antecubital veins of both forearms, one for infusion of ^{51}Cr-EDTA and sodium nitroprusside and one for withdrawal of blood samples. An oral water load of 175 ml every 30 min was initiated at 07.00 h. Urine was collected in the sitting or standing position, otherwise the subjects were kept in supine position.

At 08.30 h, a priming dose of ^{51}Cr-EDTA was administered followed by sustained infusion. After 60 min of equilibration the study continued with six clearance periods of 30 (periods 1–4) or 60 (periods 5 and 6) min each. Two baseline periods were conducted from 09.30 h to 10.30 h. At 10.45 h the cold pressor test was performed: One hand of the subject was immersed in ice water to the wrist for 2 min. Blood samples were drawn from the contralateral side for measuring NE 1 min prior to immersion and 1 min after emersion. BP was measured immediately before the immersion and after 1 and 2 min of immersion. The subjects were instructed to breathe normally and not perform Valsalva-like manoeuvres or to speak during the process.

At 11.30 h the infusion of sodium nitroprusside (SNP) was initiated. BP was measured before the start of the infusion and every 5 min during the first 30 min of the infusion, thereafter every 15 min. Start infusion rate was 0.5 µg kg^{-1} min^{-1}. This rate was adjusted with 0.1–0.3 µg kg^{-1} min^{-1} after each blood pressure measurement aiming at a reduction of the mean arterial pressure of 10 mmHg. The infusion rate was not allowed to exceed 1.9 µg kg^{-1} min^{-1}.

HR was measured continuously from 09.30 h to 13.30 h.

Urine collection was performed for each clearance period and was analyzed for Na, Li, creatinine, osmolality, AQP2 and ^{51}Cr-EDTA.

Blood samples are drawn every 0.5 h from 09.30 h to 11.30 h, at 12.30 h and 13.30 h, and were analyzed for Na, Li, creatinine, osmolality and ^{51}Cr-EDTA. In addition, analysis of NE, AVP, ANP, BNP, PRC, Ang II and Aldo were performed from blood samples drawn at 10.30 h, 11.30 h, 12.30 h and 13.30 h.

Measurements

All blood samples were centrifuged for 15 min at 3000 rev min^{-1} at 4°C. Plasma was separated from blood cells and kept frozen at −20°C until assayed.

Glomerular filtration rate was measured using the constant infusion clearance technique with ^{51}Cr-EDTA as reference substance.

NE was determined by a commercial RIA assay (IBL, Hamburg, Germany). The minimal detection concentration was 50 pg ml^{-1}. The coefficients of variation were 14.9% (interassay) and 7.9% (intra-assay).

ANP, BNP, AVP and Ang II were extracted from plasma with C_{18} September–Pak (Water associates, Milford, MA, USA) and thereafter determined by radioimmunoassay [21, 22]. Rabbit anti-ANP antibody was obtained from Department of Clinical Chemistry, Bispebjerg Hospital, Denmark. The minimal detection concentration was 0.5 pmol l^{-1}, coefficients of variation were 12% (interassay) and 10% (intra-assay). Rabbit anti-BNP antibody without cross reactivity with urodilatin and α-ANP was used. The minimal detection concnetration was 0.5 pmol l^{-1} plasma. The coefficients of variation were 11% (interassay) and 6% (intra-assay). The antibody against AVP was a gift from Professor Jacques Dürr, Miami, FL., USA. The minimal detection concentration was 0.5 pmol l^{-1}. The coefficients of variation were 13% (interassay) and 9% (intra-assay). The antibody against Ang II was obtained from the Department of Clinical Physiology, Glostrup Hospital, Denmark. The minimal detection concentration was 2 pmol l^{-1}. The coefficients of variation were 12% (interassay) and 8% (intra-assay).

Aldo was determined by a commercial RIA assay (Diagnostic Systems Laboratories Inc., Webster, Texas, USA). The minimal detection concentration was 22 pmol l^{-1}. The coefficients of variation were 8.2% (interassay) and 3.9% (intra-assay).

PRC was determined by a commercial RIA assay (CIS bio international, GIF-SUR-YVETTE CEDEX, France). The minimal detection concentration was 1 pg ml^{-1}, the within-run reproducibility CV was 4.5% and the between-run reproducibility was CV 14.5%.

U-AQP-2 was measured by radioimmunoassay as previously described [23]. Urine samples were centrifuged for 5 min at 3000 rev min^{-1} and 125–3000 µl supernatant was freeze dried and kept frozen at −20°C until assayed. Rabbit anti-AQP2 antibody for radioimmunoassay was obtained from Søren Nielsen (The Water and Salt Research Centre, Institute of Anatomy, Aarhus University, Denmark). The minimal detection concentration was 32 pg $tube^{-1}$. The coefficients of variation were 11.7% (interassay) and 5.9% (intra-assay). Serum and urinary concentrations of lithium were measured by atomic absorption spectrometry (AAnalyst 100 spectrometer, Perkin Elmer, Boston, MA). Plasma and urinary osmolality were measured by freezing-point

depression (Advanced Model 3900 multisampling osmometer). C_{H2O} was determined according to the formula $C_{H2O} = V - C_{Osm}$, where V is urine output, and C_{Osm} is the osmolality clearance.

Blood pressure was measured with UA-743 digital blood pressure meter (A & D Company, Tokyo, Japan). Heart rate was measured continuously by a heart rate monitor (Polar S810i, Polar Electro Oy, Kempele, Finland). Plasma and urinary concentrations of sodium and creatinine were measured by routine methods at the Department of Clinical Biochemistry, Holstebro Hospital, Denmark. All clearances were standardized to a body surface area of 1.73 m^2.

Statistics

Statistical analyses were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL). All estimates are given as means with 95% confidence intervals or ±SD, unless stated otherwise.

Paired-sample *t*-tests were used with Bonferroni correction for multiple comparisons where appropriate. The systemic haemodynamic response to SNP infusion was analyzed with multivariate repeated measures analysis of variance (ANOVA) with eprosartan/placebo administration and time as within subject factors.

The statistical level of significance was $P < 0.05$ in all analyses.

Results

Demographics

Nineteen subjects were included in the study, nine women and 10 men. Two participants were excluded, both due to lack of compliance.

Table 1 shows clinical and laboratory data of the 17 subjects completing the study.

24 h urine collection

Table 2 shows the results of the 24 h urine collection during administration of eprosartan and placebo. No significant differences were observed. The total sodium excretion was in the lower range during both administrations.

Cold pressor test

Blood pressures at baseline were significantly lower after eprosartan compared with placebo, while HR was unchanged (systolic BP: 108.2 ± 8.1 *vs.* 116.5 ± 8.7 mmHg, $P < 0.001$, diastolic BP: 60.6 ± 6.6 *vs.* 67.4 ± 6.5 mmHg, $P < 0.001$, MAP: 76.5 ± 6.1 *vs.* 83.8 ± 6.4 mmHg, $P < 0.001$ and HR: 59.3 ± 5.6 *vs.* 58.4 ± 7.6 beats min^{-1}, $P = 0.58$)

Figure 1 depicts changes from baseline values in HR, MAP and NE during the cold pressor test (CPT) after eprosartan and placebo. Compared with placebo eprosartan significantly reduced the rise in HR during the first minute of the CPT, while no differences were observed in the increase in MAP after the first minute. After 2 min of the CPT, the increase in MAP was significantly lower after eprosartan compared with placebo. The increase in NE was unaffected by eprosartan.

Table 3 shows U_{Na}, FE_{Na}, C_{Li}, FE_{Li}, GFR, urine output, C_{H2O} and u-AQP2 before and the changes during the CPT, and before and the changes during the SNP infusion. No differences were observed between eprosartan and placebo before the CPT. During the CPT GFR, urine output and C_{H2O} were significantly reduced while FE_{Na} was significantly increased after both eprosartan and placebo administration. While the changes in GFR and FE_{Na} were similar after both administrations, the changes in urine output and C_{H2O} were significantly more pronounced during eprosartan compared with placebo. U_{Na}, C_{Li}, FE_{Li} and urinary excretion of AQP-2 corrected for creatinine excretion were unchanged during CPT, and urinary excretion rate of

Table 1

Baseline clinical and laboratory characteristics of the 17 healthy subjects. Values are means ± SD

Age (years)	26.9 ± 4.8
Body mass index (kg m^{-2})	23.8 ± 2.6
Daytime ambulatory systolic BP (mmHg)	125.8 ± 6.7
Daytime ambulatory diastolic BP (mmHg)	75.8 ± 4.4
Haemoglobin (mmol l^{-1})	8.7 ± 0.8
Plasma sodium (mmol l^{-1})	140.0 ± 1.7
Plasma potassium (mmol l^{-1})	4.0 ± 0.3
Plasma creatinine (μmol l^{-1})	79.4 ± 14.6
Plasma alanine aminotransferase (U l^{-1})	24.4 ± 16.0
Plasma alkaline phosphatase (U l^{-1})	59.4 ± 15.9
Plasma bilirubin (μmol l^{-1})	9.7 ± 3.3
Plasma glucose (mmol l^{-1})	5.1 ± 0.6
Plasma cholesterol (mmol l^{-1})	4.3 ± 0.8

Table 2

Urine output, urine osmolality, total sodium excretion (Total Na), fractional excretion of sodium (FE_{Na}), and urinary excretion of aquaporin-2 with correction for creatinine excretion (u-AQP-2_{CR}) in 17 healthy subjects in the 24 h urine collection

		24 h urine collection
Urine output (ml)	Eprosartan	2620 (2260, 2995)
	Placebo	2534 (2174, 2894)
	Difference	–86 (–504, 331)
Urine osmolality (mosm kg^{-1})	Eprosartan	328.4 (249.4, 407.4)
	Placebo	325.3 (257.6, 393.0)
	Difference	–3.1 (–67.2, 61.0)
Total Na (mmol)	Eprosartan	79.7 (59.5, 99.8)
	Placebo	71.4 (57.9, 84.9)
	Difference	–8.3 (–26.6, 10.1)
FE_{Na} (%)	Eprosartan	0.32 (0.23, 0.40)
	Placebo	0.29 (0.23, 0.35)
	Difference	–0.03 (–0.09, 0.04)
u-AQP-2_{CR} (ng $mmol^{-1}$)	Eprosartan	0.089 (0.080, 0.098)
	Placebo	0.083 (0.075, 0.091)
	Difference	–0.006 (–0.016, 0.005)

Values are means (95% confidence interval). Paired samples *t*-test.

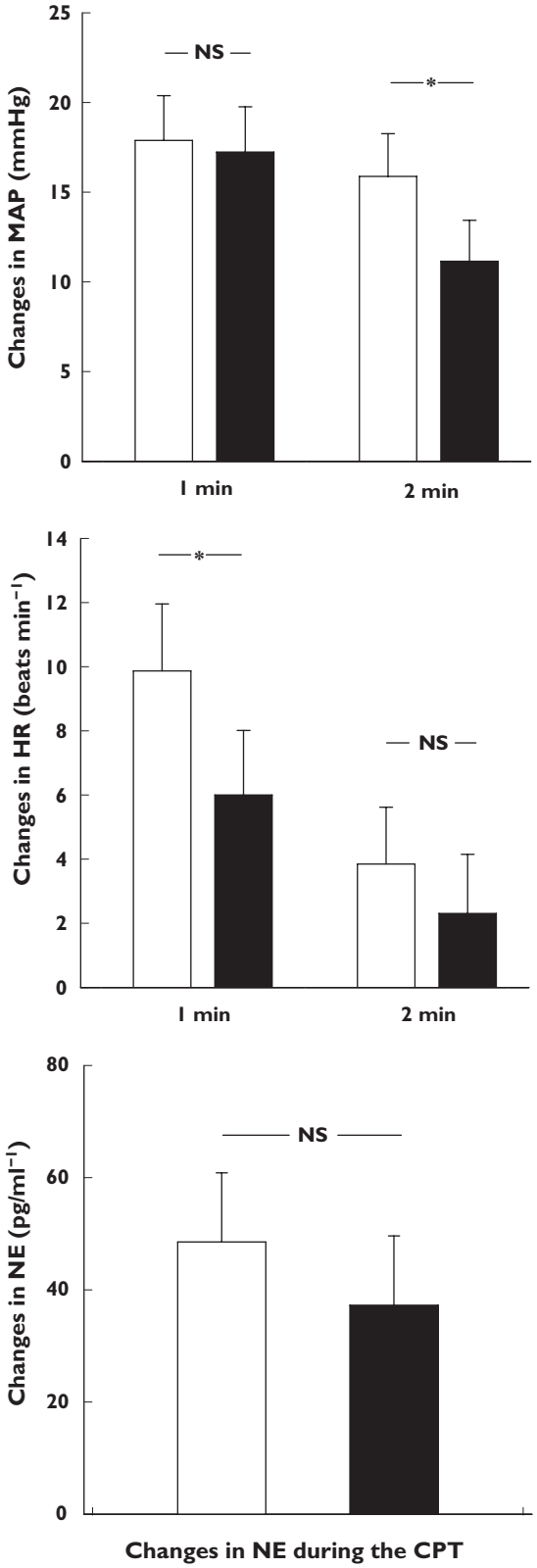

Figure 1

Changes in mean arterial pressure (MAP), heart rate (HR) and plasma concentrations of norepinephrine (NE) during the cold pressor test (CPT), displayed as means + SEM. Open bars: placebo. Black bars: eprosartan. *$P < 0.05$ paired sample t-test. Placebo (□); Eprosartan (■)

AQP-2 and serum osmolality were as well unchanged during the CPT (data not shown).

Concentrations of plasma hormones are shown in Table 4. Ang II and PRC were significantly increased and ANP and BNP were significantly reduced after eprosartan compared with placebo before the CPT.

Sodium nitroprusside infusion

Changes in systolic BP, diastolic BP, MAP and HR during the SNP infusion are depicted in Figure 2. MAP and systolic BP were reduced to similar extents during the SNP infusion after both administrations, whereas diastolic BP was reduced significantly less during this process after eprosartan compared with placebo. HR tended to increase more during SNP infusion after eprosartan administration compared with placebo. The infusion rate used attempting to lower by MAP 10 mmHg was not significantly different during placebo compared with eprosartan administration (data not shown).

Significant reductions in FE_{Na} along with U_{Na}, C_{Li} and FE_{Li} were observed after administration of eprosartan compared with placebo during SNP infusion (Table 3). GFR, urine output and C_{H2O} were not significantly changed compared with placebo. Urinary excretion of AQP-2 corrected for creatinine excretion was significantly higher during eprosartan before the SNP infusion and this difference disappeared during the SNP infusion. Urinary excretion rate of AQP-2 was unchanged during the SNP infusion (data not shown). Serum osmolality was similar before the SNP infusion (286.0 ± 2.2 *vs.* 286.7 ± 2.8 mosm kg^{-1}, $P = 0.32$), but was significantly reduced during the SNP infusion and this reduction tended to be more pronounced after eprosartan compared with placebo (-2.1 ± 1.1 *vs.* -1.4 ± 1.0 mosm kg^{-1}, $P = 0.12$).

AVP increased significantly during the SNP infusion after eprosartan administration compared with placebo (Table 4). ANP and BNP were both significantly reduced, while Aldo was significantly increased during the SNP infusion and these changes were not affected by eprosartan. Significant increases were also noted for PRC and Ang II during the SNP infusion, but the relative increases did not differ between eprosartan and placebo. NE increased significantly during the SNP infusion, and the increase tended being higher during eprosartan administration compared with placebo ($P = 0.09$).

Discussion

This report comprises a randomized, placebo-controlled, crossover study of the effects of the AT_1 antagonist eprosartan during sodium restriction on renal tubular function, systemic haemodynamics and vasoactive hormones during two different sympatho-excitatory manoeuvres.

The main findings are that eprosartan attenuated the increases in HR and MAP during the CPT. Furthermore, for

Table 3

Urine excretion of sodium (U_{Na}), lithium clearance (C_{Li}), fractional excretions of sodium (FE_{Na}) and lithium (FE_{Li}), glomerular filtration rate (GFR), urine output (U V), free water clearance (C_{H20}), and urinary excretion of aquaporin-2 corrected for creatinine excretion (U-AQP-2_{CR}) after eprosartan and placebo administration in 17 healthy subjects before the CPT and changes during the cold pressor test (CPT) and before and changes during the infusion of sodium nitroprusside (SNP)

		Before the CPT	Changes during the CPT	Before SNP infusion	Changes during SNP infusion†
FE_{Na} (%)	Eprosartan	0.87 (0.59, 1.15)	0.21 (0.12, 0.29)**	1.13 (0.89, 1.37)	–0.51 (–0.76, –0.27)**
	Placebo	0.80 (0.51, 1.09)	0.23 (0.06, 0.40)**	0.97 (0.66, 1.29)	–0.05 (–0.29, 0.18)
	Difference	0.07 (–0.31, 0.44)	0.01 (–0.16, 0.18)	0.16 (–0.18, 0.50)	–0.46 (–0.77, –0.14)**
U_{Na} (μmol min^{-1})	Eprosartan	119.5 (83.0, 156.0)	15.7 (2.9, 28.4)	155.0 (120.6, 189.4)	–72.5 (–107.2, –37.9)**
	Placebo	111.6 (69.3, 153.9)	22.6 (–1.0, 46.1)	130.5 (87.8, 173.3)	–9.9 (–47.3, 27.6)
	Difference	7.9 (–44.4, 60.2)	–6.9 (–34.3, 20.5)	24.5 (–21.9, 70.8)	–62.7 (–107.8, –17.6)**
C_{Li} (ml min^{-1})	Eprosartan	24.0 (21.1, 26.9)	–2.2 (–3.5, –0.8)	25.0 (21.5, 28.6)	–7.2 (–10.2, –4.2)**
	Placebo	22.4 (20.1, 24.7)	–1.2 (–2.4, 0.0)	22.5 (20.5, 24.6)	–1.7 (–3.1, –0.4)*
	Difference	1.6 (–1.4, 4.6)	–1.0 (–2.8, 0.9)	2.5 (–0.6, 5.6)	–5.5 (–8.8, –2.2)**
FE_{Li} (%)	Eprosartan	24.3 (21.6, 26.9)	–0.4 (–1.5, 0.8)	25.5 (22.6, 28.5)	–6.0 (–8.1, –3.8)**
	Placebo	22.7 (20.1, 25.4)	0.4 (–0.5, 1.3)	23.7 (20.9, 26.6)	–0.8 (–2.0, 0.3)
	Difference	1.5 (–1.7, 4.8)	–0.8 (–2.2, 0.7)	1.8 (–1.1, 4.7)	–5.1 (–7.6, –2.5)**
GFR (ml min^{-1})	Eprosartan	99.3 (92.0, 106.6)	–8.3 (–12.6, –4.1)**	98.5 (90.0, 107.0)	–7.3 (–16.5, 1.9)
	Placebo	99.7 (92.3, 107.1)	–6.5 (–9.5, –3.5)**	96.5 (90.0, 103.1)	–3.7 (–7.6, 0.2)
	Difference	–0.4 (–3.8, 3.1)	–1.9 (–6.9, 3.2)	2.0 (–3.5, 7.5)	–3.5 (–13.7, 6.6)
U V (ml min^{-1})	Eprosartan	8.34 (7.33, 9.36)	–4.15 (–5.16, –3.13)**	4.98 (3.72, 6.25)	–2.13 (–3.92, –0.34)*
	Placebo	7.91 (7.33, 8.48)	–2.76 (–3.38, –2.14)**	5.93 (4.88, 6.99)	–1.55 (–3.13, 0.03)
	Difference	0.43 (–0.68, 1.54)	–1.39 (–2.39, –0.38)**	–0.95 (–2.29, 0.39)	–0.59 (–2.82, 1.65)
C_{H20} (ml min^{-1})	Eprosartan	5.56 (4.57, 6.55)	–3.92 (–4.86, –2.99)**	2.05 (1.02, 3.08)	–1.12 (–2.66, 0.42)
	Placebo	5.06 (4.56, 5.56)	–2.61 (–3.17, –2.05)**	3.13 (2.19, 4.07)	–1.10 (–2.54, 0.34)
	Difference	0.50 (–0.56, 1.56)	–1.32 (–2.24, –0.39)**	–1.08 (–2.19, 0.02)	0.08 (–1.90, 2.07)
U-AQP-2_{CR} (ng mmol^{-1})	Eprosartan	0.090 (0.078, 0.102)	–0.003 (–0.009, 0.003)	0.089 (0.080, 0.099)	–0.013 (–0.019, –0.006)**
	Placebo	0.083 (0.071, 0.094)	0.001 (–0.004, 0.006)	0.079 (0.072, 0.087)	–0.004 (–0.008, 0.000)
	Difference	0.007 (–0.005, 0.020)	–0.004 (–0.013, 0.005)	0.010 (0.004, 0.016)*	–0.008 (–0.017, –0.000)*

Values are means (95% confidence interval). *$P < 0.05$, **$P < 0.01$, paired samples *t*-test. †Calculated from mean values of the two consecutive clearance periods during the SNP infusion.

the same reduction in BP (or less reduction regarding the diastolic BP) during the SNP infusion eprosartan led to an increase in tubular sodium and lithium reabsorption and tended to increase the elevation of HR and NE.

The CPT has been used for decades for nonbaroreflex mediated activation of the SNS leading to increases in HR, muscle sympathetic activity and NE. HR increases to peak levels within the first 30–60 s, while muscle sympathetic activity increases with a latency of 20–30 s after the immersion. BP is elevated mainly due to an increase in peripheral resistance except for the first 30 s where minor increases in cardiac output contribute [24, 25]. Given that our hypothesis of a sympatho-inhibitory effect of eprosartan was true then attenuated responses in HR, MAP and NE during the CPT would be expected. Indeed this was observed regarding the two former parameters, but NE was unaffected by eprosartan. The reduction in MAP after 2 min of CPT is in agreement with the findings of another study of the effect of AT_1 antagonism on systemic haemodynamics during a CPT [9]. Since the increases in HR during the CPT can be abolished with β-adrenoceptor blockade [24] and the fact that MAP after 2 min of the CPT is increased due to sympathetic mediated vasoconstriction the current observations are suggestive of a small sympatho-inhibitory effect of eprosartan on systemic haemodynamics during this procedure.

Plasma concentrations of endothelin-1 (ET-1) also increase during the CPT and may contribute to the pressor response [26]. Furthermore, AT_1 receptor blockade inhibits ET-1 induced vasoconstriction in the skin microcirculation [27]. It is not known, whether this interaction between the endothelin system and the RAS exists beyond the skin microcirculation in humans. If it does, we cannot rule out that ET-1 may contribute to the reduction in MAP during the CPT after eprosartan administration.

To our knowledge, this is the first study measuring the effect of a CPT on GFR and renal tubular function. As expected, the CPT led to decreases in GFR and urine water excretion, but surprisingly also to increases in FE_{Na}. The fact that FE_{Na} increased while FE_{Li} remained unchanged indicates that the reduction in tubular sodium reabsorption took place in the distal part of the nephron. As renal sympathetic nerve activity also influences proximal sodium reabsorption [28], other mechanisms must be involved in this change in tubular function. Thus, GFR and renal tubular function hardly reflect sheer increases in renal sympathetic nerve activity, and we can therefore not attribute the more pronounced decrease in renal water excretion during the

Table 4

Plasma concentrations of arginine vasopressin (AVP), atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), renin, angiotensin II (Ang II), aldosterone (Aldo) and norepinephrine (NE) in 17 healthy subjects before the cold pressor test (CPT) and before and changes during the infusion of sodium nitroprusside (SNP)

		Before the CPT	Before SNP	Changes during SNP infusion†
AVP (pg ml^{-1})	Eprosartan	0.76 (0.68, 0.85)	0.71 (0.61, 0.81)	0.27 (0.06, 0.48)*
	Placebo	0.75 (0.65, 0.85)	0.77 (0.65, 0.89)	0.01 (–0.12, 0.14)
	Difference	0.02 (–0.07, 0.11)	–0.06 (–0.17, 0.06)	0.26 (0.07, 0.45)*
ANP (pmol l^{-1})	Eprosartan	6.5 (4.9, 8.1)	6.2 (4.5, 7.8)	–1.8 (–2.6, –1.0)**
	Placebo	8.3 (6.3, 10.4)	8.5 (6.1, 11.0)	–3.1 (–4.7, –1.5)**
	Difference	–1.9 (–2.8, –0.9)**	–2.4 (–3.8, –0.9)**	1.3 (–0.1, 2.6)
BNP (pmol l^{-1})	Eprosartan	0.64 (0.38, 0.89)	0.62 (0.36, 0.88)	–0.05 (–0.10, –0.01)*
	Placebo	0.82 (0.50, 1.14)	0.88 (0.50, 1.25)	–0.15 (–0.29, –0.01)*
	Difference	–0.19 (–0.34, –0.03)*	–0.26 (–0.44, –0.08)**	0.10 (–0.01, 0.21)
Renin (mU l^{-1})	Eprosartan	208.3 (97.7, 318.9)	157.2 (81.9, 232.5)	86.5 (37.0, 136.1)**
	Placebo	18.3 (10.7, 25.9)	16.6 (12.1, 21.2)	9.8 (3.8, 15.8)**
	Difference	190.0 (86.2, 293.7)**	140.5 (68.8, 212.3)**	76.7 (27.3, 126.1)**
Ang II (pmol l^{-1})	Eprosartan	147.1 (100.4, 193.9)	109.8 (72.4, 147.2)	85.8 (56.2, 115.4)**
	Placebo	19.9 (13.0, 26.8)	17.6 (12.1, 23.2)	7.6 (1.9, 13.3)**
	Difference	127.2 (82.3, 172.1)**	92.2 (55.2, 129.1)	78.2 (49.1, 107.3)**
Aldo (pmol l^{-1})	Eprosartan	397.2 (298.0, 496.3)	400.2 (288.5, 512.0)	362.7 (161.0, 564.4)**
	Placebo	441.1 (311.8, 570.4)	409.4 (289.9, 528.9)	168.9 (33.4, 304.4)*
	Difference	–43.9 (–172.2, 84.4)	–9.2 (–104.9, 86.6)	193.8 (–16.4, 404.0)
NE (pg ml^{-1})	Eprosartan	94.4 (78.0, 110.7)	87.5 (73.8, 101.2)	126.8 (78.0, 175.7)**
	Placebo	90.8 (71.6, 109.9)	90.6 (72.4, 108.9)	93.7 (47.9, 139.5)**
	Difference	3.6 (–10.2, 17.4)	–3.1 (–19.1, 12.8)	33.1 (–5.8, 72.1)

Values are means (95% confidence interval). *$P < 0.05$, **$P < 0.01$, paired samples t-test. †Calculated from mean values of the two consecutive clearance periods during the SNP infusion.

CPT, after eprosartan administration compared with placebo, to increased renal sympathetic nerve activity. Water excretion was reduced despite unchanged urinary AQP-2 excretion, suggesting that the CPT increases non-AVP mediated water reabsorption [23] and that eprosartan pronounces this effect. Our study provides no explanation for this phenomenon.

In spite of the likely involvement of confounding factors, the fact that the eprosartan and placebo responses for FE_{Na}, FE_{Li} and GFR during the CPT did not differ speaks against a difference in renal sympathetic nerve activity. For this reason, we conclude that eprosartan does not seem to exert a sympatho-inhibitory action on renal tubular function and GFR during the CPT.

The second reflex sympatho-excitatory manoeuvre used in the study was the unloading of the arterial baroreceptors with infusion of SNP. SNP has been used extensively for the evaluation of the human sympathetic and vagal baroreflex responses together with phenylephrine in various protocols such as the modified Oxford technique that consists of sequential intravenous bolus injections of these vasoactive drugs [29]. We used sustained infusion of SNP for 120 min to achieve sufficient impact on GFR and renal tubular function.

The findings were that for the same reduction in BP (or less reduction regarding the diastolic BP) during the SNP infusion eprosartan, compared with placebo, led to an increase in tubular sodium and lithium reabsorption and tended to increase the elevation of HR and NE.

These observations are not indicative a sympatho-inhibitory effect of eprosartan on systemic haemodynamics and renal tubular function. In fact, they suggest increased levels of sympathetic nerve activity, which probably can be attributed to changes in the arterial baroreflex.

At baseline, eprosartan significantly reduced blood pressure, with no concurrent changes in HR, plasma concentrations of NE and fractional sodium excretion. This represents a resetting of the arterial baroreflex to a lower blood pressure level, a well known effect of AT_1 receptor antagonism [12, 13] and angiotensin converting enzyme (ACE) inhibition [30, 31].

Eprosartan not only resets the arterial baroreflex, but it most likely also affects the sensitivity of the arterial baroreflex. This is reflected by the increased impact of the 10 mmHg MAP reduction on proximal tubular sodium reabsorption during the SNP infusion, which strongly suggests a significant increase in the sensitivity of the arterial baroreflex in the control of renal sympathetic nerve activity. The increase in HR only tended to be influenced by eprosartan during the SNP infusion, supporting the notion that the sensitivity of the arterial baroreflex in the control of HR is not significantly changed.

This proposed change in arterial baroreflex sensitivity contrasts with the finding of Grassi and colleagues, who

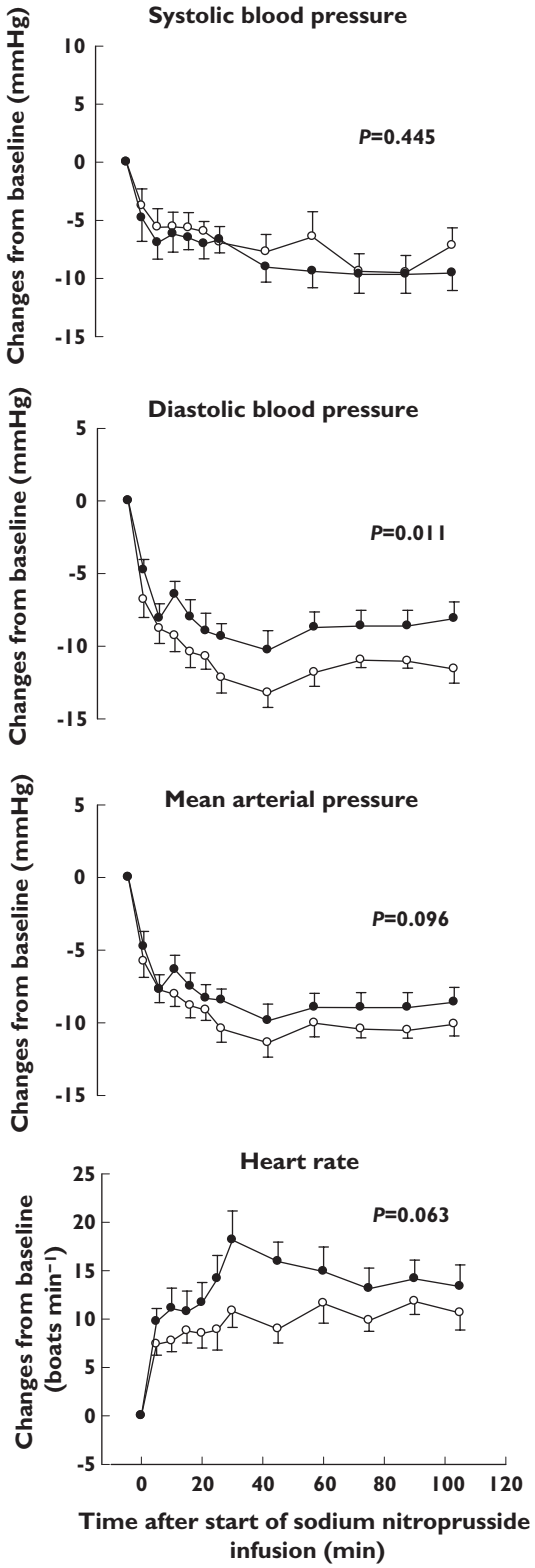

Figure 2

Changes in systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate from baseline during the sodium nitroprusside infusion, displayed as means ± SEM. Open circles: placebo. Black circles: eprosartan. *P* value: effect of eprosartan/placebo in the multivariate repeated measures ANOVA

reported that chronic ACE-inhibitor treatment did not alter the ability of the arterial baroreflex to alter HR and muscle sympathetic nerve activity in patients with essential hypertension [30]. The discrepancy can probably be attributed to the mild sodium restriction employed in our study, since sodium restriction increases the concentrations of Ang II. In both animal and human studies Ang II reduces the sensitivity of the arterial baroreflex [1, 32]. In accord with this, studies in rats have confirmed that the effect of sodium restriction on baroreceptor function can be reversed by an AT_1 antagonist [33].

Two reports of the effects of dietary sodium restriction in patients with essential hypertension have shown that sodium restriction attenuates arterial baroreflex control of muscle sympathetic nerve activity, but not (or only to a minor degree) arterial baroreflex control of HR [34, 35]. Bearing in mind that muscle sympathetic nerve activity correlates well with renal sympathetic nerve activity during arterial baroreflex perturbations [36], these studies are completely in line with the observed differential effects of an AT_1-antagonist on arterial baroreflex function during restrictions in sodium intake in our study.

We therefore reason that the mild sodium restriction utilized in our study impairs arterial baroreflex sensitivity and that this effect is reversed by eprosartan.

Thus, we find no evidence of a sympatho-inhibitory effect of eprosartan on the reflex activation of the SNS through unloading of the arterial baroreceptors. Although a small effect cannot be ruled out, the impact of the increased baroreflex sensitivity is by far the most decisive in our experimental setting.

The natriuretic peptides were significantly reduced by eprosartan during the entire study day. This contrasts with other reports on the effect of AT_1 blockade on concentrations of ANP, as ANP increased after treatment with irbesartan for 30 days in patients with essential hypertension [37] and also after administration of candesartan for 8 weeks in the rat [38]. The reason for this discrepancy is unclear, but could be related to fact that we evaluated only the very short term effect of eprosartan during sodium restriction. Regardless of the underlying mechanism, the reduced concentration of ANP after eprosartan administration could act as a confounder on arterial baroreflex function and sympathetic nerve activity, since ANP has been demonstrated to decrease the sensitivity of arterial baroreflex [39, 40] and also to inhibit sympathetic nerve transmission [41]. The majority of these experiments were performed with infusion of ANP leading to 10–20 fold increases in plasma concentrations of ANP. A study using only moderate doses of the natriuretic peptides in sheep showed no effect on arterial baroreflex sensitivity [42]. Consequently, the relatively minor differences between eprosartan and placebo in the concentrations of ANP and BNP probably play no major role in the proposed change in the function of the arterial baroreflex in our study.

We used the lithium clearance method to evaluate the site of action of eprosartan on renal tubular sodium handling during the sympatho-excitatory manoeuvres [43]. Lithium clearance is an index of filtrate and sodium delivery from the proximal tubules, since lithium under most conditions is solely absorbed in the proximal tubules in proportion to the reabsorption of sodium and water. This method can, in humans, be used with confidence also during restrictions in sodium intake [44, 45].

SNP infusion causes not only arterial vasodilatation, but also dilation of the veins thereby reducing central venous pressure and consequently unloading of the cardiopulmonary baroreceptors [46]. This modulates the overall integrative function of the arterial baroreflex [47], and could, if the reduction in central venous pressure were more pronounced after eprosartan, also be a confounder in our conclusion regarding the impact of eprosartan on arterial baroreflex function. Our data do not provide exact information on this issue, but the fact that nonosmotic AVP release occurred during the SNP infusion after eprosartan administration suggests some impact of this modulation, since nonosmotic vasopressin release is the likely result of predominantly cardiopulmonary baroreceptor unloading given the relative small decreases in MAP in our study.

In summary, the very short-term administration of eprosartan during mild sodium restriction reduces the systemic haemodynamic response of the CPT, while increasing sodium reabsorption in the proximal tubules during arterial baroreflex mediated SNS activation by the SNP infusion.

These findings suggest that eprosartan during mild sodium restriction has a small inhibitory effect on non-baroreflex mediated activation of the sympathetic nervous system. During arterial baroreflex mediated activation of the sympathetic nervous system this effect is, however, completely overruled by an increased sensitivity of the arterial baroreflex.

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