



HAL
open science

Exaggerated natriuresis during clamping of systemic NO supply in normal man

Jane Angel Simonsen, Mona Sommer Rasmussen, Werner Vach, Poul Flemming Høilund-Carlsen, Peter Bie

► **To cite this version:**

Jane Angel Simonsen, Mona Sommer Rasmussen, Werner Vach, Poul Flemming Høilund-Carlsen, Peter Bie. Exaggerated natriuresis during clamping of systemic NO supply in normal man. *Clinical Science*, 2011, 122 (2), pp.63-73. 10.1042/CS20110144 . hal-00733887

HAL Id: hal-00733887

<https://hal.science/hal-00733887>

Submitted on 20 Sep 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Exaggerated natriuresis during clamping of systemic NO supply in normal man

Jane Angel Simonsen^{1,3}, Mona Sommer Rasmussen¹, Werner Vach²,

Poul Flemming Højlund-Carlsen³, Peter Bie¹

¹Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark, ²Clinical Epidemiology, Institute of Medical Biometry and Medical Informatics, University Medical Center Freiburg, Germany, ³Department of Nuclear Medicine, Odense University Hospital, Odense, Denmark.

Running title: NO mediated sodium excretion in man

Key words: aldosterone, AngII, arterial blood pressure, natriuresis, nitrite/nitrate, renin, volume expansion

Corresponding author:

Jane Angel Simonsen, MD
Department of Nuclear Medicine,
Odense University Hospital,
DK-5000 Odense C,
Phone: + 45 6 541 2981
Fax: + 45 6 590 6192
Email: Jane.Simonsen@ouh.regionyddanmark.dk

Abstract

Nitric oxide (NO) may be involved in fluid homeostasis. We hypothesized that increases in NO synthesis contribute to acute, saline-induced natriuresis which, therefore, should be blunted when NO availability is stabilized.

Young men were studied during simultaneous infusions of N^ω-nitro-L-arginine methyl ester (L-NAME, 750 µg·(kg body weight)⁻¹ bolus and 8.3 µg·kg⁻¹·min⁻¹) and sodium nitroprusside, the latter at a rate preventing the L-NAME from increasing total peripheral resistance ('NO-clamping'). Slow volume expansion (saline, 20 µmol NaCl·kg⁻¹·min⁻¹ for 3 h) was performed with and without concomitant NO-clamping.

NO-clamping *per se* decreased renal plasma flow (RPF, $P \sim 0.02$) and tended to decrease arterial blood pressure (MABP). Volume expansion markedly decreased the plasma levels of renin, angiotensin II (AngII), and aldosterone (all $P < 0.001$), while MABP (oscillometry), heart rate, cardiac output (impedance cardiography), RPF (by p-aminohippurate), glomerular filtration rate (GFR by ⁵¹Cr-ethylenediaminetetraacetate), plasma [Na⁺] and [K⁺] remained constant. Volume expansion increased sodium excretion ($P < 0.02$) at constant filtered load; however, more so during NO-clamping than during control (+184% vs. 52%, $P < 0.0001$). Urinary nitrate/nitrite excretion increased during volume expansion; plasma cGMP and plasma vasopressin were unchanged.

The results demonstrate that NO-clamping augments sodium excretion in response to volume expansion at constant MABP and GFR, reduced RPF, and decreased renin system activity, a response termed hypernatriuresis. The results indicate that mediator(s) other than MABP, RPF, GFR, and renin system activity contribute significantly to the homeostatic response to saline loading, but the specific mechanisms of the hypernatriuresis remain obscure.

Introduction

The mechanisms responsible for the physiological control of sodium balance are not clearly identified, see review [1]. Following the classical experiments of Guyton and coworkers in the seventies [2;3], it has been widely accepted that the renin angiotensin aldosterone system (RAAS) and the pressure natriuresis mechanism are the major determinants of normal electrolyte balance and pivotal to blood pressure control [4]. Nevertheless, an increasing number of results seem to indicate that the pressure natriuresis mechanism is not the more important controller of renal sodium excretion under normal conditions. Modest sodium loading may cause marked natriuresis without any change in arterial blood pressure, sometimes even against minor decreases in mean arterial pressure. In these situations, changes in blood pressure cannot be the primary signal to the kidney, cf. recent reviews [1;5].

Volume expansion leads to deactivation of the RAAS and natriuresis, and the former seems to be a necessary precondition for the latter [6]. The RAAS is believed to be regulated by parallel inputs to renin secretion via (i) renal arterial pressure, (ii) renal nerve activity to the juxtaglomerular cells mediated by β_1 adrenoreceptors, and (iii) the concentration of chloride in the tubular fluid at the macula densa. The composition of the tubular fluid at the macula densa is a complex function of glomerular filtration rate (GFR) and proximal tubular functions including glomerulo-tubular balance and tubulo-glomerular feedback [7;8]. Recent experiments (designed to identify renal nerve activity as the primary controller) showed unexpectedly that a large fraction of the homeostatic control of secretion of the RAAS may be exerted by factors other than arterial blood pressure, GFR, and signaling via β_1 -adrenoreceptors [9;10]. This unexplained control element could include the rate of synthesis of nitric oxide (NO). NO has multiple roles in the control of renal function, and its actions under physiological conditions are complex [11]. However, L-arginine (a substrate for NO synthases) increases renal blood flow, filtration rate, and sodium excretion [12], while inhibition of NO synthases reduces renal plasma flow and sodium excretion [13]. It seemed attractive to hypothesize that changes in NO synthesis play an important role in the corrective natriuresis in response to increases in body fluid volume.

We investigated the role of NO in the renal response to a modest sodium load known not to change arterial blood pressure or GFR. Simple blockade of NO synthesis by a NO synthase (NOS) inhibitor is unattractive because of the associated increases in peripheral resistance and arterial blood pressure. Therefore, we combined the administration of N^o-nitro-L-arginine methyl ester (L-NAME) with an infusion of nitroprusside adjusted to eliminate the increase in total peripheral resistance generated by the NOS inhibition. Under these conditions, we performed volume expansion with isotonic saline at a modest rate. Unexpectedly, NO-clamping did not decrease the natriuretic response to volume expansion.

Methods

Nine healthy, male volunteers, average age 26 years (range 24-28 years), height 181 cm (173-192 cm), body weight 81 kg (74-88 kg), were assigned to three separate experiments carried out in random order with intervals of at least two weeks. The subjects denied intake of any medication for two weeks prior to and during the investigation. The experiments were performed in accordance with the Declaration of Helsinki after approval by the Danish Medicines Agency (J.No. 2612-1988) and the regional scientific ethical committee (ref. no. 20020035). All participants gave written informed consent.

For four days prior to each experiment, the participants ingested a standardized low salt diet containing up to 30 mmol Na⁺ per day. Compliance was checked by analysis of urine collected during the last 24 hours. In case daily excretion was higher than 40 mmol of Na⁺, the experiment was rejected. On the day of the study, the subjects presented at 07:50 a.m. having refrained from caffeine for the past 12 hours. They were weighed and seated, and short catheters (Venflon 18G,

Becton Dickinson, Helsingborg, Sweden) for infusions and blood sampling, respectively, were inserted into the left and right superficial cubital vein.

Glomerular filtration rate was measured as the clearance of chromium labelled ethylenediaminetetraacetic acid (^{51}Cr -EDTA, GE Healthcare, Little Chalfont, UK). An intravenous bolus injection ($27.8 \text{ kBq} \cdot (\text{kg body weight})^{-1}$ in 10 ml 0.9% NaCl) was followed by a continuous intravenous infusion ($1.04 \text{ MBq} \cdot \text{h}^{-1}$ in 5.5% glucose solution at $25 \text{ mL} \cdot \text{h}^{-1}$). Effective renal plasma flow (RPF) was measured by the clearance of para-aminohippurate sodium (PAH, Merck, West Point, PA, USA). An intravenous priming dose of $10 \text{ mg} \cdot \text{kg}^{-1}$ in 10 ml 0.9% NaCl was followed by infusion of $1.65 \text{ g} \cdot \text{h}^{-1}$ in 5.5% glucose solution at $25 \text{ mL} \cdot \text{h}^{-1}$. Clearances of PAH and ^{51}Cr -EDTA were calculated as rate of excretion divided by plasma concentration.

Electrodes for measurements of electrocardiogram (ECG) and impedance cardiogram (ICG) were applied at the neck and chest, and the ICG device (PhysioFlow PF-05, Manatec Biomedical, Paris, France) was started. Brachial blood pressure was measured every 10 min by use of an automatic oscillometric sphygmomanometer (Colin Press-Mate BP-8800, ViCare Medical A/S, Birkeroed, Denmark) transferring data to the ICG apparatus. Subjects ingested 400 mL of 10% glucose hourly.

In each individual subject three investigations were planned (for overview, see Fig. 1): i) infusion of isotonic NaCl solution for three hours ($n = 8$) (NaCl series), ii) clamping of the NO level by simultaneous infusion of a NOS inhibitor and a NO donor for five hours ($n = 7$) (ClampCon series), and iii) a series of combined interventions ($n = 7$) (ClampNaCl series).

In the clamp series (ClampCon and ClampNaCl), administration of L-NAME (Merck Biosciences AG, Clinalfa, L aufelfingen, Switzerland) was started at $t = -45 \text{ min}$ with a priming dose of $750 \mu\text{g} \cdot \text{kg}^{-1}$ in 10 mL saline followed by continuous infusion of $8.3 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in $1 \text{ mL} \cdot \text{min}^{-1}$ of 5.5% glucose as previously described [14]. After 30 min ($t = -15 \text{ min}$), SNP (sodium nitropruside, Abbott Laboratories, Abbott Park, Illinois, USA) was added at a rate of $0.5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in 5.5% glucose, delivered at $5 \text{ mL} \cdot \text{h}^{-1}$ by a syringe pump.

In the NaCl series (NaCl and ClampNaCl), a sodium load of $20 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (7.8 mL of 0.9% NaCl $\cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) was given over three hours ($t = 60\text{-}240 \text{ min}$) by a volumetric infusion pump.

At time $t = 300 \text{ min}$, L-NAME was withdrawn. The infusion of SNP was stopped after an additional 30 min. Blood and urine samples were obtained hourly. Urine was collected by voluntary micturition; the subject was allowed to stand during voiding if necessary.

Laboratory methods

Blood samples were centrifuged immediately at 4°C . Plasma and urine samples were analysed at the end of the experiment by flame photometry (IL 943 Flame Photometer, Instrumentation Laboratory, Lexington, MA, USA) and osmometry (model 3D3, Advanced Instruments, Needham Heights, MA, USA), respectively. The activity of ^{51}Cr in plasma and urine was measured in a well counter (1480 WIZARD, Wallac, Turku, Finland). Plasma and urine samples for analysis of hormones, nitric oxide metabolites (NOx), cGMP, PAH, and creatinine were stored at -20°C .

The plasma renin concentration (PRC) was assessed by the antibody trapping method of Poulsen & Jorgensen [15] and the activity standardized to the WHO International Reference Reagent (Human Renin, NIBSC, Hertfordshire, UK) which was analyzed in each assay run. Results are expressed in milli International Units per litre ($\text{mIU} \cdot \text{L}^{-1}$).

Concentrations of peptide hormones in plasma were measured by radioimmunoassay using specific antibodies and ^{125}I -labeled tracer after extraction performed by use of Sep-Pak C_{18} columns (Waters, Millipore Corporation, Milford, MA, USA) [16]. Immunoreactivity of angiotensin II (ANG II) in plasma extracts was measured using antibody Ab-5-030682 as described earlier [6] and ^{125}I -labelled ANG II (kindly provided by Dept. Clinical Physiology, Glostrup Hospital, Glostrup, Denmark). Vasopressin (AVP) immunoreactivity was determined by use of an antibody

(AB3096) produced in this laboratory [17]. The concentration of aldosterone was measured with a commercial radioimmunoassay kit (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA, USA).

Quantification of NO_x was performed with a commercial kit (850-001-KI01, Nitrate/Nitrite Colorimetric Assay Kit, Cayman Chemical Company, Ann Arbor, MI, USA) by use of a conventional plate reader (Elx808, Biotek Instruments Inc, Winooski, VT, USA).

A commercial kit (Biotrak, cGMP Enzyme Immunoassay, Amersham Pharmacia Biotech, Buckinghamshire, UK) was used for determination of concentrations of cGMP.

PAH and creatinine concentrations were determined by conventional spectrophotometry.

Statistics

Results are presented as mean \pm SEM. Differences between mean values at baseline between the three groups were assessed by a one way ANOVA with pair wise comparisons based on the Tukey-Kramer method. The development over time within each series was analysed by a repeated measurement ANOVA using a random intercept model with an AR(1) error structure using PROC MIXED. Pair wise comparisons between all time points and baseline were performed using the Tukey-Kramer method to adjust for multiple comparisons. To take random baseline imbalances between the groups into account, comparisons of the two NaCl groups are based on dividing each measurement with the baseline value of each individual. The comparison is then based on a repeated measurement ANOVA of the remaining time points using the same model as above.

In presenting our results for the single outcome measures, we start with focusing on differences between the baseline measurements and values obtained during or after the infusion in each series. Then we report on testing for systematic differences in the overall course between the two NaCl series.

Results

The slow saline loading was carried out under steady-state conditions of low sodium intake. On the fourth day of the diet, baseline rates of urinary sodium excretion were low (ClampCon: 10 ± 3 , ClampNaCl: 12 ± 4 , and NaCl: 13 ± 2 mmol 24h^{-1}) while the rates of potassium excretion were 7-10 fold higher (113 ± 8 , 89 ± 9 , and 112 ± 11 mmol 24h^{-1} in ClampCon, ClampNaCl, and NaCl, respectively). One participant was excluded on the basis of apparent lack of compliance with the diet (24 h sodium excretion rates of 48 mmol and 42 mmol on separate occasions). Data from eight participants are reported; six subjects underwent all procedures.

Natriuretic response

The saline loading increased renal sodium excretion (Fig. 2, Table 1). Without NO-clamping (NaCl series), the rate of sodium excretion within the infusion period showed a strong tendency towards an increase when compared to baseline (23 ± 5 $\mu\text{mol} \cdot \text{min}^{-1}$ to 30 ± 6 $\mu\text{mol} \cdot \text{min}^{-1}$, $P \sim 0.06$), and the excretion rate after the infusion (35 ± 7 $\mu\text{mol} \cdot \text{min}^{-1}$) was increased significantly compared to the first hour of infusion ($P < 0.05$). When the same saline load was administered during NO-clamping (ClampNaCl series), there was a robust increase in the rate of sodium excretion (19 ± 5 to 54 ± 10 $\mu\text{mol} \cdot \text{min}^{-1}$ post infusion, $P < 0.001$). Without NO-clamp, the change in sodium excretion was approximately 50%. During NO-clamping, sodium excretion rose by a factor of 3 (Fig. 2), and the difference between the two series is highly significant ($P < 0.0001$). The baseline values of sodium excretion and of filtered load of sodium in the two sessions were similar (Table 1). Thus, during NO-clamping, the sodium loading elicited a natriuretic response markedly larger than that measured without clamping (Fig. 2).

Other renal functions

Clamping of NO *per se* was associated with a decrease in renal plasma flow (Table 1); during NO blockade, the renal plasma flow at baseline was 24% smaller ($P \sim 0.02$) than the corresponding value obtained in the absence of L-NAME (Table 1). Clamping of NO did not change the

baseline values of GFR or filtered load of sodium (FiLoad- Na^+ , NaCl vs. ClampNaCl and ClampCon, see Table 1).

Neither with nor without NO-blockade did the saline loading change renal plasma flow (Table 1); there was not even a tendency towards an increase. Also, the FiLoad- Na^+ and the GFR remained constant during the saline loading (Table 1, Fig. 2). Therefore, changes in renal plasma flow, GFR, or FiLoad- Na^+ do not appear to be involved in the present natriuretic responses. In terms of relative changes in GFR and FiLoad- Na^+ , the data from the clamp series showed a transient decrease during the saline loading (ClampNaCl vs. NaCl: FiLoad- Na^+ : $P < 0.02$, GFR: $P < 0.01$, Fig. 2); however, the decrease was relatively small and occurred only during the middle hour of infusion. In the absence of NO-clamping, the saline loading increased the renal excretion of NO metabolites (NOx) significantly ($P < 0.02$) (Table 1) despite a fall in the plasma concentration (see below). The urinary excretion rate of cGMP did not change measurably during any of the three series of experiments (Table 1). Fractional potassium excretion increased in all three studies in a very similar way (Table 1).

In summary, NO-clamping *per se* decreased renal plasma flow, but changed neither GFR nor FiLoad- Na^+ . Saline loading increased the rate of sodium excretion significantly more during NO-clamping than without NO-clamping, i.e. NO-clamping is associated with a hypernatremia. Saline loading did not increase renal plasma flow, GFR, or FiLoad- Na^+ , either with or without NO-clamping. In contrast, transient decreases in GFR and FiLoad- Na^+ occurred during clamping. In other words, during NO-clamping, the saline loading elicited hypernatremia despite sustained suppression of renal plasma flow, and despite transient decreases in GFR and FiLoad- Na^+ . This pattern indicates that renal haemodynamic changes cannot explain the hypernatremia occurring during NO-clamping.

Hormones and other mediators

During saline loading without and with NO-clamping (NaCl and ClampNaCl, respectively), PRC, plasma AngII, and plasma aldosterone decreased simultaneously (Table 2, Fig. 3) and to very similar extents. Plasma AngII and aldosterone decreased by approximately 50%. During the time control protocol (ClampCon), PRC, plasma AngII, and plasma aldosterone did not change significantly. Plasma NOx concentrations showed a similar pattern: During the time control protocol (ClampCon), values were approximately constant around $40 \mu\text{mol} \cdot \text{L}^{-1}$. During saline loading, plasma NOx concentrations fell by about $8\text{-}9 \mu\text{mol} \cdot \text{L}^{-1}$ irrespective of NO-clamping. Plasma cGMP concentrations were stable throughout all the experiments (Table 2). Plasma vasopressin showed no significant differences in any series. In summary, changes in renin system variables, vasopressin, NO metabolites, and cGMP do not seem to be related to the hypernatremia.

Systemic haemodynamics

The experiments were designed to provide inhibition of NOS by L-NAME infusion under conditions of unaltered peripheral resistance. The results show that this goal was achieved as total peripheral resistance did not deviate significantly from the baseline values, e.g., the final values of peripheral resistance in the NaCl and ClampNaCl series were 1.13 ± 0.04 and $1.15 \pm 0.06 \text{ mmHg} \cdot \text{s} \cdot \text{mL}^{-1}$, respectively (Table 3). Mean arterial blood pressure (MABP) was measured every 10 min, and the results averaged to hourly values. NO-clamping tended to reduce MABP (Table 3), but the 6-9 mmHg decreases in mean values were not statistically significant. MABP did not deviate significantly from baseline during either of the saline loading protocols; the mean values showed trends towards decreases (2-6 mmHg) but none were statistically significant. A small decrease in MABP in the time control series was significant, but transient (-7 mmHg, ClampCon series, first 2/3 of infusion period, $P < 0.05$, Table 3). Heart rates averaged 59 to 66 $\text{beats} \cdot \text{min}^{-1}$ under baseline conditions in the different series; significant differences between or within the series could not be detected (Table 3). Similarly, the baseline values of cardiac output were uniform (5.4 to $5.8 \text{ L} \cdot \text{min}^{-1}$), and the cardiac output data did not show any differences within or between series (Table 3).

Therefore, the saline loading was so slow that it was unable to produce measurable changes in MABP, heart rate, and cardiac output, with or without NO-clamping.

Side effects

During NO-clamping, most participants reported some degree of nausea and drowsiness. A total of five sessions were terminated prematurely and thus excluded from the data analysis. Remarkably, plasma vasopressin concentrations, normally sensitive to nausea, were constant during the sessions (Table 2).

In summary, under conditions of stable haemodynamics, RPF, GFR, and filtered sodium load, slow saline loading induced natriuresis along with a decrease in PRC, plasma AngII, and plasma aldosterone. However, the increase in the sodium excretion in response to the saline loading during NO-clamping (ClampNaCl) was 3-4 times that occurring without clamping (NaCl).

Discussion

We hypothesized that stabilization of the systemic supply of NO by constant infusion of L-NAME in combination with nitroprusside would limit the ability of the kidneys to respond to a modest saline load. Our results do not support this working hypothesis. Unexpectedly, the slow saline loading produced a clearly exaggerated natriuretic response during NO-clamping, a hypernatriuresis, despite no concurrent changes in arterial blood pressure, heart rate, cardiac output, renal plasma flow, GFR, filtered load of sodium, and cGMP excretion, and despite very similar excursions in renin system activity in plasma and plasma NO_x levels. The hypernatriuresis is remarkable also because it occurred under conditions of constantly reduced renal blood flow and relatively low arterial blood pressure.

The reason for the hypernatriuresis during NO-clamping is uncertain. It is possible that increases in the rate of NO synthesis do play an essential role in the normal natriuretic response to an increase in total body sodium, but that the design of the present investigation did not enable us to unveil this role. Alternatively, the normal physiological adjustments of renal sodium excretion are independent of acute changes in the rate of NO synthesis in the kidney.

The model

NO may participate in the regulation of renal excretory function through several pathways. An increase in systemic NO availability decreases peripheral vascular resistance, reduces arterial blood pressure and in this way reduces sodium excretion. In contrast, increased availability of NO in the kidney reduces renal vascular resistance, decreases tubular sodium reabsorption, and inhibits intrarenal control mechanisms such as TGF, thereby favouring the excretion of sodium. In humans, NO inhibition has been found to increase blood pressure by 7-10% concomitant with robust decreases in renal plasma flow of 13-20% [18-20], and infusion of L-arginine, a NOS substrate, may increase renal plasma flow, GFR, and sodium excretion in the absence of systemic haemodynamic changes [12]. It is a major advantage of the present model that the effects of stabilized NO availability throughout the body are measured at arterial blood pressures very similar to baseline.

It is evident that the intrarenal availability of NO, affecting the vasculature, the tubular transport, and the intrarenal control systems, not necessarily is restored quantitatively by the present co-infusion of nitroprusside. In the kidney, nNOS is expressed in the efferent arteriole, in the macula densa, and in the medulla [21-23], and eNOS is present in the proximal tubule, the thick ascending part of the loop of Henle, and in the collecting ducts in addition to the vessels [22]. It is difficult to obtain data elucidating the local tensions of NO involved in the function of these structures. Therefore, our results may reflect circumstances where local NO levels deviate from those of baseline, but it remains a valid assumption that the excursions in NO availability are limited under the present conditions.

Several studies in normal humans have shown that acute inhibition of NO synthesis decreases renal sodium excretion [13;24-28]. In addition, other findings may support the notion of a regulatory role of NO. Administration of L-arginine, a NOS substrate, increases sodium excretion [12;29]

and augments the renal response to volume expansion [30]. From such results it can be argued (i) that if NO is involved in the normal control of sodium excretion, then a slow volume expansion should increase NO production, and (ii) that stabilization of the supply of NO, therefore, should reduce the natriuretic response to this intervention. Although the renal effects of experimental perturbations of NO synthesis are clear, the role of NO in normal fluid homeostasis has been very difficult to assess due to the concomitant, large changes in renal blood flow and GFR occurring during such perturbations.

The role of NO in sodium homeostasis is likely to include an effect of NO on renin secretion. Although the conclusions from the multiple studies of the effect of NO on renin secretion under physiological conditions are not completely unanimous, the data supporting the view that NO stimulates renin secretion appear strong [24;31-35]. Therefore, it seems that under *in vivo* conditions NO may accelerate both sodium excretion and renin secretion, i.e., it elicits natriuresis and activates a powerful antinatriuretic mechanism at the same time. Against this background, it appeared attractive to try to stabilize the systemic supply of NO, and study the effects of modest saline loading on renin levels and sodium excretion with and without a stabilized NO supply. The variable with which to gauge the effect of L-NAME, and, therefore, to be used for adjustment of the rate of administration of NO donor, was vascular resistance. It was assumed that arterial blood pressure would not be an appropriate indicator of the L-NAME effects because blood pressure is heavily regulated by other control systems, and it was decided, therefore, to use total peripheral resistance as the L-NAME effect to be eliminated by administration of the NO donor [14]. The main methodological questions seem to be the extent of the blockade of NO synthesis in the kidney, and the somewhat related issue of sensitivity of the renal NOS isoforms relative to the eNOS of the peripheral circulation determining total peripheral resistance.

In humans, inhibition of NOS by infusion of L-NAME has been investigated by infusion of a wide range of doses from $1 \mu\text{g} \cdot \text{min}^{-1} (\text{kg body mass})^{-1}$ [24] to $133 \mu\text{g} \cdot \text{min}^{-1} (\text{kg body mass})^{-1}$ [36] for periods of 30 min [24;36], 40 min [13;34;35], 60 min [37] and 90 min [25;26]. Priming doses have not been applied in these investigations. As the onset of the effects of even large doses may be slow [37], and the aim of the present study was to maintain a new steady state for hours, we applied bolus injection plus continuous infusion of L-NAME, quickly followed by continuous infusion of nitroprusside.

There is no generally accepted method for assessment of the systemic NOS activity. Exhaled amounts of NO may serve as an indicator [38], but it is not clear to what extent this reflects the NO involved in vascular endothelial function [39]. In the absence of specific measures we can conclude only that judged from the single effect of the L-NAME on the total peripheral resistance (+33%; [14]), the protocol provided substantial, but probably incomplete inhibition of NOS activity. This leaves open the possibility that NOS located in different renal structures may be inhibited to different extents, but at present it is very difficult to assess such incompleteness of inhibition, particularly in normal human subjects.

The hypernatriuresis

The main new finding is that the clamping of the NO supply was associated with a hypernatriuresis irrespective of a sustained decrease in renal plasma flow, and unaltered values of (and, if anything, decreasing trends in) arterial blood pressure and GFR. The significant reduction in renal plasma flow in combination with the clear trend towards reduction in arterial blood pressure would be expected to reduce the rate of sodium excretion. The hypernatriuresis occurred despite these antinatriuretic conditions, and inasmuch as the clamp-mediated changes in 'renal haemodynamics' favoured antinatriuresis, the results indicate that changes in tubular sodium reabsorption are the most likely cause(s) of the hypernatriuresis. L-NAME inhibits the NO synthases, of which eNOS is expressed in the renal endothelial cells and nNOS predominantly in the macula densa cells. Both seem to be regulated by a variety of physiological stimuli (see review by Kone [40]). Endogenous NO may enhance proximal fluid reabsorption in normal man [25] but under experimental conditions

in rats it seems to promote excretion of sodium ions and water via an effect on medullary blood flow [41-44]. This apparent discrepancy may be due to species specific and/or environmental differences. Routine daily sodium intakes in rats scale to very large sodium intakes in humans.

Although seeming to support the notion that the renal haemodynamic changes cannot be responsible for the observed effects, the present results must be interpreted with caution. The renal medullary circulation constitutes a minor fraction of the renal vascular bed, so that changes in renal haemodynamics specific to the medulla are unlikely to be detected by measurements of total renal blood flow by the clearance methods applied in the present study. The results of a recent study indicate that the renal medullary circulation is indeed particularly sensitive to changes in NO availability [45]. Interference with the NO supply to this relatively small region may have major effects on renal water and solute excretion to the extent that hypertension is generated by NOS inhibition and abrogated by infusion of excess substrate [41-44]. Therefore, the present results do not conclusively indicate that changes in tubular reabsorption are the cause of the observed hypernatremia. Changes in medullary haemodynamics may be a plausible cause of the present findings.

A significant secondary result - that NO-clamping did not reverse the L-NAME-mediated decrease in renal plasma flow - seems to indicate that the renal vascular bed is more sensitive to NOS inhibition by L-NAME than the remainder of the peripheral vasculature. Alternatively, the results show that the amount of NO required to return total peripheral vascular resistance to baseline is not able to revert renal vascular resistance. Previous results have been interpreted to indicate that in humans the renal vascular bed may be more sensitive to NO inhibition than other vascular beds [24]. The present data are compatible with this notion, but our design did not allow a detailed analysis of the changes in renal perfusion.

Contrary to our expectations, the present data do not contribute significantly to a clarification of the role of changes in NO availability for the homeostatic reaction to slow saline loading. The finding that the hypernatremia occurred under conditions of depressed renal plasma flow, constant arterial pressure, and changes in systemic RAAS activity similar to those of control experiments indicates only that mechanisms other than arterial pressure and systemic RAAS variables are important. If, in addition, it is considered unlikely that a functionally relevant increase in medullary flow occurred under the present 24% decrease in total renal plasma flow, then the results indicate that changes in renal haemodynamics may not be a plausible explanation either. The finding that during NO-clamping GFR remained unchanged despite a robust decrease in renal plasma flow and a trend towards a decrease in arterial blood pressure is compatible with a condition of relative hyperfiltration. This could indicate that the efficacy of the TGF was reduced during the NO-clamping. An increase in the NO availability in the juxtaglomerular apparatus (JGA) would inhibit TGF (see reviews [46;47]) and thereby enhance a natriuretic response [48]. If the nNOS of the macula densa were less sensitive to inhibition by L-NAME than the eNOS of the renal endothelium, then the level of NO in the JGA would be higher than in the rest of the kidney. As mentioned above, this would most likely be associated with an increase in renin secretion (at least locally). Therefore, NO-clamping may be associated with reduced sensitivity of the TGF, promoting sodium excretion, and elevated (intrarenal) RAAS activity increasing sodium reabsorption. It may be speculated that in a dynamic situation, the reduced TGF activity leads to hypernatremia, but that in steady states, the effects of the elevated (intrarenal) RAAS activity prevail. At present, however, there is little evidence to support this hypothesis of selective sensitivity of different NOS isoforms to L-NAME resulting in imbalance between TGF and (intrarenal) RAAS activities. Future studies have to be designed more specifically to clarify the relative importance of the different renal NO signalling mechanisms in the control of total body sodium.

Perspectives

The present results clearly demonstrate the existence of hypernatremia during NO-clamping. Although the mechanisms remain obscure, the response is interesting phenomenologically. It has

been known for many years, e.g. [49;50] and recently confirmed [51] that mild, monosymptomatic, essential hypertension is associated with hypernatruuresis. Other studies have demonstrated reduced renal perfusion and reduced glomerular filtration in hypertension, e.g. [52]. If NO-clamping in normal subjects is associated with renal hypoperfusion, hypofiltration and hypernatruuresis, and these phenomena also are characteristics of patients with essential hypertension, it is possible that hypertension is associated with reduced renal NO availability. Notably, the hypernatruuresis present during NO-clamping and in essential hypertension may reflect abnormal NO-mediated modulation of the TGF. During NO-clamping, the enzyme involved in TGF regulation (nNOS, NOS1) could be particularly sensitive to L-NAME increasing the eNOS/nNOS activity ratio in the kidney during clamping, and in hypertension an increase in renal afferent arteriolar tone could be a primary element of disease causing chronic suppression of TGF responses. It is possible to test the hypotheses that the enzyme activity ratio changes during NO-clamping and that renal - rather than extrarenal - vasoconstriction is present in essential hypertension.

Author contribution

This manuscript represents truthful original work without fabrication, fraud, or plagiarism. All authors have made an important scientific contribution to the study and are thoroughly familiar with the primary data. All authors have read the complete manuscript and take responsibility for the content and completeness of the manuscript.

Acknowledgments

The concept of the present investigation emerged in discussions with Dr. Scott Thomson, San Diego, CA. The skilled technical assistance of Ulla Melchior Hansen and Bodil Kristensen is gratefully acknowledged.

Funding

The work was supported by grants from The Danish Heart Association [01-2-2-89B-22951], The Beckett Foundation [20924], Aase and Ejnar Danielsen's Foundation [103730], Director Ib Henriksen's Foundation, The European Organisation of Circulatory Diseases, and The L. F. Foght Foundation [019955-0001 12-fg].

Conflicts of interest

None.

References

1. Bie,P. (2009) Blood volume, blood pressure and total body sodium: internal signalling and output control. *Acta Physiol (Oxf)* **195**, 187-196.
2. DeClue,J.W., Guyton,A.C., Cowley,A.W., Jr., Coleman,T.G., Norman,R.A., Jr. and McCaa,R.E. (1978) Subpressor angiotensin infusion, renal sodium handling, and salt-induced hypertension in the dog. *Circ.Res.* **43**, 503-512.
3. Hall,J.E., Guyton,A.C., Smith,M.J., Jr. and Coleman,T.G. (1980) Blood pressure and renal function during chronic changes in sodium intake: role of angiotensin. *Am.J.Physiol* **239**, F271-F280.
4. Le,T.H., Crowley,S.D., Gurley,S.B. and Coffman,T.M. (2008) The Renin-Angiotensin System. In Seldin and Giebisch's *The Kidney Physiology and Pathophysiology* (Alpern,R.J. and Hebert,S.C., eds.), pp. 343-357, Elsevier, Amsterdam
5. Bie,P., Wamberg,S. and Kjolby,M. (2004) Volume natriuresis vs. pressure natriuresis. *Acta Physiol Scand.* **181**, 495-503.
6. Bie,P. and Sandgaard,N.C. (2000) Determinants of the natriuresis after acute, slow sodium loading in conscious dogs. *Am.J.Physiol Regul.Integr.Comp Physiol* **278**, R1-R10.
7. Thomson,S.C. and Blantz,R.C. (2008) Glomerulotubular balance, tubuloglomerular feedback, and salt homeostasis. *J.Am.Soc.Nephrol.* **19**, 2272-2275.
8. Bie,P. and Damkjaer,M. (2010) Renin secretion and total body sodium: Pathways of integrative control. *Clin.Exp.Pharmacol.Physiol* **37**, e34-e42.
9. Bie,P., Molstrom,S. and Wamberg,S. (2009) Normotensive sodium loading in conscious dogs: regulation of renin secretion during beta-receptor blockade. *Am.J.Physiol Regul.Integr.Comp Physiol* **296**, R428-R435.
10. Molstrom,S., Larsen,N.H., Simonsen,J.A., Washington,R. and Bie,P. (2009) Normotensive sodium loading in normal man: regulation of renin secretion during beta-receptor blockade. *Am.J.Physiol Regul.Integr.Comp Physiol* **296**, R436-R445.
11. Weinstein,A.M. (2008) Sodium and Chloride Transport. Proximal nephron. In Seldin and Giebisch's *The Kidney Physiology and Pathophysiology* (Alpern,R.J. and Hebert,S.C., eds.), pp. 793-847, Elsevier, Amsterdam
12. Schlaich,M.P., Jacobi,J., John,S., Delles,C., Fleischmann,I. and Schmieder,R.E. (2000) Is l-arginine infusion an adequate tool to assess endothelium-dependent vasodilation of the human renal vasculature? *Clin.Sci.(Lond)* **99**, 293-302.
13. Kielstein,J.T., Impraim,B., Simmel,S. et al. (2004) Cardiovascular effects of systemic nitric oxide synthase inhibition with asymmetrical dimethylarginine in humans. *Circulation* **109**, 172-177.

14. Simonsen, J.A., Rasmussen, M.S., Johansen, J.B., Hoiland-Carlson, P.F. and Bie, P. (2010) Systemic nitric oxide clamping in normal humans guided by total peripheral resistance. *Acta Physiol (Oxf)* **198**, 125-132.
15. Poulsen, K. and Jorgensen, J. (1974) An easy radioimmunological microassay of renin activity, concentration and substrate in human and animal plasma and tissues based on angiotensin I trapping by antibody. *J.Clin.Endocrinol.Metab* **39**, 816-825.
16. Emmeluth, C. and Bie, P. (1992) Effects, release and disposal of endothelin-1 in conscious dogs. *Acta Physiol Scand.* **146**, 197-204.
17. Emmeluth, C., Drummer, C., Gerzer, R. and Bie, P. (1994) Natriuresis in conscious dogs caused by increased carotid [Na⁺] during angiotensin II and aldosterone blockade. *Acta Physiol Scand.* **151**, 403-411.
18. Bech, J.N., Svendsen, K.B., Nielsen, C.B. and Pedersen, E.B. (1999) The systemic and renal response to NO inhibition is not modified by angiotensin-II-receptor blockade in healthy humans. *Nephrol.Dial.Transplant.* **14**, 641-647.
19. Delles, C., Klingbeil, A.U., Schneider, M.P., Handrock, R., Schaufele, T. and Schmieder, R.E. (2004) The role of nitric oxide in the regulation of glomerular haemodynamics in humans. *Nephrol.Dial.Transplant.* **19**, 1392-1397.
20. Schlaich, M.P., Oehmer, S., Schneider, M.P., Delles, C., Schmidt, B.M. and Schmieder, R.E. (2007) Effects of nitric oxide synthase inhibition and L-arginine on renal haemodynamics in young patients at high cardiovascular risk. *Atherosclerosis* **192**, 155-160.
21. Mundel, P., Bachmann, S., Bader, M. et al. (1992) Expression of nitric oxide synthase in kidney macula densa cells. *Kidney Int.* **42**, 1017-1019.
22. Bachmann, S., Bosse, H.M. and Mundel, P. (1995) Topography of nitric oxide synthesis by localizing constitutive NO synthases in mammalian kidney. *Am.J.Physiol* **268**, F885-F898.
23. Kakoki, M., Zou, A.P. and Mattson, D.L. (2001) The influence of nitric oxide synthase 1 on blood flow and interstitial nitric oxide in the kidney. *Am.J.Physiol Regul.Integr.Comp Physiol* **281**, R91-R97.
24. Broere, A., Van Den Meiracker, A.H., Boomsma, F., Derkx, F.H., Veld, A.J. and Schalekamp, M.A. (1998) Human renal and systemic hemodynamic, natriuretic, and neurohumoral responses to different doses of L-NAME. *Am.J.Physiol* **275**, F870-F877.
25. Montanari, A., Tateo, E., Fasoli, E. et al. (1998) Dopamine-2 receptor blockade potentiates the renal effects of nitric oxide inhibition in humans. *Hypertension* **31**, 277-282.
26. Perinotto, P., Biggi, A., Carra, N. et al. (2001) Angiotensin II and prostaglandin interactions on systemic and renal effects of L-NAME in humans. *J.Am.Soc.Nephrol.* **12**, 1706-1712.
27. Bech, J.N., Nielsen, C.B. and Pedersen, E.B. (1996) Effects of systemic NO synthesis inhibition on RPF, GFR, UNa, and vasoactive hormones in healthy humans. *Am.J.Physiol* **270**, F845-F851.

28. Bech,J.N., Nielsen,E.H., Pedersen,R.S., Svendsen,K.B. and Pedersen,E.B. (2007) Enhanced sodium retention after acute nitric oxide blockade in mildly sodium loaded patients with essential hypertension. *Am.J.Hypertens.* **20**, 287-295.
29. Herlitz,H., Jungersten,L.U., Wikstrand,J. and Widgren,B.R. (1999) Effect of L-arginine infusion in normotensive subjects with and without a family history of hypertension. *Kidney Int.* **56**, 1838-1845.
30. Andersen,J.L., Sandgaard,N.C. and Bie,P. (2002) Volume expansion during NOS substrate donation with L-arginine: regulatory offsetting of renal response? *Am.J.Physiol Regul.Integr.Comp Physiol* **282**, R1149-R1155.
31. Johnson,R.A. and Freeman,R.H. (1994) Renin release in rats during blockade of nitric oxide synthesis. *Am.J.Physiol* **266**, R1723-R1729.
32. Persson,P.B., Baumann,J.E., Ehmke,H., Hackenthal,E., Kirchheim,H.R. and Nafz,B. (1993) Endothelium-derived NO stimulates pressure-dependent renin release in conscious dogs. *Am.J.Physiol* **264**, F943-F947.
33. Schricker,K., Hamann,M. and Kurtz,A. (1995) Nitric oxide and prostaglandins are involved in the macula densa control of the renin system. *Am.J.Physiol* **269**, F825-F830.
34. Van Den Meiracker,A.H., van der Linde,N.A., Broere,A., Derkx,F.H. and Boomsma,F. (2002) Effects of L-arginine and L-NAME on the renal function in hypertensive and normotensive subjects. *Nephron* **91**, 444-451.
35. van der Linde,N.A., Boomsma,F. and Van Den Meiracker,A.H. (2005) Potentiation of L-NAME-induced systemic and renal vasoconstrictor responses by alpha1-adrenoceptor antagonism. *J.Hypertens.* **23**, 1017-1024.
36. Buus,N.H., Bottcher,M., Hermansen,F., Sander,M., Nielsen,T.T. and Mulvany,M.J. (2001) Influence of nitric oxide synthase and adrenergic inhibition on adenosine-induced myocardial hyperemia. *Circulation* **104**, 2305-2310.
37. Sander,M., Chavoshan,B. and Victor,R.G. (1999) A large blood pressure-raising effect of nitric oxide synthase inhibition in humans. *Hypertension* **33**, 937-942.
38. (2005) ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am.J.Respir.Crit Care Med.* **171**, 912-930.
39. Pietropaoli,A.P., Perkins,P.T., Perillo,I.B. and Hyde,R.W. (2000) Exhaled nitric oxide does not provide a marker of vascular endothelial function in healthy humans. *Am.J.Respir.Crit Care Med.* **161**, 2113-2114.
40. Kone,B.C. (2004) Nitric oxide synthesis in the kidney: isoforms, biosynthesis, and functions in health. *Semin.Nephrol.* **24**, 299-315.
41. Mattson,D.L., Lu,S., Nakanishi,K., Papanek,P.E. and Cowley,A.W., Jr. (1994) Effect of chronic renal medullary nitric oxide inhibition on blood pressure. *Am.J.Physiol* **266**, H1918-H1926.

42. Mattson,D.L. and Higgins,D.J. (1996) Influence of dietary sodium intake on renal medullary nitric oxide synthase. *Hypertension* **27**, 688-692.
 43. Mattson,D.L. and Wu,F. (2000) Control of arterial blood pressure and renal sodium excretion by nitric oxide synthase in the renal medulla. *Acta Physiol Scand.* **168**, 149-154.
 44. Zou,A.P. and Cowley,A.W., Jr. (1997) Nitric oxide in renal cortex and medulla. An in vivo microdialysis study. *Hypertension* **29**, 194-198.
 45. Damkjaer,M., Vafae,M., Moller,M.L. et al. (2010) Renal cortical and medullary blood flow responses to altered NO availability in humans. *Am.J.Physiol Regul.Integr.Comp Physiol* **299**, R1449-R1455.
 46. Schnermann,J. and Briggs,J.P. (2008) Tubuloglomerular feedback: mechanistic insights from gene-manipulated mice. *Kidney Int.* **74**, 418-426.
 47. Wilcox,C.S. (1998) Role of macula densa NOS in tubuloglomerular feedback. *Curr.Opin.Nephrol.Hypertens.* **7**, 443-449.
 48. Singh,P. and Thomson,S.C. (2010) Renal homeostasis and tubuloglomerular feedback. *Curr.Opin.Nephrol.Hypertens.* **19**, 59-64.
 49. PAPPER,S., BELSKY,J.L. and BLEIFER,K.H. (1960) The response to the administration of an isotonic sodium chloride-lactate solution in patients with essential hypertension. *J.Clin.Invest* **39**, 876-884.
 50. THOMPSON,J.E., SILVA,T.F., KINSEY,D. and SMITHWICK,R.H. (1954) The effect of acute salt loads on the urinary sodium output of normotensive and hypertensive patients before and after surgery. *Circulation* **10**, 912-919.
 51. Damkjaer, M., Jacobsen, I. A., Hoiland-Carlsen, P. F., and Bie, P. Exaggerated natriuresis in patients with essential hypertension. *Acta Physiol* 198(Suppl 677), 184. 2010.
- Ref Type: Abstract
52. London,G.M., Safar,M.E., Sassard,J.E., Levenson,J.A. and Simon,A.C. (1984) Renal and systemic hemodynamics in sustained essential hypertension. *Hypertension* **6**, 743-754.

Figure legends

Figure 1

Time line of the experimental protocol. Prep: Preparation. Bas: Baseline. Inf: Infusion. Rec: Recovery. Mon: Monitoring. Arrow with dot: Blood sample. Arrow with triangle: Urine collection. BP: Arterial blood pressure measurements. ICG: Impedance cardiography. L-NAME: Infusion of N^o-nitro-L-arginine methyl ester. SNP: Sodium nitroprusside infusion. For details, see text.

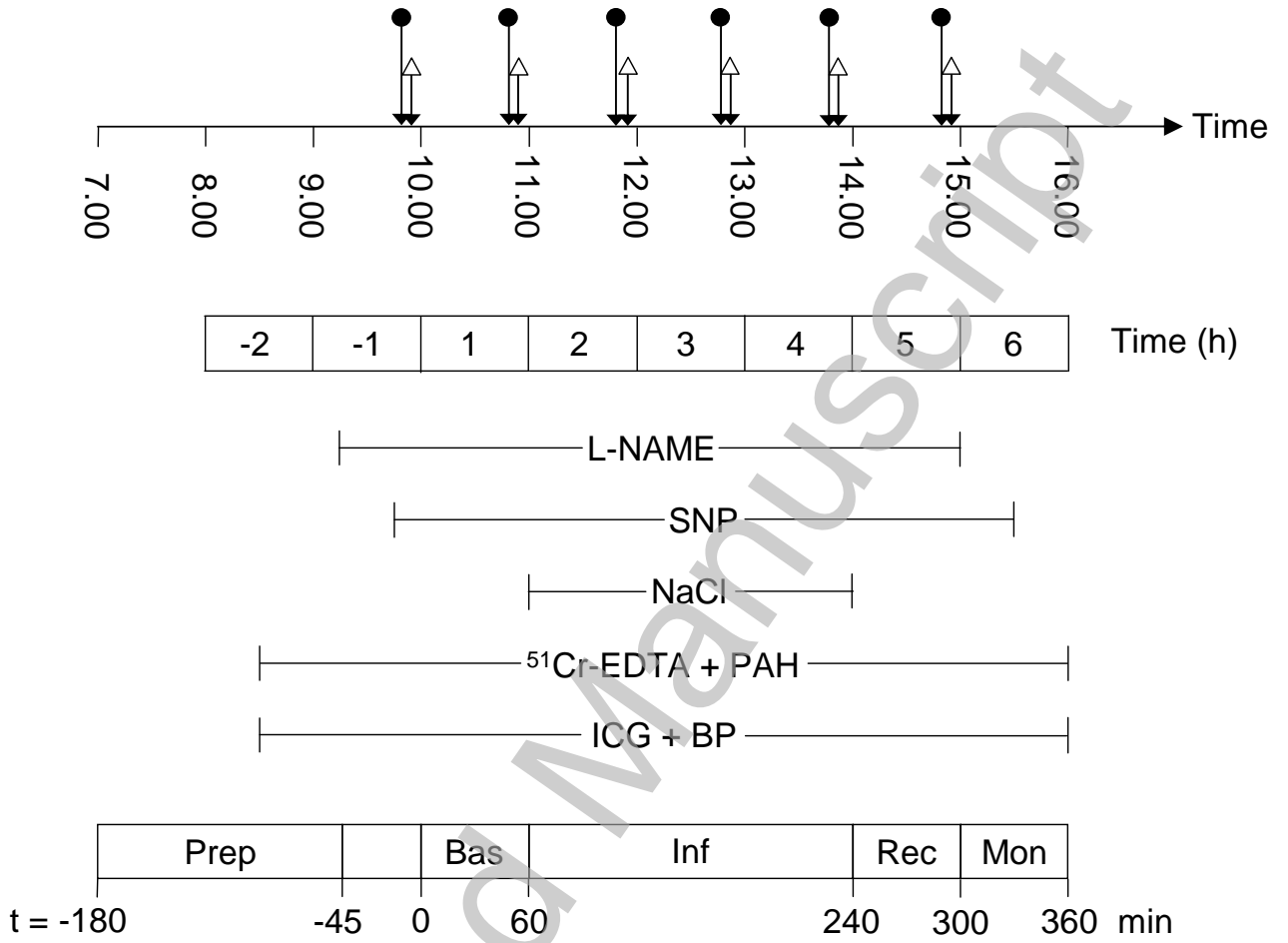
Figure 2

Relative changes in renal sodium handling in the series of saline loading with and without NO-clamping; (a) Sodium excretion, (b) Filtered load of sodium, (c), Fractional sodium excretion, and (d) Glomerular filtration rate. Circles: NaCl-series (n=8). Triangles: ClampNaCl-series (n=7). Mean values \pm SEM. *: Significantly different from baseline value. [§]: Significantly different from the value in the first hour of infusion.

Figure 3

Plasma aldosterone concentrations during slow sodium loading. Circles: NaCl-series. Triangles: ClampNaCl-series. Squares: Time control with NO-clamping. *: Significantly different from baseline value at t = 50 min. Mean values \pm SEM, n = 6 or 7.

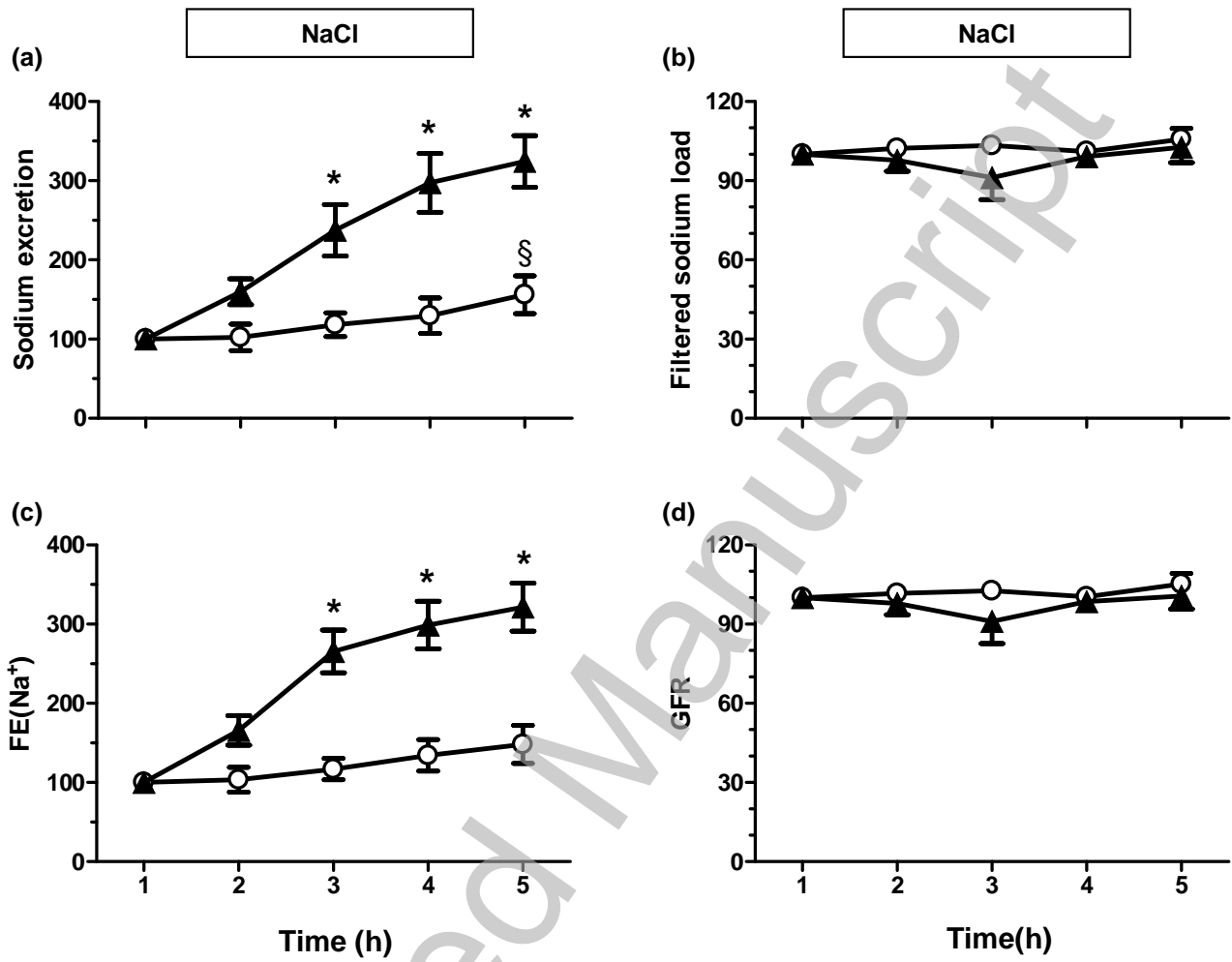
Figure 1



THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/CS20110144

Accepted Manuscript

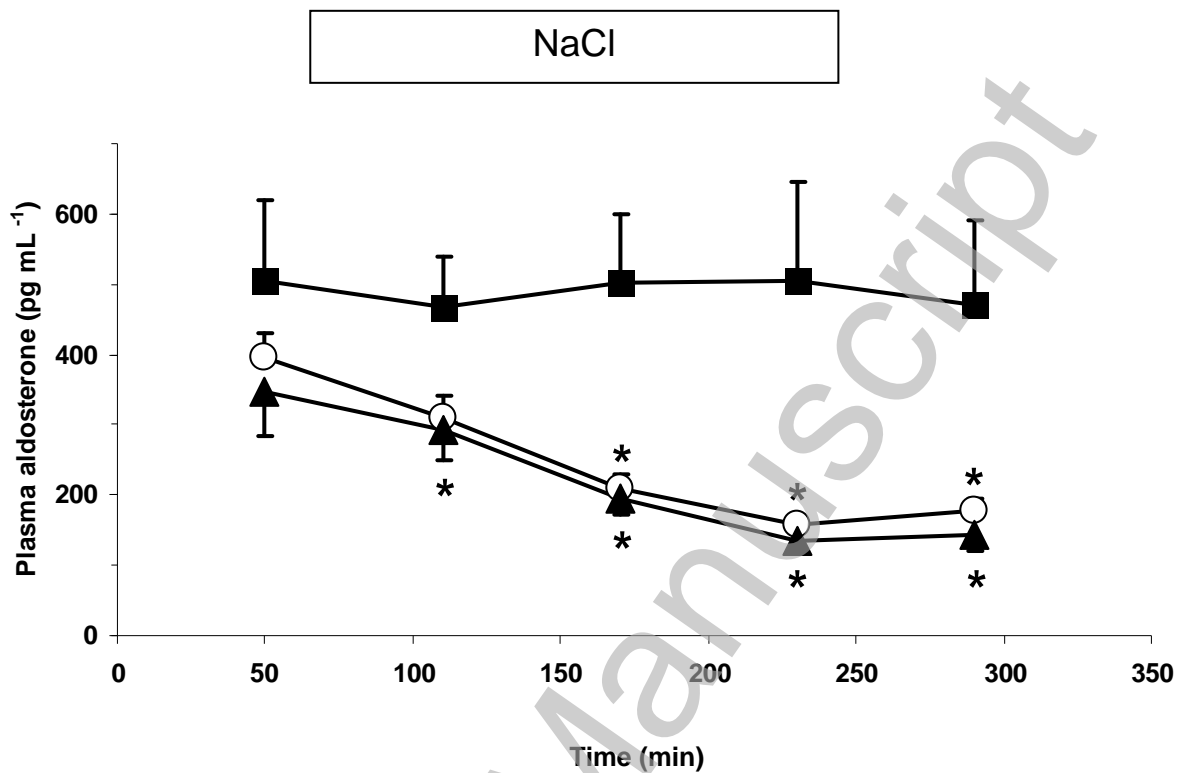
Figure 2



THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/CS20110144

Accepted Manuscript

Figure 3



THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/CS20110144

Accepted Manuscript

Table 1. Renal variables

Time (min)	Baseline	Infusion period			Post infusion
		60-119	120-179	180-239	
		Renal plasma flow (mL · min ⁻¹)			
ClampCon	414 ± 31 [†]	398 ± 28	347 ± 11	333 ± 62	363 ± 18
NaCl	552 ± 35	547 ± 67	562 ± 63	575 ± 16	586 ± 63
ClampNaCl	427 ± 35 [†]	405 ± 18	391 ± 27	431 ± 21	416 ± 21
		Glomerular filtration rate (mL · min ⁻¹)			
ClampCon	110 ± 5	111 ± 5	107 ± 6	100 ± 15	108 ± 5
NaCl	113 ± 3	115 ± 4	116 ± 3	114 ± 5	119 ± 4
ClampNaCl	116 ± 7	111 ± 4	106 ± 9	114 ± 3	112 ± 4
		Filtered load of sodium (mmol · min ⁻¹)			
ClampCon	14.9 ± 0.7	15.0 ± 0.7	14.6 ± 0.8	13.5 ± 2.1	14.7 ± 0.6
NaCl	15.4 ± 0.5	15.8 ± 0.6	15.9 ± 0.4	15.6 ± 0.8	16.3 ± 0.6
ClampNaCl	15.8 ± 1.0	15.2 ± 0.6	14.4 ± 1.2	15.6 ± 0.5	15.5 ± 0.7
		Sodium excretion (µmol · min ⁻¹)			
ClampCon	10 ± 2	12 ± 4	13 ± 5	17 ± 7	18 ± 7
NaCl	23 ± 5	24 ± 5	27 ± 6	30 ± 6	35 ± 7 [§]
ClampNaCl	19 ± 5	27 ± 5	37 ± 8*	49 ± 7*	54 ± 10*
		Fractional sodium excretion (%)			
ClampCon	0.07 ± 0.02	0.08 ± 0.03	0.09 ± 0.04	0.12 ± 0.05	0.12 ± 0.05
NaCl	0.15 ± 0.04	0.16 ± 0.04	0.17 ± 0.04	0.21 ± 0.04	0.23 ± 0.05
ClampNaCl	0.12 ± 0.03	0.18 ± 0.03	0.25 ± 0.05*	0.32 ± 0.05*	0.35 ± 0.06*
		Potassium excretion (µmol · min ⁻¹)			
ClampCon	59 ± 9	60 ± 10	63 ± 10	60 ± 12	72 ± 11
NaCl	55 ± 9	67 ± 10	80 ± 10	84 ± 12	84 ± 10
ClampNaCl	55 ± 6	56 ± 6	62 ± 7	71 ± 6	72 ± 7
		Fractional potassium excretion (%)			
ClampCon	13.9 ± 2.1	14.3 ± 2.5	15.5 ± 2.7	17.0 ± 3.1*	18.2 ± 3.0*
NaCl	12.9 ± 1.7	14.9 ± 1.8*	17.7 ± 2.0*	18.9 ± 1.8*	18.4 ± 1.9*
ClampNaCl	12.5 ± 1.3	13.3 ± 1.5	15.4 ± 1.6	16.1 ± 1.4	16.7 ± 1.4*
		NOx excretion (µmol · min ⁻¹)			
ClampCon	0.43 ± 0.11	0.41 ± 0.10	0.33 ± 0.09	0.30 ± 0.10	0.27 ± 0.09
NaCl	0.64 ± 0.08	0.74 ± 0.09	0.84 ± 0.07*	0.89 ± 0.09*	0.82 ± 0.08
ClampNaCl	0.50 ± 0.12	0.52 ± 0.12	0.62 ± 0.10	0.73 ± 0.12	0.66 ± 0.08
		cGMP excretion (pmol · min ⁻¹)			
ClampCon	396 ± 33	395 ± 31	376 ± 42	369 ± 83	395 ± 67
NaCl	368 ± 53	344 ± 46	371 ± 45	360 ± 37	377 ± 40
ClampNaCl	498 ± 53	426 ± 43	364 ± 45	403 ± 39	397 ± 47

Values are means ± S.E.M. NaCl was infused (20 µmol kg⁻¹ min⁻¹) from t = 60 min to t = 240 min. **ClampCon**: Control series. **NaCl**: NaCl series. **ClampNaCl**: NaCl infusion during clamp. *: Significantly different from baseline value (*P* < 0.05). †: Significantly different from the corresponding NaCl series (only applied at baseline) (*P* < 0.05). §: Significantly different from the value in the first hour of infusion (*P* < 0.05).

THIS IS NOT THE VERSION OF RECORD - see doi:10.1042/CS20110144

Table 2. Plasma electrolytes, osmolality, and hormones

Time (min)	Baseline	Infusion period			Post infusion
	50	110	170	230	290
		Plasma Renin Concentration (mIU · L ⁻¹)			
ClampCon	68 ± 11	53 ± 7	53 ± 9	46 ± 6	50 ± 10
NaCl	86 ± 13	67 ± 13*	54 ± 7*	46 ± 6*	50 ± 8*
ClampNaCl	64 ± 9	40 ± 5*	35 ± 4*	31 ± 4*	31 ± 4*
		Plasma Angiotensin II (pg · mL ⁻¹)			
ClampCon	28 ± 5	25 ± 4	25 ± 5	21 ± 4	21 ± 4
NaCl	35 ± 7	24 ± 5*	18 ± 3*	17 ± 2*	18 ± 3*
ClampNaCl	23 ± 4	17 ± 4*	12 ± 2*	12 ± 2*	10 ± 2*
		Plasma NOx (µmol · L ⁻¹)			
ClampCon	42 ± 6	41 ± 6	40 ± 5	40 ± 6	38 ± 6
NaCl	36 ± 4	35 ± 3	32 ± 3	31 ± 4	27 ± 3*
ClampNaCl	38 ± 4	37 ± 4	35 ± 4	32 ± 3*	30 ± 3*
		Plasma cGMP (pmol · mL ⁻¹)			
ClampCon	3.1 ± 0.4	2.9 ± 0.3	2.8 ± 0.3	3.1 ± 0.3	2.8 ± 0.3
NaCl	3.0 ± 0.4	3.3 ± 0.4	3.0 ± 0.3	3.2 ± 0.2	3.1 ± 0.3
ClampNaCl	3.3 ± 0.4	3.5 ± 0.4	3.4 ± 0.3	3.2 ± 0.4	3.3 ± 0.4
		Plasma osmolality (mOsm)			
ClampCon	287 ± 1	286 ± 1	285 ± 1	285 ± 2	285 ± 1
NaCl	286 ± 1	285 ± 1	287 ± 1	287 ± 1	287 ± 1
ClampNaCl	287 ± 1	288 ± 1	288 ± 1	288 ± 1	289 ± 1
		Plasma vasopressin (pg · mL ⁻¹)			
ClampCon	2.0 ± 0.3	2.0 ± 0.3	2.0 ± 0.4	1.9 ± 0.3	2.2 ± 0.4
NaCl	1.3 ± 0.3	1.3 ± 0.3	1.1 ± 0.2	1.2 ± 0.2	1.4 ± 0.3
ClampNaCl	1.8 ± 0.3	1.7 ± 0.2	1.4 ± 0.2	1.4 ± 0.2	1.5 ± 0.2
		Plasma sodium (mmol · L ⁻¹)			
ClampCon	136.3 ± 0.9	135.9 ± 0.7	135.8 ± 0.8	136.2 ± 0.8	135.6 ± 1.1
NaCl	136.2 ± 0.9	136.9 ± 1.0	137.1 ± 0.9	136.5 ± 1.4	137.2 ± 1.4
ClampNaCl	136.2 ± 0.9	136.0 ± 0.8	136.5 ± 0.8	137.3 ± 0.7	139.4 ± 2.0
		Plasma potassium (mmol · L ⁻¹)			
ClampCon	3.88 ± 0.05	3.86 ± 0.06	3.96 ± 0.08	3.82 ± 0.07	3.75 ± 0.09
NaCl	3.83 ± 0.08	3.90 ± 0.07	3.87 ± 0.08	3.84 ± 0.08	3.82 ± 0.07
ClampNaCl	3.81 ± 0.06	3.86 ± 0.11	3.87 ± 0.07	3.87 ± 0.07	3.85 ± 0.07

Values are means ± S.E.M. NaCl was infused (20 µmol kg⁻¹ min⁻¹) from t = 60 min to t = 240 min. **ClampCon**: Control series. **NaCl**: NaCl series. **ClampNaCl**: NaCl infusion during clamp. *: Significantly different from the baseline value (*P* < 0.05).

Table 3. Haemodynamic variables measured with sphygmomanometry and impedance cardiography

Time (min)	Baseline 0-59	Infusion period			Post infusion 240-299
		60-119	120-179	180-239	
Mean arterial blood pressure (mmHg)					
ClampCon	87 ± 3	81 ± 3*	80 ± 4*	82 ± 3	82 ± 3
NaCl	93 ± 3	91 ± 3	91 ± 3	90 ± 3	87 ± 2
ClampNaCl	84 ± 4	83 ± 3	83 ± 3	82 ± 3	84 ± 3
Heart rate (min ⁻¹)					
ClampCon	66 ± 3	66 ± 3	64 ± 3	63 ± 3	62 ± 4
NaCl	59 ± 2	57 ± 1	57 ± 2	57 ± 2	56 ± 2
ClampNaCl	65 ± 4	62 ± 4	61 ± 4	58 ± 3	59 ± 4
Cardiac output (L · min ⁻¹)					
ClampCon	5.8 ± 0.3	5.8 ± 0.3	5.9 ± 0.5	5.7 ± 0.4	5.8 ± 0.6
NaCl	5.5 ± 0.3	5.5 ± 0.4	5.3 ± 0.5	5.1 ± 0.4	4.9 ± 0.3
ClampNaCl	5.4 ± 0.2	5.5 ± 0.4	5.2 ± 0.3	4.8 ± 0.2	4.6 ± 0.2
Total peripheral resistance (mmHg · s · mL ⁻¹)					
ClampCon	0.94 ± 0.06	0.87 ± 0.06	0.86 ± 0.07	0.91 ± 0.07	0.93 ± 0.10
NaCl	1.04 ± 0.04	1.05 ± 0.06	1.08 ± 0.06	1.12 ± 0.06	1.13 ± 0.04
ClampNaCl	0.96 ± 0.04	0.95 ± 0.05	0.99 ± 0.06	1.07 ± 0.06	1.15 ± 0.06

Values are means ± S.E.M. NaCl was infused (20 μmol kg⁻¹ min⁻¹) from t = 60 min to t = 240 min. **ClampCon**: Control series. **NaCl**: NaCl series. **ClampNaCl**: NaCl infusion during clamp. *: Significantly different from the baseline value (*P* < 0.05).