Normotensive sodium loading in normal man:

Regulation of renin secretion during beta-receptor blockade

Mølstrøm, S.¹, Larsen, N.H.², Simonsen, J.A.³, Washington, R.¹, and Bie, P.¹

¹Department of Physiology and Pharmacology, Institute of Medical Biology, University of Southern Denmark, ²Department of Anesthesiology and Intensive Care, Odense University Hospital; ³Department of Nuclear Medicine, Odense University Hospital, DK-5000 Odense, Denmark

Running title:

Beta receptor blockade and renin secretion in man

Corresponding author:

Professor Peter Bie, MD, DMSc,
Department of Physiology and Pharmacology
University of Southern Denmark
21 Winslowparken, Odense, DK-5000, Denmark
Phone: +45 6 550 3799
Fax: +45 6 613 3479
Email: pbie@health.sdu.dk
Abstract

Saline administration may change renin system (RAAS) activity and sodium excretion at constant mean arterial pressure (MAP). We hypothesized that such responses are elicited mainly by renal sympathetic nerve activity by β₁-receptors (β₁-RSNA), and tested the hypothesis by studying RAAS and renal excretion during slow saline loading at constant plasma sodium concentration (Na-loading: 12 μmol Na⁺ kg⁻¹ min⁻¹ for 4 h). Normal subjects were studied on low-sodium intake with and without β₁-adrenergic blockade by metoprolol.

Metoprolol per se reduced RAAS activity as expected. Na-loading decreased plasma renin (PRC) by ⅓, AngII by ⅓, and aldosterone (pAldo) by ⅓, (all p<0.05); surprisingly, these changes were found without as well as during acute metoprolol administration. Concomitantly, sodium excretion increased indistinguishably with and without metoprolol (16±2 to 71±14 μmol min⁻¹; 13±2 to 55±13 μmol min⁻¹, respectively). Na-loading did not increase plasma atrial natriuretic peptide (pANP), glomerular filtration rate (GFR by ⁵¹Cr-EDTA), MAP, or cardiac output (CO by impedance cardiography), but increased central venous pressure (CVP) by approximately 2.0 mmHg (p<0.05). During Na-loading, sodium excretion increased with CVP at an average slope of 7 μmol/min per mmHg. Concomitantly, plasma vasopressin decreased by 30-40% (p<0.05).

In conclusion, β₁-adrenoceptor blockade affects neither the acute saline-mediated deactivation of RAAS nor the associated natriuretic response, and the RAAS response to modest saline loading seems independent of changes in MAP, CO, GFR, β₁ mediated effects of norepinephrine, and ANP. Unexpectedly, the results do not allow assessment of the relative importance of RAAS dependent and independent regulation of renal sodium excretion. The results are compatible with the notion that at constant arterial pressure, a volume-receptor elicited reduction in RSNA, via receptors other than β₁-adrenoceptors, decreases renal tubular sodium reabsorption proximal to the macula densa leading to increased NaCl concentration at the macula densa and subsequent inhibition of renin secretion.
INTRODUCTION

The renin-angiotensin-aldosterone system (RAAS) is an essential regulator of fluid balance and arterial blood pressure. The secretion of renin is adjusted to body fluid status by changes in renal arterial pressure, in renal sympathetic nerve activity (RSNA), and in the composition of the fluid to which the macula densa (MD) cells are exposed (15; 22) through a complex interaction of a number of signaling pathways (30). RSNA stimulates renin secretion via $\beta_1$-receptors (10). However, the relative contribution of the three classical mechanisms under normal conditions in man, and the possibility that other mechanisms, e.g. hormonal systems, also contribute significantly, remain poorly elucidated.

Years ago, experiments in conscious dogs showed that a robust volume expansion reduces RSNA via a reflex mediated by the vagus nerve (24). Subsequently, other experiments in conscious dogs have shown that renin secretion is markedly inhibited by modest salt loading, also under conditions where blood pressure or glomerular filtration rate (GFR) did not change (28). It was tempting to assume that the well-known effect of volume expansion on renin secretion is mediated by RSNA via $\beta_1$ receptors (hereafter called $\beta_1$ mediated renal nerve activity). However, we have volume-expanded dogs during the administration of the adrenergic $\beta_1$ receptor antagonist metoprolol [Bie et al. accompanying paper] and found that this is probably not the case. During metoprolol administration, the relative decreases in plasma renin, AngII, and aldosterone caused by salt loading were very similar to the decreases occurring without metoprolol. Similarities between the deactivation patterns of the RAAS were found at regular as well as low sodium diets. These results are strong evidence in favor of the notion that the homeostatic regulation of renin secretion is independent of renal nerve activity expressed via $\beta_1$ receptors, also under conditions where arterial blood pressure is constant.
We have previously shown in humans that marked natriuretic responses may be initiated by saline loading without any changes in arterial pressure (2; 4; 27). Actually, in one of these studies, natriuresis occurred together with a small decrease in arterial pressure (2) clearly indicating that an increase in blood pressure is not a necessary precondition for the natriuresis. In the studies of humans, the natriuresis caused by sodium loading consistently occurred concomitant with a decrease in RAAS activity, and in contrast to an increase in arterial pressure, deactivation of the RAAS seems to be a necessary condition of the development of a natriuretic response. So far, the human results appear congruent with the dog data.

With the present study, we wanted to elucidate whether homeostatic regulation of renin secretion in humans also occurs unimpeded when changes in blood pressure, GFR and $\beta_1$ mediated effects of norepinephrine do not contribute. We tested the hypothesis that in man the deactivation of the RAAS with slow saline loading at constant blood pressure and GFR is reduced by $\beta_1$-adrenoceptor blockade. In the dog study [Bie et al. accompanying paper], we applied hypertonic saline to minimize the volume element of the perturbation. The present investigation was designed to avoid any increase in the plasma sodium concentration, and central venous pressure was measured to ascertain the volume effect. Based on the expectation that RAAS activity in man is a log-linear function of sodium intake, cf. (19), the investigations were conducted after several days of low-salt diet to increase the control values of RAAS activity.

**METHODS**

*Subjects*

Experiments were carried out in eight healthy male volunteers aged 23-28 years, weighing 75 to 91 kg (81.4±2.3 kg; mean ± SE) with a body mass index of 22.1 to 26.3 kg m$^{-2}$ (23.6±0.5 kg m$^{-2}$; mean ± SE). All subjects gave written informed consent. The study was approved by the local ethics committee (file no. VF 20050155) and was performed in compliance with the Declaration of Helsinki. Prior to the study, a clinical examination was
performed in each subject. None of the subjects provided any history or showed any signs of renal disease, arrhythmia, diabetes or hypertension, none was using any kind of medication for two weeks prior to or during the study. All subjects were normotensive and had normal plasma creatinine concentrations (62-134 µmol L^{-1}).

**Experimental protocols**

Each subject was investigated four times and the study days were at least 14 days apart. The subject consumed a standardized diet containing 30 mmol NaCl day^{-1} for 4 days prior to the investigation, i.e. the time normally required to obtain a new steady state (18). The diet was prepared by the kitchen for special diets of the university hospital in collaboration with a clinical dietician. The volunteers were allowed to drink water and tea as desired. They agreed not to exercise on the day before the experiment. To assess compliance with the prescribed diet 24-h urinary sodium excretion (07.00-07.00 h) was measured on the day prior to investigation.

Before the experiment, the subject was awakened at 06.30 h. At 06.45 h he consumed a light standardized, low-salt breakfast. At 07.00 h, urine was collected, and at 07.10 h, the subject was transported to the laboratory. He was weighed, and placed in a sitting position (back at approximately 60 degrees) in an armchair for the duration of the experiment and allowed to stand up only for micturition. He was given 200 ml of glucose solution (10%) and 50 ml water *per os* every 60 min throughout the experiment in order to maintain the standardized degree of hydration, to generate a uniform diuresis facilitating the clearance measurements, and to suppress the feeling of hunger otherwise occurring during the experiment.

GFR was measured by the clearance of chromium-labeled ethylenediaminetetraacetic acid (^{51}Cr-EDTA, code CJ13PD, Amersham Healthcare, Denmark). Before 08.15 h a bolus injection of ^{51}Cr-EDTA (0.0278 MBq kg^{-1}) in 5% glucose solution was given immediately followed by a constant infusion of ^{51}Cr-EDTA (1 MBq h^{-1}). Plasma activity was approximately 500 cpm ml^{-1}. 

5
A catheter (B. Braun, Cavafix Certo, 0.8/18G x 700 mm) was inserted into the cubital vein (v. basilica) preferably on the left side and advanced to a position estimated to be the superior vena cava. Intrathoracic placement of the tip of the catheter was confirmed by typical pressure waveforms and characteristic responses to respiratory maneuvers. The catheter was kept patent by infusion of heparinized saline (5 IU ml⁻¹ at 7 ml h⁻¹). The transducer was positioned and zeroed at the level of the right atrium (IC4) and connected to a monitor (Dialogue 2000, Danica Electronics, Rodovre, Denmark). Short catheters were inserted into a vein on each forearm, one catheter for infusion and the other for blood sampling.

Cardiac output (CO) and systemic vascular resistance (SVR) were measured non-invasively by an impedance cardiograph (PhysioFlow PF-03, Manatec Biomedical, Macheren, France). The mean difference between the CO values obtained in normal subjects at rest by the direct Fick and PhysioFlow methods has been found to be negligible (0.07 L min⁻¹) (9).

Each experiment lasted for 7 h divided into instrumentation and equilibration (120 min), control (60 min), four infusion periods (240 min) min, and post-infusion (60 min) (Fig. 1). Arterial pressures, heart rate and CO were determined at 15 min intervals. Central venous pressure data was obtained every min and averaged over 60 min periods. MAP and HR were measured by an automatic blood pressure monitor (Colin Europe BP8800, Courbevoie, France).

The study included four experimental protocols during low-salt regimen, time control experiments (Con), metoprolol control experiments (MetCon), salt loading experiments (Salt) and salt loading with concomitant metoprolol infusion experiments (MetSalt). In theory, standard physiological saline (154 mM) elevates the concentration of sodium when infused into normal extracellular fluid. We have previously found marginal, but significant increases in plasma sodium concentration when saline was infused to subjects on low-salt diet providing control sodium concentrations of 135±1 or 137±1 mM (27). In the present study, physiological saline was diluted slightly with water to obtain a sodium concentration very similar to that of plasma.
From 1 liter of normal saline 123 ml was removed and replaced by 123 ml of sterile water creating a sodium concentration of 135 mM in the infusate.

*Time control series (Con).* All procedures were similar to the intervention series (Salt and MetSalt), except that neither saline nor metoprolol infusions occurred.

*Metoprolol control series (MetCon).* Forty-five min before start of measurements a bolus of metoprolol (0.3 mg per kg body weight) was given followed by continuous intravenous infusion (1 μg kg⁻¹ min⁻¹) throughout the experiment which was otherwise identical to the Con series.

*Sodium loading series (Salt).* An intravenous infusion of saline was given for 240 min at a rate of 12 μmol kg⁻¹ min⁻¹ corresponding to 0.09 ml kg⁻¹ min⁻¹ or ~360 ml h⁻¹ (70 kg)⁻¹.

*Sodium loading series with metoprolol (MetSalt).* The saline load was performed during metoprolol administration as described above.

At regular intervals ~20 ml blood samples were obtained for measurement of electrolytes, osmolality, activity of Cr⁵¹ and hormone determinations (renin, AngII, aldosterone). In addition, atrial natriuretic peptide (ANP) and vasopressin (AVP) concentrations in plasma were determined at t = 55, 235 and 355 min. The total volume of blood sampled was less than 200 ml. The urinary bladder was emptied at 09.00 hours, and urine collected hourly thereafter by voluntary micturition. Analyses of urine samples included sodium and potassium concentrations, osmolality, and activity of Cr⁵¹.

The efficacy of the metoprolol protocol was investigated in five separate experiments. The PRC response to step-up administration of isoprenaline (2 and 4 μg min⁻¹ for 15 min each) was measured before and after administration of metoprolol following the protocol described above.

*Analyses*

Blood samples were centrifuged immediately at 4 °C. Measurements of electrolytes, osmolality, and of the activity of Cr⁵¹ were made on the day of the experiment. Ion concentrations were measured by flame photometry (ILS-943, Instrumentation Laboratory,
Lexington, MA). Osmolality was determined by freezing point depression (model 3D3, Advanced Instruments, Needham Heights, MA). The activity of $^{51}$Cr in plasma and urine samples was determined in a gamma counter.

**Plasma hormones**

**Plasma renin concentration.** PRC was determined by the antibody trapping method of Poulsen & Jørgensen (26) followed by a radioimmunoassay of the angiotensin I formed per unit time as described recently (20). Results are reported as milli international units (mIU l$^{-1}$) of the activity of the WHO International Standard (ref. #68-356, National Institute for Biological Standards and Control (NIBSC), Hertfordshire, UK) measured along with the plasma samples in each assay. Intra- and interassay variation coefficients were 6.2 and 5.6 %, respectively.

Peptide hormone concentrations in plasma were measured by radioimmunoassay after extraction as described previously (8; 20). The immunoreactivity of AngII in extracts was measured using a specific rabbit antibody (AB-5-030682). Intra- and interassay coefficients of variation were 6.6 and 9.0 %, respectively. Aldosterone was measured by a commercial kit (COAT-A-COUNT, Diagnostic Products, Los Angeles, CA). The detection limit was 11 pg ml$^{-1}$, and the mean recovery of unlabelled aldosterone was 89 %. The intra- and interassay variation coefficients were 2.9 and 3.5 % respectively. For AVP determinations, a specific rabbit antibody (AB3096) was used. The detection limit of AVP was 0.15 pg ml$^{-1}$ and the mean recovery of unlabelled vasopressin was 67 %. The intra- and interassay variation coefficients were 2.2 and 10.7 %, respectively. Antibody against ANP (RAS8798) was purchased from Peninsula Laboratories. The detection limit was 1.6 pg ml$^{-1}$, and the mean extraction recovery of unlabelled ANP added to plasma was 71 %. The intra- and interassay variation coefficient were 3.9 and 6.5 % respectively.

**Statistics**

All values are presented as mean ± standard error (SE). Comparisons were performed by one-way analysis of variance (ANOVA) for repeated measures. In case of significant differences
post hoc Newman-Keuls’ test were performed. Specific comparisons between two groups were performed by subsequent use of Student’s paired t-test. CVP was measured in one min averages and the results characterized by asymmetric outliers, e.g., increases associated with movements. Therefore, the 30 data points from each sampling period were trimmed by removal of a fixed and equal number of lowest and highest data points. Statistical calculations were performed with GraphPad Prism (GraphPad Software, San Diego, USA). Differences were considered significant at p< 0.05.

RESULTS

For seven subjects, the values of 24 h renal sodium output prior to the experiments were low and similar in all series (Con 17±4 mmol (24 h)⁻¹; MetCon 19±6 mmol (24 h)⁻¹; Salt 20±6 mmol (24 h)⁻¹; MetSalt 25±6 mmol (24 h)⁻¹) indicating compliance with the low-salt protocol. Baseline levels of all three RAAS variables were also uniform: PRC (Con: 59±8 mIU l⁻¹; Salt: 65±13 mIU L⁻¹), plasma AngII (Con 24±5 pg ml⁻¹; Salt 25±6 pg ml⁻¹) and plasma aldosterone (Con 202±27 pg ml⁻¹; Salt 199±32 pg ml⁻¹). For one subject, the values of the 24 h renal sodium excretion prior to the experiments were high on several occasions, up to 98 mmol (24 h)⁻¹; all data for this subject were excluded from the data analysis.

Verification of the effect of metoprolol

Intravenous isoprenaline infusion (4 µg/min) increased PRC from 37±6 mIU ml⁻¹ to 71±19 mIU ml⁻¹ (p<0.01). During administration of metropolol (injection of 0.3 mg (kg body weight)⁻¹ plus i.v. infusion of 1 µg kg⁻¹ min⁻¹) the same dose of isoprenaline did not change PRC at all (35±5 to 28±5 mIU ml⁻¹). Therefore, the present protocol of metoprolol administration completely blocked the isoprenaline mediated stimulation of renin secretion.

Systemic hemodynamics

MAP was measured every 15 min and remained stable during the time control series (Con, ~89 mmHg and MetCon ~76 mmHg). The β₁-adrenergic blockade reduced the control values of
MAP (MetCon 77±2 and MetSalt 76±1 mmHg compared to Con 90±2 and Salt 84±3 mmHg) (Fig. 2). Averaging the pre-intervention data, metoprolol decreased MAP by 9.5±1.9 mmHg. Saline loading did not change MAP (Salt 84±3 to 83±2 mmHg; MetSalt 76±1 to 75±2 mmHg, respectively). Notably, there were no trends at all towards increases in MAP.

Heart rate (HR) was constant during the time control series (Con); metoprolol initially reduced HR (11±2 beats min\(^{-1}\), p<0.001), followed by a further 9% decrease over time. The saline load generated a 5-6 bpm (9%) decrease in HR (Salt), but during metoprolol (MetSalt) HR remained unchanged at ~53 beats min\(^{-1}\) (Fig. 2).

Cardiac output (CO) was unchanged during the time control series (Con) with a first hour mean value was 7.2±0.5 l min\(^{-1}\), but decreased slightly in the three other series. Metoprolol per se decreased CO by an average of 16% (Student’s t, p<0.01, Fig. 2), from this reduced level, a small but significant decrease occurred in the metoprolol control series (MetCon: 6.4±0.4 to 5.7±0.5 l min, Δ=11%). CO also decreased during the two series with saline loading (Salt: 7.5±0.5 l min\(^{-1}\) to 6.7±0.4 l min\(^{-1}\) and MetSalt: 6.1±0.4 l min\(^{-1}\) to 5.6±0.4 l min\(^{-1}\) ) (Fig.2). However, these changes are similar the mentioned 11% decrease over time observed in CO during the time control series (MetCon). Therefore, the saline loading did not elicit specific changes in CO.

Significant changes in systemic vascular resistance (SVR) were not observed, except during the saline loading series (Salt), where a marginal, significant increase occurred, generated mainly by unusually low control values in two subjects.

Changes CVP were absent in both time control series (Con and MetCon). CVP increased with saline loading (Salt series, Δ = 1.9±0.6 mmHg, p<0.005; MetSalt series, Δ = 2.0±1.0 mmHg, p<0.05, Fig. 3).
Plasma electrolytes and osmolality

Plasma osmolality and plasma sodium concentration were very stable in all series (Table 1). Plasma potassium concentration decreased slightly in experiments without metoprolol but was constant in the two series with metoprolol administration (Table 1).

Plasma hormone concentrations

Metoprolol treatment decreased PRC and AngII by 30-40% (Fig. 4). The decrease in plasma aldosterone was less consistent. During saline infusion, PRC decreased markedly from 59±13 to 34±6 mIU L⁻¹ (by 43 %). After metoprolol pretreatment, the saline loading inhibited PRC to a similar, if not larger, extent (by 57 %). Similarly, the saline loading decreased plasma AngII equally with and without metoprolol (by 59 and 53 %, respectively). Likewise, plasma aldosterone decreased with or without metoprolol (215±34 to 86±18 mmol L⁻¹ and 143±29 pg ml⁻¹ to 86±9 pg ml⁻¹, Fig. 4). Taken together the data clearly show that the β₁-adrenergic blockade decreased the control values of the PRC and AngII, but did not affect the acute suppression of RAAS elicited by the saline loading.

Plasma AVP concentrations did not change in either control series, but decreased appreciably during saline infusion without (3.0±0.4 to 1.8±0.1 pg ml⁻¹) and with metoprolol (3.0±1.1 to 2.1±0.3 pg ml⁻¹, Table 3). Therefore, the present protocol, which generated a marginal increase in central venous pressure at constant plasma osmolality and sodium concentration, nevertheless reduced vasopressin secretion.

Plasma ANP remained constant during Con series (24±2 pg ml⁻¹ to 22±2 pg ml⁻¹). There was no change due to or during the β₁-adrenergic blockade (average ~23 pg ml⁻¹). ANP did not change significantly during saline loading with or without metoprolol (Salt: 24±3 to 24±3 pg ml⁻¹; MetSalt: 31±5 to 30±5 pg ml⁻¹, Table 3). The control values of the different series of investigations were not significantly different.

Renal variables and excretory patterns

11
Neither the $\beta_1$-adrenoceptor blockade nor the saline loading elicited any significant changes in GFR (Fig. 5). During time control experiments, GFR was stable at $\sim$105 ml min$^{-1}$ and $\sim$103 ml min$^{-1}$, in the Con and MetCon experiments, respectively.

Urine flows were stable during control experiments at $\sim$4.8 ml min$^{-1}$ and $\sim$4.9 ml min$^{-1}$. Saline loading caused a trend towards an increase in urine flow (Salt 4.6±1.2 to 7.0±0.6 ml min$^{-1}$, Table 2). In both saline infusion series (Salt and MetSalt), peak urine flows (6.9 and 7 ml min$^{-1}$) were different from the corresponding values in the appropriate control series. Urine osmolality decreased considerably in intervention series (Salt 292±71 to 157±17 mOsm kg$^{-1}$ and MetSalt 207±33 to 128±12 mOsm kg$^{-1}$), but not to values significantly different from the respective control experiments (Con and MetCon, Table 2).

The control values of the rate of excretion of sodium were very similar in the different series and remained constant in the absence of intervention (Fig. 5). In both saline loading series, sodium excretion increased gradually with the infusion (Fig. 5). The statistically indistinguishable increases continued into the recovery period reaching excretion rates of 55±13 µmol min$^{-1}$ and 71±14 µmol min$^{-1}$. Saline loading augmented fractional sodium excretion $F_{ENa^+}$ gradually in a pattern similar to absolute renal sodium excretion, as GFR remained unchanged (Salt 0.09±0.02 to 0.36±0.08 % and MetSalt 0.10±0.01 to 0.43±0.07 %).

The measured changes in the CVP were small. In one of the seven subjects, data from the control period are not available due to a technical error. For the six other subjects, it was possible to generate lines of regression with respect to CVP and rate of sodium excretion (Fig. 6). The mean slope indicates an increase of 7 µmol min$^{-1}$ in sodium excretion for each mmHg change in CVP.

**DISCUSSION**

We tested the hypothesis that $\beta_1$ mediated effects of norepinephrine participate in salt-mediated RAAS suppression and RAAS-mediated natriuresis, i.e., that the inhibition of renin
secretion and the associated natriuresis occurring during acute saline loading under constant blood pressure and GFR is reduced by $\beta_1$-adrenoceptor blockade. The major findings were that (i) metoprolol significantly decreased the control values of PRC and AngII (30-40%), (ii) saline loading decreased PRC, pAngII and pAldo by 50-60%, and (iii) this deactivation of the renin system was unimpeded by metoprolol administration. Therefore, the results are not compatible with the hypothesis. The overall result is that the acute RAAS deactivation and the RAAS-mediated natriuretic responses to modest, slow salt loading are independent of changes in blood pressure, glomerular filtration rate, and $\beta_1$-receptor-mediated nerve activity.

The efficacy of the metoprolol treatment was tested in separate experiments, which showed that the present metoprolol administration completely abolished isoprenaline-mediated renin secretion. Therefore, there is little doubt that the changes in RAAS activity measured during metoprolol administration were not mediated via $\beta_1$-receptors.

In recent dog experiments [Bie et al. accompanying paper], extracellular volume and sodium concentration were increased simultaneously as small doses of hypertonic saline were applied, and the deactivation of the RAAS could be due to both volume and concentration changes. In the present investigation, extracellular volume was increased at constant plasma sodium concentration. The present deactivation of the RAAS, therefore, cannot be due to changes in sodium concentration or osmolality as both were very stable.

The present results may be influenced by several details of the present protocol. Firstly, the subjects were investigated in the reclined, but sitting position (angle of back-support 50-60 degrees). Previous investigations in this laboratory have shown that in the supine position, normal subjects may show a three-fold increase in sodium excretion over a few hours without any intervention (4). Therefore, any change in sodium excretion in supine humans should be evaluated relative to the results of stringent time control experiments. In the present experiments, all variables measured (except plasma potassium) were shown to be stable during the time control series. Another advantage of the sitting position is that the orthostatic alterations
associated with micturition are minimized. The present male subjects emptied their bladder while briefly standing up. The hemodynamic changes (e.g. baroreceptor deactivation) associated with standing up from sitting are much smaller than with standing up from the supine position. Secondly, the saline was infused at a very low rate for an extended period in time in order to minimize the rate of change e.g. in hemodynamic variables. This intention was fulfilled as blood pressure and heart rate remained very stable through the perturbation. Thirdly, the infusate was physiological saline slightly diluted by water in order to avoid differences between the concentrations of sodium in infusate and plasma. In a previous, similar study of slow sodium loading (27) a higher dose rate (20 µmol NaCl kg⁻¹ min⁻¹) was given as normal saline (154 mmol l⁻¹) with the consequence that plasma sodium concentration increased marginally, but significantly. In the present study, normal saline was diluted about 12% lowering the sodium concentration to ~135 mmol l⁻¹, and plasma sodium concentrations were very stable during the interventions at 138-139 mmol l⁻¹. Finally, the inhibition of the renin system occurred from elevated levels generated by a low-sodium diet. It is evident that this standardized, elevated starting level provides a favorable basis for quantitative comparisons of deactivation of the RAAS.

*Metoprolol infusion.* The acute hemodynamic and renal effects of metoprolol were expected (average decrease in blood pressure of ~11% ± 3% concomitant with a similar change in HR without significant alterations in peripheral resistance). The overall metoprolol effect on GFR, urine flow and renal excretory pattern of sodium and potassium was negligible. Similar changes have been described by others (34; 35). As discussed in the preceding paper [Bie et al. accompanying paper], the effects of systemic β₁-adrenergic blockade in the intact animal may be complex, particularly if results of renal nerve recordings in anesthetized rabbits before and after administration of the atenolol and propranolol (12; 13) are interpreted to indicate that β₁-adrenergic antagonists decrease renal nerve activity. If so, metoprolol, in addition to the decrease in renin secretion, may have changed the nervous setting, in which the kidney responded to salt
the loading by decreasing sympathetic tone to the kidney. However, as indicated by the similarity between the control measurements obtained prior to salt loading with and without administration of metoprolol, any inhibitory effect of metoprolol on renal sympathetic tone has little if any effect on renal function.

Renin secretion. It is well known that renin secretion is inhibited by an increase in renal perfusion pressure via intrarenal baroreceptor activity, by a decrease in renal sympathetic nerve activity, and by enhanced delivery of sodium chloride to the MD region (25; 30; 32). Based on previous studies in humans (4; 6; 27) and dogs (28) including the preceding paper [Bie et al. accompanying paper], the present protocol was designed to investigate the effect of $\beta_1$-adrenoceptor blockade under conditions where MAP and GFR were constant. Consequently, the inhibition of the renin system under the present conditions was assumed sensitive to such blockade. However, our data clearly show that elimination of the $\beta_1$ mediated effects do not affect the acute, regulatory deactivation of the RAAS in response to a modest sodium load. Under the present conditions, it is not possible to assess tubular fluid solute concentrations at the MD. Alterations of GFR are only one of the determinants of the amount of NaCl delivered to MD. The absence of changes in GFR does not necessarily indicate that the MD NaCl concentration remained stable; it only demonstrates that one contributor to deviations in MD NaCl concentration was inoperative. It seems likely, that changes in proximal sodium reabsorption occurred and modified the distal load to MD, and thereby influenced on renin secretion. The renal clearance of lithium ions can be used for calculation of proximal sodium reabsorption (31). However, this measure is valid only under the assumption that the rate of reabsorption of lithium in nephron segments distal to the pars recta of the proximal tubule is negligible. The assumption is reasonable only when distal sodium to lithium concentration ratio is high, but this is not necessarily the case during exposure to a low-salt diet. For this reason, proximal sodium reabsorption was not measured in the present investigation, and it cannot be excluded that proximal reabsorption was decreased, that MD solute concentrations increased
during our interventions, and that MD-mediated mechanisms, therefore, played a major role in
the control of renin secretion and of sodium excretion under the present conditions. However, if
this were to be the case, the primary homeostatic control of the rate of excretion of sodium, i.e.
the regulation of total body sodium, is mediated via changes in proximal reabsorption. This may
seem less likely or at least at odds with current concepts of relative constancy of distal tubular
solute load and final regulation of renal sodium excretion being exerted by adjustments in distal
tubular reabsorption of sodium (11; 29).

MAP and GFR remained stable during our saline loading and it is unlikely that increased
renal afferent arteriolar pressure increased and decreased renin secretion under the present
conditions. Previous findings in our laboratory indicate that, at modest perturbations, the
regulation of renin is not primarily dependent of MAP (27). Earlier studies have shown that
changes in arterial pressure, i.e. pressure natriuresis, is a powerful controller of sodium excretion
(14; 17). Our data do not address the role of pressure natriuresis, but in the light of the present
results, it seems valid to assume that MAP is not a critical factor in the control of renin secretion
and sodium secretion under physiological conditions in humans.

With the present protocol, receptors were blocked throughout the body, and in the
kidney, this blockade may eliminate the effect of locally released as well as circulating
norepinephrine. More specifically, the separate experiments showed that the $\beta_1$-receptors
involved in renin secretion were effectively blocked. However, the deactivation of the RAAS
and the natriuresis in response to saline loading were unimpeded. Although incompatible with an
important role of $\beta_1$-receptor-mediated control of renin secretion, our data do not exclude that
other components of RSNA are involved. Micropuncture studies may indicate possible
mechanisms; the work of Cogan et al. provided direct evidence that the active sodium chloride
reabsorption in the early proximal tubule is selectively blocked by prazosin, a selective $\alpha_1$-
adrenoceptor antagonist (33). Consequently, our results may indicate either that an unknown
regulator of renin secretion is essential, or that known components of the regulation of renin
secretion are active in an unexpected way, e.g., that an $\alpha_1$-receptor-mediated decrease in proximal reabsorption is the primary regulator of sodium balance via changes in renin secretion.

Homeostatic renin secretion could be applied to describe the unknown regulation of renin secretion occurring in the absence of changes in MAP, GFR, and $\beta_1$ mediated effects of norepinephrine. The most likely candidate for a sensor mechanism involved in the homeostatic renin secretion seems to be the low-pressure (cardiovascular) receptors supposedly triggered by the 2 mmHg increase in CVP. The finding, that plasma vasopressin decreased at very stable values of plasma sodium and osmolality, supports the assumption that volume receptor signals were indeed augmented by the present small change in CVP. The receptors could be atrial type B receptors (16) and these may be located at the left side of the heart (1) as isolated changes on the right side of the heart at a magnitude much larger than the present increases appear ineffective with respect to renin secretion (3). The efferent pathway is not known. It is reasonable to assume that all $\beta_1$-receptors were blocked during the present experiments. If so, the efferent mechanism linking the central nervous system to deactivation of the RAAS activity could be either a decrease in RSNA not operating via $\beta_1$-adrenoceptors, e.g. $\alpha$-receptors, causing a decrease in proximal reabsorption eventually leading to a decrease in renin secretion (see above) or an increase in a humoral substance directly inhibiting the secretion from the granular cells. On one side, it is well known that a decrease in renal sympathetic nerve activity elicited e.g. by the saline loading, is able to decrease NaCl reabsorption by a direct action on both proximal tubule and the loop of Henle (10). On the other side, circulating renin inhibitor(s) may be involved. In this context, the recent results obtained by Lohmeier and coworkers in conscious dogs are interesting; they found that renin secretion from denervated kidneys remained unchanged during hypotension due to baroreceptor activation seemingly indicating that a least one major, non-nervous controller of renin secretion and RAAS-dependent blood pressure remains unknown (23). The present experiments were not designed to address this issue.
Natriuretic mechanisms. The regulation of the renal sodium excretion is complex and the natriuretic response to volume loading is considered - at least under experimental conditions - to be mediated by a combination of physical, neural, and endocrine factors. In our experiments, the decrease in RAAS activity seems a suitable explanation of the increase in sodium excretion. The unsolved puzzle is the mechanism by which renin secretion is deactivated. Recent studies in conscious rats may provide suggestions in this regard. Renal denervation did not affect the renin inhibitory or natriuretic responses to a sodium load (50 µmol kg⁻¹ min⁻¹) which elicited a slight increase (5-8 mmHg) in MAP (21) compatible with the notion that RSNA plays only a minor role when MAP increases measurably. However, preliminary results have shown (i) that a lower infusion rate (20 µmol kg⁻¹ min⁻¹), which did not change MAP, nevertheless reduced RSNA (measured by implanted electrodes), and (ii) that the natriuresis induced by this sodium load was eliminated by renal denervation (7). These results in rats seem to indicate that non-β₁ components of RSNA are involved in homeostatic renin secretion. However, other modulators of proximal reabsorption and/or renin secretion may well be important. There is no paucity of candidates (e.g., α-adrenergic and dopaminergic control of proximal reabsorption, adenosine concentration changes in the renal interstitium driven by proximal reabsorption, prostaglandin and NO mediated control of renin secretion). The challenge is to document a homeostatic function, i.e. a link between minor changes in total body sodium and the operation of a signaling pathway operating on renin secretion without changes in blood pressure, glomerular filtration rate, or β₁-adrenoceptor sensitive mechanisms. Convincing results seem to require carefully designed investigations of human subjects or conscious animals.

It has been suggested that salt loads administered via the gastro-intestinal tract elicit specific signals important to sodium homeostasis in analogy to the incretin effect of glucose metabolism. We investigated this notion a few years ago by administering a sodium load equivalent to 10% of that contained in the individually measured extracellular volume (5). The load was given either as isotonic or as hypertonic saline intravenously or by gastric tube. The
results were not compatible with an incretin like effect (5). On this basis, the present conclusions may seem representative for the response of normal man to acute salt and fluid excess irrespective of mode of administration.

Summary

Our results indicate that $\beta_1$-adrenergic nerve activity does not have an essential role in the acute PRC response to sodium loading occurring in normal man in the absence of changes in blood pressure and GFR. Consequently they suggest that the homeostatic, e.g. meal related, control of sodium excretion occurs independent of the classical regulators of renin secretion. It cannot be excluded that MD is of major importance, but if so, the mechanisms seem to involve changes in proximal tubular sodium reabsorption not normally assumed to play a major role in the homeostatic regulation of sodium excretion.

Perspectives

The mechanisms of suppression of the renin system are pivotal to the regulation of sodium balance, extracellular fluid volume, and arterial blood pressure. The present results indicate that a comprehensive analysis of the physiological control of renin secretion must include a fourth factor in addition to renal arterial pressure, $\beta_1$ mediated effects of norepinephrine, and GFR-related MD electrolyte exposure or an unrecognized homeostatic mode of operation of one or more of these classical regulators, notably renal nerve activity mediated via $\alpha$-receptors. This factor or mode of operation is a primary defense against increases in total body sodium and, therefore, of major interest to the analysis of normal blood pressure regulation and of the pathophysiology of major diseases including abnormal fluid volume and pressure control.
ACKNOWLEDGEMENTS

The expert technical assistance of Bodil Aarup Kristensen and Ulla Melchior Hansen is gratefully appreciated. We also acknowledge the assistance of the staff at the kitchen of Odense University Hospital for providing the special diets. The work was supported by the Danish Medical Research Council and the Danish Heart Foundation.


18. **Humphreys MH.** Salt intake and body fluid volumes: have we learned all there is to know? *Am J Kidney Dis* 37: 648-652, 2001.


Figures legends

Fig. 1
Time line of investigations

Fig. 2
Acute effects of saline infusion on mean arterial blood pressure (MAP), cardiac output (CO) and heart rate (HR). Dashed line: Time control. Dotted line: Metoprolol time control. Squares: Saline loading. Dots: Saline loading during metoprolol. Asterisk (*): significant difference from pre-infusion value by ANOVA and Newman-Keuls’ test. Significant deviations did not occur in the two time control series. Values are mean ± SE, n = 7.

Fig. 3
Acute effects of saline infusion on central venous pressure (ΔCVP). Saline was infused from t = 60-300 min and results are deviations from control values. Gray: Time control. White: Metoprolol time control. Black: Saline loading. Striped: Saline loading during metoprolol. Control values were 4.0±0.7, 6.2±0.6, 5.7±0.8, and 6.9±0.9 mmHg, respectively. Asterisk (*): significant difference zero by ANOVA and Newman-Keuls´ test. Values are mean ± SE, n = 7.

Fig. 4
Acute effects of saline infusion on plasma renin concentration (PRC), plasma AngII and plasma aldosterone. Dashed line: Time control. Dotted line: Metoprolol time control. Squares: Saline loading. Dots: Saline loading during metoprolol. Asterisk (*): significant difference from pre-infusion value by ANOVA and Newman-Keuls´ test. Significant deviations did not occur in the two time control series. Values are mean ± SE, n = 7.
Fig. 5

Measurements of systemic hemodynamics and renal function

Instrumentation  Control  Infusion  Recov.
Fig. 3

![Graph showing CVP (mmHg) changes over time for different groups: Con, MetCon, Salt, MetSalt. The graph indicates significant changes at certain time points with stars (*) indicating statistical significance.](image-url)
Sodium loading

GFR (ml min^{-1})

Sodium excretion (µmol min^{-1})

Potassium excretion (µmol min^{-1})

Time (min)
Table 1.

Effects of saline infusion on plasma parameters with and without metoprolol.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Preinfusion</th>
<th>Sodium loading</th>
<th>Postinfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-5</td>
<td>55</td>
<td>115</td>
</tr>
<tr>
<td>Con</td>
<td>284 ± 1</td>
<td>284 ± 1</td>
<td>286 ± 4</td>
</tr>
<tr>
<td>MetCon</td>
<td>286 ± 1</td>
<td>284 ± 1</td>
<td>283 ± 1</td>
</tr>
<tr>
<td>Salt</td>
<td>285 ± 1</td>
<td>284 ± 1</td>
<td>284 ± 1</td>
</tr>
<tr>
<td>MetSalt</td>
<td>285 ± 1</td>
<td>284 ± 1</td>
<td>287 ± 3</td>
</tr>
</tbody>
</table>

**Plasma Osmolality (mOsmol kg⁻¹)**

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>MetCon</th>
<th>Salt</th>
<th>MetSalt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>138.6 ± 0.6</td>
<td>137.7 ± 0.6</td>
<td>137.6 ± 0.6</td>
<td>137.6 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>138.3 ± 1.0</td>
<td>137.9 ± 1.0</td>
<td>137.7 ± 0.9</td>
<td>137.4 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>138.2 ± 0.4</td>
<td>137.9 ± 0.6</td>
<td>137.4 ± 0.7</td>
<td>138.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>139.0 ± 0.7</td>
<td>138.8 ± 1.0</td>
<td>140.9 ± 1.8</td>
<td>139.1 ± 1.1</td>
</tr>
</tbody>
</table>

**Plasma Sodium (mmol l⁻¹)**

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>MetCon</th>
<th>Salt</th>
<th>MetSalt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.3 ± 0.1</td>
<td>4.2 ± 0.1</td>
<td>4.1 ± 0.1</td>
<td>4.0 ± 0.1*</td>
</tr>
<tr>
<td></td>
<td>4.2 ± 0.1</td>
<td>4.2 ± 0.1</td>
<td>4.2 ± 0.1</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>4.2 ± 0.1</td>
<td>4.2 ± 0.1</td>
<td>4.1 ± 0.1</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>4.1 ± 0.1</td>
<td>3.6 ± 0.6</td>
<td>4.2 ± 0.1</td>
<td>4.2 ± 0.1</td>
</tr>
</tbody>
</table>

Table 2.

Effects of saline infusion on renal parameters with and without metoprolol.

<table>
<thead>
<tr>
<th></th>
<th>Preinfusion</th>
<th>Sodium loading</th>
<th>Posinfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time (min)</strong></td>
<td>0-59</td>
<td>60-119</td>
<td>120-179</td>
</tr>
<tr>
<td><strong>Urine Flow (ml min⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>5.0 ± 0.6</td>
<td>5.0 ± 0.4</td>
<td>5.5 ± 0.5</td>
</tr>
<tr>
<td>MetCon</td>
<td>4.3 ± 0.9</td>
<td>4.7 ± 0.7</td>
<td>6.2 ± 0.6</td>
</tr>
<tr>
<td>Salt</td>
<td>4.6 ± 1.2</td>
<td>6.2 ± 0.9</td>
<td>6.9 ± 0.6</td>
</tr>
<tr>
<td>MetSalt</td>
<td>5.1 ± 0.7</td>
<td>6.3 ± 0.8</td>
<td>6.5 ± 0.5</td>
</tr>
<tr>
<td><strong>Urine Osmolality (mOsm kg⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>211 ± 35</td>
<td>161 ± 26</td>
<td>141 ± 31</td>
</tr>
<tr>
<td>MetCon</td>
<td>350 ± 111</td>
<td>210 ± 41</td>
<td>133 ± 15*</td>
</tr>
<tr>
<td>Salt</td>
<td>292 ± 71</td>
<td>162 ± 24*</td>
<td>127 ± 13*</td>
</tr>
<tr>
<td>MetSalt</td>
<td>207 ± 33</td>
<td>156 ± 33</td>
<td>131 ± 16</td>
</tr>
<tr>
<td><strong>Free-Water clearance (ml min⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>1.6 ± 0.7</td>
<td>2.4 ± 0.5</td>
<td>2.9 ± 0.6</td>
</tr>
<tr>
<td>MetCon</td>
<td>0.7 ± 1.0</td>
<td>1.8 ± 0.7</td>
<td>3.5 ± 1.3</td>
</tr>
<tr>
<td>Salt</td>
<td>1.3 ± 1.0</td>
<td>3.1 ± 0.7*</td>
<td>4.0 ± 0.5*</td>
</tr>
<tr>
<td>MetSalt</td>
<td>1.8 ± 0.7</td>
<td>3.3 ± 0.7</td>
<td>3.7 ± 0.6</td>
</tr>
</tbody>
</table>

Table 3.

Effects of saline infusion on plasma hormones with and without metoprolol.

<table>
<thead>
<tr>
<th></th>
<th>Preinfusion</th>
<th>Sodium loading</th>
<th>Postinfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
<td>55</td>
<td>235</td>
<td>355</td>
</tr>
<tr>
<td><strong>Plasma AVP (pg ml⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>2.9 ± 0.6</td>
<td>2.8 ± 0.6</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>MetCon</td>
<td>3.6 ± 0.8</td>
<td>2.7 ± 0.9</td>
<td>3.1 ± 1.0</td>
</tr>
<tr>
<td>Salt</td>
<td>3.0 ± 0.4</td>
<td>2.0 ± 0.2*</td>
<td>1.8 ± 0.1*</td>
</tr>
<tr>
<td>MetSalt</td>
<td>3.0 ± 0.6</td>
<td>2.3 ± 0.3*</td>
<td>2.1 ± 0.3*</td>
</tr>
<tr>
<td><strong>Plasma ANP (pg ml⁻¹)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Con</td>
<td>24 ± 2</td>
<td>19 ± 2*</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>MetCon</td>
<td>24 ± 2</td>
<td>21 ± 3</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>Salt</td>
<td>24 ± 3</td>
<td>25 ± 4</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>MetSalt</td>
<td>31 ± 5</td>
<td>29 ± 4</td>
<td>30 ± 5</td>
</tr>
</tbody>
</table>