CLINICAL STUDY

Direct effect of methylprednisolone on renal sodium and water transport via the principal cells in the kidney

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Abstract

Background: Glucocorticoids influence renal concentrating and diluting ability. We tested the hypothesis that methylprednisolone treatment increased renal water and sodium absorption by increased absorption via the aquaporin-2 (AQP2) water channels and the epithelial sodium channels (ENaCs) respectively.

Methods: The effect of methylprednisolone was measured during fasting in a randomized, placebo-controlled, single-blinded cross-over study of 15 healthy humans. The subjects received a standardized diet on day 1, fasted on day 2, and received 500 mg methylprednisolone intravenously on day 3. The effect variables were urinary excretions of AQP2 (u-AQP2), urinary excretion of the β -fraction of the ENaC (u-ENaC $_{\beta}$), cAMP (u-cAMP), prostaglandin E₂ (u-PGE₂), free water clearance (C_{H₂O}), and fractional excretion of sodium (FE_{Na}), and plasma vasopressin (p-AVP), angiotensin II (p-Ang II), aldosterone (p-Aldo), atrial natriuretic peptide (p-ANP), and brain natriuretic peptide (p-BNP).

Results: Methylprednisolone treatment increased u-AQP2, u-ENaC $_{\beta}$, and p-AVP significantly, but did not change u-cAMP, C_{H₂O}, and FE_{Na}. P-ANP increased during methylprednisolone treatment, but after the increase in u-AQP2 and u-ENaC $_{\beta}$, U-PGE₂, p-Ang II, and p-BNP were unchanged. Heart rate increased and diastolic blood pressure fell.

Conclusions: Methylprednisolone increased u-AQP2 and u-ENaC. Neither the AVP–cAMP axis nor changes in the renin–angiotensin–Aldo system, or the natriuretic peptide system seems to bear a causal relationship with the increase in either u-AQP2 or u-ENaC. Most probably, the effect is mediated via a direct effect of methylprednisolone on the principal cells. The lack of decrease in urinary output and sodium reabsorption most likely can be attributed to the diuretic and natriuretic properties of the increased secretion of ANP.

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Introduction

Glucocorticoids modulate the secretion of vasopressin (AVP). AVP secretion is inhibited in Cushing's syndrome and during methylprednisolone treatment, which results in a reduced urinary concentrating ability. In Addison's disease, AVP secretion is stimulated, and urinary diluting ability is reduced. However, in healthy subjects treated with methylprednisolone, plasma concentration of AVP (p-AVP) was severely suppressed, but urinary concentrating ability remained unchanged (1). Thus, glucocorticoid treatment has an AVP-independent effect on the renal capacity to excrete water.

Aquaporin-2 (AQP2) trafficking mediates water transport across the apical cell membrane in the principal cells of the collecting ducts in the kidneys (2). The short-term regulation by AVP involves activation of V₂ receptors and subsequent trafficking of AQP2 vesicles to the apical plasma membrane,

resulting in increased water permeability and absorption. The long-term regulation is due to a rapid change in AQP2 mRNA expression followed by a more slow AQP2 synthesis. From a theoretical point of view, several mechanisms could be involved in a non-AVP-mediated increase in urinary concentrating ability. First, inhibition of the prostaglandin receptors on the principal cells has a synergistic effect on AVP (3). Secondly, direct stimulation of cAMP in the principal cells will increase AQP2 trafficking to the apical membrane and facilitate water transport. Thirdly, glucocorticoids might directly stimulate AQP2 trafficking and/or AQP2 synthesis. Lastly, the activity of the renin–angiotensin–Aldo system and the natriuretic peptide system might be changed during glucocorticoid treatment, and subsequently renal water reabsorption.

Cellular trafficking and synthesis of the epithelial sodium channels (ENaCs) are the pathways for sodium transport across epithelia, including the kidney

collecting ducts. The epithelial sodium transport is regulated to a great extent by trafficking mechanisms that control ENaC expression at the cell surface. Delivery of channels to the cell surface is regulated by Aldo (and corticosteroids) and AVP, both of which increase ENaC synthesis and exocytosis respectively (4).

In mineralocorticoid receptor knockout mice, treatment with glucocorticoids induced an amiloride-sensitive sodium absorption in renal cortical collecting ducts, indicating that glucocorticoids might have a direct effect on amiloride-sensitive sodium absorption in renal cortical collecting ducts (5). The increased sodium absorption in renal cortical collecting ducts of mice might be due to an increased number of ENaCs. Thus, glucocorticoids might induce an increased urinary excretion of ENaCs. However, ENaC excretion might also be increased due to other mechanisms, e.g. increased shear stress by urinary flow rate. Methylprednisolone, a synthetic glucocorticoid, was chosen for the study due to its rapid inserting effect with a very modest mineralocorticoid effect.

We wanted to test the hypothesis that methylprednisolone stimulated water and sodium absorption in the kidney via the AQP2 water channels and ENaCs. The degree of water transport via AQP2 is reflected by the level of urinary excretion of AQP2 (u-AQP2) (6). Correspondingly, the sodium transport via ENaCs is supposed to be reflected by the level of urinary excretion of the β -fraction of ENaC (u-ENaC $_{\beta}$). This is the first study to investigate the effect of methylprednisolone on urinary AQP2 and ENaC excretion in humans.

We performed a randomized, placebo-controlled, cross-over study in healthy humans to examine the effect of methylprednisolone on renal handling of water and sodium during fasting. We measured the effect of methylprednisolone/placebo on u-AQP2, u-ENaC, fractional urinary excretion of sodium (FE $_{Na}$), urinary excretion of prostaglandin E $_2$ (u-PGE $_2$), urinary excretion of cAMP (u-cAMP), and free water clearance (C $_{H_2O}$), and on plasma concentrations of renin (p-PRC), angiotensin II (p-Ang II), Aldo (p-Aldo), p-AVP, atrial natriuretic peptide (p-ANP), and brain natriuretic peptide (p-BNP).

Materials and methods

Participants

Inclusion criteria Inclusion criteria were age 18–65 years and body mass index < 30.

Exclusion criteria Exclusion criteria were clinical signs or history of disease in the heart, lungs, kidneys, or endocrine organs; abnormal laboratory tests (blood hemoglobin, white cell count, platelet counts, plasma concentrations of sodium, potassium, creatinine, albumin, bilirubin, alanine aminotransferase, and

cholesterol; blood glucose; and albumin and glucose in urine); malignancies; arterial hypertension (i.e. casual blood pressure > 140/90 mmHg); alcohol abuse (more than 21 drinks per week for males and more than 14 drinks for females); medical treatment; pregnancy; breast-feeding; lack of oral contraceptive treatment for women in the fertile age; intercurrent diseases; problems with blood sampling or urine collection; medicine abuse; donation of blood < 1 month before the study; and unwillingness to participate.

Withdrawal criteria Withdrawal criteria were development of one or more of the exclusion criteria.

Ethics

The local medical ethics committee approved the study. All the participants received written information, and gave their written consent.

Design

The study was randomized, placebo-controlled, single-blind, and over-crossed. There was a time interval of 2 weeks between the two examinations. Each examination lasted for 3 days.

Recruitment

Participants were recruited by advertisements in public and private institutions.

Diet and fluid intake

The normal energy requirement was calculated using the formula: weight (kg) $\times$ 100 (kJ) $\times$ activity factor (AF). AF ranged from 1.3 to 2.4, with a possible extra of 0.3 for physical activity in the spare time, e.g. 30 min of sports 5–6 times a week. AF of 1.3 indicates no physical activity. AF of 1.4–1.5 indicates secretarial work without physical activity in the spare time. AF of 1.6–1.7 indicates secretarial work with walking during working hours and/or physical activity in the spare time. AF of 1.8–1.9 corresponds to shop assistant job (standing/walking all day), and AF of 2.0–2.4 indicates hard physical activity with or without physical activity in the spare time. The food had a specified amount of energy with carbohydrates (55% of the total energy), protein (15% of the total energy), and fat (30% of the total energy). The diet consisted of three main meals and three small meals. The participants were not allowed to add any spices or sodium to the meals or to divide the meals into bigger or smaller portions. The participants drank tap water, 35 ml/kg each, for 24 h and nothing else. They maintained normal physical activity during the study.

Procedure

Day 1 (24 h) The participants ate the specified diet, drank tap water, 35 ml/kg body weight, and maintained normal physical activity.

Day 2 (24 h) The participants fasted, drank tap water, 35 ml/kg body weight, maintained normal physical activity, and collected urine during 24 h.

Day 3 The participants arrived at 0730 h to the laboratory. An intravenous catheter was placed in fossa cubiti on each side: one for the collection of blood samples, and the other for the infusion of ^{51}Cr -EDTA and hypertonic saline. Urine was collected during the following seven periods: 0700–0930 h (P-0), 0930–1000 h (P-1), 1000–1030 h (P-2), 1030–1100 h (P-3), 1100–1130 h (P-4), 1130–1200 h (P-5), 1200–1230 h (P-6), and 1230–1300 h (P-7). Urine was analyzed for u-AQP2, u-ENaC, u-Osm, u-Na, u-Crea, u-cAMP, u-PGE₂, and u- ^{51}Cr -EDTA. The subjects voided in the standing or sitting position. Otherwise, they were in the supine position during the examination. Blood samples were taken every 30 min, starting at 0930 h for the analysis of p-AVP, p-Osm, p-Na, p-Crea, p-albumin, and p- ^{51}Cr -EDTA. In addition, blood samples for the measurements of p-Ang II, p-ANP, p-BNP, p-PRC, and p-Aldo were drawn at 0800, 1100, 1200, and 1300 h. A total amount of 350 ml of blood was drawn during each of the study days. The blood drawn at blood sampling was immediately substituted with isotonic saline. From 1100 to 1130 h (P-4), 500 mg of methylprednisolone or placebo were infused intravenously. Blood pressure and pulse rate were measured every 30 min during the examination.

The participants were weighed before (day 3 at 0730 h) and after the trial (1330 h).

Effect variables

The main effect variables were u-AQP2 and u-ENaC. The other effect variables were u-PGE₂, u-cAMP, p-AVP, C_{H₂O}, urine volume, FE_{Na}, p-Osm, p-PRC, p-Ang II, p-Aldo, p-ANP, and p-BNP.

Number of participants

Using a significance level of 5% and a power of 90%, it was calculated that 14 subjects needed to be included, while the minimal relevant difference in u-AQP2 was estimated to be 0.20 ng/min and s.d. was estimated to be 0.15 ng/min.

Test substance

Methylprednisolone (Solu-Medrol) was dissolved in benzyl alcohol according to the instructions given by the pharmaceutical company, and was then further

diluted in 100 ml of 0.9% saline before infusion. Placebo was 100 ml of 0.9% saline. The test substance was prepared 15 min before infusion according to the randomization list.

Measurements

U-AQP2 was measured by RIA as described previously, and antibodies were raised in rabbits to a synthetic peptide corresponding to the 15 COOH-terminal amino acids in human AQP2 to which an NH₂-terminal cysteine was added for conjugation and affinity purification (7). Minimal detection level was 32 pg/tube. The coefficients of variation (CV) were 11.7% (inter-assay) and 5.9% (intra-assay).

U-cAMP was measured using a kit obtained from R&D Systems, Minneapolis, MN, USA. Minimal detection level was 12.5 pmol/tube. The CV were 6.9% (inter-assay) and 5.3% (intra-assay).

ENaC_β was measured by a newly developed RIA. Urine samples were kept frozen at -20°C until assayed. ENaC_β was synthesized by and purchased from Lofstrand Labs Ltd, Gaithersburg, Maryland, USA. The β-ENaC antibody was raised against a synthetic peptide in rabbits and affinity purified as described previously (8). Iodination of ENaC_β was performed by the chloramine T method using 40 μg ENaC_β and 37 MBq ^{125}I . The reaction was stopped by the addition of 20% human serum albumin. ^{125}I -labeled ENaC_β was separated from the iodination mixture using a Sephadex G-25 Fine column. The assay buffer contained 40 mM sodium phosphate (pH=7.4), 0.2% human albumin, 0.1% Triton X-100, and 0.4% EDTA. A 1.5% solution of γ-globulins from pig (Sigma) and 25% polyethylene glycol 6000 (Merck) also containing 0.625% Tween 20 (Merck) was prepared using 0.4 M phosphate buffer. Urine samples were kept frozen at -20°C . After thawing out, urine samples were centrifuged for 5 min at $1.6 \times 100\text{ g}$ (3000 r.p.m.). The supernatant was extracted using Sep-Pak C₁₈. The elution fluid used was 4 ml of a mixture comprising 90% methanol, 0.5% acetic acid, and 9.5% demineralized water. The eluates were freeze-dried and kept at -20°C until assayed. A mixture of 300 μl of standard or freeze-dried urine eluates redissolved in 300 μl of assay buffer and 50 μl of antibody was incubated for 24 h at 4°C . Thereafter, 50 μl of the tracer were added, and the mixture was incubated for a further 24 h at 4°C . γ-Globulin from pigs (100 μl) and 2 ml of polyethylene glycol 6000 were added. The mixture was centrifuged at 2000 g for 20 min at 4°C . The supernatant (free fraction) was poured off, and the precipitate (bound fraction) was counted in a gamma counter. The unknown content in urine extracts was read from a standard curve. For 13 consecutive standard curves, the zero standard was $70 \pm 1.6\%$, and for increasing amounts of the ENaC_β standard, the binding inhibition was $69 \pm 1.4\%$ (15.6 pg/tube),

66 ± 1.5% (31.25 pg/tube), 62 ± 1.6% (62.5 pg/tube), 54 ± 1.5% (125 pg/tube), 40 ± 1.4% (250 pg/tube), 26 ± 1.2% (500 pg/tube), 14 ± 0.6% (1000 pg/tube), 8.2 ± 0.4% (2000 pg/tube), and 5.1 ± 0.3% (4000 pg/tube). The ID50, i.e. the concentration of standard needed for 50% binding inhibition, was 322 ± 12 pg/tube ($n=13$). The non-specific binding determined by performing RIA without antibody was 1.3 ± 0.3% ($n=13$). The inter-assay variation was determined by quality controls from the same urine pool spiked with the ENaC β standard. In consecutive assays, the CV were as follows: 12% (12 assays) at a mean level of 78 pg/tube, 10% (12 assays) at a mean level of 155 pg/tube, and 17% (10 assays) at a mean level of 394 pg/tube. The intra-assay variation was determined using samples from the same urine pool in several assays at different concentration levels. At a mean level of 180 pg/tube ($n=10$) and 406 pg/tube ($n=10$), the CV were 6.4 and 9.0% respectively. In addition, CV were calculated on the basis of duplicate determinations in different assays to 9.1% ($n=22$) in the range 58–101 pg/tube, 8.6% ($n=26$) in the range 143–203 pg/tube, 8.7% ($n=20$) in the range 205–421 pg/tube, and 10.0% ($n=68$) in the whole range 58–421 pg/tube. The sensitivity calculated as the smallest detectable difference at the 95% confidence limit was 10 pg/tube in the range 58–101 pg/tube ($n=22$), 20 pg/tube in the range 143–203 pg/tube ($n=26$), 48 pg/tube in the range 205–421 pg/tube ($n=20$), and 28 pg/tube in the whole range 58–421 pg/tube ($n=68$). The lower detectable limit of the assay was 34 pg/tube. It was calculated using the average zero binding for 13 consecutive assays -2 s.d. The volume of urine used for extraction from the same pool was varied (18 different volumes in the range 250–6000 μ l), and the mean concentration measured was 89 ± 6 pg/ml. There was a highly significant correlation between the extracted volume of urine and the amount of pg/tube ($r=0.99$, $n=18$). Recovery of the labeled tracer during the extraction-freeze drying procedure was 94 ± 3% ($n=13$), 95 ± 3% ($n=13$), 95 ± 2% ($n=10$), and 95 ± 2% ($n=7$) in four different pools used in several extraction procedures. When ENaC β in the range 62.5–250 pg was added to urine, a highly significant correlation was found between the measured and the expected values ($r=0.981$, $n=12$, $P<0.001$). We measured u-ENaC β in 12 patients with arterial hypertension treated with amiloride. During the study day, a urine sample was collected at 0800 and 1100 h for measurement of u-sodium/u-creatinine (u-Na/u-Crea). No medication was given in the morning before the collection of the first urine sample. Immediately afterwards, the usual doses of 5 or 10 mg amiloride were given. A significantly negative correlation was found between the changes in u-Na/u-Crea and changes in u-ENaC β /u-Crea ($\rho=-0.724$, $n=11$, $P<0.012$).

U-PGE $_2$ was measured using a kit obtained from Assay Designs, Inc., Ann Arbor, MI, USA. The CV were 10.9% (inter-assay) and 6.3% (intra-assay).

Blood samples were centrifuged for 15 min at 1600 g at 4 °C. Plasma was separated from blood cells, and kept frozen at -20 °C until assayed. AVP, ANP, BNP, and Ang II were extracted from the plasma with Sep-Pak C $_{18}$ (Water Associates, Milford, MA, USA), and were subsequently determined by RIAs (9, 10). The antibody against AVP was a gift from Prof. Jacques Dürr, Miami, FL, USA. Minimal detection level was 0.5 pmol/l. The CV were 13% (inter-assay) and 9% (intra-assay). Rabbit anti-ANP antibody was obtained from the Department of Clinical Chemistry, Bispebjerg Hospital, Denmark. Minimal detection level was 0.5 pmol/l, and CV were 12% (inter-assay) and 10% (intra-assay). Rabbit anti-BNP antibody without cross-reactivity with urodilatin and α -ANP was used. Minimal detection level was 0.5 pmol/l plasma. The CV were 11% (inter-assay) and 6% (intra-assay). The antibody against Ang II was obtained from the Department of Clinical Physiology, Glostrup Hospital, Denmark. Minimal detection level was 2 pmol/l. The CV were 12% (inter-assay) and 8% (intra-assay).

Aldo in the plasma was determined by RIA using a kit obtained from the Diagnostic Systems Laboratories Inc., Webster, Texas, USA. Minimal detection level was 22 pmol/l. The CV were 8.2% (inter-assay) and 3.9% (intra-assay).

PRC was determined by RIA using a kit obtained from CIS Bio International, Gif-Sur-Yvette Cedex, France. Minimal detection level was 1 pg/ml. The CV were 14.5% (inter-assay) and 4.5% (intra-assay).

Plasma and urinary osmolality were measured by freezing point depression (Advanced Model 3900 multi-sampling osmometer).

Blood pressure was measured using a UA-743 digital blood pressure meter (A&D Company, Tokyo, Japan).

Plasma and urinary concentrations of sodium were measured by routine methods at the Department of Clinical Biochemistry, Holstebro Hospital, Denmark.

Fractional excretion of substance X was calculated as the clearance of X divided by glomerular filtration rate (GFR). Free water clearance (C_{H_2O}) was calculated using the formula: $C_{H_2O} = V \times C_{Osm}$, where V is the urine flow and C_{Osm} is the osmolar clearance. All clearances were standardized to a body surface area of 1.73 m 2 .

Statistical analysis

Statistical level of significance was $P<0.05$ in all the analyses. We used a general linear model with repeated measures for comparison between methylprednisolone and placebo treatments when several measurements were done during the examination. A paired t -test was used for comparison between two groups. The Bonferroni correction was used when appropriate. Values are given as mean ± s.d.

Results

Demographics

Eighteen subjects were allocated to the study. Three subjects withdrew their consents. Fifteen participants were included in the study, nine women and six men, with a mean age of 29 ± 8 years. Blood pressure was $124/72 \pm 14/9$ mmHg. Blood samples showed b-hemoglobin 8.5 ± 0.8 mmol/l, p-sodium 139 ± 2 mmol/l, p-potassium 3.9 ± 0.3 mmol/l, p-albumin 44 ± 4 g/l, p-creatinine 70 ± 9 μ mol/l, p-bilirubin 9 ± 6 μ mol/l, p-transaminase 18 ± 6 U/l, p-glucose 4.9 ± 0.9 mmol/l, and p-cholesterol 4.6 ± 0.9 mmol/l.

Baseline levels of urinary output and urinary sodium excretion during 24 h

Table 1 shows that urinary output, u-Na excretion, u-AQP2, u-ENaC β , and u-PGE $_2$ were the same during the last 24 h before intervention on the study days with either methylprednisolone or placebo.

Effect of methylprednisolone infusion on urinary water excretion

Table 2 shows the effect variables before, during, and after methylprednisolone infusion. We found no significant changes in C $_{H_2O}$ and urine volume between methylprednisolone and placebo treatments.

U-AQP2 increased significantly by 32% ($P < 0.001$) after methylprednisolone infusion. U-AQP2 remained significantly ($P < 0.001$) elevated compared with placebo during the rest of the investigation. Treatment with methylprednisolone did not change p-Osm. P-AVP increased significantly after methylprednisolone infusion, i.e. 28% ($P < 0.05$) at 30 min, and remained significantly increased compared with placebo during the following periods ($P < 0.02$). We found no significant changes in either u-cAMP or u-PGE $_2$ after methylprednisolone treatment.

Table 1 Urine volume, urinary excretion of prostaglandin E $_2$ (u-PGE $_2$), aquaporin-2 (u-AQP2), epithelial sodium channels (u-ENaC), and total urinary sodium (u-Na) excretion during 24 h of fasting in a randomized, placebo-controlled, cross-over study of 15 healthy subjects. Results are shown as mean (s.d).

	Methylprednisolone group	Placebo group	Level of significance
Urine volume (ml/24 h)	2688 (683)	2693 (890)	NS
U-PGE $_2$ (ng/24 h)	620 (301)	535 (262)	NS
U-AQP2 (ng/24 h)	1089 (251)	1009 (217)	NS
U-ENaC (ng/24 h)	139 (31)	123 (24)	NS
U-Na (mmol/24 h)	114 (34)	105 (34)	NS

A paired *t*-test was used for comparison between groups.

Effect of methylprednisolone on urinary sodium excretion

Table 3 shows the effect variables before, during, and after methylprednisolone infusion. We found no significant changes in GFR, urinary sodium excretion, u-Na, and FE $_{Na}$ between methylprednisolone and placebo treatments. At 60 and 90 min after infusion, FE $_{Na}$ increased slightly during both methylprednisolone and placebo treatments, but no significant differences existed between the groups. U-ENaC β increased significantly by 95% ($P < 0.001$) after methylprednisolone infusion, and remained significantly ($P < 0.001$) elevated compared with placebo during the rest of the investigation.

Effect of methylprednisolone on vasoactive hormones

The effect of methylprednisolone on vasoactive hormones is shown in Table 4. Methylprednisolone treatment resulted in a 25% increase in p-ANP after 120 min ($P < 0.01$). Sixty minutes after infusion, p-ANP was increased by 19% ($P < 0.08$). Methylprednisolone treatment increased p-Aldo by 17% ($P < 0.05$) at 60 min after the infusion. However, 120 min post infusion, this difference had disappeared. P-Renin, p-Ang II, and p-BNP were unchanged by methylprednisolone infusion.

Blood pressure and pulse rates

At baseline, blood pressure was $111/63 \pm 11/5$ mmHg, and pulse rate was 60 ± 11 beats/min during methylprednisolone treatment. The corresponding values during placebo treatment did not deviate significantly, blood pressure was $109/62 \pm 9/7$ mmHg and pulse rate was 59 ± 10 beats/min.

Systolic blood pressure did not change significantly after methylprednisolone infusion or placebo infusion. Diastolic blood pressures were significantly reduced after methylprednisolone infusion 90 min after baseline and the rest of the study. The reduction was 2.6 mmHg ($P < 0.02$) at 90 min and 3.8 mmHg ($P < 0.02$) at 120 min. At 30 and 60 min, there were clearer tendencies to reduced diastolic blood pressure. The diastolic blood pressure reduction was 1.9 mmHg ($P < 0.08$) at 30 min and 2.0 mmHg ($P < 0.07$) at 60 min. Diastolic blood pressure did not change statistically significantly during the placebo treatment.

Pulse rate increased significantly during methylprednisolone treatment compared with the placebo treatment (ANOVA; $P < 0.02$). The increase was 3 beats/min ($P < 0.02$) at 30 min, 6 beats/min ($P < 0.001$) at 60 min, 7 beats/min ($P < 0.001$) at 90 min, and 10 beats/min ($P < 0.001$) 120 min after baseline (Fig. 1).

Table 2 Effect of i.v. methylprednisolone infusion during 30 min (500 mg) on urinary excretion of aquaporin-2 (u-AQP2), plasma arginine vasopressin (p-AVP), urine osmolality (u-Osm), urinary excretion of prostaglandin E₂ (u-PGE₂), free water clearance (C_{H₂O}), urinary excretion of cAMP (u-cAMP) in a randomized, placebo-controlled, cross-over trial in healthy subjects (*n* = 15). U-AQP2, p-AVP, p-Osm, u-PGE₂, C_{H₂O}, and urine volume were measured at baseline (−60 to 0 min), during infusion (0–30 min), and post infusion (30–60, 60–90, and 90–120 min). Values are means and s.d.

	Baseline	0–30 min	30–60 min	60–90 min	90–120 min	<i>P</i>
U-AQP2 (ng/min)						
Methylprednisolone	0.84 (0.22)	1.11 (0.29)	1.06 (0.23)	1.05 (0.28)	1.13 (0.25)	<0.001
Placebo	0.84 (0.21)	0.87 (0.19)	0.86 (0.19)	0.88 (0.20)	0.90 (0.22)	
<i>P</i> value		<0.001*	<0.001*	<0.02*	<0.001*	
P-AVP (pg/ml)						
Methylprednisolone	0.92 (0.21)	1.18 (0.45)	1.27 (0.44)	1.09 (0.25)	1.03 (0.19)	<0.02
Placebo	0.88 (0.17)	0.89 (0.19)	0.93 (0.21)	0.92 (0.26)	0.99 (0.26)	
<i>P</i> value		<0.02*	<0.02*	<0.02*	NS*	
P-Osm (mOsm/kg H ₂ O)						
Methylprednisolone	282 (3.4)	281 (5.1)	282 (3.4)	281 (4.5)	281 (4.2)	NS
Placebo	283 (4.0)	284 (3.2)	283 (3.0)	283 (3.1)	282 (3.7)	
U-PGE ₂ (pg/min)						
Methylprednisolone	440 (247)	422 (249)	466 (287)	447 (331)	463 (371)	NS
Placebo	402 (209)	388 (171)	362 (185)	368 (188)	386 (203)	
C _{H₂O} (ml/min)						
Methylprednisolone	3.5 (1.4)	2.4 (1.4)	3.6 (1.1)	3.7 (1.3)	3.2 (1.9)	NS
Placebo	4.5 (2.0)	3.2 (1.6)	3.3 (1.4)	2.4 (1.3)	2.8 (1.2)	
Urine (ml/min)						
Methylprednisolone	6.15 (1.68)	5.11 (1.59)	6.27 (1.23)	6.52 (1.65)	6.38 (2.23)	NS
Placebo	7.27 (2.21)	6.09 (1.67)	6.23 (1.47)	5.33 (1.45)	5.89 (1.67)	
U-cAMP (pmol/min)						
Methylprednisolone	3850 (754)	3515 (591)	3592 (575)	3735 (796)	4022 (832)	NS
Placebo	3811 (601)	4024 (879)	3853 (694)	3888 (674)	3649 (605)	

P value indicates significant difference between methylprednisolone and placebo treatments using a general linear model with repeated measures. A paired *t*-test was used for comparison of means when differences were found between the two treatments *.

Discussion

The present results show that methylprednisolone given intravenously in an acute study did not change free water clearance and FE_{Na}. However, both u-AQP2 and u-ENaC_β increased significantly just as p-AVP, p-ANP, and pulse rate.

The amount of ENaC_β in urine is supposed to reflect the activity of sodium transport via the ENaCs, just as u-AQP2 reflects the functional status of the AQP2 water channels (6). Our analyses showed that the assay has a satisfactory reliability. In addition, we demonstrated a significantly negative correlation between changes in urinary sodium excretion and changes in u-ENaC_β during amiloride treatment. This means that the decrease in u-ENaC_β was related to the increase in urinary excretion of sodium during amiloride treatment. Thus, our results are in accordance with u-ENaC_β being a biomarker of the transport of sodium via ENaCs during acute studies, presumably reflecting up- and down-regulation of β-ENaC expression and sodium transport via ENaCs. However, further studies are necessary to elucidate more precisely to what degree u-ENaC_β reflects the activity of ENaCs. Furthermore, it is not clarified which of the protein components, α-, β-, or γ-fraction, in the ENaC will be the best biomarker for the channel.

We found that methylprednisolone clearly increased p-AVP by a non-osmotic mechanism, since methylprednisolone did not change p-Osm. This is in disagreement with other studies in animals and healthy humans, in which p-AVP was pronouncedly reduced (1, 11). However, we have studied the effect of a large single i.v. dose of methylprednisolone in an acute study, whereas glucocorticoid was given orally for a longer period, i.e. several days in healthy humans, and in a smaller dose (1, 11). These differences in design and doses may explain the discrepancy. The central mechanism for the antidiuretic action of AVP is the exocytotic insertion of AQP2 from intracellular vesicles into the apical membrane of the principal cells, an event initiated by an increase in cAMP and activation of protein kinase A (12). In our study, we found no increase in u-cAMP despite increased levels of p-AVP, indicating that the AVP–cAMP pathway for up-regulation of AQP2 expression was not activated during methylprednisolone treatment. In addition, we measured no changes in u-PGE₂. Thus, it is unlikely that our results could be explained by possible antagonistic effect of prostaglandins on the vasopressinergic effect on the distal part of the nephron. The principal cells in the distal part of the nephron seem to be refractory to AVP during methylprednisolone treatment, and the increase in u-AQP2 must be attributed to a non-AVP-mediated mechanism.

Table 3 Effect of i.v. methylprednisolone infusion during 30 min (500 mg) on urinary excretion of the β -fraction of the epithelial sodium channel (u-ENaC $_{\beta}$), fractional excretion of sodium (FE $_{Na}$), and glomerular filtration rate (GFR) in a randomized, placebo-controlled, cross-over trial in healthy subjects ($n=15$). U-AQP2, FE $_{Na}$, and GFR were measured at baseline (–60 to 0 min), during infusion (0–30 min), and post infusion (30–60 min, 60–90 min, and 90–120 min). Values are means and s.d.

	Baseline	0–30 min	30–60 min	60–90 min	90–120 min	P
U-ENaC $_{\beta}$ (pg/min)						
Methylprednisolone	86.6 (20)	160.5 (57)	185.6 (29)	143.7 (26)	155.8 (27)	<0.001
Placebo	84.6 (22)	86.8 (17)	80.3 (16)	78.8 (19)	87.7 (13)	
P value	NS	NS	0.055	0.0001	0.004	
FE $_{Na}$						
Methylprednisolone	1.04 (0.43)	1.08 (0.39)	1.17 (0.43)	1.18 (0.35)	1.10 (0.36)	NS
Placebo	1.16 (0.48)	1.20 (0.36)	1.36 (0.38)	1.36 (0.40)	1.26 (0.48)	
GFR (ml/min)						
Methylprednisolone	108 (19)	108 (13)	101 (13)	103 (15)	115 (16)	NS
Placebo	107 (13)	109 (15)	101 (11)	105 (14)	111 (16)	

P value indicates significant difference between methylprednisolone and placebo treatments using a general linear model with repeated measures. A paired *t*-test was used for comparison of means when differences were found between the two treatments.

In addition to AVP, the activity in the renin–angiotensin–Aldo system and the natriuretic peptide system can influence the expression of AQP2 (13–19). We measured an increase in p-ANP after methylprednisolone treatment. This is in agreement with previous studies, in which glucocorticoids up-regulated ANP in myocytes from rats (20) and modulated the ANP response to different stimuli (11, 21). In rats, ANP infusion had no immediate effect on the intracellular localization of AQP2, but apical targeting of AQP2 increased after 90 min of infusion (22). We found increased levels of u-AQP2 120 min before any changes in ANP, and due to this time dissociation, we consider it unlikely that increased ANP could be responsible for the up-regulation of AQP2 and subsequent increase in u-AQP2 during methylprednisolone treatment.

According to the results from experiments with denervated rat kidneys, a reduction in sympathetic nervous stimulation reduced AQP2 expression (23), and the authors suggest that renal sympathetic nerve activity may play an excitatory role in AQP2 regulation. Methylprednisolone treatment induced changes in systemic hemodynamics, i.e. a decrease in diastolic blood pressure and an increase in pulse rate. The increase in pulse rate most likely reflects enhanced sympathetic nerve activity. Thus, increased renal sympathetic nerve activity during methylprednisolone treatment could play a role in the up-regulation of AQP2 water channels in our study. We did not measure an increase in plasma renin concentration, but this does not exclude that increased sympathetic adrenergic activity influences AQP2 expression as shown in animal

Table 4 Effect of i.v. methylprednisolone infusion during 30 min (500 mg) on plasma atrial natriuretic peptide (p-ANP), plasma brain natriuretic peptide (p-BNP), plasma renin, plasma angiotensin II (p-Ang II), and p-aldosterone (p-Aldo) in a randomized, placebo-controlled, cross-over trial in healthy subjects ($n=15$). The hormones were measured at baseline and 30 and 90 min after methylprednisolone infusion. Values are means and s.d.

	Baseline	30 min post	90 min post	P value
P-ANP (pmol/l)				
Methylprednisolone	6.3 (3.5)	7.5 (4.6)	7.9 (4.7)	<0.01
Placebo	6.3 (3.0)	5.9 (2.8)	5.1 (2.1)	
P value			0.004*	
P-BNP (pmol/l)				
Methylprednisolone	1.7 (2.3)	1.8 (2.3)	1.8 (2.5)	NS
Placebo	1.4 (1.3)	1.4 (1.2)	1.3 (1.2)	
P-Renin (mU/l)				
Methylprednisolone	7.6 (4.3)	15.6 (25.9)	13.0 (25.3)	NS
Placebo	7.5 (3.2)	8.8 (7.5)	10.6 (10.1)	
P-Ang II (pmol/l)				
Methylprednisolone	12.3 (6.3)	13.0 (8.5)	12.3 (8.0)	NS
Placebo	11.5 (6.3)	13.0 (8.8)	11.9 (6.5)	
P-Aldo (pmol/l)				
Methylprednisolone	284 (147)	334 (107)	269 (96)	NS
Placebo	283 (228)	249 (180)	258 (131)	
		0.05*		

P value indicates significant difference between methylprednisolone and placebo treatments using a general linear model with repeated measures. A paired *t*-test was used for comparison of means when differences were found between the two treatments *.

Figure 1 Effect of i.v. methylprednisolone infusion during 30 min (500 mg) on the pulse rate in a randomized, placebo-controlled, cross-over trial in healthy subjects ($n=15$). U-AQP2 was measured at baseline, immediately after infusion (0 min), and post infusion (30, 60, and 90 min). P value indicates significant difference between methylprednisolone and placebo treatments using a general linear model with repeated measures.

studies. The effect of increased renal sympathetic nerve activity has not been studied with regard to ENaC expression.

Although the activity of ENaCs is mainly regulated by mineralocorticoids, AVP has also some effect on sodium reabsorption via ENaCs via a cAMP/protein kinase A-mediated mechanism (24). However, the increase in p-AVP with the lack of increase in cAMP indicates that the AVP–cAMP pathway cannot be responsible for the increased u-ENaC in our study during methylprednisolone treatment.

We did not measure any decrease in either C_{H_2O} or FE_{Na} . This might be due to the fact that methylprednisolone has only a weak mineralocorticoid effect and a strong glucocorticoid effect. Thus, methylprednisolone has more than fourfold larger glucocorticoid effect than cortisol, whereas its mineralocorticoid effect is only 2% of the effect of Aldo when measured in cell cultures (25). However, our results clearly indicated that u-AQP2 and u-ENaC $_{\beta}$ were increased during treatment with methylprednisolone. Thus, an increased tubular absorption of water and sodium must have taken place in the early phase of the treatment via the principal cells in the distal part of the nephron. Consequently, other mechanisms must have antagonized this water- and sodium-retaining effect. We measured an increase in p-ANP. Most probably, the increase in p-ANP is responsible for a compensatory increase in both urinary water and sodium excretions, and the increased anti-natriuretic effect of ANP could have masked and neutralized the expected decrease in urinary water output and sodium excretion.

In our study, the subjects fasted for 1 day. This might have increased endogenous production of glucocorticoids. However, the glucocorticoid effect of methylprednisolone is much higher than the effect of the endogenously produced cortisol. Thus, our results primarily reflect the effect of methylprednisolone.

It is the strength of our study that test conditions were very well defined regarding energy intake, diet, and fluid intake. The study was done during fasting conditions during which prostaglandin synthesis was stimulated (13). Our measurements of u-PGE $_2$ showed that we avoided a confounding inhibition of prostaglandin synthesis by methylprednisolone. It cannot be excluded that stress during the examination increased the endogenous steroid level, and thereby had an effect on values in the control period. This might have reduced the differences between the control and methylprednisolone periods.

We did not measure any differences in the effect variables between the group of women and the group of men in the study population. Thus, although we have studied both women and men, our results do not seem to be influenced by gender.

In conclusion, methylprednisolone clearly increased u-AQP2 and u-ENaC. Neither the AVP–cAMP axis nor changes in the activity in the renin–angiotensin–Aldo system, or the natriuretic peptide system seem to bear a causal relationship with the increase in either u-AQP2 or u-ENaC. Most probably, the effect is mediated via a direct effect of methylprednisolone on the principal cells in the distal part of the nephron. The lack of increase in urinary output and sodium excretion most likely can be attributed to the diuretic and natriuretic properties of the increased secretion of ANP shortly after the infusion of methylprednisolone.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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