

Nocturnal polyuria in monosymptomatic nocturnal enuresis refractory to desmopressin treatment

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Kamperis, K., S. Rittig, K. A. Jørgensen, and J. C. Djurhuus. Nocturnal polyuria in monosymptomatic nocturnal enuresis refractory to desmopressin treatment. *Am J Physiol Renal Physiol* 291: F1232–F1240, 2006. First published June 27, 2006; doi:10.1152/ajprenal.00134.2006.—The transition from day to night is associated with a pronounced decline in diuresis with reductions in the amount of excreted water, electrolytes, and other end products of our metabolism. Failure to do so leads to a large urine output at night, a condition known as nocturnal polyuria, encountered in a large proportion of children with nocturnal enuresis. The aim of this study was to clarify the mechanisms responsible for the nocturnal polyuria seen in enuretics with inadequate response to desmopressin (dDAVP). Forty-six enuretics (7–14 yr of age) and fifteen age-matched controls were admitted for a 24-h protocol with standardized fluid and sodium intake, comprising urine collections, blood sampling, and blood pressure monitoring. We included patients with severe enuresis (5 ± 1 wet nights/wk) showing <50% reduction in wet nights on dDAVP. We characterized the patients on the basis of their nocturnal urine production. The children with nocturnal polyuria excreted larger amounts of sodium and urea at night than nonpolyurics and controls. Solute-free water reabsorption as well as urinary arginine vasopressin and aquaporin-2 excretion were normal in polyurics, and no differences were found in atrial natriuretic peptide, angiotensin II, aldosterone, and renin levels. Urinary prostaglandin E₂ (PGE₂) excretion was significantly higher in polyurics. The nocturnal polyuria in children with dDAVP-resistant nocturnal enuresis seems to be the result of augmented sodium and urea excretion. The high urinary PGE₂ levels found in these children point toward a role for increased prostaglandin synthesis in the pathogenesis of enuresis-related polyuria.

hypernatremia; urea excretion; natriuresis; osmotic diuresis; prostaglandins

NOCTURNAL ENURESIS is second only to asthma in terms of prevalence, affecting 7–10% of 7-yr-old children and distressing up to 1–2% of adults (9, 38). Although benign in its nature and to a large degree self-limited (13), bed-wetting can have substantial negative emotional consequences, impeding proper psychosocial development.

The mismatch between the capacity of the bladder to accommodate urine during the night and the nocturnal urine production together with the failure of the child to awake when this happens are the prerequisites for an enuretic episode. As early as the 1950s, the importance of the nighttime urine output was noted in the etiology of enuresis (22), but a biochemical basis for these observations would only come much later with the identification of arginine vasopressin (AVP) as being responsible for the enuresis-related nocturnal polyuria (1, 21,

24). These observations offered the rationale for treatment of the condition with the AVP synthetic analog desmopressin (dDAVP). dDAVP treatment proves successful in the majority of enuretics that present with nocturnal polyuria (33). However, a substantial number of enuretics with excess urine production at night do not become dry in response to dDAVP. Recently, other etiological factors for the nocturnal polyuria of enuresis have been hypothesized. Increased sodium and potassium excretion both have been implicated in the pathogenesis of monosymptomatic nocturnal enuresis (MNE) (23, 36), whereas indications of hypercalciuria have been demonstrated in enuretics (34). Of the hormones governing renal water and solute handling, atrial natriuretic peptide does not seem to be involved in the etiology of enuresis (23), but a study of the nocturnal levels of aldosterone and angiotensin II indicates a role for these hormones in MNE-related nocturnal polyuria (25). Furthermore, renal prostaglandins have emerged as a possible key molecule in the etiology of enuresis-related polyuria (18), and it has been demonstrated that children with dDAVP-resistant enuresis nocturna may excrete larger amounts of prostaglandin E₂ (PGE₂), the major renal autacoid, in urine (19). Because prostaglandin synthesis inhibition effectively treats bed-wetting (2, 20, 29), it seems reasonable to suggest a role for prostaglandins in the physiological mechanisms causing MNE.

In the present study, we evaluate renal water and solute handling in children with dDAVP-resistant MNE with and without nocturnal polyuria, comparing them with age-matched healthy children under standardized conditions regarding water and sodium intake.

MATERIALS AND METHODS

Study subjects. The study protocol was approved by the local Ethics Committee, and informed consent was obtained from all participants. The protocol conformed to the recommendations for good clinical practice (CPMP/ICH/135/95).

Forty-six children with enuresis (7–14 yr) having at least 3 wet nights per week and 15 matched controls were included in the study. Enuretics were recruited from the outpatient clinics of the Center for Child Incontinence, Aarhus University Hospital, Skejby Section, a tertiary referral center. Healthy controls were recruited through the personnel of the center and their acquaintances. Inclusion criteria for enuretics were the lack of daytime symptoms such as incontinence and urgency and frequency (defined as >10 voidings per day), a normal bladder capacity [defined as >70% of the expected for age using the following formula: bladder capacity (in ml) = $30 \times \text{age (in yr)} + 30$], a lack of clinical or laboratory signs suggestive of an

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underlying disease other than MNE, a normal uroflowmetry with residual urine assessment, an unremarkable clinical examination, normal urine dip-stick analysis, and inadequate response to dDAVP (<50% reduction in wet nights). For the characterization of enuretics on the basis of their nocturnal urine production, 2-wk frequency volume charts were used (16). If an average nocturnal urine output on wet nights exceeded 130% of the expected bladder capacity for age, the child was defined as having nocturnal polyuria. Of the initial population, 27 children had nocturnal polyuria, and the remaining 19 were nonpolyurics. A 2-wk titration period with incremental doses of either dDAVP spray (20–40 µg) or tablets (0.2–0.4 mg) was used to define response rates.

Study design. Participants were admitted to the Department of Pediatrics for 24 h following an adaptation night used for acclimatization to the hospital environment.

At 0730 (24-h clock) on the day after the adaptation night, venous access was established with a heparinized cannula via a cubital vein for blood sampling. Starting at 0800, blood samples (17 ml) were taken every 4 h during the entire experimental protocol. Participants were ambulatory and upright during the daytime but were asked to remain seated for at least 10 min before blood sampling. A volume of 5 ml of isotonic saline and 0.5 ml of heparin (100 IE) was injected subsequent to blood sampling to prevent catheter clotting. Care was taken not to wake the children during blood sampling at night.

Urine was fractionally collected with 4-h intervals following spontaneous voiding. Participants were asked to empty their bladders just after blood sampling at bedtime and on waking in the morning. All other voidings were at free will.

For the purpose of urine collection at night from enuretics and to avoid waking up the children, a urine collecting bag attached to a Conveen Security⁺ Uridom (boys) or an adhesive stomia (Assura, Coloplast, Denmark) was used. A diaper was used to measure urine losses in case of system failure. This was the case for four children.

Normal activity was allowed from 0800 to 2100; hereafter, participants were asked to retire to their beds. The exact bedtime for each participant was noted.

During the stay in the hospital, both water and sodium intake was standardized to 30 ml/kg and 3 mmol/kg, respectively, and supervised and recorded by a clinical dietitian. Meals were served at 0815, 1200, and 1730. Fluid intake was distributed as follows: two-thirds before 1600 and the remaining one-third until bedtime.

The following determinations were made in plasma: sodium, potassium, creatinine, urea, osmolality, atrial natriuretic peptide (ANP), ANG II, aldosterone (Aldo), and activated renin. Urine was analyzed for the following: sodium, potassium, urea, creatinine, osmolality, AVP, PGE₂, and aquaporin-2 (AQP2). Blood samples were immediately centrifuged at +4°C and stored at –20°C unless immediately analyzed.

Blood pressure was measured every hour for the entire 24-h experimental period by means of an ambulatory blood pressure monitor (Spacelab, model no. 90207).

Biochemistry determinations. Determination of plasma sodium, potassium, creatinine, and urea measurements were carried out at the Department of Clinical Biochemistry on a Kodak Ektachem 700XRC analyzer. In urine, sodium and potassium concentrations were measured by use of flame photometry (Eppendorf FCM6341 and Eppendorf MFM6350). Plasma and urine osmolalities were measured by the freezing-point depression method (osmometer 3900, Advanced Instruments).

ANP was measured in plasma following extraction in Sep-Pak C₁₈ cartridges (Water Associates, Milford, MA) by means of RIA as previously described (31) and using a rabbit ANP-specific antibody showing no cross-reactivity with other natriuretic peptides (Eurodiagnostica, Malmö, Sweden). The limit of detection was 1.5 pg/ml, and the intra- and interassay coefficients of variation were, respectively, 10 and 12%.

ANG II was determined in plasma by RIA as previously described (17) with modifications, using a rabbit anti-ANG II antibody (Ab-5-030682; P. Christensen, Department of Clinical Physiology, Glostrup Hospital, Denmark). ANG II was extracted from plasma using Sep-Pak C₁₈ cartridges, methanol, and water. Intra- and interassay coefficients of variation were 9 and 13%, respectively, and the limit of detection was 1.4 pg/ml.

RIA was used to determine Aldo in EDTA-plasma (Active Aldosterone Coated Tube RIA kit; Diagnostic Systems Laboratories, Webster, TX). The assay was performed with an intra-assay coefficient of variation of 7%, an interassay coefficient of variation of 13%, and a minimum detection limit of 25 pg/ml.

Activated renin was directly measured in EDTA-plasma by a commercially available kit (Nichols Institute Diagnostics) utilizing a monoclonal antibody against active renin showing 0.2% cross-reactivity with prorenin. Intra- and interassay coefficients of variation were 2 and 8%, respectively, and detection limit was 1.4 µU/ml.

Urine PGE₂ was measured by way of an enzyme immunoassay for metabolites (Cayman Chemical) that allowed us to reliably estimate the actual PGE₂ levels in urine at the time of collection. To avoid bias due to the rapid PGE₂ conversion to unstable metabolites, the assay is based on the conversion of PGE₂ and its metabolic products to a single, stable, and measurable derivative (bicyclo-PGE₂). Urine was diluted with a factor of 25 before analysis. Interassay and intra-assay coefficients of variation were, respectively, ~15 and 10%. The detection limit was 2 pg/ml.

AVP was directly measured in urine using a highly specific antibody generously supplied by Dr. P. Bie (AB3096; Dept. of Physiology and Pharmacology, University of Southern Denmark) and incorporated in an RIA (27). For the analysis, urine was diluted according to its osmolality and probed. The interassay and intra-assay coefficients of variation were, respectively, 13.6 and 4.9%.

Urine AQP2 measurements were carried out using the dot blot technique as previously described (16a). The antibody used was a rabbit anti-human AQP2 (AN-368AP), identifying the 15 COOH-terminal amino acids. The detection limit was 0.0035 U/ml.

Other determinations. For the purpose of residual urine measurements, a Bladderscan BVI 2500+ (Diagnostic Ultrasound) was used.

Calculations and statistical analysis. On the basis of the measurements, excretions (E) and clearances (C) were calculated for electrolytes, creatinine, urea, and osmoles using standard formulas. Solute-free water reabsorption (T^H₂O) was calculated using the formula T^H₂O = urine flow (U_{flow}) – osmolar clearance (C_{osm}).

Fractional excretions (FE) were defined according to the formula FE_i (%) = [C_i/glomerular filtration rate (GFR)] × 100. The clearance of creatinine was used for GFR approximations. To calculate the filtered sodium load (F_{Na}), the formula F_{Na} = plasma sodium (P_{Na}) × GFR was used.

Results were tested for normal distribution and are presented as means ± SE. To evaluate variations over time, data were subjected to analysis by repeated-measurement ANOVA. Gender and age were used as covariates to assess their influence on the parameters tested. One-way ANOVA and paired Student's *t*-test were used for selected comparisons between groups. For overall analysis of blood pressure and heart rate measurements, areas under the curve were calculated using the trapezoid method and subjected to analysis. The Pearson test was used for correlation analysis. SPSS 10.0 (SPSS) was used for all statistical inference. Statistical significance was defined by a *P* value <0.05.

RESULTS

Of the initial population of enuretics, nine children (5 polyurics and 4 nonpolyurics) did not experience an enuresis

Table 1. Demographic data and clinical characterization of the participants

	Controls	Nonpolyuric	Polyuric	Dry Nights	ANOVA
Age, yr	11.1±0.6	10.4±0.5	9.6±0.3	10.8±0.7	<i>P</i> = 0.16
Gender	10 M, 5 F	9 M, 6 F	15 M, 7 F	7 M, 2 F	<i>P</i> = 0.85
Body wt, kg	42±3	43±2	38±2	44±5	<i>P</i> = 0.45
Height, cm	153±5	150±3	142±2	148±5	<i>P</i> = 0.15
MVV, ml	NA	292±93	292±68	371±84	<i>P</i> = 0.06
FBC/FBC _E	NA	0.88±0.32	0.92±0.22	1.06±0.34	<i>P</i> = 0.35
NUv home, ml	NA	301±65	419±82	429±141	<i>P</i> < 0.01
NUv inpatient, ml	250±105	294±90	456±120	242±82	<i>P</i> < 0.001
<i>n</i>	15	15	22	9	

Values are means ± SE. Maximal voided volume (MVV), functional bladder capacity (FBC) and its ratio to the expected bladder capacity for age (FBC/FBC_E), and average nocturnal urine output on wet nights at home (NUv home) and during the circadian study (NUv inpatient). Enuretics with nocturnal polyuria excreted comparable amounts of urine during the study as in the home setting. On the contrary, children experiencing dry nights had a considerably lower urine production during the study. M, male; F, female; NA, not applicable.

episode during the circadian study. These children having dry nights were not excluded from further analysis but constituted a separate group used for comparisons. Table 1 presents demographic and home recording data of the participants. There were no differences between the groups of participants in terms of age, weight, and height or gender composition.

Circadian urine production and steady state. Polyurics shared higher total 24-h urine output compared with the rest of the groups (controls 31 ± 3 ml/kg, nonpolyurics 29 ± 3 ml/kg, polyurics 40 ± 3 ml/kg, and dry nights 28 ± 4 ml/kg; *P* < 0.05). We found no difference between groups for the overall 24-h excretion of sodium, potassium, creatinine, or urea (Table 2). Age and gender did not have a significant influence on the variations of the parameters tested over time.

Circadian variations in urine output were evident for all groups, with urine production having its peak during the period between 1600 and 2000 and the lowest urine output being at night (Fig. 1). There was no significant interaction between

group type and the variations of urine production during daytime. However, polyurics excreted approximately twice as much urine during the night (1.22 ± 0.09 compared with 0.63 ± 0.06 ml·kg⁻¹·h⁻¹ for controls and 0.68 ± 0.04 ml·kg⁻¹·h⁻¹ for nonpolyurics; *P* < 0.001, Fig. 1).

All groups showed a significant circadian rhythm in urine osmolality (Uosm), with the lowest values between 1600 and 2000 (Fig. 1). No differences were seen between the groups in Uosm during daytime, but polyurics excreted significantly less concentrated urine during sleep compared with the rest of the participants. Despite this, first morning Uosm values did not significantly differ among groups, indicating that polyurics were capable of maximal urine concentration at least in the last hours before arousal (Fig. 2). Enuresis episode Uosm was significantly lower than first morning Uosm in the polyurics (enuresis Uosm 519 ± 46 mosmol/kgH₂O and morning Uosm 699 ± 39 mosmol/kgH₂O; *P* < 0.01). A similar discrepancy was not evident for nonpolyurics (enuresis Uosm 772 ± 57

Table 2. Day, night, and 24-h values for the different parameters in controls and nonpolyuric and polyuric MNE children

Period	Controls			Nonpolyuric			Polyuric		
	Day	Night	24-h	Day	Night	24-h	Day	Night	24-h
Uflow, ml·kg ⁻¹ ·h ⁻¹	1.78±0.25	0.63±0.06 ³	1.32±0.15	1.65±0.22	0.68±0.04 ³	1.23±0.13	2.00±0.15	1.22±0.09 ^{3,6}	1.67±0.10 ⁴
Uosm, mosmol/kgH ₂ O	572±71	801±63 ³	606±67	516±53	780±50 ¹	587±54	493±46	560±40 ⁶	510±44
GFR, ml/min	126±9	98±7	117±8	108±7	100±9	103±8	99±4 ⁵	93±5	96±4
E _{Na} , mmol·kg ⁻¹ ·h ⁻¹	0.13±0.02	0.06±0.008 ²	0.10±0.01	0.11±0.01	0.06±0.01 ²	0.09±0.01	0.14±0.01	0.10±0.01 ^{2,5}	0.12±0.01
C _{Na} , ml/min	0.64±0.08	0.34±0.05 ²	0.53±0.06	0.59±0.08	0.32±0.04 ²	0.46±0.05	0.65±0.06	0.45±0.04 ^{2,4}	0.57±0.04
FE _{Na} , %	0.53±0.08	0.34±0.04 ¹	0.47±0.06	0.54±0.05	0.32±0.04 ²	0.44±0.03	0.65±0.05	0.50±0.04 ^{1,6}	0.59±0.04
E _K , mmol·kg ⁻¹ ·h ⁻¹	0.09±0.01	0.03±0.01 ³	0.07±0.01	0.08±0.01	0.03±0.01 ³	0.06±0.01	0.10±0.01	0.04±0.01 ³	0.07±0.01
C _K , ml/min	15.8±1.2	4.58±0.49 ³	11.4±0.8	14.4±1.2	5.80±0.59 ³	10.7±0.7	15.5±1.1	5.96±0.54 ³	11.3±0.9
FE _K , %	12.9±0.8	4.81±0.46 ³	9.9±0.4	13.5±0.9	6.23±0.87 ³	10.7±0.7	15.9±1.1	6.75±0.61 ³	12.0±0.9
E _{Ur} , mmol·kg ⁻¹ ·h ⁻¹	0.37±0.04	0.23±0.01 ³	0.31±0.03	0.30±0.03	0.23±0.02 ¹	0.26±0.02	0.36±0.02	0.31±0.02 ⁵	0.32±0.02
C _{Ur} , ml/min	59.9±5.4	42.1±3.3 ²	53.6±4.4	51.3±3.3	42.7±3.4 ¹	47.5±3.5	50.5±1.9	44.0±2.4 ¹	46.9±1.8
FE _{Ur} , %	47.5±1.6	43.03±1.65 ²	46.0±1.6	47.7±1.2	43.03±1.61 ²	46.2±1.3	51.6±1.4	48.03±1.71 ¹	49.4±1.3
E _{osm} , mosmol·kgH ₂ O ⁻¹ ·h ⁻¹	0.83±0.07	0.47±0.03 ³	0.69±0.05	0.74±0.06	0.48±0.03 ²	0.61±0.05	0.87±0.04	0.66±0.04 ^{3,6}	0.76±0.04
C _{osm} , ml/min	1.96±0.13	1.14±0.09 ³	1.65±0.09	1.84±0.15	1.23±0.09 ²	1.55±0.11	1.95±0.10	1.47±0.08 ^{3,4}	1.72±0.08
T ⁰ H ₂ O, ml/min	0.81±0.17	0.71±0.08	0.79±0.12	0.70±0.12	0.76±0.06	0.73±0.09	0.69±0.13	0.72±0.08	0.68±0.11
E _{AVP} , ng·kg ⁻¹ ·h ⁻¹	0.12±0.01	0.09±0.01	0.11±0.08	0.10±0.08	0.09±0.01	0.10±0.05	0.17±0.04	0.10±0.01	0.15±0.03
EPGE ₂ , ng·kg ⁻¹ ·h ⁻¹	0.22±0.02	0.14±0.02 ²	0.19±0.02	0.21±0.02	0.13±0.03 ¹	0.17±0.03	0.21±0.02	0.20±0.02 ⁴	0.21±0.02

Values are means ± SE. MNE, monosymptomatic nocturnal enuresis; Uflow, uroflowmetry; Uosm, urine osmolality; GFR, glomerular filtration rate; E_{Na}, sodium (Na) excretion; C_{Na}, Na clearance; FE_{Na}, fractional Na excretion; E_K, potassium (K) excretion; C_K, K clearance; FE_K, fractional K excretion; E_{Ur}, urea excretion; C_{Ur}, urea clearance; FE_{Ur}, fractional urea excretion; E_{osm}, osmolar excretion; C_{osm}, osmolar clearance; T⁰H₂O, solute-free water reabsorption; E_{AVP}, arginine vasopressin excretion; EPGE₂, prostaglandin E₂ excretion. Day vs. night: ¹*P* < 0.05, ²*P* < 0.01, and ³*P* < 0.001. Between groups: ⁴*P* < 0.05, ⁵*P* < 0.01, and ⁶*P* < 0.001.

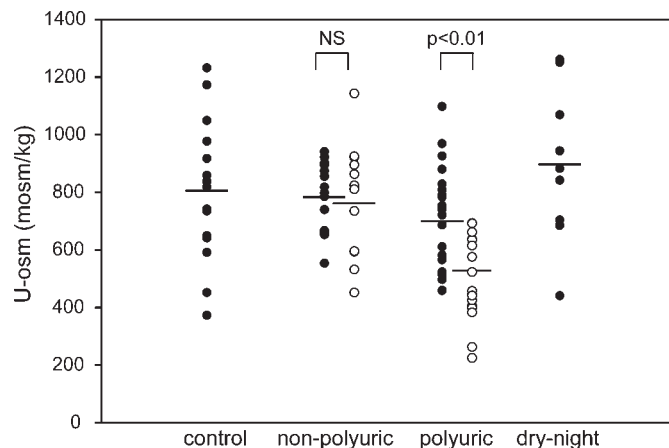
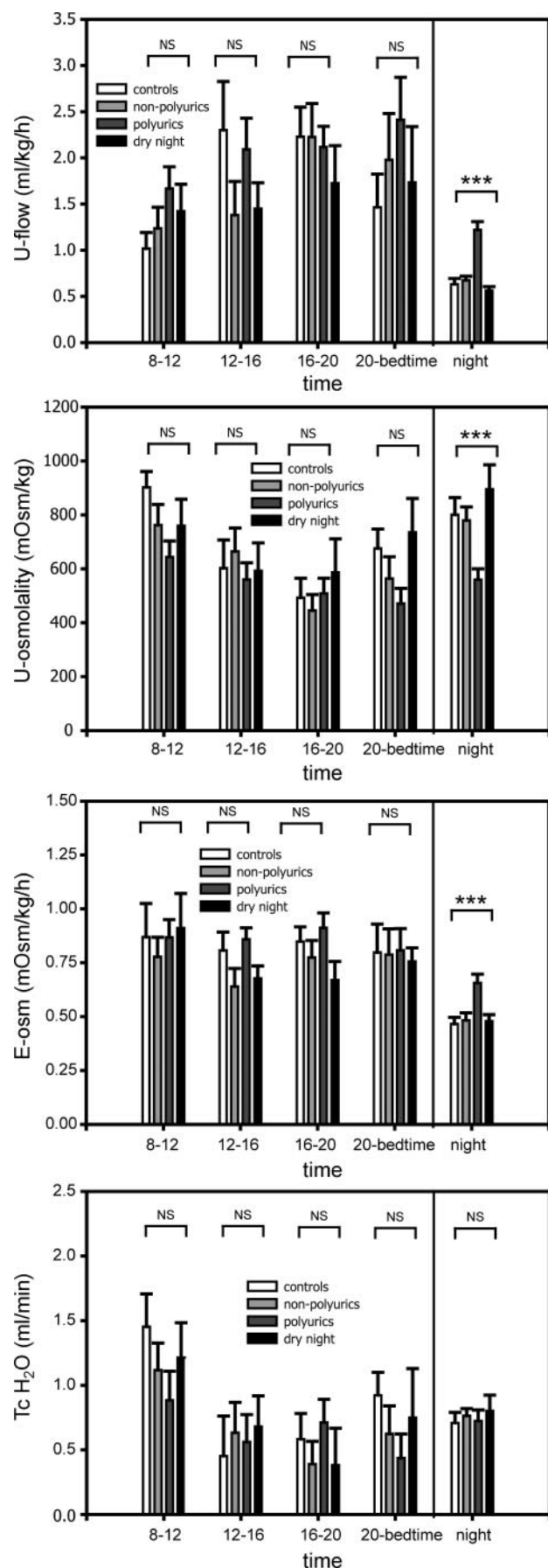


Fig. 2. Scatter plot of Uosm from enuresis episodes (○) and first morning voidings (●). Patients with nocturnal polyuria excreted more dilute urine before the occurrence of an enuresis episode. A similar trend was not seen in nonpolyurics. First morning urine was not significantly different between groups.

mosmol/kgH₂O and morning Uosm 788 ± 31 mosmol/kgH₂O; not significant).

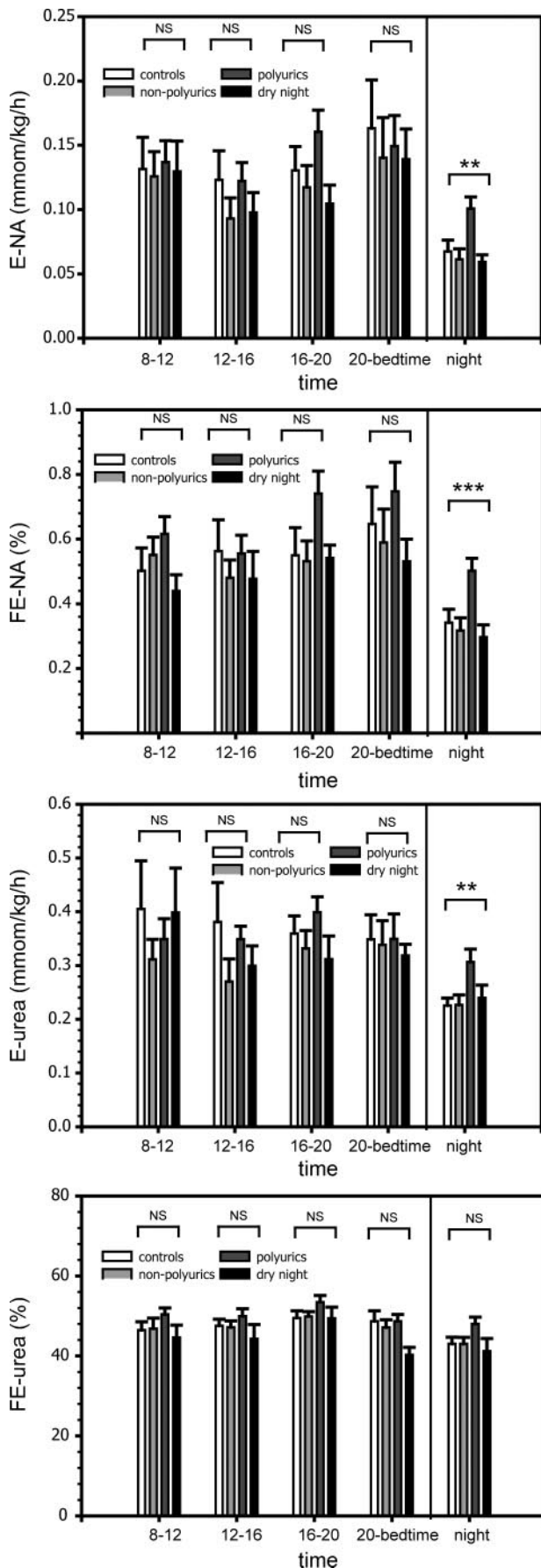
GFR as estimated by the clearance of creatinine showed little variation throughout the day with a marginal, nonsignificant decline at night. GFR levels were comparable between the groups at all clearance periods, although polyurics shared a tendency toward lower values (Table 2).

Urine content of solutes. A nycthemeral variation was found in sodium excretion (E_{Na}) in all groups (Fig. 3) with a marked decrease in E_{Na} during the night. Polyurics had a significantly higher E_{Na} during nighttime, whereas controls, nonpolyurics, and children experiencing dry nights had comparable nocturnal E_{Na} values both during daytime and at night. Similar results were obtained for sodium clearance (C_{Na}), with polyurics showing higher values at night compared with controls and nonpolyurics (0.45 ± 0.03 compared with 0.33 ± 0.05 ml/min for controls, 0.31 ± 0.04 ml/min for nonpolyurics, and 0.32 ± 0.05 ml/min for dry nights; $P < 0.05$), as well as for sodium fractional excretion (FE_{Na} ; $0.50 \pm 0.03\%$ for polyurics compared with $0.34 \pm 0.04\%$ for controls, $0.31 \pm 0.03\%$ for nonpolyurics, and $0.29 \pm 0.03\%$ for dry nights; $P < 0.001$, Fig. 3). There was a significant correlation between E_{Na} and Uflow (see Fig. 6).

We also found a diurnal variation in excretion, clearance, and fractional excretion of potassium (E_K , C_K , and FE_K , respectively) in all groups, with a consistent reduction at night. We found, however, no significant differences among the four groups regarding the circadian variations in E_K , C_K , or FE_K (Table 2).

The excretion of urea (E_{UR}) decreased significantly at night in all groups (Fig. 3), but this decline was somewhat blunted for polyurics, who exhibited significantly higher E_{UR} values at night compared with the other groups (0.30 ± 0.02 mmol·kg⁻¹·h⁻¹ for polyurics, 0.22 ± 0.01 mmol·kg⁻¹·h⁻¹ for controls, 0.22 ± 0.01 mmol·kg⁻¹·h⁻¹ for nonpolyurics,

Fig. 1. Circadian variation in diuresis (urine flow; Uflow), urine osmolality (Uosm), osmolar excretion (E_{osm}), and solute-free water reabsorption (T^*H_2O) among the different groups of participants. NS, not significant; 8, 0800; 12, 1200; 16, 1600; 20, 2000 (24-h clock). *** $P < 0.001$.



and $0.24 \pm 0.02 \text{ mmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for dry nights; $P < 0.01$). Urea clearance (C_{Ur}) and fractional excretion (FE_{Ur}) showed similarly lower values at night in all groups, indicating a consistent renal urea handling in both enuretics and controls (Table 2).

The overall excretion of osmotically active solutes is shown in Fig. 1. Osmolar excretion (E_{osm}) was generally constant during daytime but decreased significantly at night in all groups, although to a lesser degree in polyurics who excreted $\sim 40\%$ more solutes during sleep (0.65 ± 0.04 compared with $0.46 \pm 0.03 \text{ mosm} \cdot \text{kgH}_2\text{O}^{-1} \cdot \text{h}^{-1}$ for controls, $0.48 \pm 0.03 \text{ mosm} \cdot \text{kgH}_2\text{O}^{-1} \cdot \text{h}^{-1}$ for nonpolyurics, and $0.47 \pm 0.03 \text{ mosm} \cdot \text{kgH}_2\text{O}^{-1} \cdot \text{h}^{-1}$ for dry nights; $P < 0.001$). Similar differences were evident in C_{osm} at nighttime ($1.47 \pm 0.08 \text{ ml/min}$ for polyurics compared with $1.13 \pm 0.09 \text{ ml/min}$ for controls, 1.23 ± 0.08 for nonpolyurics, and $1.20 \pm 0.13 \text{ ml/min}$ for dry nights; $P < 0.05$).

TcH_2O varied during the day, peaking in the first hours after arousal and reaching the lowest values after noon for controls and polyurics and somewhat later for the remaining two groups (Fig. 1). At night, TcH_2O was not significantly different from the corresponding daytime values in any of the groups studied. Furthermore, no significant differences were found between the various groups with regard to TcH_2O in any of the clearance periods, indicating consistent renal water handling among the different subgroups of participants. In accordance with that, the nocturnal excretion of AQP2 showed no difference among groups ($78.8 \pm 10.6 \text{ U} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for controls, $100.4 \pm 20.9 \text{ U} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for nonpolyurics, $95.8 \pm 12.1 \text{ U} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for polyurics, and $64.4 \pm 9.9 \text{ U} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for dry nights; $P = 0.39$).

Measurements in plasma. P-Na concentrations varied slightly but significantly and with no significant differences between the groups at any point (Fig. 4). Potassium levels consistently followed a circadian rhythm with the lowest values around midnight. Plasma osmolality was also consistently higher at the very start of the experimental period, reaching the lowest levels at 1200 and 0400.

Plasma urea (P-urea) measurements peaked around 1600 for both controls and nonpolyurics, decreasing significantly thereafter (Fig. 4). In polyurics, P-urea had a tendency to remain at higher levels during both the evening hours and the initial hours of the night, and the difference between the polyurics and controls reached statistical significance at both 2000 and 2400.

Hormone measurements. Figure 4 shows the diurnal variations of the hormones studied. Apart from a consistent nocturnal peak at 2400, the profile of plasma ANP (P-ANP) was stable throughout the 24-h experimental period. Plasma ANG II (P-ANG II) levels were higher during the night, with a peak at 0400. No significant differences were apparent between groups.

A clear circadian rhythmicity was also evident for plasma Aldo (P-Aldo) levels. The plasma levels of the hormone markedly declined toward the evening and the first hours of the night to continuously rise thereafter until the early morning

Fig. 3. Circadian variation in excretion and fractional excretion of sodium (E_{Na} and FE_{Na} , respectively) and urea (E_{Ur} and FE_{Ur} , respectively) among the different groups of participants. ** $P < 0.01$. *** $P < 0.001$.

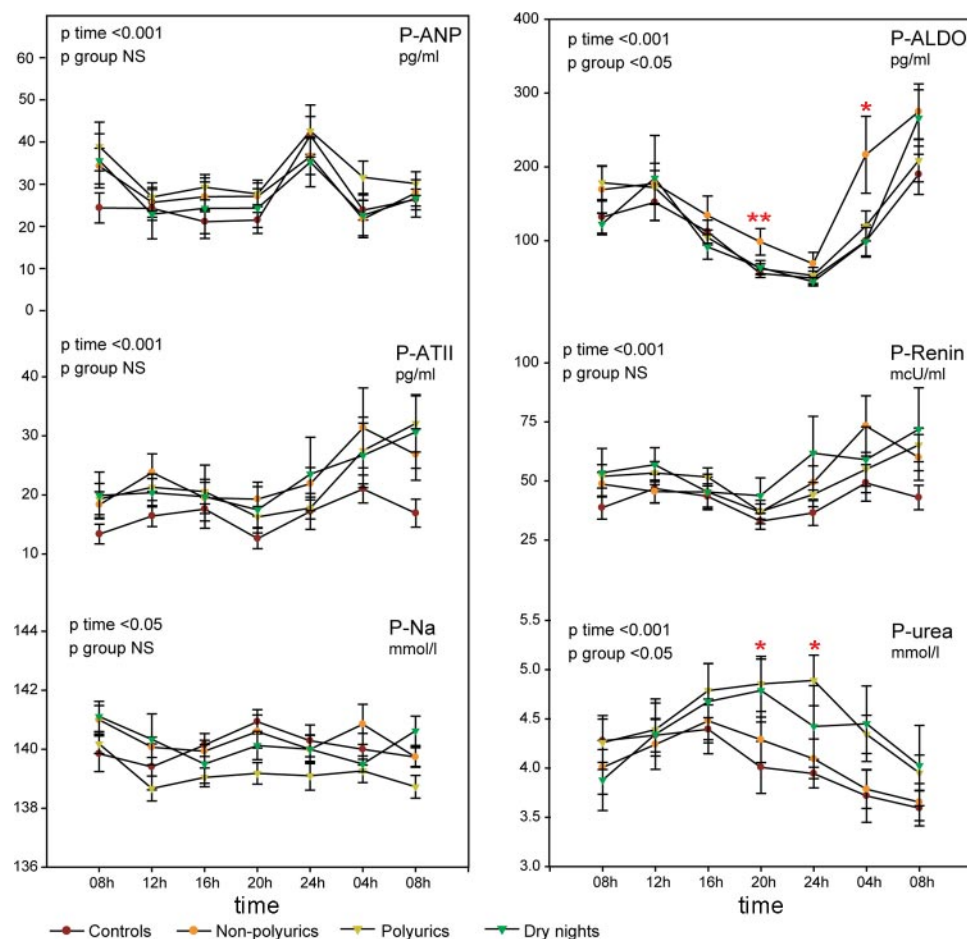


Fig. 4. Diurnal variations in the plasma (P-) levels of atrial natriuretic peptide (ANP), aldosterone (ALDO), angiotensin II (ATII), and activated renin as well as in P-Na and P-urea concentrations for all groups of participants. * $P < 0.05$. ** $P < 0.01$.

(Fig. 4). Nonpolyurics had higher P-Aldo levels than the other groups at 2000 and 0400.

Plasma renin (P-renin) showed circadian rhythmicity, reaching the lowest levels at 2000 in all groups. The highest values were seen at 0400 and 0800. We found no difference in P-renin among groups.

The four groups of participants did not differ in any of the experimental clearance periods in AVP excretion (Fig. 5). Nocturnal levels were lower than the corresponding daytime excretion levels of the hormone in all groups.

The urinary PGE_2 excretion (E_{PGE_2}) was particularly stable throughout the day but declined significantly at night in all groups (Fig. 5). Polyurics excreted larger amounts of this eicosanoid at night (0.20 ± 0.02 compared with 0.13 ± 0.01 $\text{ng} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for controls, 0.13 ± 0.02 $\text{ng} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for non-polyurics, and 0.09 ± 0.09 $\text{ng} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for dry nights; $P < 0.05$). A significant correlation was evident between E_{PGE_2} and E_{Na} levels at night (Fig. 6).

Hemodynamics. Arterial blood pressure levels were significantly lower during sleep for all groups of participants (Fig. 6). We did not find any significant differences in the levels of the systolic and diastolic mean blood pressure between groups.

Heart rate followed the rhythm of blood pressure, showing a significant decline at night. All groups of participants exhibited the same circadian variations in heart rate with their measurements being similar at all times (Fig. 6). For further analysis,

area under the curve was calculated for both mean arterial pressure (MAP) and heart rate and compared among the groups, showing no significant differences.

DISCUSSION

When the transition from day to night is not associated with the normal decline in diuresis, nocturnal polyuria occurs. This blunted circadian rhythm in diuresis is commonly seen in nocturnal enuresis (24) as well as nocturia (5). The absence of the normal nycthemeral AVP rhythm with water diuresis as a result is still considered the physiological basis of the nocturnal polyuria in enuresis (24, 35). Several other factors outside the AVP-AQP2 axis have been implicated in the etiology of MNE-related nocturnal polyuria, but we still lack knowledge on the exact mechanisms responsible.

The present study concerns a highly specific population of children with dDAVP-resistant MNE with and without nocturnal polyuria. Because daytime symptoms and low bladder capacity were both exclusion criteria, the bladder reservoir function of the included enuretics can be considered normal. The two groups of enuretics recruited for the present study were rather homogenous and well characterized.

Our aim was to elucidate the mechanisms responsible for the nocturnal polyuria of dDAVP-resistant MNE. Enuretics with nocturnal polyuria excreted markedly larger amounts

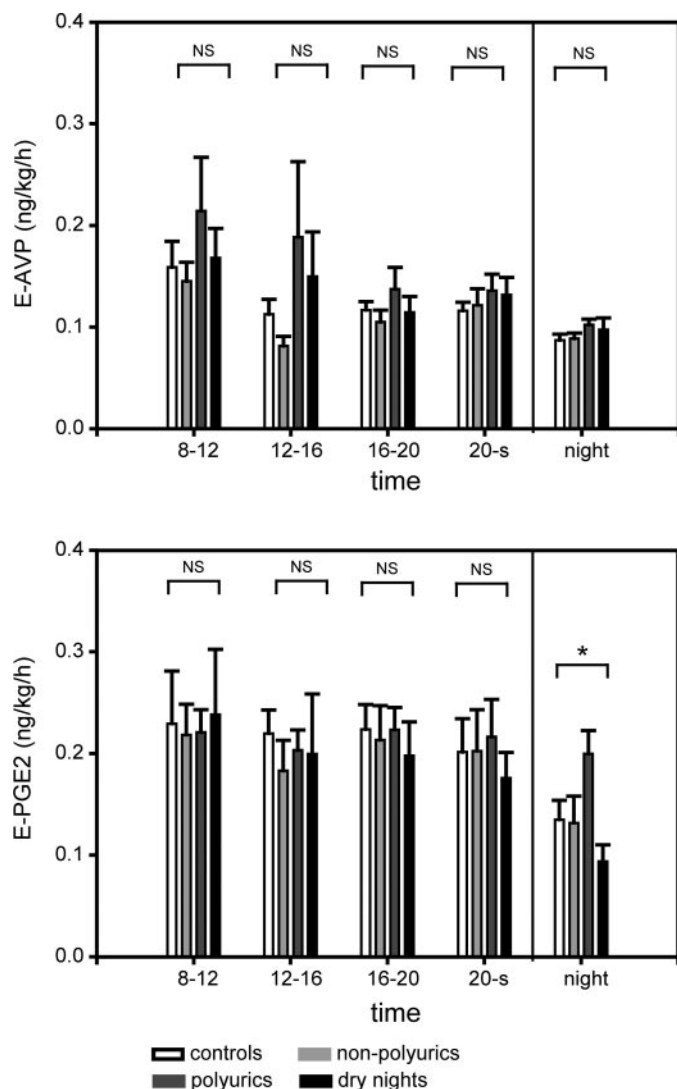


Fig. 5. Diurnal variation in arginine vasopressin (AVP) and prostaglandin E_2 (PGE_2) excretion (E_{AVP} and E_{PGE_2} , respectively) for all groups of participants. 20-s, 2000 to sleep/bedtime. * $P < 0.05$.

of urine at night compared with nonpolyurics and controls despite the standardized conditions regarding sodium and water intake and with a nocturnal urine output that was similar to the corresponding values of their home recordings (Table 1).

The fact that the 24-h diuresis values in the same group were significantly higher may indicate differences in fluid balance between groups, as fluid intake was standardized. Enuretics experiencing dry nights during the study passed much less urine during the night than polyurics, with diuresis values similar to those of controls and nonpolyurics. This observation is in accordance with previous findings that urine output on dry nights is markedly lower than during nights with enuresis (26). Because of methodological difficulties that make urine collection from enuresis episodes a particularly demanding task, data on the constituents of urine from enuresis episodes are virtually absent from enuresis research, and the nocturnal urine output of enuretics has previously been approximated through urine

from first morning voidings (12). This study clearly shows that urine from enuresis episodes of patients with nocturnal polyuria is significantly less concentrated compared with urine from first morning voidings. The first morning urine was found comparable in children with and without polyuria in terms of osmolality. Because osmolalities of urine from enuresis episodes differed between children with and without polyuria, one could hypothesize that any concentration defects in polyurics occur in the first hours of sleep. Furthermore, these observations prove any approximations of the nocturnal urine output in children with enuresis inadequate unless they take into account urine from enuresis episodes.

The nocturnal polyuria of enuretics in this study was not related to differences in renal water handling. This could explain the failure of these children to respond to the AVP analog dDAVP. Although patients with nocturnal polyuria were excreting less concentrated urine at night, they had similar nocturnal TcH_2O , urine AVP, and urine AQP2 profiles as controls and nonpolyurics. Enuretics with dDAVP-resistant nocturnal polyuria may constitute a particular subgroup with no evidence of a nocturnal lack of AVP.

We found the enuresis-related polyuria to be largely due to an abnormal nocturnal renal handling of solutes and in particular sodium. Because P_{Na} and GFR levels were not different among groups, and sodium intake was standardized, the sodium load reaching the initial ultrafiltrate was identical among groups (approximated to 13.7 ± 3.6 mmol/min for controls, 14.0 ± 4.2 mmol/min for nonpolyurics, and 12.8 ± 3.3 mmol/min for polyurics; $P = 0.59$) and therefore cannot account for the higher E_{Na} . Hence, it is the difference in the renal tubular sodium reabsorption profile that is responsible for the high E_{Na} . Indeed FE_{Na} was almost 50% higher in polyurics compared with controls. The levels of the sodium-regulating hormones measured in the present study were not different among groups, indicating that none of these hormones is responsible for this phenomenon. A similar conclusion can be drawn for hemodynamics evaluated by blood pressure, heart rate, and GFR. Children with and without nocturnal polyuria showed the same circadian profile in MAP and heart rate, observations challenging the hypothesis that hemodynamics and increased E_{Na} in polyurics are related.

PGE_2 , the most abundant renal autacoid, counteracts the effects of AVP on tubular water reabsorption (3, 8, 11) but also directly influences renal tubular sodium handling, exerting potent natriuretic properties (4, 10). The markedly higher nocturnal urinary E_{PGE_2} seen in patients with nocturnal polyuria may be responsible for their increased FE_{Na} . The significant correlation between E_{PGE_2} and E_{Na} shown in Fig. 6 is supportive of such a conjecture. Furthermore, because PGE_2 modulates the renal effects of AVP through cAMP downregulation (30, 32), the high luminal PGE_2 levels may be related to the failure of dDAVP to effectively diminish the nocturnal urine output in these children. The mechanisms responsible for this apparent overproduction in PGE_2 are elusive, but speculations should include humoral, neuronal, and other modulators of renal function such as nitric oxide. AVP and ANP, both implicated in enuresis pathophysiology, influence renal prostaglandin synthesis. AVP directly stimulates PGE_2 production in what seems to be part of a negative feedback mechanism (37). ANP-stimulated generation of PGE_2 (7) and similar

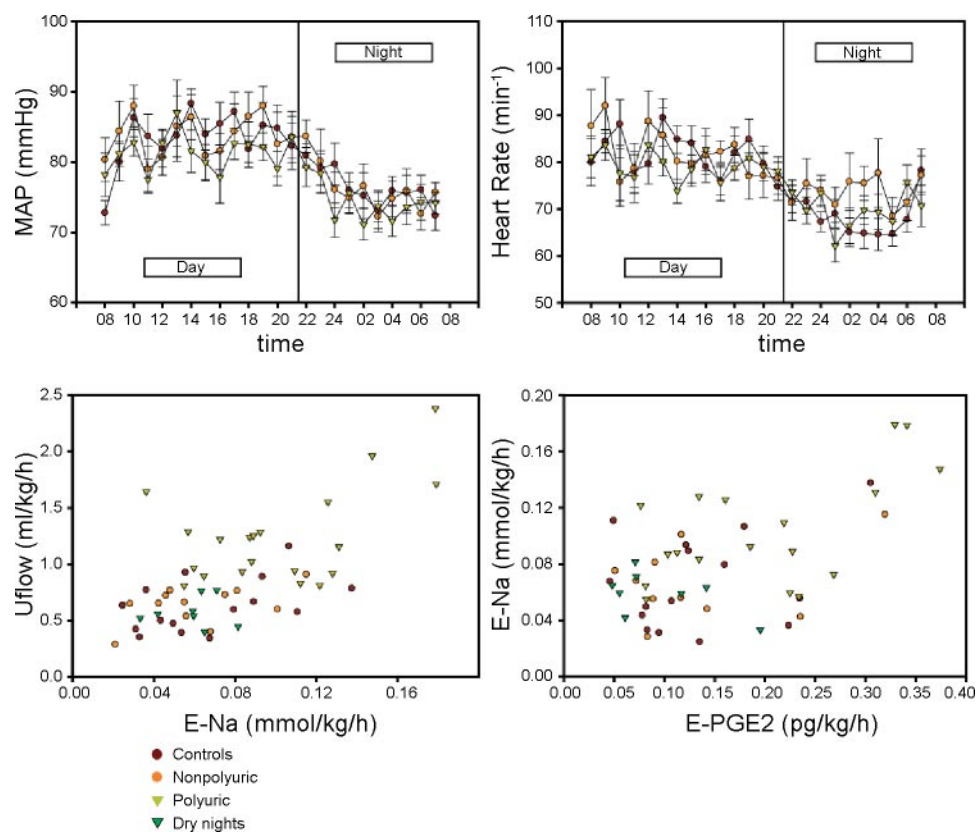


Fig. 6. Diurnal variation in mean arterial pressure (MAP) and heart rate for polyurics, nonpolyurics, and healthy controls. *Bottom*: scatter plots showing the correlation between nocturnal E_{Na} and nocturnal diuresis as well as the relationship between E_{Na} and nocturnal E_{PGE2} .

properties have been demonstrated for other natriuretic peptides (15). Any aberrations in these physiological processes that could influence the sensitivity of the renal PGE_2 production systems to incoming stimuli could account for the excess PGE_2 production found in polyurics. Sleep-related neuronal factors may also be part of the mechanisms resulting in natriuresis in patients with nocturnal polyuria. Although the lack of differences in the present study in the nocturnal heart rate levels among polyurics, nonpolyurics, and controls contradicts this hypothesis, parasympathetic hyperactivity has previously been postulated in enuretics (14).

Although we were able to confirm the previously described nycthemeral variations in E_K (6) with a dramatic reduction at night, we cannot substantiate that renal potassium handling is implicated in the etiology of enuresis-related polyuria, as suggested by past studies (23, 36). Conversely, urea seems to be implicated in the osmotic diuresis of polyurics, as indicated by the present findings. Apart from being an end product of protein metabolism excreted in urine, urea, with its renal reabsorption clearly regulated by AVP (39), plays an important role in renal concentrating mechanisms aiding the buildup of the renal medullary osmotic gradient (28). To our knowledge, this is the first study to evaluate the role of E_{Ur} in enuresis, and we clearly demonstrate that patients with nocturnal polyuria have higher E_{Ur} than nonpolyurics and controls, a phenomenon that may be secondary to the higher P-urea levels that these children showed in the late evening hours and the start of the night. C_{Ur} and FE_{Ur} were identical among the groups, pointing toward uniform renal urea handling. The importance of these findings in the etiology of enuresis needs to be elucidated through future studies.

In conclusion, the present study attempts to find insight into the mechanisms responsible for the nocturnal polyuria of a highly selected and well-characterized population of children with dDAVP-resistant enuresis.

Patients with nocturnal polyuria excrete larger amounts of sodium and urea at night compared with healthy controls and nonpolyurics despite a normal rhythm in renal water handling and normal urine AVP and urine AQP2 excretion profiles. This natriuresis found in polyurics does not seem to reflect abnormalities in either hemodynamics or the circadian rhythm of sodium-regulating hormones such as ANP, Aldo, ANG II, and renin but may be secondary to augmented E_{PGE2} , as became evident in the population of polyurics. This excess PGE_2 generation found in this subgroup of enuretics may also be responsible for the failure of dDAVP to control their polyuria.

The mechanisms responsible for the higher nocturnal E_{Ur} in polyurics are unclear and may be secondary to higher plasma levels of this catabolic product. Further studies are needed to evaluate the exact role of renal sodium and urea handling in nocturnal enuresis-related polyuria.

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GRANTS

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