CLINICAL STUDY

Urinary aquaporin-2 excretion in nocturnal enuresis

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Abstract

Objective: To evaluate the role of the arginine vasopressin (AVP)–aquaporin-2 (AQP-2) axis in the pathogenesis of nocturnal enuresis.

Study participants: Twelve children (seven male and five female), aged 11.6 ± 4.3 (6.7–15.6) years, suffering from primary monosymptomatic nocturnal enuresis and 12 healthy children, matched for sex and age. Enuretic children were further subdivided into responders and non-responders to treatment with 1-desamino-8-D-AVP (DDAVP).

Methods: Serum concentrations of AVP, and plasma and urine osmolality were measured at night (0100, 0400 and 0700 h), together with nocturnal urinary excretion of AQP-2 (2000–0800 h). Magnetic resonance imaging (MRI) of the pituitary gland was carried out to evaluate the amount of AVP stored in the posthypophysis.

Results: Mean AVP serum concentrations were similar in patients and controls. Urinary AQP-2 was also similar in patients and controls, but responders had a significantly lower level of AQP-2 than non-responders ($P < 0.005$). Plasma osmolality was greater in patients than in controls ($P < 0.001$), whereas urinary osmolality was similar in both groups. No difference in the ratio of the signal intensity of the posterior lobe of the hypophysis to that of the pons (AVP content) was found between patients and controls or between responders and non-responders.

Conclusion: A decreased urinary excretion of AQP-2 is associated with, and seems to have a role in, nocturnal enuresis, at least in some children, and this could also explain why only some of them respond to DDAVP treatment.

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Introduction

Nocturnal enuresis is a relatively common disorder, affecting about 15–20% of 5-year-old and 5% of 10-year-old children, 2–3% of adolescents and 1% of adults (1–4). It seems to be a multifactorial entity and several factors have been implicated. Genetic (5), neurological (6, 7) and hormonal factors (8) can influence the balance between bladder capacity and nocturnal urinary production, which ultimately appears to be the determinant in inducing enuresis. In the past few years the discovery of an absent circadian rhythm of production of antidiuretic hormone (arginine vasopressin, AVP) in monosymptomatic patients with enuresis (9, 10) led to treatment with 1-desamino-8-D-arginine vasopressin (DDAVP), an antidiuretic hormone analogue; about one-third of the patients, however, do not respond to this kind of therapy (11). The ultimate pathophysiological explanation for this variation in DDAVP response remains unknown. Aquaporin 2 (AQP-2), a vasopressin-regulated protein that mediates transmembrane water

transport in the collecting ducts (12), might also have a role in nocturnal enuresis. This prompted us to evaluate the role of the AVP–AQP-2 axis in groups of enuretic and normal children.

Patients and methods

Twelve children (seven male and five female), aged 11.6 ± 4.3 (6.7–15.6) years and suffering from primary monosymptomatic nocturnal enuresis with at least six episodes of bedwetting over 2 weeks were chosen for the study. Monosymptomatic nocturnal enuresis refers to the lack of diurnal urinary incontinence, with normal blood and urine biochemistry, urine culture and urine flowmetry, and normal kidney and urinary tract ultrasound without any residual urine, and therefore the absence of any urological and neurological disorders. All the children had been regularly attending the enuresis outpatient clinic and none of them was previously treated with DDAVP.

They were admitted to our department at 2000 h and retired to a darkened and quiet room. The patients

were allowed to drink up to 100 ml of fluids until 2130 h and then nothing till morning. The patients remained in bed for the duration of the study. The same modalities were applied to controls. A non-thrombogenic catheter was used for blood sampling, performed without the need to wake up the patients. At 0100, 0400 and 0700 h, blood samples were taken for AVP and plasma osmolality assay. Urine was collected with a plastic bag during the night (between 2000 and 0800 h) for the evaluation of AQP-2 and urinary osmolality.

The following day, all patients underwent magnetic resonance imaging (MRI) of the pituitary gland, in order to assess the amount of stored AVP. The signal intensity of the posterior lobe on T1-weighted image is believed to reflect the content of neurosecretory granules containing AVP (13). Furthermore, it is known that this hyperintense signal may disappear in situations characterised by AVP depletion, such as diabetes insipidus (14) or in the case of persistent AVP hypersecretion (15).

As a control group we investigated, before discharge, 12 healthy children, matched for sex and age, who had been admitted to our department because of a minor illness.

As a control group for the MRI findings, we selected 24 normal MRI brain images of children matched for age and sex, taken at the radiological department of our hospital.

All patients were then treated with DDAVP, up to 0.4 mg/day, orally at bedtime, and they were followed up for several months. Children with less than 50% reduction in the number of wet nights after at least 6–8 weeks of treatment were classified as non-responders and the others as responders. Informed consent for the study was obtained from all parents.

Methods

AVP was measured by an RIA (Arginine Vasopressin DSL-1800, Diagnostic System Laboratories, TX, USA) that has a sensitivity of 0.5 pg/ml and intra- and interassay coefficients of variation of 8% and 13% respectively.

The RIA for urinary AQP-2 was performed by the method described in our previous report (16). Urinary AQP-2-like immunoreactivity was measured by a specific RIA using the polyclonal antibody against a synthetic portion of the C terminus of human AQP-2 (V257-A271), raised in rabbits. A synthetic peptide (Tyr⁰-aquaporin-2 (V257-A271)) corresponding to the 15-amino acid sequence of the C terminus of AQP-2 was radioiodinated with iodine-125 (New England Nuclear, Boston, MA, USA) by the chloramine-T method. For the assay, 0.1 ml of assay buffer (0.05 mol/l sodium phosphate (pH 7.4), 0.08 mol/l sodium chloride, 0.01 mol/l EDTA, 0.5% BSA, 0.5% NP-40, and 0.01% sodium azide), and 0.1 ml of

antibody (final dilution, 1: 12 000) were incubated at 4 °C for 48 h, followed by addition of 0.1 ml of radiolabelled synthetic peptide (approximately 10 000 c.p.m.) and further incubation at 4 °C for 48 h. Bound and free quantities of radiolabelled ligand were separated by the double antibody method. The serial dilution curve of the urine samples was parallel to that of the standard. Each sample was analysed in duplicate. Intra- and interassay coefficients of variation were less than 10%. The minimal detectable quantity of AQP-2 was 0.86 pmol/tube, and an amount equivalent to 6.9 pmol/tube caused a 50% inhibition of binding of the radiolabelled ligand.

Serum for AVP and urine for AQP-2 assay were immediately frozen to –20 °C until required for analysis.

Plasma and serum osmolality were also evaluated.

For MRI, a ratio of the signal intensity of the posterior lobe of the hypophysis to that of the pons was measured on a display monitor. The mean signal intensity of three regions of interest in the posterior lobe was calculated and compared with that in the pons. MRI was performed with a 0.5 T superconducting unit (Gyrosan T5, Phillips Medical Systems, Eindhoven, the Netherlands). Sagittal T1-weighted images were obtained with the three sagittal SE/T1 method: 570 ms of repetition time, 25 ms of echo time, 90° flip angle, 18 cm field of view, 256 × 256 matrix, four excitations, 13 slices and 3 mm slice thickness.

Statistics

Unpaired Student's *t*-test was used to verify differences between patients and controls and the area under the curve (AUC) was calculated according to the trapezoidal rule. Multivariate analysis was used to verify correlations between AVP, urinary AQP-2 and plasma osmolality. A *P* value <0.05 was considered significant.

Results

AVP

The mean serum concentration of AVP and the AUC were similar in patients and controls (Table 1). Also, when enuretic children were subdivided according to their response to DDAVP treatment, no difference was found (Table 2).

Urinary AQP-2

No significant difference was found between patients and controls, but responders had a significantly lower concentration of urinary AQP-2 than non-responders (*P* < 0.005).

Table 1 Hormonal evaluation in patients and controls.

	Mean AVP (pg/ml)	AVP AUC (pg/ml per 6 h)	AQP-2 (fmol/mg Cr)	Mean plasma osmolality (mosmol/kg)	Mean urinary osmolality (mosmol/kg)
Patients (<i>n</i> = 12)	8.6±2.3	34.6±6.3	277±69	289.1±4.9***	720±132
Controls (<i>n</i> = 12)	7.9±4.1	30.9±12.0	360±200	281.2±5.1	824±265

Cr, creatinine. *** *P* < 0.001 for the difference between patients and controls.

Table 2 Hormonal evaluation in DDAVP responders and non-responders.

	Mean AVP (pg/ml)	AVP AUC (pg/ml per 6 h)	AQP-2 (fmol/mg Cr)	Mean plasma osmolality (mosmol/kg)
Responders (<i>n</i> = 7)	8.3±2.2	32.7±5.6	234±47§	289.9±3.9
Non-responders (<i>n</i> = 5)	9.2±2.6	37.2±7.5	338±32	288.7±5.8

§ *P* < 0.005 for the difference between responders and non-responders.

Table 3 MRI ratio of signal intensity of the hypophysis posterior lobe to that of the pons.

	All patients	Controls	Responders	Non-responders
Signal intensity ratio	1.70±0.27	1.52±0.23	1.72±0.32	1.69±0.21

Osmolality

Plasma osmolality was greater in patients than in controls (*P* < 0.001), but urinary osmolality was not different between these groups. No correlation was found between AVP serum concentrations and urinary AQP-2 and serum and urinary osmolality.

MRI findings

No difference in the signal intensity ratio of the posterior lobe to the pons was found between patients and controls or between responders and non-responders.

Discussion

If a deranged function of the AVP–AQP-2 axis were involved in the pathogenesis of nocturnal enuresis, then a reduced production of AVP, a defective secretion or a tissue insensitivity to AVP should be present.

In our investigation we could not detect any defect in AVP production, as the MRI signal intensity ratio of the posterior lobe of the hypophysis, which is a good marker of stored AVP (13), was similar in both patients and controls. Also the ability to secrete AVP was not impaired, as mean AVP concentrations and the AUC were similar in both patients and controls. The findings concerning AVP secretion are similar to those of Hunsballe *et al.* (17, 18) but are different from those of Rittig *et al.* (9) and Aikawa *et al.* (19). This seems to suggest that some other factors, such as age, number of

patients studied, and intra- and interindividual variability of AVP rhythm might explain these differences among studies.

Urinary AQP-2, which can be considered a good marker of tissue responsiveness to AVP (12), was found to be slightly, but not significantly lower in enuretic children. However, when we considered the enuretic children according to their response to DDAVP treatment, then statistically significant lower concentrations of urinary AQP-2 were found in the responders. We believe that this finding can also explain the tendency of the enuretic individuals to exhibit lower concentrations of urinary AQP-2 compared with controls. Our results are in agreement with those of Valenti *et al.* (20), who also found low concentrations of urinary AQP-2 in enuretic children; however, this was in the presence of low-normal AVP concentrations.

Our findings seem to suggest that enuretic children who respond to DDAVP treatment have a tendency to secrete less AQP-2 in response to AVP stimulation, probably because of an immature or altered function at receptor or postreceptor level, as previously suggested (21). DDAVP treatment in these patients might increase AQP-2 secretion, thus reducing nocturnal water loss. In agreement with previously reported findings (22), we also did not find any difference in urine osmolality between patients and controls. Genetic linkage of familial nocturnal enuresis to chromosome 12q has led to the speculation that the AQP-2 gene, which maps on 12q13-q21, might be involved in the pathogenesis of the disorder (23). More data are needed, however, to confirm this hypothesis and to explain why nocturnal

enuresis is mainly a self-limiting disturbance, despite the presence of a genetic defect. Other mechanisms independent of the AVP–AQP-2 axis are presumably involved in the non-responders group (5–8).

In conclusion, our study shows that a decreased urinary AQP-2 excretion in response to AVP stimulation might play a part in nocturnal enuresis, at least in some children, and this could also explain why only some of them respond to DDAVP treatment.

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