Desmopressin for nocturnal enuresis in nephrogenic diabetes insipidus

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Abstract

We investigated two unrelated families, in which two children had inherited primary nocturnal enuresis, and nephrogenic diabetes insipidus caused by new mutations in the aquaporin-2 gene (AQP2). The mutant AQP2 proteins were inactive, suggesting that administration of desmopressin could not concentrate the urine in these patients. However, treatment with desmopressin resolved PNE completely. This observation questions the notion that desmopressin resolves primary nocturnal enuresis through pharmacological manipulation of renal concentrating ability only. Desmopressin might also act on extrarenal targets, such as the central nervous system.

Research letter

Primary nocturnal enuresis (PNE), a frequent complaint in pediatric practice, is defined by the persistence of bedwetting at least three nights a week after the fifth year of age. Children do not wake up during or after voiding the bladder. Genetic investigations[1] have suggested that in about 50% of cases, primary nocturnal enuresis is transmitted in an autosomal dominant mode of inheritance, but the underlying molecular defect remains unknown.

For more than a decade, most patients with primary nocturnal enuresis were thought to have reduced endogenous secretion of antidiuretic hormone arginine-vasopressin at night which in turn led to increased urinary volume thereby exceeding the functional capacity of the bladder[2]. Exogenous administration of the arginine-vasopressin analogue desmopressin can compensate for the deficit in this hormone and thus, desmopressin is widely prescribed for treatment of primary nocturnal enuresis, with success rates up to 80%. Here, however, we provide clear evidence against the hteory that desmopressin works solely through renal concentrating ability.

In patients with congenital nephrogenic diabetes insipidus, mutations in the vasopressin-2 receptor (*V2R*) or aquaporin-2 (*AQP2*) water-channel genes inactivate these proteins. Since arginine-vasopressin regulates the concentration of urine by binding to V2R in renal principal cells, which redistribute AQP2 from intracellular vesicles to the apical membrane, patients are unable to concentrate their urine in response to secretion of arginine-vasopressin or exogenous administration of desmopressin.

Serum osmolality is thus high and urinary osmolality low, despite high plasma arginine-vasopressin levels. The standard diagnostic test consists of a combined fluid restriction and

desmopressin test, in response to which patients with congenital nephrogenic diabetes insipidus have persistently low urinary osmolality.

We identified two unrelated families with two children who had primary nocturnal enuresis primary nocturnal enuresis and congenital nephrogenic diabetes insipidus. A further two children presented with isolated congenital nephrogenic diabetes insipidus, and another child had isolated PNE **(**figure 1**)**. When tested, the child with primary nocturnal enuresis only showed a notable increase of urinary osmolality (from 588 mosmol/kg H_2O before desmopressin and 1259 mosmol/kg H_2O after) whereas urine osmolality of the two children with primary nocturnal enuresis and congenital nephrogenic diabetes insipidus remained unchanged (245 to 261 mosmol/kg H_2O and 230 to224 mosmol/kg H_2O).

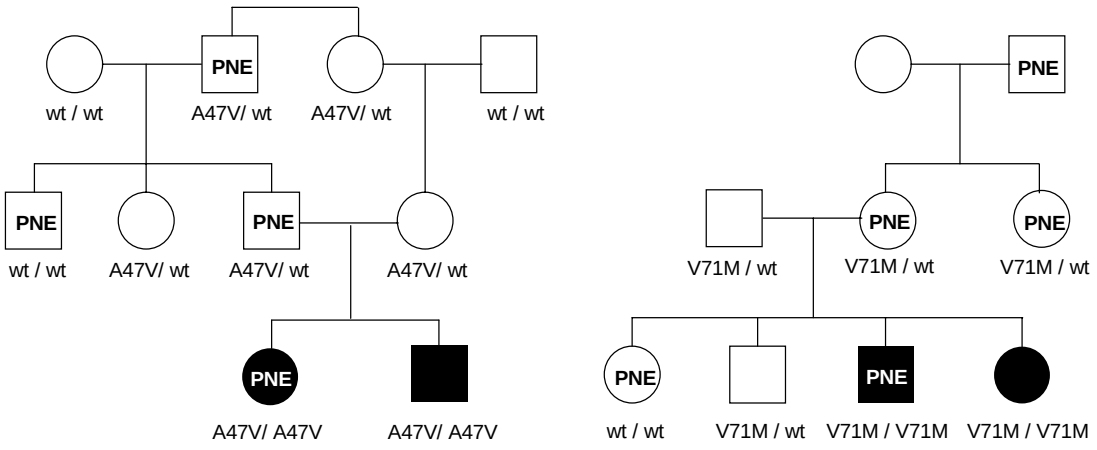

Figure 1. Disease pedigrees of the families with primary nocturnal enuresis and congenital nephrogenic diabetes insipidus. PNE=primary nocturnal enuresis. Individuals affected by congenital nephrogenic diabetes insipidus are shown as black boxes (male) and circles (female). For the individuals tested, their haplotype is given for each allel separately.

We obtained patient and parental consent to start treatment with desmopressin (20 µg intranasally) in three children affected by primary nocturnal enuresis. Despite clear differences in the desmopressin test, PNE resolved in all three children within 2 days: The child with primary nocturnal enuresis slept through the night without wetting the bed, whereas the children with primary nocturnal enuresis and congenital nephrogenic diabetes insipidus

woke up and went to the toilet to empty their bladder. To verify that treatment with desmopressin was the cause, we requested consent to to temporarily halt treatment. Withdrawal of desmopressin restored the primary nocturnal phenotype, and reinstitution of the drug promptly reproduced the relief of PNE in all children.

Our results suggest that desmopressin did not resolve primary nocturnal enuresis through its renal concentrating capacity, and we therefore used molecular tools to further support this conclusion. We did mutation screenings of the *V2R* and *AQP2* genes, which showed new homozygous mutations in the *AQP2* gene in the affected members of family A (C 140T) and family B (G211A), coding for mutant AQP2-A47V and AQP2-V71M proteins, respectively. To ascertain the effect of these mutations on AQP2 functioning, both mutants were expressed in *Xenopus* (frog) oocytes which were then fixed in paraformaldehyde and embedded in paraffin.

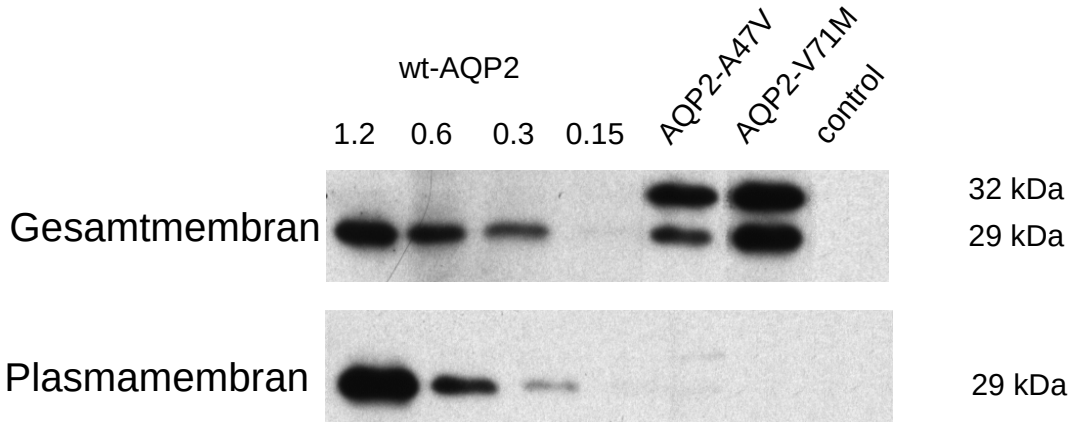

Figure 2 (A) Immunoblot of expressed AQP2 proteins. Total membranes (TM) or plasma membranes (PM) of 12 non-injected oocytes or those expressing AQP2-A47V, AQP2-V71M or wt-AQP2.

Sections were incubated with rabbit antibodies to AQP-2 and then with Alexa594-conjugated rabbit antibodies. AQP-2 proteins were seen with confocal laser scanning microscopy. Swelling assays showed that the water permeability conferred by either mutant did not differ from non-injected controls (20 µm/s [SE 12] and 15 µm/s [7] *vs* 12 µm/s [4], respectively; p=0.4 for AQP2-A47V and p=0.5 for AQP2-V71M), whereas oocytes expressing different amounts of wild-type (wt) AQP2 showed increased water permeabilities. Oocytes with 1.2 ng of cDNA coding for wt-AQP2 had a mean water permeability of 700 µm/s (SE30) ($p<0.001$),

those with 0.6 ng cDNA 222 µm/s (45) ($p<0.0001$), those with 0.3 ng cDNA 141 µm/s (16) ($p<0.0001$), and those with 0.15 ng cDNA 77 µm/s (16) ($p<0.0001$).

Immunoblotting of all membranes of the oocyte showed that both mutants were expressed at high concentrations, but blotting of the plasma membranes showed that the mutant proteins were not on the cell surface, by contrast with wt-AQP2 (figure 2). As in other AQP2 mutants in recessive congenital nephrogenic diabetes insipidus, the expression as 29 and 32 kDa bands indicated that AQP2-A47V and AQP2-V71M are not transported to the plasma membrane. The dispersed immunocytochemical staining of both proteins in the oocytes (figure 2) is in agreement with this theory[3].

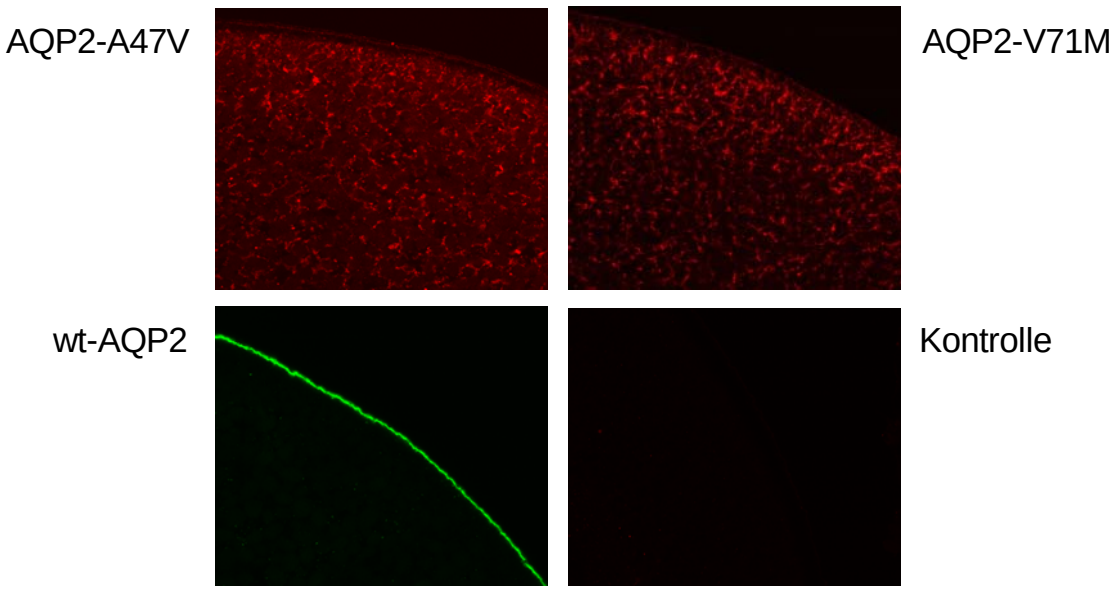

Figure 2(B) Immunocytochemistry of oocytes expressing AQP2 proteins. Oocytes expressing AQP2-A47V (1), AQP2-V71M (2), wt-AQP2 (3) or non-injected oocytes (4). Arrows: Plasma membranes.

Our analysis showed that congenital nephrogenig diabetes insipidus in both families was caused by intracellular retention of presumably misfolded AQP2 mutant proteins, explaining the absence of urinary concentrating in patients with congenital nephrogenic diabetes insipidus who were given desmopressin.

The complete relief of bedwetting in our patients with desmopressin in our patients with congential nephrogenic diabetes insipidus and primary nocturnal enuresis clearly showes that the therapeutic benefit of this drug is not due to the pharmacological manipulation of renal concentrating ability suggesting that desmopressin acts, at least in part, through another organ than the kidney. Metabolites and synthetic analogues of endogenous arginine-vasopressin are potent neurotransmitters that have an effect on memory and locomotor activity[4,5]. Therefore, extrarenal central vasopressin receptors such as V1R or V2R, may represent the proteins activated by desmopressin treatment in primary noctzrnal enuresis. This central effect of desmopressin is also supported by the fact that use of tricyclic antidepressants and the bell pad are as effective as desmopressin in the treatment of primary nocturnal enuresis.

Our clinical and experimental data demonstrate that a major action of desmopressin to resolve primary nocturnl enuresis might reside outside the kidney and we suggest the central nervoussystem as the alternative site. Such action would account not only for the pathophysiological but also for the pharmacological basis of desmopressin treatment.

Acknowledgements

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1. Eiberg H, Berendt I, Mohr J. Assignment of dominant inherited nocturnal enuresis (ENUR1) to chromosome 13q. *Nat Genet* 1995; **10**: 354-6.
2. Rittig S, Knudsen UB, Norgaard JP, Pedersen EB, Djurhuus JC. Abnormal diurnal rhythm of plasma vasopressin and urinary output in patients with enuresis. *Am J Physiol* 1989; **25**: 664-1.
3. Deen PMT, Knoers NVAM. Physiology and pathophysiology of the aquaporin-2 water channel. *Curr Opin Nephrol Hypertens* 1998; **7**: 37-42.

4. De Wied, Gaffon O, van Ree JM, de Jong W. Central target for the behavioural effects of vasopressin neuropepetides. *Nature* 1984; **308**: 276-8.
5. Weingartner H, Gold P, Beallenger JC et al. Effects of vasopressin on human memory functions. *Science* 1981; **211**: 601-3.

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Nocturnal enuresis in patients with nephrogenic diabetes insipidus

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Sir—Dominik Müller and colleagues [1] report two unrelated families with congenital nephrogenic diabetes insipidus. Two children had coexistent nephrogenic diabetes insipidus and primary nocturnal enuresis, and one child had primary nocturnal enuresis only. This report supports this group's previous hypothesis that desmopressin is effective in patients with nocturnal enuresis because of a mechanism other than an effect on renal concentrating ability. Jonat and colleagues [2] reported a boy aged 8 years with coexistent primary nocturnal enuresis and nephrogenic diabetes insipidus. Treatment with hydrochlorothiazide and dietary measures reduced urine output to a third of the pretreatment volume, but nocturnal enuresis persisted. Daily intranasal desmopressin did not reduce the urine output further, but strikingly improved nocturnal enuresis.

In Müller and colleagues' study, nocturnal enuresis resolved on treatment with desmopressin in all three children with primary nocturnal enuresis. The children with primary nocturnal enuresis and nephrogenic diabetes insipidus woke and went to the toilet to void, and the child with primary nocturnal enuresis only slept through and was dry. They suggest that the therapeutic action of desmopressin might be consequent to a CNS effect. We agree that the therapeutic action of desmopressin in the two children with nephrogenic diabetes insipidus

was not due to an effect on renal concentrating ability. However, a therapeutic effect of desmopressin on renal concentrating ability is still possible in the child who had primary nocturnal enuresis only. Primary nocturnal enuresis is a genetically and clinically heterogeneous disorder. Pathogenic factors include difficulty with arousal, small nocturnal bladder capacity, and nocturnal polyuria [3]. The two children with coexisting nephrogenic diabetes and primary nocturnal

enuresis substituted a pattern of nocturia for that of nocturnal enuresis. One interpretation for this change is a combination of small nocturnal bladder capacity and difficulty with arousal. The therapeutic effect of the desmopressin would presumably be to help arousal, and the nocturia reflects the continued presence of a small nocturnal bladder capacity. Since the child with primary nocturnal enuresis slept through without nocturia only after treatment, the nocturnal enuresis could have been due to a difficulty with arousal, nocturnal polyuria, or both but was less likely to be due to small nocturnal bladder capacity. The pedigrees provided by Müller and colleagues suggest that six other members of the two families have primary nocturnal enuresis only.

We would like to know whether the primary nocturnal enuresis spontaneously resolved in these patients, whether they slept through or substituted nocturia for this nocturnal enuresis, and how the pattern and resolution of wetting correlated with heterozygous status for nephrogenic diabetes.

References

1. D. Müller, N. Marr, T. Ankermann, P. Eggert and P.M.T. Deen.

Desmopressin for nocturnal enuresis in nephrogenic diabetes insipidus.

Lancet 359 (2002), pp. 495–497.

2. S. Jonat, R. Santer, R. Schneppenheim, T. Obser and P. Eggert.
Effect of DDAVP on nocturnal enuresis in a patient with nephrogenic diabetes inspidus.
Arch Dis Child 81 (1999), pp. 57–59.
3. W.L.M. Robson, Nocturnal enuresis. Adv Pediatr 48 (2001), pp. 409–438.
4. A. von Gontard, H. Schaumburg, E. Hollmann, H. Eiberg and S. Rittig , The
genetics of enuresis: a review. J Urol 166 (2001), pp. 2438–2443.

Nocturnal enuresis in patients with nephrogenic diabetes insipidus

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Sir—Dominik Müller and colleagues[1] suggest a new extrarenal mechanism of action for desmopressin. We have reported decreased nocturnal urinary vasopressin excretion in enuretic children, which was normalised by short-term imipramine [2].

Treatment induced a striking reduction in 24 h fluid intake, greater than the reduction in urine output, pointing to the possibility of another, possibly central, mechanism of action for imipramine, vasopressin, or both. Our observation supports Müller and colleagues' hypothesis. On the other hand, their statement that the complete stop of bedwetting after desmopressin treatment in the patients with nephrogenic diabetes insipidus and primary nocturnal enuresis clearly shows that the therapeutic benefit of this drug is not due to the pharmacological manipulation of renal concentration ability even in normal patients, is

probably too drastic. After treatment, their patients with nephrogenic diabetes and primary nocturnal enuresis could not sleep through the night, unlike the child with primary nocturnal enuresis only, but woke up and emptied their bladders. This finding is consistent with two different mechanisms of action for desmopressin and vasopressin, the first related to increased water resorption through classic vasopressin-2 receptor/aquaporin-2 water channel, and the second through decreased fluid intake, increased awareness of a full bladder, or both. Children with enuresis seem to be less sensitive to bladder-neck distention [3] and half of our children with enuresis had occult spinal abnormalities on radiography [2].

References

1. D. Müller, N. Marr, T. Ankermann, P. Eggert and P.M.T. Deen. Desmopressin for nocturnal enuresis in nephrogenic diabetes insipidus. Lancet 359 (2002), pp. 495–497.

2. P.A. Tomasi, S. Siracusano, A.M. Monni, G. Mela and G. Delitala. Decreased nocturnal urinary antidiuretic hormone excretion in enuresis is normalized by imipramine. BJU Int 88 (2001), pp. 932–937.

3. H. Ringertz. Bladder capacity, urethral sensation and lumbosacral anomalies in children with enuresis. Acta Radiol 25 (1984), pp. 45–48.

Nocturnal enuresis in patients with nephrogenic diabetes insipidus

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Authors' reply

Sir—On the basis of current knowledge, the only existing renal concentration mechanism is realised by the vasopressin-2 receptor/aquaporin-2 system located in the renal collecting duct. Mutations in this mechanism cause nephrogenic diabetes insipidus. There is no evidence for a second significant urine-concentrating pathway. If there were a mechanism that could compensate for the non-functioning of the system vasopressin-2 receptor/aquaporin-2, patients with mutations on the corresponding genes would not have nephrogenic diabetes. Consequently, our patients with both nephrogenic diabetes insipidus and primary nocturnal enuresis had, like patients with nephrogenic diabetes only, a high urinary volume with low urinary osmolality. Thus, the increased voiding frequency is due to the high urinary output rather than to a small bladder capacity.

The administration of desmopressin led to relief of nocturnal enuresis in the patients with primary nocturnal enuresis alone and coexisting with nephrogenic diabetes. Due to their increased urinary volume (nephrogenic diabetes insipidus was not cured), the patients with both disorders still needed to empty their bladder at night. Therefore, we prefer not to speak of a replacement of primary nocturnal enuresis by nocturia, as suggested by WLane and colleagues, since nocturia is one of the pathophysiological consequences of untreated nephrogenic diabetes insipidus in patients who have achieved bladder control. As mentioned by Lane and colleagues, we cannot rule out a therapeutic action of desmopressin on the urinary concentration in patients who have primary nocturnal enuresis only. We have shown

that these patients do have reduced urinary volume while taking desmopressin because of their normally functioning vasopressin-2 receptor/aquaporin-2 system. We did not, however, aim to show this effect. We aimed to show that desmopressin relieves nocturnal enuresis in patients who do not have a functional renal concentrating system.

Thus, the therapeutic benefit must be provided by the means of other mechanisms than the simple manipulation of renal concentration ability. Additionally, the heterozygous status for V47A and V71M in the pedigrees did not affect the achievement of dryness; nocturia was not reported. We did a mutational analysis in a cohort of patients with primary nocturnal enuresis in whom the disease cosegregated with the aquaporin-2 locus on chromosome 12. The open reading frame and exon-flanking sequences of the AQP2 gene did not differ between patients and controls [1]. We have also noted the positive effect of desmopressin on short-term memory in patients with primary nocturnal enuresis. Since short-term memory is a function of arousal (awareness), this finding again supports the central effect of desmopressin on sensitivity to signals from the bladder [2].

We are aware that our data for two families cannot hold for all primary nocturnal enuresis. However, our and others' data provide evidence that the success of desmopressin treatment and the pathophysiology of primary nocturnal enuresis might be attributed to a nervous, possibly central, mechanism [3].

References

1. P.M.T. Deen, N. Dahl and M.J. Caplan. The aquaporin-2 water channel in autosomal dominant primary nocturnal enuresis. J Urol 167 (2002), pp. 1447–1450.

2. D. Müller, H. Florkowski, K. Chavez-Kattau, G. Carlsson and P. Eggert.

The effect of desmopressin on short-term memory in children with primary nocturnal enuresis. J Urol 166 (2001), pp. 2432–2434.

3. N.M. Wolfish, R.T. Pivik and K.A. Busby. Elevated sleep arousal thresholds in enuretic boys: clinical implications. Acta Pediatr 86 (1997), p. 381.