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Regulation of Arginine Vasopressin in Enuretic Children Under Fluid Restriction

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ABSTRACT. *Background.* **Treatment of primary nocturnal enuresis using 1-deamino-8-D-arginine-vasopressin is based on the hypothesis that antidiuretic hormone (arginine vasopressin [AVP]) secretion is insufficient during the night. Persisting doubts about the theoretical background of this treatment and first results pointing to a different AVP regulation in children with nocturnal enuresis were the motives for the present study.**

Objective. **To determine if children with primary nocturnal enuresis have different AVP levels during fluid restriction when compared with normal controls.**

Methods. **Twenty-three children with nocturnal enuresis (median age, 11 years) were compared with a corresponding control group of 18 healthy children. Plasma osmolality, urine osmolality, and plasma AVP concentrations were determined before and after a defined fluid restriction.**

Results. **Regarding plasma and urine osmolality, no differences were found between the two groups. AVP levels after fluid restriction, however, showed significant differences. To maintain osmolality, the plasma AVP concentrations of the controls rose to a median value of 5.7 pg/mL (range: 0.9–29.0 pg/mL) in comparison to a median of 14.0 pg/mL (range: 3.5–64.0 pg/mL, $P = .015$) for the enuretic children.**

Conclusion. **The results are consistent with the established fact that AVP secretion is a function of plasma osmolality. They contradict the hypothesis that enuretic children have a AVP deficiency that has to be supplemented. Rather, the results point to a defect at the AVP receptor level or of the signal transduction pathway.** *Pediatrics* **1999;103:452–455;** ***nocturnal enuresis, AVP regulation, desmopressin treatment.***

ABBREVIATIONS. 1-deamino-8-D-arginine-vasopressin, desmopressin; AVP, arginine vasopressin; CI, confidence interval.

Treatment of primary nocturnal enuresis with 1-deamino-8-D-arginine-vasopressin (desmopressin) has become a standard medical regimen.[1] In different studies, depending on the definition of efficacy, the success rate has been reported to range from 10% to 91%.[2] The theoretical basis for this type treatment is the hypothesis that a missing circadian rhythm of arginine vasopressin (AVP) secretion results in nocturnal hormone deficiency in enuretic individuals.[3] Despite the fact that the therapeutic effect is generally accepted, controversy continues about this theory. The major point of criticism is that this hypothesis ignores the role of AVP in the "plasma osmolality–AVP secretion" feedback loop or at least limits the effects of AVP to daytime.[4] Motivated by this contradiction, investigators in a previous study[5] have shown that individuals with primary nocturnal enuresis do not have an increased nocturnal urine production when compared with a control group. There were no differences between the two groups regarding plasma osmolality and the rhythm of AVP secretion. There was one aspect, however, in which enuretics did differ from the controls: to maintain a normal plasma osmolality, enuretic individuals seemed to require a markedly higher plasma AVP concentration. Because these preliminary findings were based on a relatively small number of cases, the present study was performed to confirm the results in a larger group of patients.

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METHODS

The study included 23 children (group 1) with primary nocturnal enuresis (15 boys, 8 girls) and 18 healthy volunteers (group 2; 14 boys, 4 girls). The criteria for participation of patients in the study was a history of at least 3 wet nights per week. None of the patients had ever received medical treatment or a treatment with bell pads. The age of the enuretic children and controls was 6.5 to 15 years (median, 11 years) and 7.7 to 12.9 years (median, 10.3 years), respectively. No significant differences between enuretics and controls regarding age, weight, sex ratio, and blood pressure were observed. Complete clinical examinations and comprehensive laboratory investigations(including plasma urea, creatinine, liver enzyme, red and white blood cell counts, urine microscopy, and culture) were normal in all children. All patients had a normal ultrasound of bladder and kidneys. To stimulate AVP secretion, restriction of fluid intake was started in the evening. On the next day, blood samples were drawn for plasma osmolality and plasma AVP determination. Fluid restriction continued until urine osmolality reached a plateau. At this point a second blood sample was collected. To avoid nonosmotic stimulation of AVP secretion, all blood samples were drawn after the patients had been in a supine position for at least 10 minutes. Blood for the AVP assay was collected in tubes containing ethylenediaminetetraacetic acid that were immediately placed in iced water. Centrifugation at 4°C and storage of the supernatant at −22°C was performed immediately after sampling. Plasma AVP concentration was measured by radioimmunoassay after extraction (Robertson et al[6] modified according to Rascher et al[7]; measurements were performed in the laboratory of Dr Rascher (Department of Pediatrics, University Hospital of Giessen, Germany). The analysts did not know to which group the samples were assigned). A Roebling osmometer was used for the determination of serum osmolality.

The results are presented as range, median values, and the 95% confidence interval (CI) of the median. Differences between groups were tested using the nonparametric *U* test (Wilcoxon, Mann, and Whitney). The level of significance was set at $P = .05$.

The study was approved by the ethics committee of the Chris-

tian-Albrechts-University, School of Medicine. After having receiving detailed information, the parents gave written consent to the study.

RESULTS

Osmolality

Before the restriction of fluid intake, plasma osmolality in group 1 ranged from 264 to 315 mosmol/kg with a median of 298 mosmol/kg. (CI: 292–300). There was no significant difference with the control group, whose initial values ranged from 259 to 329 mosmol/kg(median 290 mosmol/kg, CI: 282–319).

At the end of the thirst test a slight increase in osmolality was found in both groups: in group 1 osmolality ranged from 293 to 323 mosmol/kg; the respective range of the control group was 261 to 335 mosmol/kg. Median values were 302 mosmol/kg (CI: 300–305) for the enuretic children and 290 mosmol/kg (CI: 288–303) for group 2. Comparing the groups revealed no significant difference.

Urine osmolality rose from a median of 936 mosmol/kg (range: 449-1009, CI: 614–1000) to 1057 mosmol/kg (range: 882–1379, CI: 934–1363) in group 1 and from a median of 946 (range: 888–1104, CI: 890–990) to 1128 mosmol/kg (range: 975–1279, CI: 975–1264) in group 2. Differences before and after fluid restriction between the two groups were not statistically significant.

AVP

Before fluid intake was restricted, enuretic children showed slightly higher plasma AVP concentrations than the controls (2.0–64.0 pg/mL, median 5.2 pg/mL and CI 4.2–7.7 in group 1 vs 1.8–13.1 pg/mL, median 4.0 pg/mL and CI 2.2–5.4 in group 2). The differences, however, were not significant. After the thirst test plasma AVP concentrations rose in both groups. Although the median value for the controls slightly increased to 5.7 pg/mL, the median for the enuretic children went up to 14.0 pg/mL. Range and confidence interval of the AVP concentrations after fluid restriction are given in a box plot (Fig 1). The differences between the groups are statistically significant ($P = .015$).

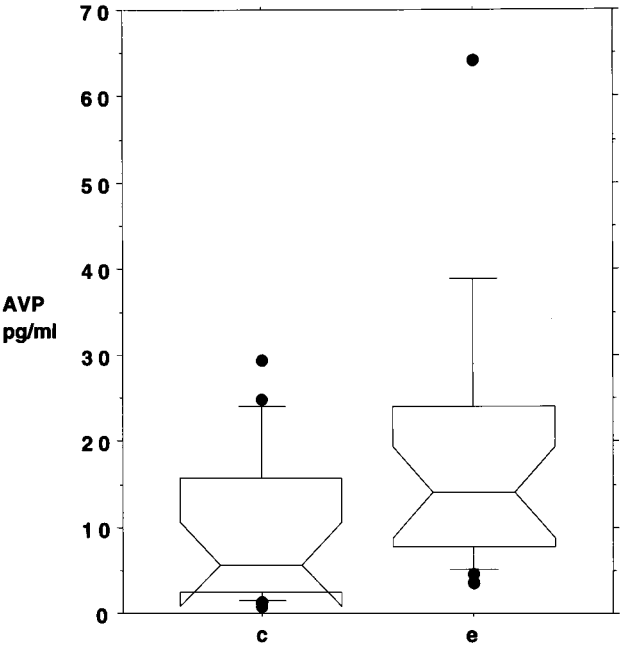

Fig 1. Arginine vasopressin concentrations after fluid restriction (e, enuretics; c, controls). The values are given in box plots showing the 10th, 25th, 50th (median), 75th, and 90th percentiles. The notch shows the 95% confidence interval of the median.

DISCUSSION

The results presented in this article confirm the hypothesis of differences in the AVP regulation pattern in children with nocturnal enuresis and healthy controls. The findings indicate that enuretic children need significantly higher AVP plasma levels to maintain constant plasma osmolality. The results show as well that these children do not differ from controls in regard to urine osmolality.

Before evaluating these results, it is necessary to analyze whether systematic errors could have had an impact. Both groups were sufficiently well defined, with the only difference being pronounced, monosymptomatic nocturnal enuresis. As far as the AVP assay is concerned, there is some evidence that differences of the sensitivity of different methods result in different values for normal plasma AVP concentrations in children.[8] The blood sampling procedure, the handling of these specimens, and the laboratory procedures were identical for all samples; furthermore, the analysts did not know to which group the blood samples were assigned. Therefore, although it may be possible that the absolute AVP concentrations are not directly comparable to other published data, the relationship between the two groups does remain relevant. Because time of blood sampling could have played a role because AVP secretion is pulsatile,[9] it is necessary to exclude the possibility that differences in AVP plasma concentrations are simulated by collecting blood samples in the rhythm of secretion. However, because the rhythmicity of secretion is not known and because samples were collected randomly in both groups, the significant result only points to the fact that it can not be explained by the time of blood sampling.

This detailed methodologic evaluation seems necessary because the findings presented here contradict the generally accepted concept of the efficacy of desmopressin for enuretic children. According to this concept, enuretic children have insufficient AVP secretion at night,[3] resulting in increased urine production, which in turn exceeds bladder capacity,[3,10] Although this hypothesis does seem convincing at first glance, it also calls for considerable criticism.

Until now, it could not be proven convincingly that nocturnal urine production is indeed increased in enuretics. Rittig et al[3] have reported a higher urine output of enuretic children at night time, but by using several urine samples of each patient included in their study they were mixing dependent and independent data, and the conclusions drawn by the authors have to be regarded with caution. Therefore, Vuilliamy's statement[11(page 442)] that "it is disappointing to find that a distinct impression can not be confirmed when put to the test" is still valid. In his

comprehensive study, the author did not find differences in the nocturnal urine volumes of enuretic children and controls. Thus, this finding is not in accordance with a lack of AVP at night time. More recent studies[12] provided evidence that enuretic children have a smaller functional bladder capacity when compared with controls. Again, from this perspective using desmopressin seems justified to restrict urine volume. On the other hand, based on this theory, a reduction in liquid intake in the evening should prove equally effective in children suffering from nocturnal enuresis. Scrupulous studies[13] and, last but not least, the experience of innumerable parents have disproved this measure.

The main point to be criticized in the "nocturnal-lack-of-AVP" hypothesis is that it ignores the role of AVP in the feedback mechanism of osmoregulation. It is difficult to imagine that the dependence of AVP secretion on plasma osmolality exists during day time only, whereas it is lost during the night.[4]

These major points of criticism constitute a striking contrast to the undisputed efficacy of desmopressin in treating primary nocturnal enuresis.[1,2,15]

The findings presented here show that enuretic children—and this is hardly surprising—are capable of maintaining constant plasma osmolality within narrow limits and that an increased AVP secretion is their response to liquid restriction. No significant differences were found for urinary osmolality between the two groups, pointing to the undisturbed fluid management in enuretic children. The increase in plasma AVP, however, is significantly higher than in comparable controls. Enuretic children, therefore, do not show an AVP deficiency but rather an increased hormone secretion when compared with controls. This finding suggests that the AVP-plasma osmolality feedback mechanism is less sensitive in children suffering from enuresis. This result prompted us to formulate a new hypothesis on the effect of desmopressin in enuretic children.

To introduce this hypothesis, the three currently accepted concepts for the treatment of nocturnal enuresis have to be mentioned. In 1953, Poulton and Hinden[16] reported on the efficacy of ephedrine and amphetamine in the treatment of nocturnal enuresis and gave way to the widely accepted use of tricyclic antidepressive agents. The methods of treatment have then been extended by the conditioning alarm with bell pads, treating the exceptional deep sleep of these patients. Finally, during the last years, the administration of desmopressin has emerged to an equivalent form of therapy. All these methods have been reported to be effective,[1] although their theoretical concepts at first glance seem to be different. A closer look, however, shows that the stimulating agents as well as the conditioning alarms aim at the central nervous system. If one looks at the effect of desmopressin in reducing urinary volume, this therapy is incompatible with the concepts mentioned above. This contradiction can be solved if we presume that the effect of desmopressin is at least in part caused by its action on the central nervous system. It is noteworthy to recall that different types of vasopressin receptors exist, with the renal V2 receptor being the main target for AVP-mediated renal water handling. Mediated mainly through the action of aquaporin-2, water is reabsorbed when AVP levels rise.[17] Another type of AVP receptor, the V1 receptor, has been shown to be located in liver, blood vessels, myocytes, and brain. In contrast to the relatively well-characterized vascular action of V1a receptors, little is known about the role of central V1b receptors. However, it has been accepted for many years that AVP and desmopressin, as well as some of their metabolites,[18] exhibit numerous effects on the central nervous system. In particular, the effect on short-term memory has been established in animal models and in humans.[19] Interestingly, middle- and long-term memory remained unaffected. This suggests that the effect of desmopressin results from an activation of the arousal system that keeps the organism more attentive but does not influence the chemical process of middle- and long-term memory. Because this would further provide an explanation why desmopressin works in enuretic children during sleep, it seems reasonable to assume that the efficacy of desmopressin is not mainly caused by its effect on V2 receptors but rather on its action on central V1 receptors. Children on desmopressin might have a higher capability to perceptuate signals from the bladder in comparison to the situation when they are off the medication and sleep is deeper. Because the arousal system is located in the brainstem, it is not surprising that electroencephalogram studies in children with nocturnal enuresis have remained inconclusive. This region of the brain is hardly accessible with this method when looking at the sleep stages alone. More advanced electroencephalogram research[20] focusing on arousability has shown an immature pattern in children with nocturnal enuresis. When considering this aspect, all the different concepts of treating nocturnal enuresis seem to have a common basis, namely the stimulation of the arousal system. A defective receptor function or an impairment of the signal transduction pathway could explain why children suffering from nocturnal enuresis have elevated AVP levels. This could further explain why fluid management is not compromised and why in enuretic children the additional application of desmopressin is effective in preventing bedwetting. We therefore suggest further studies investigating the molecular action of desmopressin. Furthermore, molecular genetic studies, possibly revealing a linkage of nocturnal enuresis to genes related to the function or regulation of central V1 receptors seem to be warranted and most promising.

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EXPERIMENTATION TRUMPS OBSERVATION

Three different meta-analyses of observational data have concluded that estrogen replacement therapy (ERT) decreases the risk of coronary heart disease (CHD), but in a . . . randomized, controlled trial of 2763 postmenopausal women with established coronary disease, treatment with estrogen plus progestin did not reduce the rate of CHD events (eg, nonfatal myocardial infarction or CHD-related death). These findings are a sobering reminder of the limitations of observational research.

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Submitted by Student