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Urinary Excretion of Immunoreactive Vasopressin in Prepubertal Children. Lack of Correlation with Urinary Excretion of Immunoreactive Neurophysins*

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Abstract. The excretion of immunoreactive vasopressin (AVP) and neurophysins was determined in 24 h urine samples from 62 normal healthy children of various ages.

Five groups of children were selected: group I ($n = 7$, aged 2 to 3 years), group II ($n = 13$, aged 3 to 5 years), group III ($n = 16$, aged 5 to 7 years), group IV ($n = 16$, aged 7 to 9 years), group V ($n = 10$, aged 9 to 11 years).

The method used for urine AVP determination consisted of an extraction using a procedure slightly modified from that of Miller and Moses [14], coupled to a radioimmunoassay.

The following urinary AVP excretions were obtained:

group I mean: 70.8 ng/m^2/24 h range: 51–150 ng
group II mean: 54.1 ng/m^2/24 h range: 17–113.6 ng
group III mean: 55.2 ng/m^2/24 h range: 18–106 ng
group IV mean: 39.9 ng/m^2/24 h range: 11.7–77.9 ng
group V mean: 39.4 ng/m^2/24 h range: 25.8–64 ng

The excretion of AVP was significantly correlated to the daily urinary osmolality ($P < 0.001$) whether expressed in ng/24 h ($r = 0.41$) or in ng/m^2/24 h ($r = 0.47$).

Neurophysins excretion ranging between 7 and 1,278 ng/24 h is too variable to allow interpretation.

Key words: AVP – Neurophysins – Urines – Prepubertal healthy children

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Introduction

Although in the last few years radioimmunoassays (RIA) have been developed to measure plasma arginine vasopressin (AVP) and neurophysins (Np), the measurement of AVP excretion remains a good method of studying neurohypophyseal function in man and has been extensively performed in adults [1, 5, 6, 9, 13, 14, 16] because few specific and sensitive plasma AVP assays are available.

Nevertheless little information on such determination in children is available and the aim of the present study was:

i) to measure the daily excretion of immunoreactive AVP in children and to investigate the relationship between AVP excretion and urinary osmolality;

ii) to find the best way to express the results (in relation to age, urinary creatinine excretion, or body surface area);

iii) to investigate a possible relationship between urinary AVP and urinary total immunoreactive neurophysins.

Material and Methods

The study was carried out in 62 boys and girls (healthy and taking no drugs) aged from 2 to 11 years who were living a normal life and ate and drank according to their usual habits. In each case the mother's informed consent was obtained.

Urine was collected over a 24 h period during which voided urine specimens were kept at + 4° C. At the end of the collection period, the weight and height of each child was recorded.

The following parameters were measured:

— total urine volume
— osmolality (Fiske osmometer)
— creatinine

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Table 1. Clinical and laboratory data of 62 normal children divided into five age groups (mean ± SEM and range)

Group	Weight (kg)	Height (cm)	Urine volume ml/day	Urine osmolality mmol/kg H_2O	Urinary creatinine mg/24 h	AVP ng/24 h	AVP pg/mg creat.	AVP $ng/m^2/day$	Np ng/24 h
I (2–3 years) ($n=7$)	12.5 ± 0.4 (12–13.5)	89 ± 1.9 (82–95)	350 ± 34.7 (200–500)	888 ± 97 (601–1202)	202.3 ± 22 (107–273)	37.8 ± 6.9 (17.7–72)	180.4 ± 15.3 (143–264)	70.8 ± 14.5 (51–150)	200 ± 67.8 (7–500) ($n=6$)
II (3–5 years) ($n=13$)	16.2 ± 0.4 (14–19)	102.8 ± 1.1 (95–110)	431 ± 43 (205–725)	886 ± 59 (534–1184)	283.1 ± 21.5 (159–450)	36.1 ± 5.2 (12–72.7)	150.4 ± 32 (34.2–452.7)	54.1 ± 8 (17–113.6)	371.8 ± 108.6 (11–1070) ($n=11$)
III (5–7 years) ($n=16$)	20 ± 0.7 (14–25)	115.7 ± 1.4 (103–124)	493 ± 31 (210–670)	879 ± 43 (500–1174)	380.1 ± 28.4 (169–638)	43 ± 6.4 (13.7–90.7)	120.7 ± 20 (30.2–203.8)	55.2 ± 8.6 (18.5–106)	254.9 ± 73.7 (11–910) ($n=13$)
IV (7–9 years) ($n=16$)	26.9 ± 1.1 (22–34)	128.9 ± 1.1 (122–137)	565 ± 42 (370–900)	842 ± 47 (458–1144)	527.1 ± 32.6 (250–758)	39.5 ± 4.8 (10.5–78.2)	77.2 ± 8.5 (18.9–130.3)	39.9 ± 4.5 (11.7–77.9)*	245 ± 63.2 (11–836) ($n=14$)
V (9–11 years) ($n=10$)	32.9 ± 1.8 (23–39)	136.7 ± 1.9 (130–148)	743.5 ± 56.5 (540–1190)	797 ± 43 (630–1058)	626.2 ± 33.5 (536–832)	41.6 ± 3 (29.7–63.2)	67.2 ± 6 (50.5–112)	39.4 ± 3.5 (25.8–64)**	373 ± 115.7 (11–1278)

* $P<0.02$ group I versus group IV; ** $P<0.05$ group I versus group V

Aliquots were acidified to pH 4.5 and frozen until AVP concentration was measured using an extraction procedure slightly modified from that of Miller and Moses [14], coupled to a radioimmunoassay described previously [1].

The validity of the method was established from firstly the mean recovery of added vasopressin (30 → 100 pg/ml; $n=25$). This averaged 90.2 ± 2%: recovery and results were therefore not corrected for this factor.

Secondly, the between-assay coefficient of variation (C.V.) was 7.9% ($n=40$) and the within-assay C.V. 7.1% ($n=19$) for urinary AVP concentrations ranging between 12 and 145 pg/ml. Thirdly, a comparison of the results obtained by the RIA and the biological method [1] using the ethanol anesthetized rat showed an excellent correlation ($r=0.96$, $P<0.001$, $n=16$).

Urinary neurophysins were measured by radioimmunoassay as described previously [11, 12], using bovine neurophysin II as standard and labelled antigen, and antiserum A_5 IV which allows the measurement of total immunoreactive neurophysins (i.e. vasopressin-carrier neurophysin and oxytocin-carrier neurophysin). This assay was affected neither by changes in osmolality between 100 and 1100 mOsm/kg nor by changes in pH between 4.5 and 9. The mean intra-assay C.V. was 5.6 ± 2.1% (SD).

For part of the statistical analysis, children were divided into five groups according to the following schedule: group I aged 2–3 yr ($n=7$); group II aged 3–5 yr ($n=13$); group III 5–7 yr ($n=16$); group IV 7–9 yr ($n=16$) and group V 9–11 yr ($n=10$) (see Table 1).

Results were expressed as mean ± SEM; statistical analysis employed the Student t test.

Results

The daily AVP excretion of the 62 children on their habitual diet, fluid intake and physical activity ranged from 10.5 to 90.7 ng/24 h with a mean of 39.8 ± 2.45 ng/24 h and was correlated with the urinary osmolality ($r=0.41$, $P<0.001$, $n=62$).

When AVP excretion was calculated taking into account the body surface area, the mean daily AVP excretion was 50.2 ± 3.6 $ng/m^2/24$ h and this also correlated with the urinary osmolality ($r=0.47$, $P<0.001$, $n=62$), as shown in Fig. 1.

When the mean daily urinary AVP was estimated in relation to the creatinine output, it reached 114 ± 10 pg/mg creatinine, demonstrating a significant inverse correlation with age expressed in months ($r=-0.5$, $P<0.001$, $n=62$).

Table 1 summarizes the results after the children had been divided into five different age groups.

Between 2 and 3 years the excretion of AVP expressed in $ng/m^2/24$ h was significantly higher ($P<0.05$) than between 7 and 9 or 9 and 11 years.

Urinary total immunoreactive neurophysins ranged between 7 and 1278 ng/24 h and no correlation was found either between urinary Np and urinary osmolality, or between urinary AVP and Np.

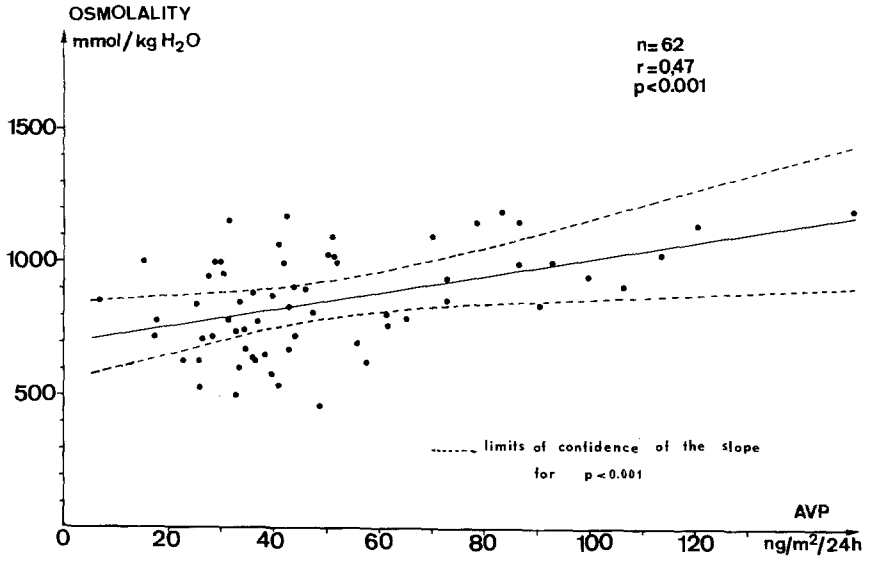

Fig. 1. Relationship between urinary osmolality and AVP excretion (ng/m^2/24 h) in 62 normal children aged from 2 to 11 years. Y = 3.12 X + 699, r = 0.47, $P < 0.001$

Discussion

The values for daily urinary AVP obtained in this study are almost the first to be reported in children. In two previous communications, the AVP excretion has been reported to be 4 to 32 mU/24 h (i.e. 10 to 128 ng/24 h where 1 μU = 2.5 pg) in 7 children without specified ages [4] and 37.1 ± 5.6 (SD) ng/m^2/day in 14 newborn infants [7]. Therefore no comparison is possible between the results of these authors and those reported in the present study.

In similar conditions we have previously found [1] AVP excretion ranging from 25 to 140 ng/24 h with a mean value of 76.4 ± 5.4 ng/24 h in 29 healthy adults aged between 22 and 42 years; there was no statistically significant difference between men and women. Consequently the mean excretion of AVP/24 h is significantly lower ($P < 0.001$) in children than in adults.

The urinary osmolality in children correlates with the daily urinary AVP excretion. We have previously found a similar correlation in adults ($r = 0.56, P < 0.001$, $n = 29$), though the regression line in the case of the adults is parallel to and below that of the children. Although the explanation is unknown, a higher urea and salt load connected with the diet of children can be suggested since it has been established that urea plays an important role in the constitution of the cortico-papillary osmotic gradient.

To find a more satisfactory way of expressing AVP excretion in children, we studied excretion in relation to various parameters:

— when daily AVP output is expressed in relation to creatinine excretion it displays a significantly inverse correlation with age ($r = -0.5$, $P < 0.001$, $n = 60$). But this is not a satisfactory mode of expression because below the age of twelve, creatinine excretion increases relatively more rapidly than does the excretion of other metabolites. This makes the normal excretion value of other metabolites expressed in relation to creatinine highly and falsely age-dependent [2].

— it has been proposed that in children the urinary output of a variety of metabolites and hormonal compounds should be assessed in relation to the body surface area. For instance, a lower excretion of aldosterone in children less than 2 years old has been found when compared to adults [15] but after correction for the surface area the aldosterone excretion of the children approximates that of the adult. So expressing AVP excretion in children in relation to body surface area shows that the mean AVP excretion reaches 50.2 ± 3.6 ng/m^2/24 h whereas the mean excretion in adults is 44 ± 3.3 ng/m^2/24 h (n = 22); these figures are not significantly different. If the children are divided into five groups, Table 1 shows that in each group the values expressed in this way, though widely scattered, decrease gradually between 2 and 11 years and that group I is statistically different from groups IV and V. On the contrary, when expressed simply in ng/24 h, the mean values range between 36.1 ± 5.2 ng/24 h for the lowest (group II) and 43 ± 6.4 ng/24 h for the highest (group III) and there is no difference between each group.

Daily immunoreactive neurophysins excretion ranged between 7 and 1,278 ng/24 h under the conditions of the assay. Such values are too variable to allow an interpretation and no correlation between urinary AVP and urinary neurophysins excretion was found. Since there is no reason to question the validity of the radioimmunoassay [12], two factors at least can be hypothesised: first, that the fate and metabolism of AVP and neurophysins are different; and second, that the RIA used measures total immunoreactive neuro-

physins. Moreover, under the same conditions a similar scatter of urinary neurophysins excretion has been described in adults [10, 12]. Therefore this measurement is of little practical interest under the described conditions.

In conclusion, as AVP secretion in blood is episodic [8, 17] a daily urinary measurement can be regarded as a good means of integrating vasopressin secretion rate, as in the case of LHRH [3]. Furthermore, urinary neuropeptide measurements are less sensitive to variations induced by various stresses.

This study is thus the first to report normal urinary AVP excretion rates in children aged between 2 and 11 years and seems to indicate that it is more useful to express the results in ng/m^2/day than in ng/24 h.

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