CLINICAL STUDY

Posterior pituitary (PP) evaluation in patients with anterior pituitary defect associated with ectopic PP and septo-optic dysplasia

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Abstract

Objective: Controversies exist about posterior pituitary (PP) function in subjects with ectopic PP (EPP) and with cerebral midline defects and/or their co-occurrence. We investigate water and electrolyte disturbances in patients at risk for PP dysfunction.

Design: The study was conducted in a single Pediatric Endocrinology Research Unit.

Methods: Forty-two subjects with childhood-onset GH deficiency were subdivided into five groups: normal magnetic resonance imaging ($n=8$, group 1); EPP ($n=15$, group 2); septo-optic dysplasia (SOD) with normal PP ($n=4$, group 3); EPP and SOD without ($n=7$, group 4), and with additional midline brain abnormalities ($n=8$, group 5). At a mean age of 16.0 ± 1.1 years, they underwent a 120 min i.v. infusion with hypertonic 5% saline and evaluation of plasma osmolality (Posm), arginine vasopressin (AVP), thirst score (in groups 1 and 2), and urinary osmolality were performed.

Results: Mean Posm and AVP significantly increased from baseline scores (284.7 ± 4.9 mosm/kg and 0.6 ± 0.2 pmol/l) to 120 min after saline infusion (300.5 ± 8.0 mosm/kg and 10.3 ± 3.3 pmol/l, $P < 0.0001$). Group 5 showed higher mean Posm and lower mean AVP at all time points ($P < 0.0001$). Mean thirst score did not show a significantly different trend between the groups 1 and 2. Urine osmolality was above 750 mosm/kg in all but seven patients after osmotic challenge.

Conclusions: Patients with midline brain abnormalities and EPP have defective osmoregulated AVP. Patients with EPP and congenital hypopituitarism have normal PP function.

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Introduction

Central diabetes insipidus (CDI), thirst abnormalities and water and electrolyte disorders have been reported in patients with structural hypothalamic–pituitary (HP) abnormalities and cerebral midline defects by magnetic resonance imaging (MRI). Controversies exist, however, regarding neurohypophyseal function in subjects with ectopic posterior pituitary (EPP), the most common condition associated with congenital anterior pituitary deficiencies (1–5). Several studies have shown abnormal PP function, ranging from reduced vasopressin release after osmotic challenge, hypodipsia, or polydipsia (6) to persistent nocturnal enuresis or symptomatic CDI, in both familial (7) and sporadic cases of hypopituitarism associated with either isolated GH deficiency (IGHD) or multiple pituitary hormone deficiencies (MPHD) (6, 8, 9). Other studies which focused on the assessment of anterior pituitary function in patients with EPP have

never reported water and electrolyte disturbances or CDI in these patient populations (1, 2, 10–15).

Osmoregulation defects have been identified in a number of congenital cerebral midline abnormalities (16–20). Specifically, septo-optic dysplasia (SOD) has been reported from its earliest descriptions as a condition associated with disorders of both anterior and PP function (4, 21–23). Moreover, several patients with SOD have a combination of midline defects and structural HP abnormalities such as anterior pituitary hypoplasia, pituitary stalk hypoplasia/agenesis with or without EPP (4) in the presence or absence of PP dysfunction; however, the diagnosis of these patients' various hypothalamic–PP disorders in the studies was based on standard procedures only. The etiology of SOD remains puzzling although the transcription factor homeobox gene expressed in embryonic stem cell (*HESX1*) might be involved (24).

The aim of our study is to investigate the relationship between PP function and its anatomy in determining

water and electrolyte disturbances in patients with or without structural HP abnormalities in the presence or absence of cerebral midline defects. As standard procedures may fail or be unreliable for the identification of subtle water and electrolyte disorders, hypertonic saline and thirst score tests were used to provide useful information on hypothalamic–PP function.

Materials and methods

Study population

Forty-two subjects (19 females, 23 males) who were diagnosed during childhood with GHD ($n=23$; eight with idiopathic GHD and normal MRI, 15 with congenital GHD and EPP, and one previously diagnosed with familial form of CDI and EPP at MRI) or with SOD and pituitary hormone deficiencies including GHD ($n=19$) were enrolled prospectively from the Pediatric Endocrinology Unit (Fig. 1). Their daily fluid intake and

daily urinary output was evaluated 48 h before dynamic PP evaluation and ranged between 1750 and 2800 ml and 1200 and 2100 ml (lower than 30 ml/kg body weight in all subjects) respectively.

All were on adequate hormonal replacement at the time of recruitment to the study. The clinical and biochemical diagnosis of hypopituitarism was followed by a detailed MRI study of the HP and brain structures. The diagnosis of SOD was made by means of three criteria: the presence of optic nerve hypoplasia (ONH), of midline neuroradiological abnormalities (such as agenesis of the corpus callosum and/or absence of the septum pellucidum), and of pituitary hypoplasia with pituitary hormone deficiency (21, 25–27). Owing to recurrent episodes of hyponatremia and seizures, treatment with desmopressin acetate (desamino-D-arginine-8-vasopressin (DDAVP)) was discontinued during the follow-up period (from 6 months to 3 years before the study) in six patients with SOD previously diagnosed with CDI; in these patients the diagnosis of CDI was made based on a random plasma osmolality (Posm) above 295 mosm/kg

Figure 1 MRI and endocrine features of 42 patients with GH deficiency. IGHD, isolated GH deficiency; MPHD, multiple pituitary hormone deficiencies; CDI, central diabetes insipidus; EPP, ectopic posterior pituitary; NPP, normally located posterior pituitary; SOD, septo-optic dysplasia; AP, anterior pituitary; PP, posterior pituitary; PS, pituitary stalk; ONH, optic nerve hypoplasia; CC, (thin) corpus callosum; OC, (absence of) optic chiasm; IM, (absence of) interthalamic mass; PH, pons hypoplasia; SP, (agenesis of) septum pellucidum.

and a low urine osmolality during childhood in other hospitals. DDAVP treatment was also discontinued in the patient with familial CDI the night before the study.

The diagnosis of GHD was made at a mean age of 6.3 ± 2.6 years, based on a peak GH of $10 \mu\text{g/l}$ after at least two pharmacological stimulation tests, including insulin tolerance test (ITT) and glucagon or arginine test. Mean IGF1 at diagnosis was -3.11 ± 0.97 standard deviation score (SDS) and mean height SDS was -3.13 ± 0.71 according to Tanner's growth standards. The clinical and biochemical characteristics of the patients are summarized in Table 1.

All subjects underwent an evaluation of pituitary function. Central hypothyroidism was defined as low/inadequately low TSH concentration (reference values $0.5\text{--}4.0 \text{ mIU/l}$) with low free thyroxine (FT_4 ; below 8.0 ng/l). ACTH deficiency was defined as either a morning serum cortisol concentration of 100 nmol/l or an impaired cortisol serum concentration peak value of 550 nmol/l during ITT. Central hypogonadism was confirmed in both sexes by lack of puberty and no increase in serum FSH and LH (or slight increase with peak $\text{LH} < \text{FSH}$) after i.v. administration of GNRH. Ultrasonography was used to identify female patients with prepubertal internal genitalia. Basal serum samples were taken for determination of IGF1.

The hormonal work-up revealed IGHD in 21 subjects and MPHD in 21 subjects, one of whom was a female with familial CDI. All patients were being treated with recombinant human GH at conventional dosages and subjects with MPHD were receiving conventional therapy for pituitary defects with good adherence as revealed by normal serum concentration of FT_4 and cortisol: 1--T_4 $75\text{--}200 \mu\text{g/day}$, hydrocortisone $15\text{--}20 \text{ mg/day}$ in two to three separate doses, testosterone enanthate $150\text{--}250 \text{ mg i.m.}$ every 2 or 3 weeks for male subjects, and ethinyl estradiol or transdermal

17β -estradiol patches with medroxyprogesterone acetate for female subjects. In the patient with familial CDI and DDAVP was administered orally three times daily.

MRI evaluation

Sagittal and coronal T1- and T2-weighted MRI images with $2\text{--}3 \text{ mm}$ sections were obtained in all patients at the time of diagnosis and evaluated by the same experienced neuroradiologist (A R), who was blinded to all clinical and hormonal findings. The study cohort was subdivided into five groups according to their MRI features (Fig. 1). Eight subjects with IGHD showed a normal HP region (group 1). An isolated EPP was found in 15 subjects (group 2, eight IGHD, six MPHD, and one MPHD with familial CDI; father affected). Four subjects (group 3, two IGHD and two MPHD) showed SOD with normal PP location. EPP was associated with SOD in 15 patients, of whom seven (three IGHD and four MPHD) without (group 4) and eight MPHD with (group 5) additional and variable combination of brain abnormalities including hypoplasia of anterior commissure and/or the mesencephalon and/or pons hypoplasia, and/or absence or hypoplastic optic chiasm and/or absence of interthalamic mass (Table 1).

Dynamic PP function evaluation

At a mean age of 16.0 ± 1.1 years, all subjects underwent an i.v. osmotic challenge with hypertonic 5% saline (NaCl , concentration 855 mmol/l) infused at a rate of 0.05 ml/kg per min, according to the method described by Baylis & Robertson (28).

To maintain adequate hydration, free access to food and fluids was allowed up to the morning of the tests.

Table 1 Clinical data of 42 subjects affected by GHD with or without septo-optic dysplasia and/or hypothalamic–pituitary abnormalities. Data are shown as mean $\pm$ s.d.

	Study population						P
	Overall	Group 1	Group 2	Group 3	Group 4	Group 5	
Subjects (M/F)	42 (23/19)	8 (5/3)	15 (9/6)	4 (2/2)	7 (5/2)	8 (2/6)	
GHD diagnosis							
Age (years)	6.3 ± 2.6	8.6 ± 1.4	5.8 ± 2.3	2.9 ± 0.5	7.2 ± 2.1	5.2 ± 2.8	0.0027
Bone age (years)	3.9 ± 2.3	6.1 ± 1.3	3.5 ± 2.2	1.0 ± 0.0	4.5 ± 2.0	3.2 ± 2.4	0.0045
Height (SDS)	-3.13 ± 0.71	-2.91 ± 0.85	-3.11 ± 0.56	-3.63 ± 0.59	-3.09 ± 0.42	-3.23 ± 1.03	NS
Hormone defects (IGHD/MPHD)	20/21	8/0	8/7	2/2	3/4	0/8	
Peak GH to ITT/glucagon ($\mu\text{g/l}$)	2.2 ± 1.3	2.2 ± 1.1	2.4 ± 1.2	1.7 ± 0.6	1.3 ± 1.1	2.9 ± 1.8	NS
Peak GH to arginine ($\mu\text{g/l}$)	2.1 ± 2.7	2.7 ± 4.0	1.5 ± 1.1	1.2 ± 0.3	1.3 ± 1.0	3.9 ± 4.1	NS
IGF1 (SDS)	-3.11 ± 0.97	-2.76 ± 0.83	-2.98 ± 0.64	-2.53 ± 0.57	-3.76 ± 0.93	-3.36 ± 1.48	NS
At time of study							
Age (years)	16.0 ± 1.1	15.9 ± 0.5	17.1 ± 1.0	17.0 ± 0.3	17.2 ± 1.6	16.9 ± 0.8	NS
BMI (kg/m^2)	21.4 ± 1.8	20.8 ± 2.0	21.5 ± 1.5	22.4 ± 1.7	21.2 ± 1.9	21.7 ± 2.0	NS

Group 1, normal MRI; Group 2, EPP; Group 3, NPP and SOD; Group 4, EPP and SOD; Group 5, EPP, SOD and brain abnormalities; MRI, magnetic resonance imaging; EPP, ectopic posterior pituitary; NPP, normally located posterior pituitary; SOD, septo-optic dysplasia; M, males; F, females; IGHD, isolated GH deficiency; MPHD, multiple pituitary hormone deficiencies; ITT, insulin tolerance test; BMI, body mass index; NS, not significant.

The six SOD patients previously treated with DDAVP were classified into groups 3 ($n=1$), 4 ($n=2$), and 5 ($n=3$).

The patients remained supine for 30 min before and throughout the test. A peripheral venous line was placed in a forearm vein and samples for Posm and arginine vasopressin (AVP) measurement were collected at time 0 and after 30, 60, 90, and 120 min of 5% saline infusion. A thirst score, based on a 10 cm long visual analog scale according to the method described by Thompson *et al.* (29, 30), was calculated in groups 1 and 2 subjects (without visual impairment and/or developmental delay). Only at the end was free access to oral fluids allowed and thirst score was calculated after 10, 20, and 30 min in groups 1 and 2. Urine samples were collected before the start of saline infusion and by the end of the test for measurement of urinary osmolality (Uosm), urine output and the Uosm/Posm ratio.

All studies were performed in the morning starting between 0800 and 0830 h. The study design was approved by local Ethics Committees and written informed consent was obtained from all patients before all procedures.

Assays methods

Posm was measured by the depression of freezing point method (Advanced Instruments Osmometer, model 3320, Norwood, MA, USA; resolution 1 mosm/kg, repeatability: s.d. ≤ 2 mosm/kg between 0 and 400 mosm/kg). Plasma AVP was determined by RIA after extraction from plasma by adsorption onto magnesium silicate (Florisil, U.S. Silica, Berkeley Springs, WV, USA), with intra- and inter-assay coefficient of variation 9.7 and 15.3%, respectively; limit of detection, 0.3 pmol/l (31). All hormonal measurements were carried out by means of standard chemiluminescence. All samples from each individual subject were analyzed together at the same time. After centrifugation at 4 °C, plasma was separated and stored at -20 °C.

Statistical analysis

Descriptive statistics were reported as means and s.d. for quantitative variables, while qualitative variables were described as absolute frequencies. Anthropometric data were expressed as absolute values or SDS, calculated with the formula (observed value – mean value for age and sex)/s.d. Pearson's correlation coefficient was estimated to evaluate the association among age, body mass index (BMI), and each clinical variable considered (namely: Posm, AVP, and thirst score). Time variation in mean values of blood concentration of clinical variables was evaluated by the ANOVA for repeated measures. A P value of <0.05 was considered statistically

significant. All tests were two-sided. Statistical analyses were performed by Stata for Windows Statistical Software (Stata release 11.0, Stata Corporation, College Station, TX, USA).

Results

PP function evaluation

The i.v. osmotic challenge was associated with an increase in Posm value above 295 mosm/kg (level requested in the diagnostic work-up of CDI) (32) with no rise in plasma AVP levels confirming CDI in the female subject of group 2 with familial CDI and EPP, who was thus excluded from the statistical analysis.

In the remaining 41 subjects, mean Posm and AVP significantly increased significantly after 120 min of saline infusion ($P<0.0001$). The temporal trend of increase was significantly different among the five groups ($P<0.0001$) as shown in Fig. 2. Group 5 subjects showed significantly higher mean Posm at all time points of infusion compared with the other groups ($P<0.0001$; Fig. 2). Group 4 subjects showed a significantly higher mean Posm at the time points between 0 and 90 min (time 0', $P=0.01$; 30', $P<0.0001$; 60', $P<0.0001$; 90', $P=0.01$; 120', $P=0.066$; Fig. 2).

While no significant difference was observed at baseline in mean AVP concentrations among the five groups, AVP reached the lowest levels in group 5 at all time points ($P<0.0001$; Fig. 2). AVP concentrations in group 4 was not statistically different from those in groups 1, 2, and 3 at each time point.

In all 41 patients, mean Posm/AVP ratio decreased significantly at 120 min ($P<0.0001$), with different temporal trends between the groups ($P=0.001$). Group 5 subjects demonstrated significantly higher Posm/AVP ratios at all time points compared with the others ($P<0.0001$; Fig. 2).

Posm, AVP and their ratio did not statistically differ between subjects with MPHD and IGHD in all study groups (data not shown). No gender differences were detected among all evaluated parameters.

Mean thirst score increased significantly in groups 1 and 2 during the osmotic challenge (time 0', 1.4 ± 0.4 cm and 120', 8.2 ± 0.9 cm) and progressively returned to near baseline levels after 30 min of free oral fluid access (1.4 ± 0.4 ; $P<0.0001$), without statistical differences (Fig. 2).

Baseline inter-individual urine osmolality was extremely variable ranging from 75 to 560 mosm/kg. Urine osmolality at the end of the hypertonic saline test was above 750 mosm/kg in all patients with the exception of one from group 4 (438 mosm/kg) and six from group 5 (425, 432, 441, 458, 482, and 564 mosm/kg respectively). In these patients, neither polydipsia nor polyuria had been previously documented. Mean Uosm and Uosm/Posm ratio were significantly lower in group 5

Figure 2 Mean plasma osmolality (Posm), vasopressin (AVP), Posm/AVP ratio, and thirst score during osmotic challenge and free fluid access. See text for all P values. Open diamond, group 1: normal MRI; open circle, group 2: isolated EPP without other HP anomalies; filled triangle, group 3: normal posterior pituitary with SOD; open triangle, group 4: EPP with SOD; open square, group 5: EPP with SOD and additional brain abnormalities.

compared with the others at both baseline and after osmotic challenge, while no differences were observed in mean urine output among the five groups (although group 5 showed a slightly higher urinary volume not reaching statistical significance). All biochemical data during the osmotic challenge are summarized in Table 2.

Correlations

A significant association between Posm and vasopressin response was found at each time point of the osmotic challenge test, independent of the MRI picture. The association was equally significant in subjects with normal MRI (group 1: $r=0.84$, $P<0.0001$), isolated EPP (group 2: $r=0.67$, $P<0.0001$), isolated SOD (group 3: $r=0.83$, $P<0.0001$), or EPP plus SOD (group 4: $r=0.79$, $P<0.0001$) with the increase in Posm never exceeding 305 mosm/kg. The association between Posm and vasopressin response was weaker in those with SOD, EPP, and additional brain abnormalities (group 5: $r=0.51$, $P=0.02$) with high baseline osmolality and a delayed (Posm >300 mosm/kg) and inadequate increase of AVP (Fig. 3). The normal range of AVP for Posm values is represented in Fig. 3 with shaded areas based on the report by Robertson (32).

No statistically significant correlation was found among Posm, AVP, and thirst score (groups 1 and 2) or between each of these variables and age or BMI.

Discussion

Although assessing PP function by determining AVP response to graded osmotic stimulation is a well-recognized procedure (33), this study is, to our knowledge, the first one designed with the purpose of systematically evaluating the relationship between PP function and brain neuroanatomy in patients at risk for various water disorders. Despite the current awareness of water homeostasis impairment ranging from subtle defects of PP function to complete forms of CDI and/or hyponatremia in patients with cerebral midline defects (including SOD) (4, 16–18, 21–23), the underlying pathophysiologic mechanisms have not yet been clearly defined (34).

The results for baseline Posm in our cohort suggest that PP function was not affected either in subjects with GHD and normal PP location or in those with EPP or SOD and normally located PP. Conversely, subjects with SOD and EPP or with SOD, EPP, and complex brain abnormalities showed baseline Posm values suggestive of osmoregulation impairment of thirst and/or water intake.

In our study, hypertonic saline infusion proved to be a reliable and very well-tolerated approach to the assessment of PP function because it requires less time than a standard water deprivation test and offers the chance to explore the hemodynamic effects of AVP osmoregulation. Subjects belonging to groups 1, 2, and 3 showed a similar curve slope with a mean Posm below 295 mosm/kg from baseline to 90 min after saline infusion with further increase at the time point of 120 min. Conversely, the increase in mean Posm documented within the first hour of saline infusion in group 5 as well as the slightly higher urine output

Table 2 Biochemical evaluation and thirst score of study subjects before and after the i.v. osmotic challenge. Data are shown as mean $\pm$ s.d.

	Study population						P
	Overall	Group 1	Group 2	Group 3	Group 4	Group 5	
i.v. osmotic challenge time 0'							
Na ⁺ (mEq/l)	139.6±5.1	136.4±1.9	136.2±1.9	136.8±3.5	139.3±3.2	144.8±2.7	<0.0001
Posm (mosm/kg)	284.7±4.9	281.6±3.0	282.1±3.3	281.8±6.2	287.3±2.1	291.4±1.8	<0.0001
AVP (pmol/l)	0.6±0.2	0.8±0.3	0.6±0.2	0.8±0.3	0.5±0.0	0.5±0.0	NS
Posm/AVP	500.9±126.9	422.8±151.6	483.9±133.3	424.0±167.8	574.6±4.1	582.8±3.5	
Thirst score (cm)	1.4±0.4	1.3±0.4	1.4±0.4	NE	NE	NE	NS
Uosm (mosm/kg)	412.4±120.6	507.2±124.3	489.5±153.6	477.6±148.5	463.8±145.9	107.8±32.7	<0.0001
i.v. osmotic challenge time 30'							
Posm (mosm/kg)	289.7±5.0	286.4±1.8	286.2±2.2	287.5±5.1	293.6±1.3	296.8±2.9	<0.0001
AVP (pmol/l)	1.8±0.6	2.1±0.5	2.0±0.5	1.5±0.4	2.2±0.4	1.1±0.6	<0.0001
Posm/AVP	193.5±125.6	141.9±34.2	147.1±31.9	204.3±64.3	135.6±19.8	371.3±195.0	<0.0001
Thirst score (cm)	2.8±0.6	2.8±0.6	2.8±0.6	NE	NE	NE	NS
i.v. osmotic challenge time 60'							
Posm (mosm/kg)	295.0±7.2	289.9±1.2	290.4±2.1	290.8±4.2	297.7±1.8	307.6±1.5	<0.0001
AVP (pmol/l)	3.6±1.0	3.8±0.7	3.5±0.7	3.8±0.6	4.4±1.3	2.6±0.7	<0.0001
Posm/AVP	89.4±27.0	77.9±12.4	85.5±14.9	79.4±15.0	72.2±20.7	127.5±30.8	<0.0001
Thirst score (cm)	5.9±1.3	5.5±1.1	6.2±1.3	NE	NE	NE	NS
i.v. osmotic challenge time 90'							
Posm (mosm/kg)	298.1±7.3	293.6±2.3	294.1±2.6	294.0±4.5	298.4±2.9	311.5±1.6	<0.0001
AVP (pmol/l)	6.6±2.1	7.6±1.1	7.8±1.6	6.1±2.1	6.9±0.6	3.5±1.2	<0.0001
Posm/AVP	52.9±27.9	39.3±6.2	39.3±8.5	53.7±22.6	43.3±3.6	98.1±32.1	<0.0001
Thirst score (cm)	7.1±0.6	7.1±0.6	7.2±0.6	NE	NE	NE	NS
i.v. osmotic challenge time 120'							
Posm (mosm/kg)	300.5±8.0	296.0±3.3	296.3±3.3	295.8±5.6	300.3±2.5	315.0±2.7	<0.0001
AVP (pmol/l)	10.3±3.3	11.9±2.0	12.1±2.2	10.1±3.2	10.9±1.3	5.2±1.9	<0.0001
Posm/AVP	35.2±22.3	25.6±4.3	25.3±4.5	31.7±11.0	28.0±3.3	70.3±30.8	<0.0001
Thirst score (cm)	8.1±0.7	8.1±0.8	8.0±0.7	NE	NE	NE	NS
Uosm (mosm/kg)	802.5±170.9	889.3±84.9	884.8±76.8	855.3±68.4	807.1±167.4	541.3±144.4	<0.0001
Urine output (ml)	261.6±70.4	247.5±89.4	251.4±69.5	263.8±62.6	256.4±75.9	296.9±53.2	NS
Uosm/Posm	2.7±0.6	3.0±0.3	3.0±0.2	2.9±0.2	2.7±0.6	1.7±0.5	<0.0001
Free oral fluid access							
Thirst score (cm)	1.4±0.4	1.3±0.4	1.4±0.4	NE	NE	NE	NS

Group 1, normal MRI; Group 2, EPP; Group 3, NPP and SOD; Group 4, EPP and SOD; Group 5, EPP, SOD and brain abnormalities; MRI, magnetic resonance imaging; EPP, ectopic posterior pituitary; NPP, normally located posterior pituitary; SOD, septo-optic dysplasia; Na⁺, plasma sodium; Posm, plasma osmolality; AVP, arginine vasopressin; Uosm, urine osmolality; NS, not significant; NE, not evaluated.

(although not significant), was compatible with impairment of hypothalamic–neurohypophyseal function. In addition, despite a significant and constant trend toward an increase in Posm values during the entire test in group 5, the slope of the curves in both groups 5 and 4 was markedly dissimilar with an earlier increase in Posm overcoming the mean value of 295 mosm/kg after 60 min in group 4 compared with groups 1, 2, and 3. We think that the pattern of Posm increase, as well as the higher Posm/AVP ratio and the early marked reduction in Posm/AVP ratio at 30 min in group 4 patients compared with the other groups may represent a subtle impairment/differing sensitivity at the level of thirst and/or vasopressin osmoreceptor regulation and/or AVP secretion. Despite the fact that baseline AVP concentration was low in all subjects, the possibility of 'chronic dehydration' cannot be entirely ruled out, although clinical features and biochemical parameters such as hematocrit, blood urea, and renin activity (data not shown) were within the normal

range; this should also exclude a relevant hemodynamic effect on AVP production at the time of the study.

Low or undetectable plasma AVP concentrations were documented at baseline in all subjects despite the presence of a significantly higher mean Posm in groups 4 and 5; this suggests that (except in the case of patients with hypernatremia and/or baseline Posm above 295 mosm/kg) neither AVP nor random Posm measurements are sensitive enough to identify patients with impairment of PP function. In addition, during hypertonic saline infusion, mean plasma AVP levels were not dissimilar among groups 1, 2, 3, and 4 at all time points, whereas group 5 patients showed the lowest AVP concentrations.

The AVP levels reached by group 5 compared with the other groups, as well as the highest Posm levels documented in this cohort, are compatible with inappropriately low AVP concentrations for the corresponding Posm suggesting a relative AVP deficiency. These data, although not supported by a thirst study for

Figure 3 Regression analyses between plasma osmolality and vasopressin in subjects with: open diamond, group 1: normal MRI; open circle, group 2: isolated EPP without other HP anomalies; filled triangle, group 3: normal posterior pituitary with SOD; open triangle, group 4: EPP with SOD; open square, group 5: EPP with SOD and additional brain abnormalities. Each graph shows the cumulative responses during osmotic challenge test. Shaded area, normal range of vasopressin values (32).

patients' visual impairment, are strongly indicative of at least a defect in osmoregulation of AVP secretion. In addition, the fact that Uosm ranged from over 400 mosm/kg to more than 750 in group 5 patients supports such a hypothesis. The highest baseline Posm/AVP ratio observed in group 5 and the different pattern of decrease during hypertonic saline infusion might indicate osmoreceptor dysregulation.

What is the underlying role of PP anatomy in the regulation of water homeostasis in our cohort? Our study demonstrated that, except for the patient with familial CDI associated with EPP and hypopituitarism, isolated EPP is not responsible *per se* for thirst and electrolyte disturbances. Our patient with familial CDI and hypopituitarism associated with EPP resembles the three cases reported by Yagi *et al.* (7), suggesting that CDI, hypopituitarism and EPP probably constitute an entirely different entity; this conclusion is based, on the one hand, in the absence of AVP and wolframin gene mutation in the family (data not shown) and, on the other hand, on normal PP function and thirst score in all group 2 patients with congenital hypopituitarism and EPP. The identification of PP hyperintensity in the patient with EPP and CDI is online with what it has been reported in the autosomal dominant forms of CDI where the presence of PP hyperintensity does not necessarily indicate that the functional integrity

of the hypothalamic–neurohypophyseal axis is preserved (15–17).

Our data in patients with hypopituitarism associated with EPP are in contrast with the few published papers on PP function which show milder to overt forms of CDI (6, 8, 9). In particular, compared with those with larger EPP or those with orthotopic PP, subjects with MPHD and small EPP size (<5 mm in T1 MRI images) have been described with various forms of PP dysfunction (8). In another report, data from an i.v. osmotic challenge showed that ten of 12 patients with EPP and anterior pituitary defects had persistent enuresis and/or nearly absent or unpredictable AVP response in the absence of overt CDI (6); CDI has also been documented after water deprivation test in 43% of patients with hypopituitarism and small EPP in a recent paper (9) (Table 3).

Systematic PP evaluation has never been carried out in patients with SOD and all reported studies focus on the presence or absence of optic nerve abnormalities, the presence or absence of septum pellucidum or corpus callosum without categorization based on additional brain defects. In our study, the 'SOD model', thanks to its heterogeneity and to the detailed neuroimaging examination, offers an excellent opportunity to evaluate the difference in osmoregulatory response in subjects with or without EPP and in those with EPP and severe brain abnormalities. While our patients with SOD and normal PP location showed PP function that was not dissimilar from patients with hypopituitarism and isolated EPP (group 2), hyperosmolality characterizes those patients with SOD and EPP and those with complex brain anomalies, indicating that SOD patients with EPP are more prone to developing osmoreceptor disturbances. In these latter patients the identification of PP hyperintensity was compatible with the diagnosis of hypothalamic dysfunction rather than with CDI, the latter being associated with a loss of PP signal (15–17).

Our data cannot be compared with those described in the literature (4, 23, 35) as the frequency of CDI in SOD has been reported in as high as 6–38% of patients with SOD (Table 3). In particular, among previously described patients, 38% of 24 patients with SOD showed CDI, although a water deprivation test was performed in only 12 of the 24 subjects (23). It is worth pointing out that five of these latter CDI patients had an impaired sense of thirst and hypernatremia treated with DDAVP and followed by at least one or more episodes of seizures and hyponatremia; this may indicate that as is the case for our six patients, the water disturbances were most likely due to osmoreceptor/thirst abnormalities rather than to a complete AVP deficiency. In a large cohort of 170 patients with congenital hypopituitarism with or without ONH, CDI was reported in 13% of patients, with a higher prevalence (27%) in those with ONH compared with the 5% of those without ONH (4). In the paper by Cameron *et al.* (35), CDI was reported in six (24%) of 25 patients with SOD with variable midline abnormalities including corpus callosum and/or septum pellucidum

Table 3 Comparison between results of previous reports evaluating posterior pituitary function in subjects with different hypothalamic–pituitary abnormalities.

Papers	Subjects	MRI	Hormone defects	Procedure	Results
Lukezic <i>et al.</i> (6)	15 (3 excluded from analysis)	APH, EPP, PSA, or thin PS	11 MPHD 4 IGHD	5% NaCl infusion	10 subjects with abnormal AVP secretion (worse in MPHD)
Yamanaka <i>et al.</i> (8)	20 complete GHD	Transected pituitary stalk and small (<5mm), large (>5mm), or no visible EPP	13 MPHD 7 IGHD	Water deprivation 5% NaCl infusion	Lower Uosm and AVP in small EPP Overt CDI when no visible EPP Lower peak AVP in small EPP
Li <i>et al.</i> (9)	125	EPP, absence of PPBS, normally located PP (CDI patients)	125 MPHD of whom 48 CDI	Water deprivation	CDI in 43% with small EPP and in all but one with absent PPBS
Mehta <i>et al.</i> (4)	170	APH, PSA, EPP, corpus callosum, and septum pellucidum abnormalities, ONH (84 subjects), in various combination	96% GH 29% FSH/LH 56% TSH 53.3 ACTH deficiency 13% CDI	Water deprivation in symptomatic patients	13% CDI (27% of those with ONH and 5% without ONH)
Masera <i>et al.</i> (22)	24	SOD ± agenesis of corpus callosum (C _T data)	20 GH 14 ACTH 10 FSH/LH deficiency	Water deprivation (performed in 12)	9 (38%) CDI, of whom 5 with hypodipsia and hypernatremia poorly responsive to DDAVP treatment, followed by hyponatremic seizures
Cameron <i>et al.</i> (32)	40	Cerebral midline abnormalities (of whom 18 SOD without and seven SOD with septum pellucidum abnormalities)	26 MPHD 10 IGHD 4 normal GH	Water deprivation in symptomatic patients Morning Posm and Uosm in asymptomatic patients	6 CDI out of 25 SOD (5 without and 1 with septum pellucidum abnormalities)
Antonini <i>et al.</i> (33)	18	Cerebral midline abnormalities (of whom 11 SOD)	72% GH 66% TSH 55% FSH/LH 27% CDI	Water deprivation	27% CDI

APH, anterior pituitary hypoplasia; EPP, ectopic posterior pituitary; PSA, pituitary stalk agenesis; PS, pituitary stalk; MPHD, multiple pituitary hormone deficiency; IGHD, isolated GH deficiency; AVP, arginine vasopressin; PPBS, posterior pituitary bright spot; CDI, central diabetes insipidus; ONH, optic nerve hypoplasia; SOD, septo-optic dysplasia; C_T, computed tomography.

involvement. Finally, in the series by Antonini *et al.* (36), in 18 patients with midline cerebral defects, five subjects (27%) showed deficiencies of both anterior and posterior function, with one of them affected by hypodipsia and hypernatremia.

The limitations of our study are the relatively small size of the cohort in such rare diseases and the low number of patients with SOD and normal PP location; the latter situation meant that it was not possible to draw a firm conclusion for this group and this in turn means that a multivariate analysis was not feasible. However, we believe that the information gleaned from the data clearly outweigh these limitations. In particular, the lack of thirst assessment due to visual impairment in patients with SOD made it impossible for us to completely rule out eventual damage of the central osmoreceptors involved in thirst perception

which are anatomically distinct from those regulating AVP (37). In addition, the clinical implications in the management of SOD patients with electrolyte disturbances and hypothalamic dysfunction are quite significant to avoid incorrect diagnoses and possibly harmful treatment. DDAVP treatment is not advisable in these patients to avoid hyponatremia leading to seizures. Regular monitoring of water intake and serum electrolytes represents the best management in the presence of relative AVP deficiency while low DDAVP dosage should be reserved to patients with adipsia or to those who might develop polyuria and polydipsia.

In conclusion, our data suggest that the most common PP dysfunction is hyperosmolality and relative AVP deficiency characterizing patients with SOD, complex brain abnormalities and EPP; this type of dysfunction seems to involve damage to the

osmoreceptors that regulate AVP synthesis and possibly the PP. Our study also confirms that patients with EPP and congenital hypopituitarism have normal PP function.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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