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Epinephrine and dDAVP administration in patients with congenital nephrogenic diabetes insipidus

Evidence for a pre-cyclic AMP V_2 receptor defective mechanism

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Epinephrine and dDAVP administration in patients with congenital nephrogenic diabetes insipidus. Evidence for a pre-cyclic AMP V_2 receptor defective mechanism. We recently showed that the administration of the antidiuretic V_2 specific agonist, 1-desamino[8-D-arginine]vasopressin (dDAVP), to seven male patients with congenital nephrogenic diabetes insipidus (CNDI) did not cause a decrease in blood pressure nor an increase in plasma renin activity or factor VIIIc or von Willebrand factor release. In normal subjects, plasma renin activity, coagulation factors and plasma cyclic AMP are stimulated not only by dDAVP but also by the administration of epinephrine. In the present study, we measured tissue plasminogen activator (activity and antigenicity), von Willebrand factor multimers, plasma and urinary cyclic AMP concentrations following dDAVP or epinephrine administration. We infused epinephrine into three male patients with CNDI. Factor VIIIc and tissue plasminogen activator augmented by 75 to 100% and von Willebrand Factor multimers were increased; plasma renin activity and plasma cyclic AMP concentration increased by 200%. None of these values changed when the same subjects as well as eleven other male patients with CNDI received dDAVP. Furthermore, dDAVP administration increased plasma cyclic AMP concentrations in normal subjects, but not in 14 male patients with CNDI. These results demonstrate the specificity of the extrarenal V_2 receptor defect expressed in our patients. The lack of a plasma cyclic AMP response to the administration of dDAVP would suggest an altered pre-cyclic AMP stimulation mechanism.

The antidiuretic hormone arginine-vasopressin (AVP) interacts with two types of receptors: V_1 receptors, which are present in blood vessels, liver and anterior pituitary, and mediate the vasopressor, metabolic and adrenocorticotrophic hormone releasing responses to the administration of AVP; and V_2 receptors, present in the kidney distal nephron, which are responsible for the antidiuretic response to AVP administration [1]. V_1 receptors are linked to the phosphoinositol second messenger system, whereas V_2 receptors activate adenylate cyclase [1]. Congenital nephrogenic diabetes insipidus is a rare X-linked disorder associated with renal tubular resistance to AVP [2]. Male patients do not give an antidiuretic response to the administration of either arginine-vasopressinor the strong antidiuretic (V_2) agonist 1-desamino[8-D-arginine]vasopressin (dDAVP); their renal V_2 receptor responses are abnormal. However, their V_1 receptor responses are apparently intact since the administration of AVP induces skin blanching, abdominal cramps, and the stimulation of the synthesis of prostaglandin E_2 [3, 4]. We recently showed that in male patients with congenital nephrogenic diabetes insipidus, blood pressure did not decrease and that plasma renin activity (PRA) and the release of the coagulation factors, Factor VIIIc (FVIIIc) and von Willebrand factor (vWF), did not increase in response to the administration of dDAVP [5]. Hemodynamic responses to the administration of [4-valine,8-D-arginine]vasopressin (VDAVP), another selective V_2 agonist, have been observed in anephric dogs [6], and an increase in the concentrations of the coagulation factors has been observed in anephric patients [7]. The lack of hemodynamic and coagulation factor responses to the administration of dDAVP in patients with congenital nephrogenic diabetes insipidus would thus suggest an abnormal extrarenal V_2 receptor response.

The specificity and the mechanism(s) underlying these altered renal and extrarenal V_2 receptor responses are unknown. The studies presented here were designed to answer the following questions: 1) are the defective coagulation factor responses specific for dDAVP administration, that is, could normal coagulation factor responses to the administration of epinephrine be shown in patients with congenital nephrogenic diabetes insipidus; and 2) are plasma cyclic adenosine monophosphate (cyclic AMP) measurements a possible indicator of a pre-cyclic AMP stimulation defect in these patients. If plasma cyclic AMP is stimulated by epinephrine and by dDAVP in normal subjects but only by epinephrine in patients with congenital nephrogenic diabetes insipidus, a defect at a pre-cyclic AMP step is implied. In our earlier study, we measured the hemodynamic and FVIIIc and vWF responses to dDAVP administration in a smaller group of male patients with congenital nephrogenic diabetes insipidus [5]. In addition, in the present study, we measured t-PA activity, antigenicity, vWF multimers, plasma and urinary

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cyclic AMP concentrations following dDAVP or epinephrine administration.

Methods

Study subjects

One of the three groups of subjects we studied consisted of 14 male patients with congenital nephrogenic diabetes insipidus (4 to 45 years old, mean age 23.6 ± 3.0 years). Seven of these patients have been described in a previous report [5]. Three new patients belong to the archetype Nova-Scotian "Hopewell" pedigree [8]. All have a documented lifelong history of polyuria and polydipsia, normal or elevated plasma concentration of AVP and dilute urine despite the administration of AVP or dDAVP. The baseline plasma concentration of arginine-vasopressin in these patients who had free access to water was 4.2 ± 1.0 pg/ml. Plasma arginine-vasopressin was measured by radioimmunoassay [9].

The second study group consisted of 11 women who were obligatory carriers of the gene for congenital nephrogenic diabetes insipidus (25 to 69 years old, mean age 42.7 ± 3.8 years). Six of these women have been described in a previous report [5]. Three, who were new mothers, belong to the "Hopewell" pedigree [8]. All these subjects were apparently in good health with no record of overt polyuria or polydipsia. The baseline plasma concentration of arginine-vasopressin in these patients was 1.31 ± 0.32 pg/ml.

The third group contained five normal subjects, one woman and four men (24 to 30 years old). Three subjects were studied two times (at least 12 months apart) and a total of eight dDAVP infusions were performed.

Epinephrine administration: Hemodynamic, coagulation factor and cyclic AMP measurements

Three brothers (aged 29 to 31 years old) with congenital nephrogenic diabetes insipidus received an intravenous infusion of epinephrine (4 to 5 μg per kg of body wt during 20 to 25 min). This dosage has been previously shown to stimulate the release of FVIIIc and tissue plasminogen activator (t-PA) in normal subjects [7]. Arterial blood pressure (by sphigmo-manometry) and pulse rate were recorded every 10 minutes before the epinephrine infusion, every two minutes during the 20 to 25 minute infusion, every five minutes during 25 to 60 minute period after the infusion, and every 10 minutes thereafter. The electrocardiogram was constantly monitored during the test. The infusion was discontinued when systolic blood pressure increased to a value higher than 150 mm Hg. The first two patients studied received 5 μg/kg body wt during 25 minutes; their systolic blood pressure remained below 145 mm Hg. In the third patient, twenty minutes after the beginning of the infusion, systolic blood pressure increased to 152 mm Hg; the epinephrine infusion was discontinued and the systolic blood pressure returned to baseline values within the next three minutes. No premature ventricular beats occurred in any of the patients.

After a 30-minute equilibration period, two to three baseline blood samples were obtained (−60, −30, and 0 minutes), epinephrine was infused from 0 to 20 or 25 minutes and additional blood samples were obtained at 30, 60, 90 and 120 minutes. Plasma concentrations of FVIIIc, vWF multimers, t-PA, cyclic AMP, glucose and potassium, and plasma renin activity were determined. Blood samples were analyzed for FVIIIc on the day of the test using a one-stage coagulation assay as previously described [5]. Alternatively, plasma samples were frozen at −80°C and analyzed later with a chromogenic method [10] (Coatest Factor VIII kit, KabiVitrum AB, Stockholm, Sweden). Samples analyzed by the two methods gave identical results (FVIIIc [one stage] = 1.039 FVIII [Coatest] − 1.672; $N = 42$, $r = 0.89$).

The von Willebrand factor multimers were analyzed by agarose electrophoresis [11] using a 0.75% stacking gel and a 1.5% running gel. Samples were diluted 1:4 in 100 mM Tris, 100 mM EDTA, 2% SDS, 8 M urea, 0.005% bromophenol blue (pH 6.8). After heating at 56°C for 15 minutes, 30 μl of treated sample was applied to the electrophoretic gel and left overnight at 4°C in running buffer solution (50 mM Tris, 0.38 M glycine, 1% SDS, pH 8.35) and a current of 8 to 10 mA. The multimers were then electroblotted at 100 mA and 4°C as described by Furlong and Peake [12] using a transfer buffer containing 0.01% SDS described by Battle et al [13]. The electroblot was washed for five minutes in Tris buffered saline + Tween 20 (TBST; 10 mM Tris, 150 mM NaCl, 0.05% Tween 20) and then blocked with 1% BSA in TBST for one hour. The membrane was then incubated in 100 ml of TBST containing 225 μl of rabbit anti-VIII/vWF (Behring Diagnostics, Marburg, FRG) for 1.5 hours, then rinsed three times in TBST for five minutes each. The membrane was then incubated in 75 ml of alkaline phosphatase conjugated, goat anti-rabbit IgG for 30 minutes, washed three times in TBST for 10 minutes each and stained with the Protoblot alkaline phosphatase color development substrates (Promega, Madison, Wisconsin, USA).

Tissue plasminogen activator antigen was measured in plasma obtained from citrated blood samples (immediately centrifuged at 4°C at 9,500 × g and stored at −80°C until analyzed). These samples were not acidified. An Elisa method, similar to the Imubind-5 kit (American Diagnostic, Inc., New York, New York, USA) was used. Miniature polystyrene test tubes (Immulon 2 Removawell strips, Dynatech Laboratories Inc., Virginia, USA) were coated with goat anti-t-PA antibodies obtained from American Diagnostic, Inc. Lyophilized t-PA (KabiVitrum, Stockholm, Sweden), goat gamma-globulins (Jackson Immunoresearch Laboratories Inc., West Grove, Pennsylvania, USA), peroxydase conjugated anti-t-PA IgG (Biopool AB, Box 1454, S-90124 Umea, Sweden) and orthophenylenediamine (Sigma Chemical Co., Montreal) were also used.

For t-PA activity determinations, the citrated blood samples were immediately acidified by mixing 1 ml of the blood with 500 μl of a 1 M sodium acetate buffer solution pH 3.9 and then centrifuged at 9,500 × g for four minutes. The acidified plasma samples were stored at −80°C. The t-PA activity was measured according to a recently described photometric assay [14, 15] with the following modifications: the chromogenic substrate was S-2390 (0.45 M; KabiVitrum) and the plasminogen (0.3 Casein U/ml; Glu-plasminogen, KabiVitrum) was activated by poly-D-lysine (0.4 μM; ICN, Montreal).

Plasma cyclic AMP was measured using a commercial kit (cAMP[^{125}I] assay system, Amersham, Oakville, Ontario, Canada). Plasma concentrations of epinephrine were measured by a radioenzymatic assay [16].

Table 1. Baseline coagulation and biochemical variables in the study groups before dDAVP infusion

Group (no)	Factor VIIIc *%*	t-PA antigen *ng/ml*	t-PA activity *IU/ml*	Plasma cyclic AMP *pmol/ml*	Urinary cyclic AMP *nmol/min/1.73 m²*	Plasma renin activity *ng/ml/hr*
Normal subjects, $N = 8$	101.3 ± 10.2	3.87 ± 0.95	0.77 ± 0.15	15.96 ± 1.52	2.9 ± 0.6	1.10 ± 0.15
Obligatory carriers, $N = 11$	125.6 ± 18.0	10.1 ± 2.5[a]	0.29 ± 0.08[a]	23.48 ± 2.31[a]	2.8 ± 1.2	1.98 ± 0.39
Patients with congenital nephrogenic diabetes insipidus, $N = 14$	121.5 ± 18.1	6.74 ± 1.4	0.46 ± 0.13	24.49 ± 1.55[a]	2.9 ± 0.5	2.68 ± 0.45[a]

Values are mean ± SE.
[a] Significant differences as compared to normal subjects.

dDAVP administration: Hemodynamic, coagulation factor and cyclic AMP measurements

After an interval of at least two weeks the three patients with congenital diabetes insipidus received an intravenous administration of dDAVP (0.3 μg/kg body wt, up to a maximum dose of 24 μg) as previously described [5]. The responses to dDAVP administration were similar to those obtained in 11 other male patients with congenital nephrogenic diabetes insipidus. The results for this entire group of 14 patients as well as the responses to dDAVP administration in 11 female obligatory carriers and in 5 normal subjects are presented here. FVIIIc and plasma renin activity were measured in all the patients. Tissue plasminogen activator responses to dDAVP administration were measured in seven male patients with congenital nephrogenic diabetes insipidus, four obligatory carriers and four normal subjects. In normal subjects blood for plasma renin activity determinations was obtained at −30, 0, 10, 20, 30, 60, 90, 120, 150 minutes after dDAVP administration.

Plasma cyclic AMP responses to dDAVP administration and cyclic AMP excretion rates were measured. Urine was obtained by spontaneous voiding. One thirty-minute collection was obtained before and three sixty-minute collections were obtained after dDAVP administration (60, 120, 150 min).

Statistical analysis

All comparisons were made by analysis of variance [17]. Repeated measurements in the same group of subjects were compared with baseline values according to the method of Dunnett [18] and measurements made in different groups of subjects during the same study period were compared by Newman-Keuls analysis. A P value less than 0.05 was considered to be statistically significant. Values reported below refer to the following periods: −60, −30, 0, baseline period; 0 to 25 minutes, epinephrine or dDAVP infusion period; 30, 60, 90, 120, 150 observation period after epinephrine or dDAVP infusion. Results are reported as the mean ± SE of either absolute values or percentages of baseline values.

Protocol approval and consent

All studies were approved by our institutional ethical review committee. The normal subjects were paid and all participants in the study gave written informed consent.

Results

Baseline values for coagulation factors, plasma renin activity, plasma cyclic AMP and urinary cyclic AMP excretion rates are presented in Table 1. Baseline values for Factor VIIIc, t-PA antigen, t-PA activity and urinary cyclic AMP excretion rates were similar between normal subjects and patients with congenital nephrogenic diabetes insipidus. Plasma cyclic AMP and plasma renin activity were slightly but significantly higher in patients with congenital nephrogenic diabetes insipidus. These differences could be related to the reflex release of renin and catecholamines secondary to a decrease in extracellular fluid volume in male patients with congenital nephrogenic diabetes insipidus. Significant differences in t-PA antigen and activity and plasma cyclic AMP were also observed in obligatory carriers compared to normal subjects. We have no explanation for these differences.

The left side (A) of Figures 1, 3 and 4 relates specifically to the three patients with congenital nephrogenic diabetes insipidus who received epinephrine. The right side (B) of these figures describes the results obtained in the three groups of patients after dDAVP administration.

Epinephrine infusion

The epinephrine infusion significantly increased heart rate (67 ± 5 to 84 ± 6 beats/min), mean arterial blood pressure (84 ± 3 to 93 ± 5 mm Hg), pulse pressure (40 ± 1 to 95 ± 8 mm Hg). Plasma potassium decreased (4.0 ± 0.1 to 2.9 ± 0.2 mEq/liter) and plasma glucose increased (92 ± 2 to 148 ± 11 mg/dl). The plasma concentration of epinephrine was 1161 ± 292 pg/ml 10 minutes after the end of the epinephrine infusion.

Epinephrine produced a twofold rise in FVIIIc and t-PA antigen (Fig. 1), t-PA activity rose by 300% (Fig. 1) and vWF multimers increased (Fig. 2). Plasma renin activity rose by 130% (Fig. 3) and plasma cyclic AMP by 250% (Fig. 4). The increases in FVIIIc and t-PA were equal to those previously reported in normal subjects after epinephrine administration [7]. Epinephrine is a difficult drug to infuse due to untoward side-effects, and, since statistically significant results were obtained with three patients, we decided not to test a larger number of subjects and to use historical normal controls [7].

dDAVP infusion

In normal subjects, dDAVP administration increased FVIIIc concentrations by 250%, t-PA antigenicity by 350% and t-PA activity by 1000% (Fig. 1), vWF multimers were increased (Fig. 2) and plasma renin activity rose twofold (Fig. 3). A significant inverse correlation ($r = -0.76$) was calculated between mean arterial blood pressure and plasma renin activity. Plasma cyclic AMP concentration increased by 60% (Fig. 4).

These responses were not observed in 14 patients with congenital nephrogenic diabetes insipidus and variable (usually

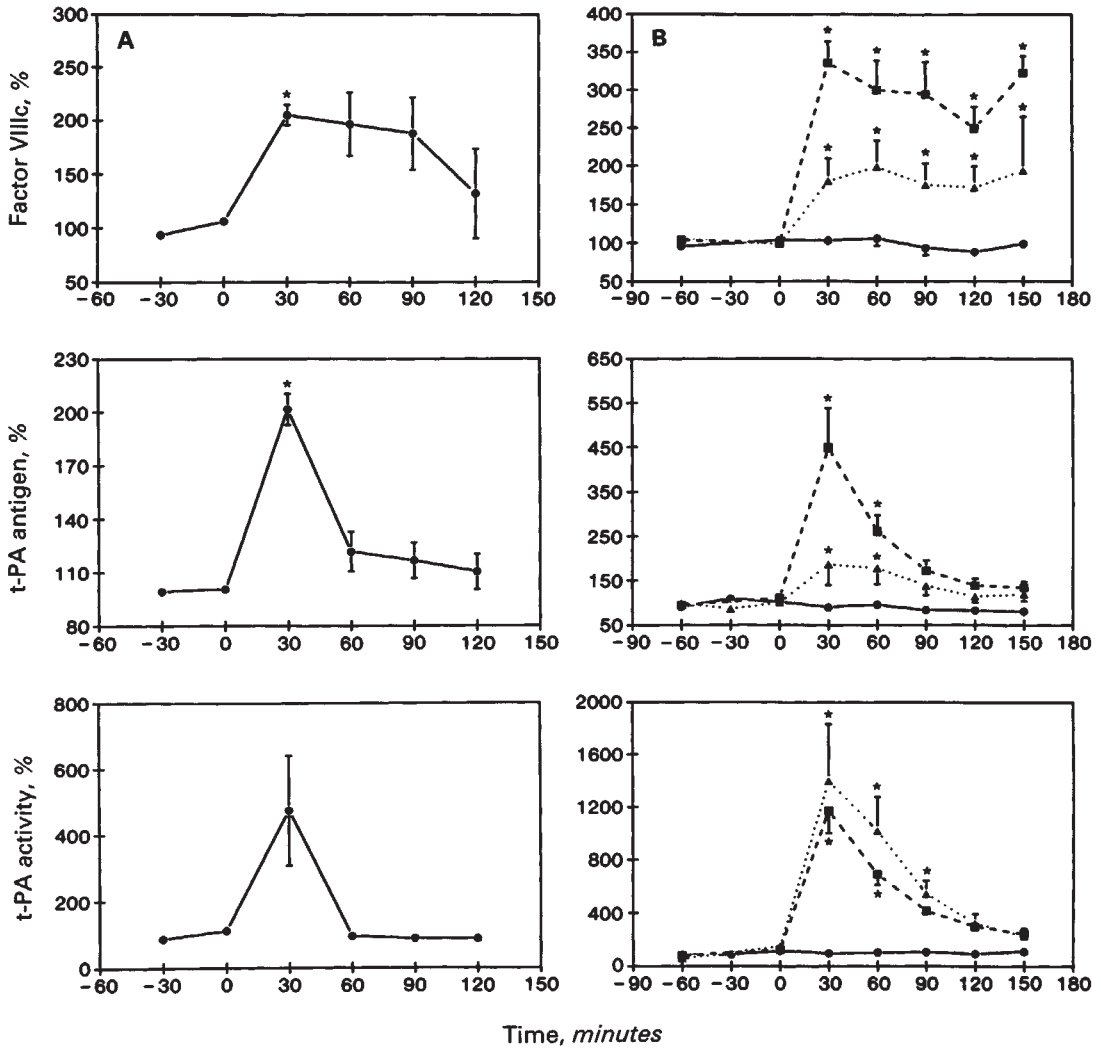

Fig. 1. *FVIIIc, t-PA antigen and t-PA activity responses to epinephrine* (**A**) *and dDAVP* (**B**) *infusions.* A. Epinephrine (4 to 5 μg per kilogram of body wt) was infused from 0 to 25 minutes into 3 patients with congenital nephrogenic diabetes insipidus (●). B. dDAVP (0.3 μg per kg of body wt) was infused from 0 to 20 minutes into 14 patients with congenital nephrogenic diabetes insipidus (●), 11 obligatory carriers of the nephrogenic diabetes insipidus gene (▲) and 5 normal subjects (8 studies) (■). Asterisks indicate significant differences from baseline values (at −60, −30 and 0 minutes). Error bars are contained within the symbol when not visible.

half) responses were observed in obligatory carriers. Plasma renin activity and t-PA activity responses were not intermediate in obligatory carriers. The changes in FVIIIc concentration, t-PA antigenicity and activity, plasma renin activity and plasma cyclic AMP concentration in the group with congenital nephrogenic diabetes insipidus were significantly different from those in the other two groups.

Urinary cyclic AMP excretion rates were unchanged after dDAVP administration in normal subjects (baseline: 2.9 ± 0.6 nmol/min/1.73 m^2; 3 consecutive 60-min urine collections post-dDAVP: 2.8 ± 0.3, 2.6 ± 0.5, 3.0 ± 0.7 nmol/min/1.73 m^2) and in patients with congenital nephrogenic diabetes insipidus (baseline: 2.9 ± 0.5 nmol/min/1.73 m^2; 3 consecutive 60-min urine collections post-dDAVP: 4.2 ± 0.6, 3.2 ± 0.5, 4.3 ± 0.6 nmol/min/1.73 m^2). Significant positive correlations were calculated in normal individuals between urinary cyclic AMP and urinary creatinine concentrations (r = 0.94) and between urinary cyclic AMP concentrations and urinary osmolality (r = 0.89).

Discussion

This study shows that in patients with congenital nephrogenic diabetes insipidus receiving epinephrine the concentrations of the coagulation factors increased to equal those previously reported in normal subjects after epinephrine administration [7]. In this regard, Mannucci et al [7] demonstrated an 80% increase in Factor VIIIc and plasminogen activator after a 6 μg/kg epinephrine infusion in six normal subjects. Similar increases were obtained in our three patients with congenital nephrogenic diabetes insipidus (Fig. 1). In contrast, there were no increases in the concentrations of the coagulation factors in response to dDAVP administration in either the same subjects or the 14 male patients with congenital nephrogenic diabetes insipidus. In an earlier study, we measured FVIIIc and vWF

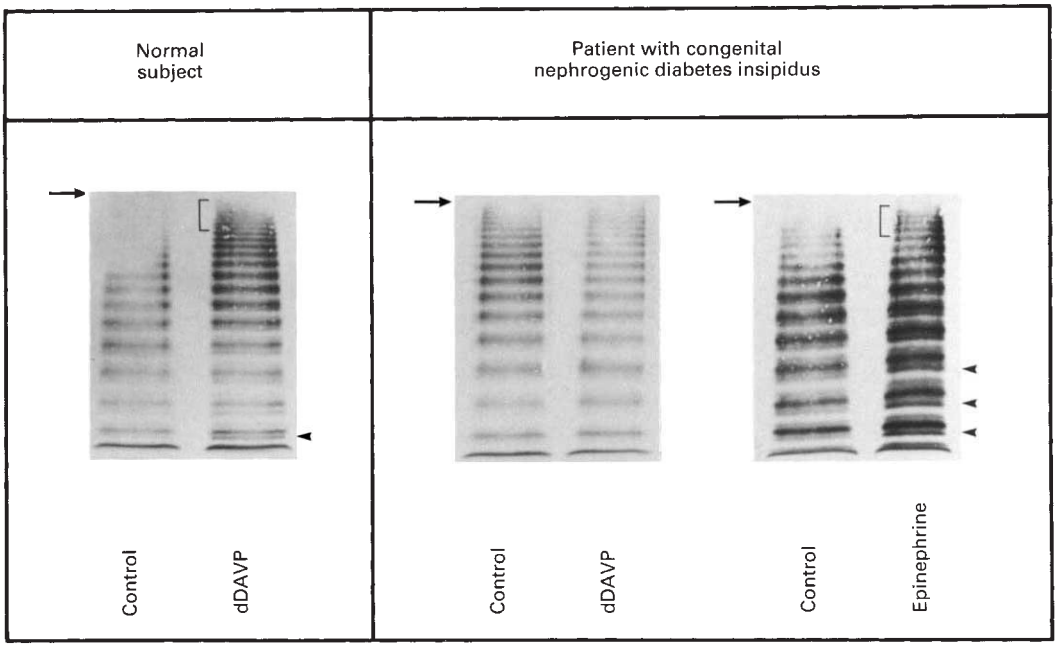

Fig. 2. *NaDodSO$_4$-agarose electrophoresis of plasma von Willebrand factor in normal subjects and patients with congenital nephrogenic diabetes insipidus.* dDAVP induced multimers (indicated by a parenthesis) in normal subjects but not in patients with congenital nephrogenic diabetes insipidus who however had an increase of multimers after epinephrine administration. dDAVP also induced the formation of doublet-like structures (arrows). Similar structures were also observed after epinephrine administration in patients with congenital nephrogenic diabetes insipidus. Plasma samples were applied at the top of the gel (arrow at the top of each figure).

responses to dDAVP administration in a smaller group of seven male patients with congenital nephrogenic diabetes insipidus [5]. In addition, in the present study we measured t-PA activity, antigenicity, vWF multimers, plasma and urinary cyclic AMP concentrations following dDAVP or epinephrine administration.

In 1961, Ingram discovered that the injection of epinephrine into healthy volunteers was followed by a short-term rise in the coagulant activity of Factor VIII [9]. Subsequently, Mannucci et al reported a transient increase in t-PA following a 6 μg/kg epinephrine infusion in normal subjects [7]. Tissue plasminogen activator, a serine proteinase with high specificity for plasminogen, is also released after dDAVP administration. The magnitude of the t-PA response to dDAVP administration usually correlates with those of FVIIIc and vWF in normal subjects [20]. The release of t-PA following dDAVP administration is not accompanied by signs of systemic activation of the fibrinolytic system or hyperplasminemia. Therefore, the release is most probably secondary to a marked decrease in the plasma concentrations of the naturally occurring, fast acting inhibitor of t-PA [21]. The administration of dDAVP also induces the appearance in plasma of larger than normal vWF multimers which could be associated with the shortening of the bleeding time observed after dDAVP administration [21]. The mechanisms by which the administration of epinephrine or dDAVP release these coagulation factors from the vascular endothelium are not well understood, but specific receptor-mediated responses seem to be involved. The t-PA and FVIIIc responses to epinephrine administration were diminished or abolished by propranolol pre-treatment [22, 23], an observation in favor of a beta-receptor-mediated response. Also the coagulation responses observed after dDAVP were not modified by propranolol, aspirin or naloxone, suggesting that beta-adrenergic mechanisms, prostaglandins and beta endorphins mediators were likely not involved [24, 25].

Male patients with congenital nephrogenic diabetes insipidus, that is, with malfunctioning renal V_2-receptor responses, give no coagulation responses to dDAVP administration [5, 26, 27]. Since in the same patients, the responses to epinephrine are not impaired, a V_2 specific receptor-mediated defect is implied. Endothelial vascular cells could bear these putative V_2 receptors. Alternatively dDAVP, through a V_2 receptor mechanism, may stimulate the release of an unknown mediator acting on the release of FVIIIc, vWF and t-PA. These extrarenal V_2 receptor mechanisms are defective in patients with congenital nephrogenic diabetes insipidus.

Plasma renin activity was stimulated by epinephrine but not by dDAVP administration in patients with congenital nephrogenic diabetes insipidus. In normal subjects, mean arterial pressure decreased after dDAVP administration and an inverse correlation was observed between mean arterial blood pressure and plasma renin activity. It is unlikely that the renin release observed after dDAVP in normal individuals might be mediated by a reflex release of catecholamines (secondary to a decrease in mean arterial blood pressure) since Schwartz et al demonstrated that [4-valine,8-D-arginine]vasopressin (VDAVP), another selective V_2 agonist decreased mean arterial blood pressure and stimulated plasma renin activity after propranolol administration [28]. The beta-adrenergic stimulation of juxtaglomerular cells is thus unlikely to account for the renin-stimulating properties of dDAVP. An alpha-adrenergic renin-stimulating mechanism is also unlikely since: 1) a decrease in

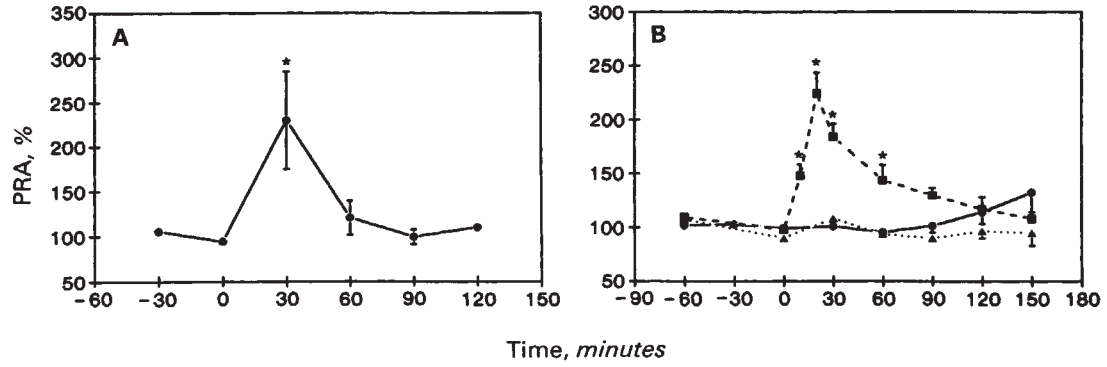

Fig. 3. *Plasma renin activity responses to epinephrine and to dDAVP.* **A.** Epinephrine (4 to 5 μg per kg of body wt) was infused from 0 to 25 minutes into 3 patients with congenital nephrogenic diabetes insipidus (●). **B.** dDAVP (0.3 μg per kg of body wt) was infused from 0 to 20 minutes into 14 patients with congenital nephrogenic diabetes insipidus (●), 11 obligatory carriers of the nephrogenic diabetes insipidus gene (▲) and 5 normal subjects (8 studies) (■). Asterisks indicate significant difference from baseline values (at −60, −30 and 0 minutes).

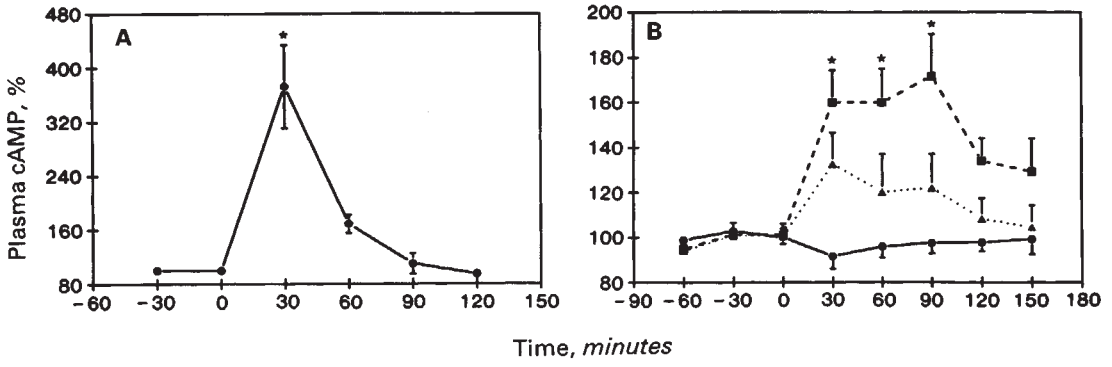

Fig. 4. *Plasma cyclic AMP responses to epinephrine and to dDAVP.* **A.** Epinephrine (4 to 5 μg per kg of body wt) was infused from 0 to 25 minutes into 3 patients with congenital nephrogenic diabetes insipidus (●). **B.** dDAVP (0.3 μg per kg of body wt) was infused from 0 to 20 minutes into 14 patients with congenital nephrogenic diabetes insipidus (●), 11 obligatory carriers of the nephrogenic diabetes insipidus gene (▲) and 5 normal subjects (8 studies) (■). Asterisks indicate significant differences from baseline values (at −60, −30 and 0 minutes).

systemic vascular resistance was observed after dDAVP or VDAVP [28]; and 2) renal blood flow (measured with radioactive microspheres) did not change after VDAVP administration [6].

Mean arterial blood pressure did not change after dDAVP administration in patients with congenital nephrogenic diabetes insipidus and plasma renin activity remained stable. However, epinephrine administration stimulated plasma renin activity. The stimulating effect of epinephrine administration is likely secondary to the hemodynamic effects and to the direct action on the juxtaglomerular cells. This stimulation of renin activity also favors a V_2-receptor specific defect in patients with congenital nephrogenic diabetes insipidus. We did not observe a stimulation of plasma renin activity after dDAVP administration in 11 female obligatory carriers of the gene for nephrogenic diabetes insipidus. The random inactivation of the X chromosome bearing the defective gene would predict a variability in the renin responses to dDAVP administration in these patients. In a large number of patients, half the normal response should be obtained. Plasma levels of AVP were within the normal range (1.31 ± 0.32 pg/ml) in our 11 obligatory carrier patients and were not in favor of a receptor desensitization mechanism [5]. We are unable to explain the complete absence of plasma renin activity stimulation in the obligatory carriers.

The antidiuretic action of vasopressin is mediated by the sequential steps of vasopressin binding to its cell surface receptor followed by the receptor mediated stimulation of adenylate cyclase. Guanine nucleotide binding proteins also intervene in the transduction process [29]. It is not known which part of the transduction process is deficient in patients with congenital nephrogenic diabetes insipidus. Mice with congenital nephrogenic diabetes insipidus have a defect in renal medullary cyclic AMP catabolism [30, 31]. However, this defect is probably not present in humans with congenital nephrogenic diabetes insipidus, since in humans the disease is an X-linked inheritance rather than a non-X-linked trait as in mice [32]. Uttley, Atkinson and Adams assessed urinary excretion of cyclic AMP in response to Pitressin (vasopressin) administration in three patients with congenital nephrogenic diabetes insipidus, four carriers and seven controls [33]. They found no significant difference in cyclic AMP excretion between the three groups nor any significant increase in urinary excretion after stimulation by Pitressin administration. They found a close correlation between urinary cyclic AMP excretion and both urinary concentration and urinary creatinine excretion, suggesting that the bulk of the cyclic AMP in the urine derives from glomerular filtration both in the waterloaded and in the urinary concentrated states [33]. In contrast, cyclic AMP uri-

nary excretion rate increased 10- to 30-fold following stimulation by the administration of parathyroid hormone. This finding proved to be a useful diagnostic test for pseudohypoparathyroidism [34]. Parathyroid hormone administration normally stimulates urinary cyclic AMP excretion in patients with congenital nephrogenic diabetes insipidus, ruling out a guanine-nucleotide-binding stimulatory protein deficient mechanism [35]. We are not aware of previous reports of plasma cyclic AMP measurements after dDAVP administration in patients with congenital nephrogenic diabetes insipidus.

In our studies, urinary cyclic AMP excretion rates were unchanged after dDAVP administration in normal subjects and in our patients with congenital nephrogenic diabetes insipidus. We thus confirmed that measurements of urinary cyclic AMP are of no value in differentiating between normal individuals and our patients resistant to AVP and, like Uttley et al [33], we found a close correlation betwen urinary cyclic AMP and both urinary concentration and urinary creatinine. In contrast, in normal subjects, plasma cyclic AMP concentrations significantly increased after dDAVP administration, but no response was observed in patients with congenital nephrogenic diabetes insipidus and a minimal response was measured in obligatory carriers. The increased plasma concentration of cyclic AMP observed after dDAVP administration was not secondary to the reflex release of epinephrine since epinephrine plasma concentrations were unchanged after dDAVP administration in normal subjects [36]. The defective plasma cyclic AMP response to dDAVP administration observed in patients with congenital nephrogenic diabetes insipidus was specific, since plasma cyclic AMP concentration increased after epinephrine administration in these patients. Our results thus suggest that a precyclic AMP stimulation mechanism is defective in patients with congenital nephrogenic diabetes insipidus. Providing that the guanine-nucleotide-binding protein step is normal [35], the defective gene in the patients we studied with congenital nephrogenic diabetes insipidus is likely to code for a defective V_2 receptor.

In summary, patients with congenital nephrogenic diabetes insipidus have a selective defect in the V_2-mediated stimulation of the release of the coagulation factors, since the release of these factors is normally stimulated by the administration of epinephrine. The administration of dDAVP, a selective V_2 agonist, stimulated plasma cyclic-AMP release in normal subjects, but not in our patients with congenital nephrogenic diabetes insipidus and minimal responses were obtained in obligatory carriers. These results favor a pre-cyclic-AMP-altered stimulation mechanism in our patients with congenital nephrogenic diabetes insipidus.

Acknowledgments

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