Renal prostaglandins and water balance: studies in normal volunteer subjects and in patients with central diabetes insipidus

R. DÜSING, R. HERRMANN, K. GLÄNZER, H. VETTER, A. OVERLACK AND H. J. KRAMER
Medizinische Universitäts-Poliklinik, Bonn, West Germany

(Received 29 September 1980/11 February 1981; accepted 19 February 1981)

Summary

1. In six healthy volunteer subjects polydipsic water loading significantly increased urine volume from 1203 ± 242 (SEM) to 5072 ± 320 ml/24 h (P < 0.001) with a significant decrease in urinary osmolality. This increase in urine volume by more than fourfold was associated with a slight increase in urinary excretion of prostaglandin E₂ from 466 ± 66 to 1017 ± 174 pmol/24 h (P = 0.05).

2. In five patients with central diabetes insipidus mean urine volume of 10 838 ± 107 ml/24 h was reduced to 1205 ± 204 ml/24 h (P < 0.001) by treatment with 1-desamino-8-arginine vasopressin (desamino-[Arg⁸]vasopressin; 15 µg/day) with a significant rise in urinary osmolality. Desamino-[Arg⁸]vasopressin treatment was associated with a significant increase of suppressed urinary excretion of prostaglandin E₂ (PGE₂) in four of these patients from 246 ± 66 to 2643 ± 677 pmol/24 h (P < 0.01).

3. Concomitant treatment with indomethacin in addition to desamino-[Arg⁸]vasopressin significantly suppressed urinary excretion of PGE₂ and significantly increased urinary osmolality as compared with treatment with desamino-[Arg⁸]vasopressin alone.

4. Desamino-[Arg⁸]vasopressin significantly increased urinary excretion of adenosine 3':5'-cyclic monophosphate (cyclic AMP). However, there was no further change in urinary excretion of cyclic AMP during concomitant indomethacin treatment.

5. The results suggest that urine flow itself is not an important determinant of urinary PGE₂ excretion. In patients with central diabetes insipidus the urinary concentrating response to desamino-[Arg⁸]vasopressin is enhanced during inhibition of prostaglandin synthesis without changes in urinary excretion of cyclic AMP.

Key words: arginine vasopressin, desamino-arginine vasopressin, diabetes insipidus, kallikrein, plasma renin activity, prostaglandins, renal function.

Abbreviations: ANG, angiotensin; cyclic AMP, adenosine 3':5'-cyclic monophosphate; desamino-[Arg⁸]vasopressin, l-desamino-8-arginine vasopressin; GFR, glomerular filtration rate; PG, prostaglandin(s); PGE, prostaglandin E; PGE₂, prostaglandin E₂; PRA, plasma renin activity; [Arg⁸]vasopressin, arginine vasopressin.

Introduction

In addition to a still controversial role of renal prostaglandins (PG) in the regulation of renal sodium excretion [1], accumulating evidence suggests that the renal PG system participates in the regulation of renal water excretion. High prostaglandin synthase activity is present in the renal medulla [2] and can be located in the renomedullary interstitial cells [3–5] and the collecting duct epithelium [6, 7]. Studies in vitro in the toad urinary bladder [8] and the isolated collecting duct [9] have demonstrated that prostaglandin E (PGE) inhibits vasopressin-induced generation of adenosine 3':5'-cyclic monophosphate (cyclic AMP) by inhibiting vaso-
pressin-activated adenyl cyclase. In addition, studies in vivo in acutely hypophysectomized dogs have shown that the renal concentrating response to exogenous arginine vasopressin ([Arg⁸]vasopressin) was markedly enhanced when the dogs were pretreated with the prostaglandin synthesis inhibitor, indomethacin [10]. Further, studies in vitro in rabbit renomedullary interstitial cell cultures [5] and the toad urinary bladder [11] have demonstrated that [Arg⁸]vasopressin itself is a stimulator of PGE biosynthesis in these tissues. Thus the concept of a short-loop feedback between [Arg⁸]vasopressin and renal PG has been proposed in which [Arg⁸]vasopressin--itself stimulates synthesis of its antago-

nist prostaglandin E₂ (PGE₂) in the collecting-
duct epithelial cells [11, 12]. This idea has received further support by the finding that rats of the Brattleboro strain with geec central diabetes insipidus excrete minimal amounts of PGE₂ in their urine which can be significantly increased when polyuria is normalized by treatment with [Arg⁸]vasopressin or the [Arg⁸]vaso-
presin-analogue 1-desamino-8-arginine vasopres-

sin (desamino-[Arg⁸]vasopressin) [13–15]. The present studies were therefore designed to investigate the renal PG system in healthy volunteer subjects during normal and excessive fluid intake and in patients with central diabetes insipidus before and during treatment with des-
amino-[Arg⁸]vasopressin without and with concomitant PG synthesis inhibition with indome-
thacin.

Methods

Studies were performed in six healthy volunteer subjects (four females and two males, aged 21–31 years) on a constant intake of 150 mmol of sodium/day and 80 mmol of potassium/day during normal and excessive fluid intake. They were first studied on a control day of fluid intake ad libitum and again when fluid intake was markedly raised by drinking approximately 1 litre of water every 4 h (10.00, 14.00, 18.00, 22.00, 02.00, 06.00, 10.00 hours). Urine was collected for 24 h from 12.00 to 12.00 hours on the control day and during water loading.

In addition, five patients with diabetes insipidus (two females and three males, aged 17–48 years), one with idiopathic central diabetes insipidus and four members of one family with hereditary central diabetes insipidus were admitted to the study. In all patients, central diabetes insipidus was proven by the water-deprivation test [16]. Except for one patient, in whom radiological evidence of bilateral ureteral obstruction was present, all patients revealed normal findings on physical examination, routine laboratory tests, chest film and intravenous pyelogram. The patients were hospitalized and equilibrated on a diet which contained 150 mmol of sodium and 80 mmol of potassium/day. Except for the patient who was diagnosed to have idiopathic central diabetes insipidus shortly before the study, all patients had previously been treated with des-
amino-[Arg⁸]vasopressin. All medication had been discontinued at least 5 days before admis-
sion so that studies were performed in patients who had been polyuric for at least 4 days. The experimental protocol consisted of a control period of 1 day which was followed by 3 days of desamino-[Arg⁸]vasopressin treatment, 7.5 μg every 12 h intranasally (12.00 hours and mid-
night). On day 3 of desamino-[Arg⁸]vasopressin treatment all patients received indomethacin (50 mg, orally at 12.00, 20.00 and 06.00 hours). Twenty-four hour urines were collected daily from 12.00 to 12.00 hours.

Urines were analysed for sodium, potassium, osmolality, creatinine, cyclic AMP and PGE₂. Blood for determination of serum sodium and potassium concentrations, creatinine, plasma osmolality, plasma renin activity (PRA), plasma aldosterone and [Arg⁸]vasopressin concentrations was drawn at 08.00 hours with subjects supine and again for PRA and plasma aldo-

sterone at 11.00 hours after they had been ambulatory for 3 h (upright). Plasma and urine concentrations of sodium, potassium and creatinine were determined by flame photometry and a conventional autoanaylsen method respect-
ively. Plasma and urinary osmolalities were determined by freezing-point depression (Knauer osmometer). Glomerular filtration rate (GFR) was estimated as clearance of endogenous creatine and creatinine (C₉₉) and free water clearances (C₁₅) were calculated according to conventional clearance formulae.

PRA was measured by radioimmunoassay of angiotensin I (ANG I) [17]. Results are expressed as pmol of ANG I generated h⁻¹ ml⁻¹ of plasma. Plasma aldosterone concentration was measured by radioimmunoassay without chromatography [18] and plasma [Arg⁸]vasopressin was deter-
mined by radioimmunoassay with a method previously described [19]. The minimal detectable concentration of [Arg⁸]vasopressin in this assay is 0.2 fmo1/ml of plasma. Urinary excretion of cyclic AMP was measured by a protein-binding assay [20]. In the polydipsia protocol and in control observations on patients with central diabetes insipidus, urine was analysed for kalli-
krein by a modified method of Claeson, Frid-
berger, Knös & Eriksson [21]. Results are expressed in terms of enzyme units (EU) per 24 h. One EU is defined as that amount of enzyme which catalyses the hydrolysis of 1 pmol of substrate per minute under the conditions of the assay (pH 8-2, 37°C). Urinary excretion of PGE₂ was measured by radioimmunoassay as described previously [22]. Volunteer subjects and patients had been informed before the study that sexual activity would interfere with PG measurements in their urine. In brief, samples of volume-adjusted urine were extracted twice with ethyl acetate. For volume adjustment, 24-h polyuric urine was evaporated to volumes equalling those during control periods in patients with diabetes insipidus were evaporated and during polydipsia and from untreated subjects during polydipsia and from untreated volunteer subjects during polydipsia and from untreated. This procedure was not associated with losses of PGE₂. The combined extracts were dried down under a stream of nitrogen and PGE₂ was separated from other PG and PG metabolites by silicic acid column chromatography. Radioimmunoassay of PGE₂ was performed with a highly specific rabbit antibody against PGE₂ (Institut Pasteur, Paris, France). [3H]PGE₂ (New England Nuclear) was a recovery tracer and in the assay. Unlabelled PGE₂ was kindly provided by Dr J. E. Pike, Upjohn Co., Kalamazoo, MI, USA.

All assays were performed in duplicate. Results were corrected for recoveries and expressed as pmol/24 h. Data are given as means ± SEM. Statistical analysis of the data was performed by Student’s paired t-test [23].

Results

Changes in renal function

In normal volunteer subjects polydipsic water loading resulted in an increase in mean urine volume from 1203 ± 242 (SEM) to 5072 ± 320 ml/24 h (P < 0.001) with a decrease in urinary osmolality from 659 ± 79 to 170 ± 19 mmol/kg (P < 0.001) and an increase in C₄O₂⁺ from -1.03 ± 0.21 to 1.39 ± 0.27 ml/min (P < 0.001). These changes were associated with a decrease in plasma osmolality from 286 ± 2 to 128 ± 2 mmol/kg (P < 0.001) and serum sodium concentration from 149 ± 1 to 143 ± 2 mmol/l (P < 0.05).

Concomitant indomethacin treatment in addition to desamino-[Arg⁸]vasopressin administration slightly and insignificantly decreased urine volume to 945 ± 131 ml/24 h as compared with 1205 ± 204 ml/24 h during desamino-[Arg⁸]vasopressin treatment alone, but significantly increased urinary osmolality to 685 ± 48 mmol/l (P < 0.002) and further decreased C₄O₂⁺ to -0.93 ± 0.14 ml/min (P < 0.05) without changes in GFR or urinary excretion of sodium and potassium (Fig. 1).

<table>
<thead>
<tr>
<th>Table 1. Effect of polydipsic water loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>V (ml/24 h)</td>
</tr>
<tr>
<td>U₄O₂⁺ (mmol/kg)</td>
</tr>
<tr>
<td>C₄O₂⁺ (ml/min)</td>
</tr>
<tr>
<td>C₄H₂O₂⁺ (ml/min)</td>
</tr>
<tr>
<td>U₄O₂⁺V (mmol/24 h)</td>
</tr>
<tr>
<td>U₄V (mmol/24 h)</td>
</tr>
<tr>
<td>C₄E₄ (ml/min)</td>
</tr>
<tr>
<td>U₄K₊ (EU/24 h)</td>
</tr>
<tr>
<td>P₄O₂⁺ (mmol/kg)</td>
</tr>
<tr>
<td>S₄Na⁺ (mmol/l)</td>
</tr>
<tr>
<td>PRA sup. (pmol h⁻¹ ml⁻¹)</td>
</tr>
<tr>
<td>PRA sup. (pmol h⁻¹ ml⁻¹)</td>
</tr>
<tr>
<td>PA sup. (nmol/l)</td>
</tr>
<tr>
<td>PA sup. (nmol/l)</td>
</tr>
</tbody>
</table>

[Arg⁸]vasopressin treatment to 1205 ± 204 ml/24 h (P < 0.001) with a significant increase in urinary osmolality from 75 ± 8 to 460 ± 72 mmol/kg (P < 0.01). This was paralleled by a decrease in C₄H₂O₂⁺ from 5.62 ± 0.68 ml/min to -0.41 ± 0.17 ml/min (P < 0.001) in the absence of changes in GFR. These renal functional changes were associated with a significant decrease in plasma osmolality from 286 ± 2 to 278 ± 2 mmol/kg (P < 0.02) and serum sodium concentration from 149 ± 1 to 143 ± 2 mmol/l (P < 0.05).

Plasma renin activity and plasma aldosterone and arginine vasopressin concentrations

In normal volunteer subjects supine and upright PRA measured 0.25 ± 0.07 and 0.48 ± 0.07 pmol h⁻¹ ml⁻¹ respectively, during the control day without a significant effect of water loading (0.27 ± 0.05 supine and 0.65 ± 0.18 pmol h⁻¹ ml⁻¹ upright). In addition, no effect of polydipsia could be observed on
FIG. 1. Effects of 1-desamino-8-arginine vasopressin (DDAVP) and DDAVP plus indomethacin (INDO) on urine volume, urinary osmolality, free water clearance, creatinine clearance, urinary cyclic AMP excretion, plasma osmolality and serum sodium concentration in five patients with central diabetes insipidus.

plasma aldosterone concentration (control: 0.26 ± 0.07 supine and 0.62 ± 0.23 nmol/l upright; water loading: 0.20 ± 0.04 supine and 0.47 ± 0.18 nmol/l upright) (Table 1).

In patients with central diabetes insipidus plasma [Arg^8]vasopressin concentration was smaller than 0.2 fmol/ml in each of the five patients studied and therefore indistinguishable from zero in the [Arg^8]vasopressin radi-immunoassay system used. In these patients control supine PRA measured 0.08 ± 0.04 pmol h^{-1} ml^{-1} and increased to 0.24 ± 0.06 pmol h^{-1} ml^{-1} with upright posture. Control PRA in the patient who had developed idiopathic central diabetes insipidus approximately 2 months before admission, and who had never been treated for this disorder, also revealed suppressed PRA with 0.04 supine and 0.20 pmol h^{-1} ml^{-1} upright. Desamino-[Arg^8]vasopressin treatment slightly but not significantly decreased upright PRA to 0.06 ± 0.02 pmol h^{-1} ml^{-1} and had no effect on supine PRA (0.05 ± 0.01 pmol h^{-1} ml^{-1}). With concomitant indomethacin administration mean supine PRA was less than 0.04 pmol h^{-1} ml^{-1}, whereas upright PRA averaged 0.05 ± 0.01 pmol h^{-1} ml^{-1}. Supine and upright plasma aldosterone concentrations during the control day were 0.20 ± 0.02 and 0.30 ± 0.11 nmol/l, respectively, and were unchanged by either desamino-[Arg^8]vasopressin treatment alone (0.22 ± 0.01 supine and 0.25 ± 0.10 nmol/l upright) or treatment with desamino-[Arg^8]vasopressin plus indomethacin (0.24 ± 0.04 supine and 0.35 ± 0.13 nmol/l upright).

**Urinary excretion of PGE_2, cyclic AMP and kallikrein**

In normal volunteer subjects on a free fluid intake urinary excretion of PGE_2 was 466 ± 66 pmol/24 h and slightly increased to 1017 ± 174 pmol/24 h (P = 0.05) during polydipsic water loading. Urinary excretion of kallikrein averaged 1.056 ± 0.210 EU/24 h during the control day and was unchanged by polydipsia-induced polyuria (1.043 ± 0.237 EU/24 h).

PGE_2 excretion was high in the patient who showed radiological evidence of bilateral ureteral obstruction (> 2500 pmol/24 h). However, in the four untreated patients with uncomplicated central diabetes insipidus urinary excretion of PGE_2 averaged 246 ± 66 pmol/24 h and was significantly (P < 0.05) suppressed compared with urinary PGE_2 excretion in the normal volunteer subjects. In these patients treatment with desamino-[Arg^8]vasopressin raised urinary PGE_2 excretion to 2643 ± 677 pmol/24 h (P < 0.01). Concomitant indomethacin treatment subsequently suppressed urinary PGE_2 excretion to 909 ± 251 pmol/24 h (P < 0.01) compared with desamino-[Arg^8]vasopressin treatment alone (Fig. 2).

In patients with central diabetes insipidus control urinary excretion of cyclic AMP was 2.55 ± 0.64 μmol/24 h and significantly increased to 4.91 ± 0.75 μmol/24 h when urine volume was normalized by treatment with des-
FIG. 2. Effect of 1-desamino-8-arginine vasopressin (DDAVP) and DDAVP plus indomethacin (INDO) on urinary excretion of prostaglandin E₂ (PGE₂) in four patients with central diabetes insipidus.

amine-[Arg₈]vasopressin. However, the significantly enhanced urinary concentrating response to desamino-[Arg₈]vasopressin in the presence of indomethacin was not associated with a further increase in urinary excretion of cyclic AMP (5.02 ± 0.59 pmo1/24 h) (Fig. 1).

In untreated patients with central diabetes insipidus urinary excretion of kallikrein was within the normal range and measured 1.160 ± 0.090 EU/24 h.

Discussion

The role of [Arg₈]vasopressin in the regulation of renal PG production in vivo is still controversial. In contrast with studies which reported decreased urinary PG excretion in the absence of [Arg₈]vasopressin in polyuric rats with hereditary central diabetes insipidus [13–15] a close correlation between increased urinary PG excretion and high urine volume has been described in healthy volunteer subjects during polydipsic water loading [24], in two patients with either nephrogenic or central diabetes insipidus [25] and in dogs during water diuresis [26, 27]. These results suggest that urine volume itself might be an important determinant of urinary PGE excretion. However, in these studies, PG measurements were performed by radioimmunooassay and receptor assay respectively, with control values of urinary PGE excretion exceeding those obtained by gas chromatography/mass spectrometry [28].

Therefore, in the present study, samples of volume-adjusted urine (see the Methods section) were assayed for PGE₂ to avoid artificial increases in urinary PGE₂ excretion calculated for excessive urine volumes, which may be due to methodological problems inherent in PG receptor and immunological assay methods respectively [29]. By this method we can show that in healthy volunteer subjects urinary excretion of PGE₂ only slightly increases with polydipsia-induced polyuria and that PGE₂ excretion is suppressed in patients with central diabetes insipidus despite their maximal water diuresis.

Studies in rabbit renomedullary interstitial cell cultures in vitro have demonstrated that [Arg₈]vasopressin is a weak stimulator of PGE₂ biosynthesis as compared with ANG II or bradykinin [11]. In accordance with these data obtained in vitro the slight rise in PGE₂ excretion, which we observed in healthy volunteer subjects despite suppression of endogenous [Arg₈]vasopressin by water loading, suggests a minor role of [Arg₈]vasopressin, at least within the physiological range, in the regulation of total renal PG synthesis in vivo. The slight increase in urinary PGE₂ excretion during polydipsic water loading therefore might rather have resulted from the slight increase in plasma renin activity which occurred during polydipsia.

In our patients with central diabetes insipidus urinary excretion of PGE₂ was significantly suppressed and treatment with the [Arg₈]vasopressin analogue desamino-[Arg₈]vasopressin significantly stimulated urinary PG excretion. This is in agreement with results obtained in rats with hereditary central diabetes insipidus [13–15, 30].

Although previous studies have suggested that PRA might be stimulated in patients with central diabetes insipidus [31], in the present study supine and upright PRA in the five patients with central diabetes insipidus was suppressed during the control period and further decreased during treatment with desamino-[Arg₈]vasopressin and with desamino-[Arg₈]vasopressin plus indomethacin. Since the therapeutic management of patients might influence PRA it should be emphasized that in this study desamino-[Arg₈]vasopressin treatment had been discontinued in four patients at least 5 days before the study. Moreover, one patient, who also revealed suppressed supine and upright PRA, had been polyuric for several weeks before admission and had not received treatment within this time period. The mechanism of suppression of PRA in
untreated patients with central diabetes insipidus remains speculative. Since [Arg⁸]vasopressin infusions have been shown to suppress PRA [32], the absence of [Arg⁸]vasopressin would be expected to be associated with increased rather than suppressed PRA. Therefore, mechanisms other than [Arg⁸]vasopressin deficiency itself, such as increased serum sodium concentration, must be considered to mediate the suppression of PRA in patients with central diabetes insipidus. Again, suppressed PRA with presumably low concentrations of circulating ANG II may have contributed to the subnormal urinary PG excretion in our patients with diabetes insipidus.

In the present study desamino-[Arg⁸]vasopressin increased urinary excretion of PGE₂ in the absence of an increase in PRA. Consequently it appears that desamino-[Arg⁸]vasopressin directly stimulated renal PG biosynthesis. However, in contrast with [Arg⁸]vasopressin, which has been shown to stimulate PGE₂ biosynthesis in the rabbit [5] and the rat [33] renomedullary interstitial cell cultures, desamino-[Arg⁸]vasopressin did not affect PGE₂ production in rat interstitial cells in vitro [33]. The exact mechanism of the desamino-[Arg⁸]vasopressin-induced stimulation of renal PG biosynthesis observed in vivo in the present study therefore remains unknown. We may speculate, however, that the nonpressor [Arg⁸]vasopressin analogue desamino-[Arg⁸]vasopressin, with its marked antidiuretic potency, selectively stimulates PG biosynthesis in the collecting duct epithelium which therefore cannot be detected in isolated interstitial cells. Our finding in patients with central diabetes insipidus that a given dose of desamino-[Arg⁸]vasopressin exerted a significantly greater urinary concentrating response during concomitant PG synthesis inhibition extends the concept that PG act as antagonists to [Arg⁸]vasopressin on collecting duct water permeability also to its analogue desamino-[Arg⁸]vasopressin. The exact mechanism of this effect, however, still remains controversial. In contrast with an increase in cyclic AMP concentration in rat renal medullary tissue observed after indomethacin [34], in the present study no further increase in urinary cyclic AMP excretion after indomethacin could be observed in patients with central diabetes insipidus despite a significant increase in urine concentration. These results are in agreement with previous studies [35, 36] and may be explained as lack of urinary cyclic AMP properly to reflect intrarenal cyclic AMP production, especially that located in the collecting duct epithelium. On the other hand, they may also point to other mechanisms by which indomethacin might affect renal concentrating ability such as enhanced sodium chloride absorption in the ascending limb of Henle [37, 38] and/or decreased medullary washout of solute, both resulting in an increase in medullary tonicity [39].

In conclusion, our results show that polyuria induced in normal subjects by polydipsic water loading is associated with a slight increase in urinary PGE₂ excretion which is of borderline statistical significance. In addition, urinary excretion of PGE₂ is markedly suppressed in patients with central diabetes insipidus and is significantly stimulated when urine volume is normalized by treatment with the [Arg⁸]vasopressin analogue desamino-[Arg⁸]vasopressin. PG synthesis inhibition significantly enhances the urinary concentrating response to desamino-[Arg⁸]vasopressin in these patients. This effect of PG inhibition is not associated with changes in urinary cyclic AMP excretion. Therefore, the desamino-[Arg⁸]-vasopressin antagonistic effect of renal PG may not solely involve inhibition of desamino-[Arg⁸]-vasopressin-activated adenylyl cyclase, but may also be due to altered intrarenal haemodynamics and/or changes in ascending loop reabsorptive capacity.

Acknowledgment

This study was supported by research grant no. FA-7604 (Kra), Ministerium für Wissenschaft und Forschung, NRW, F.R.G. The authors gratefully acknowledge the excellent technical assistance of Mrs Angela Bäcker and Mrs Helgard Stelkens. This study was in part presented at the Fourth International Prostaglandin Conference, Washington, D.C., 28–31 May, 1979.

References

PG and renal water excretion


