Renal resistance to vasopressin in poorly controlled type 1 diabetes mellitus

K. McKENNA,1 A. D. MORRIS,2 M. RYAN,2 R. W. NEWTON,2 B. M. FRIER,3 P. H. BAYLIS,4 T. SAITO,5 S. ISHIKAWA,5 AND C. J. THOMPSON1

1Beaumont Hospital, Dublin 9, Republic of Ireland; 2Nineells Hospital and Medical School, Dundee DD1 9SY, Scotland; 3Royal Infirmary of Edinburgh, Edinburgh EH3 9YW, Scotland; 4Royal Victoria Infirmary, Newcastle-upon-Tyne NE1 4LP, England, UK; and 5Jichi Medical School, Tochigi, Japan 392-04

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Renal resistance to vasopressin in poorly controlled type 1 diabetes mellitus. Am J Physiol Endocrinol Metab 279: E155–E160, 2000.—To investigate the hypothesis that diabetes induces nephrogenic diabetes insipidus, we studied the urine-concentrating ability in response to vasopressin (AVP) in 12 patients with insulin-dependent diabetes mellitus (IDDM) and 12 nondiabetic subjects. Subjects were euglycemic-clamped, and after oral water loading, AVP was infused intravenously for 150 min. AVP induced a greater (P < 0.001) rise in urine osmolality in controls (67.6 ± 10.7 to 720 ± 31.1 mosmol/kg, P < 0.001) than in IDDM patients (64.3 ± 21.6 to 516.7 ± 89.3 mosmol/kg, P < 0.001). Urinary aquaporin-2 concentrations after AVP infusion were higher in controls (611.8 ± 105.6 fmol/mg creatinine) than in IDDM (462.0 ± 94.9 fmol/mg creatinine, P = 0.003). Maximum urine osmolality in IDDM was inversely related to chronic blood glucose control, as indicated by HbA1c (r = −0.87, P = 0.002). To test the hypothesis that improved glycemic control could reverse resistance to AVP, 10 IDDM subjects with poor glycemic control (HbA1c >9%) were studied after (A) intensified glycemic control. Maximum urine osmolality in response to AVP increased with improved glycemic control (B, 443.8 ± 49.0; A, 640.0 ± 137.2 mosmol/kg, P < 0.001), and urinary aquaporin-2 concentrations after AVP increased from 112.7 ± 69 to 375 ± 280 fmol/mg creatinine (P = 0.006), with improved glycemic control. Poorly controlled IDDM is associated with reversible renal resistance to AVP.

METHODS

Study 1: Renal response to AVP in IDDM and control subjects. This study was designed to test the hypothesis that people with type 1 diabetes have renal resistance to the antidiuretic actions of AVP. Twelve male subjects with IDDM were recruited from the outpatient departments of participating hospitals, and 12 age- and sex-matched nondiabetic controls were recruited from hospital staff. The characteristics of the diabetic volunteers and the controls are shown in Table 1. Subjects who had hypertension (blood pressure >140/90 mmHg), a history of cardiac disease, established microalbuminuria (urinary albumin concentration >20 mg/l), or hematuria on urinalysis were excluded from the study. No subjects were taking any medications other than insulin. All subjects gave informed consent for the studies, which had local medical ethical committee approval.

On the morning of the study, the diabetic subjects came to the investigation unit at 0700, having fasted from midnight and having omitted their usual morning dose of insulin. Alcohol was prohibited from the evening before the study, and the ingestion of caffeine and smoking of nicotine were not allowed after 2200. Intravenous cannulas were inserted into antecubital veins of each forearm under 1% lidocaine anesthesia. An intravenous infusion was commenced of 50 units of short-acting, soluble insulin (Actrapid, Novo Nordisk) in

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Address for reprint requests and other correspondence: C. J. Thompson, Academic Dept of Diabetes, Beaumont Hospital, Dublin 9, Republic of Ireland. (E-mail: chris.thompson@beaumont.ie).

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50 ml of 0.9% sodium chloride solution, and the rate was adjusted to maintain blood glucose concentrations between 4 and 6 mmol/l. Once steady-state euclaycemia was attained, an oral water load was administered, with an initial volume of 20 ml/kg body weight plus supplementary oral loads of 150 ml, plus the equivalent volume of fluid lost as urine; this water load was given every 20 min. Urine was collected every 20 min; when steady-state diuresis was achieved (three consecutive urine volumes >12 ml/min, without an increase of >2 ml/min between samples), AVP infusion was commenced at an increasing rate of 0–15 fmol kg⁻¹ min⁻¹, in an adaptation of the method of Dixey et al (5). Nondiabetic control subjects underwent an identical protocol, except they did not require insulin infusion to maintain euclaycemia.

Blood samples were withdrawn from an indwelling canula into chilled syringes at 15-min intervals during the AVP infusion. Within 10 min of blood sampling, the blood was transferred into lithium heparin tubes before being centrifuged at 2,000 g and 4°C for 15 min. The supernatant was removed by pipette, and an aliquot was retained for measurement of plasma osmolality and plasma sodium, on the same day as the study. The remaining plasma was stored at −70°C until assayed for AVP. Urine was collected at blood sampling times, the volume was measured, and aliquots were removed for measurement of osmolarity and sodium. Urine aliquots were also stored at −70°C for later measurement of urinary aquaporins.

**Study 2: Renal response to AVP after improved glycemic control in IDDM.** The second study was designed to investigate whether an improvement in glycemic control could reverse the renal resistance to AVP in poorly controlled type 1 diabetes. Ten male subjects with type 1 diabetes (mean age 23.5 yr (range 18–34 yr), mean duration of diabetes 4.2 yr (1–11 yr), and with poorly controlled diabetes (Hb A₁c >9%, local nondiabetic reference range 3.5–5.8%, target for good glycemic control <6.5%)) were recruited for the study. Three of the subjects had previously acted as volunteers for the first study. Mean Hb A₁c was 10.6%, with the range 9.5–13.1%. The exclusion criteria were as applied for study 1, and all subjects had plasma total calcium, plasma potassium, and serum creatinine within normal reference ranges for the clinical chemistry laboratory. All patients gave informed consent, and the study had local medical ethical committee approval.

The subjects collected their urine for 24 h, to establish baseline urine output, and measured their capillary blood glucose before meals and at bedtime on the day before the study to provide a baseline blood glucose profile. On the first morning of the study, subjects underwent an intravenous infusion of AVP by use of an identical protocol to that employed in study 1. In addition to the removal of blood into lithium heparin tubes, however, blood samples were also taken into EDTA tubes, and after centrifugation as in study 1, the supernatant was removed for measurement of plasma insulin concentration.

Before discharge home at the end of the AVP infusion, subjects had their insulin schedule changed to a multiple injection regimen (soluble insulin before meals and isophane insulin at bedtime) and received specific education about adjustment of insulin dosage to maintain blood glucose concentrations between 4 and 9 mmol/l. Subjects phoned the principal investigator (C. J. Thompson) on a daily basis with the results of their home blood glucose monitoring and were given advice about insulin dose. When the blood glucose profiles had remained within the target range for 1 wk, the subjects repeated the 24-h urine collection and a concurrent blood glucose profile and re-attended the investigation unit where a repeat AVP infusion was performed. The time between institution of intensive monitoring and obtaining blood glucose within target range was 4–17 days.

**Laboratory methods.** AVP was measured by a sensitive and specific radioimmunoassay (12), with a lower limit of detection of 0.3 pmol/l and intra- and interassay coefficients of variation (CVs) for AVP standards at 2 pmol/l of 9.7 and 15.3%, respectively (1st International AVP standard, Mill Hill, London, UK). AVP was extracted from plasma before assay by magnesium silicate (Florisil) adsorption (recovery of cold AVP from plasma 80–90%, results not corrected for recovery). Plasma and urine osmolality was measured by the depression of freezing point method and plasma sodium by ion-selective electrode. Urine creatinine was measured by the Jaffe reaction. Blood glucose was measured by the glucose oxidase reaction (Yellow Springs analyzer, Clandon Scientifics, London, UK). Urinary aquaporin-2 concentration was measured by a radioimmunoassay previously described in the literature (13), which has intra- and interassay CVs of <10%. Plasma insulin was measured using the Phasedeph radioimmunoassay (Pharmacia, Uppsala, Sweden), which has an interassay CV of 9.1% and an intra-assay CV of 6.0% at a 1.65 ng/ml standard.

**Statistical methods.** All results are expressed as means ± SD or mean ± range, as appropriate. Differences between baseline characteristics were calculated by unpaired t-tests. Changes in variables with time were calculated by one-way ANOVA and those between groups by two-way ANOVA. Statistics were undertaken using Excel 7.0.

**RESULTS**

**Study 1: Renal response to AVP in IDDM patients and controls.** Plasma total calcium and plasma potassium, and baseline urine output, plasma and urine osmolality, plasma sodium, and AVP concentrations were similar in the diabetic and control groups (Table 1). Baseline blood glucose concentration was similar in the diabetic and control groups and remained unchanged throughout the study.

Plasma AVP concentrations were suppressed appropriately by water loading in both groups, and intravenous infusion of AVP caused a similar rise in plasma AVP concentrations, in the diabetes group to 1.3 ± 0.5 pmol/l (P < 0.001) and in controls to 1.1 ± 0.5 pmol/l.
The rise in plasma AVP concentrations was associated with a fall in urine flow in both the diabetic (15.1 ± 1.1 to 3.9 ± 0.9 ml/min, $P < 0.001$) and control groups (14.7 ± 1.5 to 1.9 ± 0.3 ml/min, $P < 0.001$), with a larger reduction in urine flow rate in the controls ($P = 0.01$). Urine osmolality rose in both the diabetic group (64.3 ± 21.6 to 516.7 ± 89.3 mosmol/kg, $P < 0.001$) and control group (67.6 ± 10.7 to 720 ± 31.1 mosmol/kg, $P < 0.001$), with a larger rise in urine osmolality in the controls ($P < 0.001$). Baseline free water clearance was similar in the two groups, but there was a greater ($P = 0.001$) fall in free water clearance during AVP infusion in the controls (11.2 ± 1.3 to −2.9 ± 0.4 ml/min, $P < 0.001$) than in type 1 diabetes (11.5 ± 1.2 to 0.3 ± 0.7 ml/min, $P < 0.001$). Mean urinary aquaporin-2 concentration was higher at the end of AVP infusion in controls (611.8 ± 105.6 fmol/mg creatinine) than in IDDM patients (462.0 ± 94.9 fmol/mg creatinine, $P = 0.003$). The urine sodium-to-creatinine ratio was similar at baseline in the two groups and rose in controls from 13.2 ± 12.6 to 34.7 ± 19.2 mmol/l·mmol/l−1·l−1 ($P = 0.02$) and in type 1 diabetes subjects from 18.5 ± 11.3 to 40.2 ± 20.8 mmol/l·mmol/l−1·l−1 ($P = 0.01$), with no significant differences between the groups (Fig. 1).

Plasma osmolality fell during AVP infusion in the diabetic (285.0 ± 1.5 to 281.5 ± 1.2 mosmol/kg, $P < 0.001$) and control groups (284.5 ± 1.7 to 277.8 ± 1.7 mosmol/kg, $P < 0.001$), with a larger fall in plasma osmolality in controls ($P = 0.002$). There was no relationship in the diabetic group between maximum urine osmolality at the end of the AVP infusion and the duration of diabetes ($r = 0.04, P = 0.9$), but a clear inverse relationship was identified between glycemic control, as expressed by Hb A1c, and maximum urine osmolality ($r = −0.87, P = 0.002$).

**Study 2: Renal response to AVP after improved glycemic control in IDDM patients.** Results in this section from the initial AVP infusion, performed when subjects had poor glycemic control, are referred to as pretreatment, and those results after a week of intensive glycemic control are referred to as posttreatment. The period of transient improvement in glycemic control was associated with an increase in the total daily dose of insulin (61 ± 12 to 74.8 ± 13.1 units, $P = 0.027$), a lower 24-h blood glucose profile on the day before AVP infusion (Table 2), and a lower 24-h urine output on the day before AVP infusion (pretreatment, 3,232 ± 585 ml; posttreatment, 2,112 ± 239 ml, $P < 0.001$). Plasma total calcium (2.42 ± 0.09 vs. 2.42 ± 0.11 mmol/l, $P = 0.77$) and potassium (4.42 ± 0.36 vs. 4.30 ± 0.33 mmol/l, $P = 0.45$) remained unchanged between the study days.

No differences were observed in mean baseline plasma osmolality, plasma AVP, urine osmolality, or urine flow rate at the end of water loading on the two
study days. Blood glucose did not change during the period of the AVP infusion, and the blood glucose concentration profile during the study period was similar on both study days. Water loading suppressed plasma AVP concentrations on both study days, and similar increments in plasma AVP concentration occurred after AVP infusion during pretreatment (0.4 ± 0.2 to 1.1 ± 0.3 pmol/l, P < 0.001) and posttreatment (0.4 ± 0.1 to 1.1 ± 0.4 pmol/l, P < 0.001).

The rise in plasma AVP concentration was associated with a fall in urine flow rate in both the pretreatment infusion (15.0 ± 1.5 to 3.9 ± 0.34 ml, P < 0.001) and the posttreatment infusion (15.0 ± 1.1 to 2.41 ± 0.4 ml, P < 0.001), with a greater fall in urine flow rate posttreatment (P = 0.006). Urine osmolality rose during the pretreatment infusion, from 74.5 ± 9.0 to 443.8 ± 49.0 mosmol/kg, P < 0.001, and during the posttreatment infusion, from 72.2 ± 11.0 to 640 ± 137.2 mosmol/kg, P < 0.001, with a greater rise in urine osmolality posttreatment. Baseline free water clearance was similar on the two study days, but there was a greater fall in free water clearance (P < 0.001) when subjects were well controlled posttreatment (11.2 ± 1.4 to −3.1 ± 0.6 ml/min, P < 0.001) than when poorly controlled (11.1 ± 1.4 to −2.3 ml/min, P < 0.001). Urinary aquaporin-2 concentration in response to AVP was 112.7 ± 69 at the end of pretreatment AVP infusion but was higher at the end of posttreatment infusion at 375 ± 280 fmol/mg creatinine (P = 0.006). Plasma osmolality fell on both study days but did not differ in magnitude between them (P = 0.08). There was a similar rise in the urine sodium-to-creatine ratio, both pretreatment (15.6 ± 8.9 to 32.9 ± 16.5 mmol · mmol⁻¹ · l⁻¹, P = 0.04) and posttreatment (17.4 ± 11.6 to 38.3 ± 19.4 mmol · mmol⁻¹ · l⁻¹, P = 0.02). Plasma insulin concentrations remained unchanged during both pretreatment study (4.3 ± 1.9 to 5.2 ± 2.3 ng/ml, P = 0.8) and posttreatment study (5.3 ± 2.3 to 4.7 ± 2.2 ng/ml, P = 0.8), with no difference in plasma insulin concentrations between the two study days (Fig. 2).

**DISCUSSION**

The data from these studies have shown that people with type 1 diabetes are less able than nondiabetic controls to effect antidiuresis and concentrate urine in response to a comparable rise in plasma AVP concentration. This resistance to the antidiuretic effect of

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**Table 2. Effect of intensive insulin therapy on blood glucose day curve, in study 2**

<table>
<thead>
<tr>
<th>Time of Blood Tests</th>
<th>0800</th>
<th>1230</th>
<th>1730</th>
<th>2200</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 1</strong></td>
<td>14.0 ± 4.8</td>
<td>17.0 ± 3.4</td>
<td>15.4 ± 3.5</td>
<td>13.3 ± 4.2</td>
</tr>
<tr>
<td><strong>Day 2</strong></td>
<td>8.7 ± 2.0</td>
<td>8.0 ± 2.1</td>
<td>6.6 ± 3.7</td>
<td>6.0 ± 1.9</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.016</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Results (means ± SD) are from day before initial vasopressin (AVP) infusion (day 1) and day before AVP infusion after intensive glycemic control (day 2). P value indicates significance of difference between mean blood glucose concentration values at each time point (by paired t-test).
AVP appears to be inversely related to glycemic control, in that failure to concentrate urine was most marked in those patients who had chronically poor glycemic control, as represented by elevated glycated hemoglobin. We have also shown that even a short period of good glycemic control can reverse the resistance to vasopressin in subjects with chronically poor glycemic control.

Because our subjects had been carefully screened to exclude those with microalbuminuria, which is the earliest clinical manifestation of diabetic nephropathy, it is clear that the renal resistance to AVP that we have documented is not due to preexisting renal disease. In addition, the reversal of resistance to AVP by short-term good glycemic control provides further evidence that the impaired ability to concentrate urine in poorly controlled IDDM is a function of chronically poor glycemic control, rather than a reflection of renal pathology. Because the studies were performed with blood glucose maintained in the euglycemic range, an osmotic diuresis secondary to glycosuria could not be deemed responsible for the failure to concentrate urine. Furthermore, the normal plasma potassium, calcium, and creatinine concentrations excluded some of the more common metabolic causes of renal resistance to AVP (15). The normal urinary sodium responses to AVP infusion in the IDDM group would further argue against the renal resistance to AVP representing a manifestation of generalized tubular dysfunction.

The role of insulin in the return of renal sensitivity to AVP after improved glycemic control is interesting. Insulin is recognized to have short-term effects to increase renal tubular sodium reabsorption in diabetes (4), and data from the rat show that insulin can enhance water transport, most probably by stimulating glucose transporters to act as water channels, but possibly by enhancing AVP-mediated water reabsorption (19). Data from the mouse also suggest that insulin may potentiate the actions of AVP (7). The mean daily dose of insulin in the poorly controlled patients in the second study did increase during intensive therapy, but the plasma insulin concentrations required to maintain normoglycemia were similar during the pretreatment and posttreatment days in study 2. Inasmuch as there were clear differences in renal sensitivity to AVP on the two study days, our data would suggest that it was the improvement in glycemic control, rather than an effect of insulin on the renal resistance to AVP, which was the dominant factor in restoring antidiuretic responses to AVP.

We have also shown for the first time that the severity of renal resistance to the antidiuretic actions of AVP was inversely proportional to the degree of glycemic control. Thus those diabetic subjects with the poorest glycemic control had the least ability to concentrate urine in response to the intravenous infusion of AVP. The demonstration that renal resistance to AVP occurs at physiological plasma concentrations of the hormone indicates that this impaired antidiuresis is likely to have clinically significant effects. The second study, conducted only in patients who had poor glycemic control, demonstrated that even a short period of good glycemic control, achieved by intensive insulin therapy, can largely reverse the renal insensitivity to AVP.

Temporary renal insensitivity to AVP occurs in other disease states characterized by marked polyuria, such as newly diagnosed cranial diabetes insipidus or severe compulsive water drinking (15), when patients often fail to maximally concentrate urine until they have received treatment for several days with an AVP analog. This partial resistance to AVP manifests as subnormal urinary concentration in response to administration of the AVP analog desmopressin (15). In study 2, the poorly controlled diabetic subjects clearly had a low-grade polyuria before the pretreatment AVP infusion, which was abolished by intensive insulin therapy with restoration of renal responsiveness to AVP. Although the 24-h urine volume was much lower than in patients with severe diabetes insipidus, chronic mild polyuria could have contributed to the reversible renal resistance to AVP. The reason for the resistance to AVP in states of chronic polyuria remains speculative, but our data in IDDM suggest a potential mechanism.

We have shown that urinary concentrations of aquaporin-2, the vasopressin-sensitive water channels that promote tubular water reabsorption, are lower in patients with IDDM than in the control group despite comparable plasma AVP concentrations. The failure to recruit aquaporin-2 was more marked in patients with chronically poor glycemic control. This suggests that hyperglycemia may impair binding of AVP to the V_2 receptor in the renal tubules or cause downregulation of the receptors after binding. Evidence from experiments in the rat does suggest that hyperglycemia causes downregulation of V_1 receptors in cultured aortic smooth muscle cells (8) and decreases hepatic V_1a receptor density (11), although induction of diabetes mellitus with streptozotocin does not alter the density of the renal V_2 receptor, the affinity of the receptor for AVP, or AVP-stimulated AMP production (18). It has been suggested that hyperglycemia may change the number or function of capillary wall aquaporins (14), which would reduce water reabsorption in the renal tubules (6), and our data would support the hypothesis that the V_2 receptors are less sensitive to AVP in poorly controlled diabetes. Clearly, because improved glycemic control led to increased AMP-stimulated urinary aquaporin-2 concentrations, the renal resistance to AVP is transient.

There is little information about renal sensitivity to AVP in type 2 (non-insulin-dependent) diabetes mellitus. Osmoregulation of thirst and AVP secretion have been reported to be normal in type 2 diabetes, with similar urine concentration in response to endogenous AVP, stimulated by dehydration, to that in nondiabetic controls (10), although the diabetic patient cohort studied were well controlled compared with our patients. Lithium therapy is, however, an example of a metabolic modulator of aquaporin-2 expression. Lithium causes reversible nephrogenic diabetes insipidus in ~15% of patients treated with the drug for bipolar depression (2), and
work in the rat model has demonstrated downregulation of aquaporin-2 expression associated with lithium therapy (9), which was partially reversible on discontinuation of the drug, compatible with clinical observations of slow recovery from lithium-induced nephrogenic diabetes insipidus.

The clinical implications of the renal resistance to AVP in subjects with poorly controlled diabetes mellitus are clear. Poor glycemic control renders the kidneys relatively insensitive to the main homeostatic mechanism that limits water excretion, namely, AVP-stimulated antidiuresis. People with poorly controlled IDDM will therefore be less able to compensate for dehydrating illnesses, such as acute gastroenteritis, and will therefore be more likely to develop hypernatremic dehydration and hypovolemia during intercurrent illness. Renal resistance to AVP, caused by failure of aquaporin-2 recruitment, may therefore contribute to the propensity for patients with poor glycemic control to develop more marked dehydration and poorer outcome from diabetic ketoacidosis.

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