Attenuation of vasopressin-induced antidiuresis in poorly controlled type 2 diabetes

Amar Agha,1 Diarmuid Smith,1 Francis Finucane,1 Mark Sherlock,1 Andrew Morris,2 Peter Baylis,3 and Christopher J. Thompson4

1Academic Department of Endocrinology, Beaumont Hospital, Dublin 9, Ireland; 2Division of Medicine and Therapeutics, Ninewells Hospital, Dundee DD1 9SY, Scotland; and 3Endocrine Unit, Royal Victoria Infirmary, Newcastle NEI 4LP, England

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THE COMMONEST CLINICAL MANIFESTATION of poorly controlled diabetes mellitus is polyuria, leading to polydipsia. Although the polyuria in poorly controlled diabetes is traditionally attributed to an osmotic diuresis secondary to glycosuria, the underlying pathophysiology is complex (1). Renal resistance to the antidiuretic action of arginine vasopressin (vasopressin, AVP), with failure to recruit aquaporin-2, has been shown to be associated with impaired urine-concentrating ability in type 1 diabetes without nephropathy (4) and type 2 diabetes with nephropathy (6). Marked elevations in plasma vasopressin concentration have been reported in diabetic ketoacidosis (15), acute insulin withdrawal (5), and nonketotic hyperglycemia (17), indicating that vasopressin does not exert an antidiuretic effect under conditions of severe glycosuric diuresis. When patients with type 1 diabetes are infused with hypertonic sodium chloride solution to elevate plasma osmolality, they exhibit elevation in plasma vasopressin similar to controls but do not respond with similar increases in urine osmolality (11, 12). These results suggest that in type 1 diabetes, there is renal resistance to vasopressin. This hypothesis was tested in studies where vasopressin was infused intravenously into patients with type 1 diabetes who failed to concentrate urine or reduce urine volume compared with controls (4). Resistance to vasopressin was most marked in those patients with poor glycemic control, and the resistance to vasopressin could be reversed with short-term improvements in control. Failure of urine concentration in these studies was associated with a lower urinary concentration of aquaporin-2. Chronic hyperglycemia, therefore, blunts the ability of vasopressin to generate renal aquaporin-2 in type 1 diabetes, thereby attenuating the antidiuretic response to glycosuric diuresis.

Renal responses to vasopressin have not been studied in type 2 diabetes in the absence of nephropathy, although vasopressin secretion in response to dehydration has been shown to be normal (3). In this study, we have tested the hypothesis that the urine-concentrating responses to the rise in plasma vasopressin concentrations during water deprivation would be blunted in poorly controlled patients with type 2 diabetes compared with patients with good glycemic control. Water deprivation was selected as the stimulus to endogenous vasopressin secretion in this study, as it is the closest simulation of those physiological conditions that promote vasopressin secretion.

METHODS

Subjects. Ten subjects with poorly controlled type 2 diabetes (PCDS) and 10 with well-controlled type 2 diabetes (WCDS) were recruited from outpatient diabetic clinics. The diagnosis of type 2 diabetes was based on absence of ketonuria, age over 40 yr at diagnosis, and insulin independence for at least 1 yr after diagnosis. Good control was defined by a glycosylated hemoglobin (Hb A1c) <7% and poor control as Hb A1c >9%. The laboratory reference range for Hb A1c, derived from the local nondiabetic population, was 3.7–5.7%. Exclusion criteria included the presence of diabetic nephropathy, defined by either serum creatinine outside of the laboratory reference range or microalbuminuria; prostate disease; microscopic hematuria; frequent urinary tract infections; hypertension (office blood pressure >140/90 mmHg); cardiac disease; and treatment with diuretics, lithium, or angiotensin-converting enzyme inhibitors. All patients had normal thyroid function, including three patients receiving...
ing maintenance thyroxine therapy. Three PCDS and two WCDS had background diabetic retinopathy, identified by dilated fundoscopy, but no other diabetes-related complications were present. Ten non-diabetic age-matched control subjects (NDCS) were recruited from hospital staff. Clinical characteristics are summarized in Table 1.

Protocol. Subjects were admitted to the investigation unit at 0730 on the day of study. All subjects had been advised to avoid alcohol for 48 h and nicotine and caffeine for 12 h before the study. Subjects were encouraged to allow themselves free access to tap water on the morning of study but to arrive at the unit in a fasted state. Subjects with diabetes withheld their oral hypoglycemic agents on the morning of therapy, but subjects taking thyroxine continued this treatment.

After resting recumbent for 30 min, subjects had intravenous cannulae inserted into the antecubital fossa for venesection. Diabetic subjects had intravenous cannulae sited in the contralateral antecubital fossa by which they were attached to an intravenous infusion of soluble insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) constituted in the form of 50 IU of insulin dissolved in 50 ml of 0.9% sodium chloride in a 50-ml syringe in a syringe driver. The insulin infusion was commenced if the blood glucose concentration rose above 6.0 mmol/l (110 mg/dl), and the rate of infusion was adjusted to maintain blood glucose concentrations between 4 and 6 mmol/l (70–110 mg/dl) for the duration of the study. Blood glucose was monitored at the bedside at 15-min intervals throughout the study. Blood pressure was measured using a mercury sphygmomanometer.

Once stable euglycemia (plasma glucose concentration of 4–6 mmol/l) had been achieved, patients voided their bladders; an aliquot of urine was set aside for measurement of baseline urine osmolality, and the remainder was discarded. Baseline blood samples were withdrawn, and thirst was measured on a well-validated visual analog scale (10) that has been shown to be reproducible within an individual (13). Each subject then commenced a standard 8-h water deprivation test. Blood samples were taken at 2-h intervals during the study, and, at blood sampling times, thirst was assessed using the visual analog scale, and urine volume was measured and an aliquot retained for measurement of urine osmolality.

At the end of the water deprivation period, patients were allowed free access to tap water at room temperature, and the volume drunk in a 30-min period was noted.

Blood samples were taken through free-running intravenous cannulae into chilled syringes and transferred immediately into heparinized tubes, in ice, and centrifuged immediately at 4°C for 15 min at 2,000 g; the plasma supernatant was separated and divided into aliquots. One aliquot was used immediately for the measurement of plasma osmolality and plasma sodium concentration, and the other was frozen at −70°C for later measurement of plasma vasopressin.

Blood was also taken into EDTA tubes, and, after centrifugation, the plasma supernatant was stored for measurement of plasma renin. All studies were medically supervised and subjects were weighed at 2-h intervals, with the intention that studies would be stopped if subjects lost >5% of their body weight, as per standard protocols for the conduct of water deprivation testing.

Analyses. Plasma and urine osmolalities were measured by the depression-of-freezing-point method (Fiske 2400 Osmometer, Norwood, MA) and plasma sodium by the ion-selective electrode method (Olympus 2700, Tokyo, Japan). Plasma AVP was measured by a sensitive and specific radioimmunoassay after extraction from plasma by adsorption onto magnesium silicate (Florisil) (9). The limit of detection of the assay is 0.3 pmol/l, with intra- and interassay coefficients of variation of 9.7 and 15.3%, respectively. Blood glucose was measured by the glucose oxidase method (Yellow Springs autoanalyzer; Clandon Scientifics, London, UK). Plasma renin activity was measured by commercial antibody (Soten, Milan, Italy).

Statistical analysis. Data are expressed as means ± SD. Univariate analysis was done using the Student’s t-test. Repeated-measures analysis of variance (ANOVA) models were used to compare plasma and urine osmolalities, plasma vasopressin, plasma sodium, urine volume, and thirst between groups. Multiple-comparison tests, using a Bonferroni correction factor, were used to determine whether results reached significance at the 5% level. Stata (version 8) was used for statistical analysis.

Ethical considerations. The studies were approved by the ethics committees of Beaumont and Royal Victoria Infirmary Hospitals, and all patients signed an informed consent form before the study.

RESULTS

The studies were well tolerated in all volunteers. Blood glucose remained unchanged throughout the study (Fig. 1) in NDCS (4.5 ± 0.5 to 4.5 ± 0.3 mmol/l, \(P = 0.74\)), WCDS (5.0 ± 0.9 to 4.5 ± 0.5 mmol/l, \(P = 0.74\)), and PCDS (4.5 ± 0.9 to 4.5 ± 0.7 mol/l, \(P = 0.84\)). There were no differences in blood glucose concentration among the groups (\(P > 0.05\)). Baseline plasma renin activity was higher in PCDS (1.0 ± 0.8 ng·ml⁻¹·h⁻¹) than in NDCS (0.3 ± 0.2 ng·ml⁻¹·h⁻¹, \(P = 0.017\)) and WCDS (0.4 ± 0.3 ng·ml⁻¹·h⁻¹, \(P = 0.038\)). There was no difference in basal plasma renin activity between NDCS and WCDS (\(P = 1.0\)). Water deprivation caused a significant rise in plasma renin activity in NDCS, from 0.3 ± 0.2 to 0.9 ± 0.8 ng·ml⁻¹·h⁻¹ (\(P < 0.001\)); in WCDS, from 0.4 ± 0.3 to 0.9 ± 1.0 ng·ml⁻¹·h⁻¹ (\(P < 0.001\); and in PCDS, from 1.0 ± 0.8 to 2.1 ± 1.6 ng·ml⁻¹·h⁻¹ (\(P < 0.001\)).

Water deprivation caused an elevation in plasma osmolality in the NDCS, from 287.5 ± 2.4 to 296.6 ± 2.8 mosmol/kgH₂O (\(P < 0.001\)); in WCDS, from 287.7 ± 2.6 to 295.1 ± 2.1 mosmol/kgH₂O (\(P < 0.001\)); and in PCDS, from 294.0 ± 2.9 to 300.7 ± 3.1 mosmol/kgH₂O (\(P < 0.001\)) (Fig. 1). Plasma osmolality was consistently higher throughout the study period in the PCDS than in the NDCS (\(P < 0.001\)) or the WCDS (\(P < 0.001\)). There was no difference in plasma osmolality between the NDCS and the WCDS.

The elevation in plasma osmolality caused a rise in plasma AVP concentrations (Fig. 2) in NDCS, from 0.6 ± 0.4 to 2.1 ± 1.3 pmol/l (\(P < 0.001\)); in WCDS, from 0.5 ± 0.2 to 2.9 ± 2.0 pmol/l (\(P < 0.001\)) and in PCDS, from 0.7 ± 0.5 to 3.0 ± 2.6 pmol/l (\(P < 0.01\)). There were no differences in plasma...
vasopressin levels between the groups during the study (P > 0.05 between groups).

The elevation in plasma osmolality also stimulated an elevation in thirst ratings (Fig. 2) in NDCS, from 1.4 ± 0.8 to 8.0 ± 1.3 cm (P < 0.001); in WCDS, from 1.0 ± 0.9 to 7.5 ± 1.6 cm (P < 0.001); and in PCDS, from 2.3 ± 1.6 to 8.4 ± 1.6 cm (P < 0.001). The elevation in thirst was not different between PCDS and NDCS (P = 0.09) or WCDS (P = 0.059). However, the volume of water drunk in 30 min after water deprivation was higher in PCDS (915 ± 153 ml) than in NDCS

Fig. 1. Changes in plasma osmolality, plasma sodium, and blood glucose concentration during water deprivation. T2D, type 2 diabetic subjects.
The elevation of plasma AVP concentration caused a fall in urine flow (Fig. 3) in NDCS, from 79.3 ± 27.2 to 19.2 ± 11.2 ml/h \((P < 0.001)\); in WCDS, from 104.5 ± 37.5 to 26.3 ± 7.8 ml/h \((P < 0.001)\); and in PCDS, from 105.4 ± 41.9 to 52.6 ± 12.9 ml/h \((P < 0.001)\). The change in urine flow was not different between the NDCS and the WCDS \((P = 0.1)\), but the decrease in urine flow rate in response to vasopressin was higher in NDCS \((P < 0.001)\) and WCDS \((P = 0.046)\) than in PCDS. The total urine volume passed during the 8 h of water deprivation was higher in the PCDS (794 ± 161 ml) than in either the NDCS (507 ± 224 ml, \(P = 0.004\)) or the WCDS (589 ± 138 ml, \(P = 0.014\)) but was not different between the NDCS and the WCDS \((P = 0.37)\).

Urine osmolality rose in response to vasopressin (Fig. 3) in the NDCS, from 336.0 ± 123.3 to 786.5 ± 63.3 mosmol/kgH_2O \((P < 0.001)\); in WCDS, from 360.7 ± 142.8 to 794.1 ± 77.3 mosmol/kgH_2O \((P < 0.001)\); and in PCDS, from 280.3 ± 49.7 to 594.4 ± 88.5 mosmol/kgH_2O \((P < 0.001)\). The rise in urine osmolality was less in the PCDS than in the NDCS \((P = 0.019)\) or the WCDS \((P < 0.001)\). There was no difference in urine osmolality between NDCS and WCDS \((P = 0.39)\).

Linear regression analysis was applied to define the relationship between plasma osmolality and plasma vasopressin and between plasma osmolality and thirst. This mathematical model defines two important characteristics of the relationships between plasma osmolality and plasma vasopressin and between plasma osmolality and thirst. The slope of the regression line reflects the sensitivity of the relationship, whereas the intercept corresponds to the osmotic threshold at which vasopressin and thirst begin. The results are shown in Table 2. Linear regression analysis demonstrated that the characteristics of osmotically stimulated thirst and vasopressin release were similar in WCDS and NDCS, in that the slopes and intercepts were similar in the two groups.

In contrast, the osmotic thresholds (intercepts) for both thirst and vasopressin secretion were set at a higher plasma osmolality in PCDS than in either NDCS (thirst, \(P = 0.005\); vasopressin, \(P < 0.001\)) or WCDS (thirst, \(P = 0.007\); vasopressin, \(P < 0.001\)). The slopes of the mean lines relating plasma osmolality and plasma vasopressin and plasma osmolality and thirst in PCDS were similar to NDCS and WCDS (Table 2).
DISCUSSION

Our data show that patients with poorly controlled type 2 diabetes respond to dehydration with two distinct abnormalities of osmoregulation. First, the osmotic set point at which vasopressin secretion and thirst begin is reset at a plasma osmolality threshold 5–6 mosmol/kgH₂O above that for nondiabetic controls and patients with well-controlled type 2 diabetes. This means that the homeostatic responses to dehydration are not activated in PCDS until they are significantly more hyperosmolar than NDCS or WCDS. Second, in response to equivalent plasma vasopressin concentrations, PCDS are less able to concentrate urine or limit urine flow compared with NDCS or WCDS. This is the first time that these abnormalities have been demonstrated experimentally in type 2 diabetes.

The demonstration that the osmotic thresholds for thirst and vasopressin release were higher in PCDS was unexpected. Given that the PCDS were relatively volume depleted before commencement of water deprivation, as shown by the higher baseline plasma osmolality and the higher baseline plasma renin activity, one would expect a lowering of the osmotic thresholds for thirst and vasopressin secretion. In other experimental models, acute volume depletion has been shown to lower the osmotic threshold and increase the sensitivity of osmotically stimulated vasopressin release (8). In type 2 diabetes, subjects who have biochemical evidence of volume depletion but good glycemic control have elevated plasma vasopressin concentrations compared with euvolemic patients (2). Previous studies have demonstrated normal characteristics of osmotically stimulated thirst and vasopressin release in patients with well-controlled type 1 (11) and type 2 diabetes.

Table 2. Mean linear regression lines for NDCS, WCDS, and PCDS

<table>
<thead>
<tr>
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<th>Plasma Osmolality vs. pAVP</th>
<th>Plasma Osmolality vs. Thirst</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Slope</td>
<td>Intercept</td>
</tr>
<tr>
<td>NDCS</td>
<td>0.15±0.1</td>
<td>284.1±4.7*</td>
</tr>
<tr>
<td>WCDS</td>
<td>0.35±0.4</td>
<td>286.0±3.6*</td>
</tr>
<tr>
<td>PCDS</td>
<td>0.31±0.2</td>
<td>291.1±2.9</td>
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Results are means ± SD. pAVP, plasma arginine vasopressin concentration. *P < 0.001 compared with PCDS.
(3). In light of published data, therefore, it seems that the results of our study indicate that normal osmoregulation occurs in WCDS but that chronic hyperglycemia causes upward resetting of the osmotic threshold for thirst and vasopressin secretion, rather than the downward resetting that volume depletion would ordinarily cause (2). This specific effect of chronic poor glycemic control on osmoregulation has not been previously reported in type 2 diabetes.

Chronic poor glycemic control may directly attenuate the vasopressin response to dehydration. Early studies that established the solute specificity of the osmoreceptors for vasopressin release suggested that hyperglycemia lowered plasma vasopressin secretion (16). Although we have previously shown that acute hyperglycemia, over 12–24 h, does not impair the osmoregulation of thirst or vasopressin release, it may be that chronic hyperglycemia blunts the osmoreceptor response to elevation in plasma sodium such that the osmotic thresholds for thirst and vasopressin secretion are reset above the normal levels. If this hypothesis is true, it is clearly a maladaptive response, as it would be more useful to lower the thresholds for thirst and vasopressin release in the situation of hypovolemia.

The counterproductive effect of the elevated osmotic threshold for thirst and vasopressin release in PCDS is compounded by the impaired renal response to vasopressin. At the end of the period of water deprivation, plasma vasopressin concentrations were similar in all three groups. However, although the WCDS concentrated urine appropriately in response to vasopressin, with a rise in urine osmolality similar to that in controls, the PCDS neither concentrated urine nor reduced urine flow to the same extent as controls. This indicates renal resistance to vasopressin in PCDS, a situation analogous to that which we have already reported in type 1 diabetes (4). The pathophysiology behind renal resistance to vasopressin action in poorly controlled diabetes remains speculative. Although the traditional view has been that interstitial solute is the key determinant of renal concentrating ability, we have shown that, in human type 1 diabetes, impaired renal response to vasopressin is caused by a failure to generate aquaporin-2, the renal water channel (4). The ability to generate aquaporin-2 and concentrate urine was restored after short-term (7 days) improvement in glycemic control, suggesting that hyperglycemia per se attenuated the recruitment of aquaporin-2 in response to vasopressin. In contrast to our human data, studies in animal models of diabetes have shown no convincing evidence of impaired generation of aquaporin-2. Rat studies have suggested that the density and affinity of renal V2 receptors are unchanged in diabetic rats compared with controls, yet the poorly controlled animals were clearly polyuric (14). These studies, however, did not rule out impaired generation of aquaporin-2 by the V2 receptors. In acute streptozotocin-induced diabetic rats, semiquantitative immunoblotting revealed higher concentrations of inner medullary aquaporin-2 than in nondiabetic rats (7). Diabetic rats had marked hyperglycemia, with evidence of increased water intake and urine excretion, but the changes in renal aquaporins in relation to plasma vasopressin concentrations were not reported in this study. Thus, although this study showed that volume depletion caused the generation of renal aquaporin-2 in diabetic rats, it could not comment on whether the recruitment of aquaporin-2 was appropriate to ambient plasma vasopressin concentrations. In addition, control rats were not in an equivalent state of hypovolemic diuresis, so that comparisons between the aquaporin response to equivalent vasopressin concentrations in diabetic rats and control rats could not be made.

Impaired renal generation of aquaporin-2 in response to dehydration has also been shown in one other study of human type 2 diabetes (6). Diabetic patients with significant nephropathy, characterized by elevated serum creatinine, were shown to have lower urinary excretion of aquaporin-2 in response to dehydration than control subjects. We were careful to exclude patients with diabetic nephropathy, as manifested by either elevated serum creatinine or the presence of microalbuminuria. Our data show that, even in the absence of nephropathy, urine concentration is blunted in response to vasopressin in the setting of poor glycemic control.

Over the 8 h of water deprivation, PCDS excreted a mean of 200–300 ml more urine than WCDS or controls. The PCDS drank a mean of 200–3,000 ml more than either WCDS or controls in the 30 min after dehydration, indicating appropriate thirst responses to the greater degree of fluid loss in the PCDS group. However, the failure to concentrate urine and limit urine flow in response to dehydration would correspond to an extra 0.75 liters of fluid loss per 24 h, which is significant in situations where fluid intake is limited, such as during serious intercurrent illness or if drinking was not possible because of vomiting or lack of availability. Our studies were performed with blood glucose maintained in the euglycemic range. However, it would seem reasonable to speculate that the degree of dehydration might be greater still if dehydration occurred against the background of hyperglycemia, which is a common clinical scenario. We would hypothesize that the inability to respond to the antidiuretic action of vasopressin may worsen dehydration during intercurrent illness in PCDS.

One additional clinical point should be noted. One of 10 of the WCDS and 9 of 10 of the PCDS “failed” the water deprivation test, with peak urine osmolality <700 mosmol/kgH2O, the standard criterion used in our laboratory to indicate a normal response. Poor glycemic control should therefore be corrected before people with diabetes undergo a water deprivation test to investigate polyuria (for example, investigation for diabetes insipidus after pituitary surgery or head trauma).

In summary, therefore, we have shown that type 2 diabetic patients with poor glycemic control, confirmed by HbA1c concentrations >9%, have the following osmoregulatory abnormalities: 1) elevated osmotic thresholds for thirst and vasopressin release, 2) impaired renal concentrating ability in response to vasopressin, and 3) excess urine excretion in response to dehydration, with a consequent reliance on increased water intake to prevent dehydration. These abnormalities are likely to contribute significantly to the development of dehydration in poorly controlled diabetes.

DISCLOSURES

During this study, A. Agha was in receipt of a Pfizer International Research Fellowship.

REFERENCES


