Impaired intrarenal dopamine production following intravenous sodium chloride infusion in Type 1 (insulin-dependent) diabetes mellitus

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Summary. Type 1 (insulin-dependent) diabetes mellitus is characterized by impaired sodium excretion following NaCl infusion. To investigate the possible role of dopamine in the impaired natriuresis in diabetes, intrarenal sodium handling, sodium excretion and urinary dopamine output, reflecting intrarenal dopamine formation, were studied following a 2 h 0.9% NaCl infusion (25 ml/kg) in eight diabetic patients and nine control subjects. The increase in sodium excretion in response to NaCl infusion was significantly ($p < 0.01$) reduced in diabetic patients ($19 \pm 7\%$) as compared with control subjects ($46 \pm 8\%$). Fractional proximal tubular sodium reabsorption (determined by lithium clearance) decreased in the control group ($p < 0.01$) following NaCl infusion but not in the diabetic group. Fractional distal tubular reabsorption decreased similarly in both groups. In response to NaCl urinary dopamine excretion increased by approximately 15% ($p < 0.01$) in the control group but did not change in the diabetic group. The mean urinary dopamine excretion above basal was significantly greater

in the control group (8.4 ± 2.1 nmol/h) than in the diabetic group (-2.2 ± 2.1 nmol/h; $p < 0.01$). The urinary sodium/dopamine excretion ratio did not differ significantly between the two groups in the basal state or following NaCl. Baseline plasma levels of atrial natriuretic peptide did not differ between control and diabetic patients. In the control group atrial natriuretic peptide levels increased significantly ($p < 0.01$) in response to NaCl whereas atrial natriuretic peptide levels did not change in the diabetic group. The results of this study show that patients with Type 1 diabetes have a blunted natriuresis in response to isotonic NaCl. This abnormality seems mainly to be due to impaired inhibition of proximal tubular sodium reabsorption, which may be the result of defective intrarenal dopamine mobilisation.

Key words: Type 1 (insulin-dependent) diabetes mellitus, sodium retention, dopamine, atrial natriuretic peptide, proximal tubule.

A well recognized feature of Type 1 (insulin-dependent) diabetes mellitus is the tendency to sodium retention. Several studies have shown that patients with metabolically stable Type 1 diabetes have a 10–15% increase in total exchangeable sodium [1, 2]. It has also been documented that diabetic subjects have an impaired ability to excrete sodium following intravenous salt loading [3–5] or water immersion [6] and it has been suggested that increased tubular sodium reabsorption is an early functional change in the diabetic kidney [6]. The mechanism(s) of these phenomena remain unclear, but hyperinsulinaemia [4, 7–9] and atrial natriuretic peptide (ANP) abnormalities in hormonal action and secretion [4] have been discussed as contributing factors.

Among other putative factors the role of endogenous intrarenal dopamine as a locally acting natriuretic and vasodilatory agent has attracted increasing attention in the last decade [10]. Filtered L-dopa is decarboxyl-

ated to dopamine by L-dopa-decarboxylase, an enzyme which is present in abundance in the proximal tubular cells [11]. Dopamine, by rapid and reversible inhibition of Na^+/K^+ -ATPase in the proximal tubule, thereby causes natriuresis [12]. It has recently been clearly demonstrated that all of the urinary excretion of endogenous dopamine is accounted for by uptake and decarboxylation of circulating endogenous L-dopa in proximal tubular cells [13].

In the present study the acute effect of intravenous NaCl infusion on sodium excretion, urinary dopamine output and renal haemodynamics was studied in patients with Type 1 diabetes. As compared with control subjects, diabetic patients showed delayed sodium excretion which was associated with defective urinary dopamine excretion, suggesting that impaired renal dopamine formation may be a contributing factor to sodium retention in Type 1 diabetes.

Subjects and methods

Subjects

Eight male patients with Type 1 diabetes, aged 27 ± 3 (SD) years (range 22–30 years), with a mean duration of diabetes of 9 ± 7 (SD) years (range 4–25 years) were studied. They were all normotensive according to WHO standards. Their systolic, diastolic, and mean arterial blood pressures were 121 ± 4 mmHg, 85 ± 3 mmHg and 97 ± 3 mmHg, respectively. Their HbA_{1c} levels ranged between 5.3 and 8.5% (mean $7.3 \pm 0.5\%$). Body mass index (BMI) was 24.2 ± 1.1 (body weight/length²; kg/m²). No patient was on medication other than s.c. insulin (58 ± 5 U/day) and none had any evidence of neuropathy, retinopathy or albumin excretion above 1 mg/h. Nine healthy, normotensive males aged 33 ± 1 (SD) years (range 31–40 years) were studied as control subjects. Systolic (114 ± 2 mmHg), diastolic (74 ± 2 mmHg), and mean arterial blood pressures (87 ± 2 mmHg) were slightly, but significantly lower ($p < 0.05$) in the control subjects than in the diabetic patients. BMI in the control group (23.0 ± 0.7 kg/m²) was not significantly different from that in the diabetic group. None of the control subjects were on any medication. All subjects were recommended to continue their usual diets and were maintained on an ad libitum sodium and protein diet.

Study protocol

The diabetic patients were admitted to the ward on the day prior to the investigation and were withdrawn from s.c. intermediate-acting insulin at least 24 h and short-acting insulin at least 15 h before the study period. At 17.00 hours an intravenous infusion of soluble insulin (Actrapid Human Novo A/S, Bagsvaerd, Denmark) was started, and the infusion rate was adjusted by frequent determinations of blood glucose for the purpose of maintaining stable blood glucose between 4 to 6 mmol/l during the night and throughout the study. All subjects received 16.2 mmol of lithium, as lithium carbonate, orally the evening before the clearance study and all subjects fasted overnight. On the morning of investigation the subjects drank 500 ml of tap water. At 08.00 hours infusions of 20% para-amino hippuric acid (PAH; 0.2 ml/kg body weight; rate, 0.5 ml/min) and 25% inulin (Inutest, 0.2 ml/kg body weight; rate, 0.5 ml/min) were started. After an equilibration period of one hour timed urine collections were started. To ensure adequate diuresis 300 ml of tap-water was given orally each hour. During the study period the subjects were in the supine position except when voiding urine. The subjects voided urine hourly and urine samples were analysed for inulin, PAH, lithium (Li), dopamine, sodium (Na) and potassium (K). Blood samples for inulin, PAH, lithium, atrial natriuretic peptide (ANP), renin, blood glucose, haematocrit, Na and K were taken after each clearance period. After a basal one-hour clearance period an infusion of 0.9% NaCl (154 mmol/l) was started and given for 2 h at a rate of 25 ml/kg body weight. The subjects were then observed for a further 2 h with hourly blood and urine sampling. All subjects gave informed consent and approval was obtained from the Local Ethical Committee.

Calculations and assay methods

Mean arterial pressure was calculated as diastolic blood pressure + one third of the pulse pressure. Sodium (C_{Na}), inulin (C_{In}) and lithium (C_{Li}) clearance were calculated according to the conventional formula. The mean of the inulin, PAH and Na values, respectively, at the beginning and end of each clearance period was used for clearance calculations. Lithium clearance was performed in accordance with the method described by Thomsen [14] which is based on the assumption that lithium is reabsorbed in the proximal tubule in parallel with sodium and water and that it is neither secreted nor reabsorbed beyond the proximal tubule. Hence the fractional proximal tubular reabsorption (FPR) of sodium was calculated as $1 - (C_{Li}/C_{In})$ and the fractional distal tubular (FDR) sodium reabsorption as $1 - (C_{Na}/C_{Li})$. Urinary sodium and urinary dopamine were measured as mmol/h and nmol/h, respectively.

Urinary dopamine were extracted essentially as described in [15] except that 0.1 mol/l perchloric acid was used instead of 1 mol/l acetic acid for the elution of adsorbed dopamine from alumina. In addition, alfa-methyldopamine was used as internal standard instead of 3,4-dihydroxy-bensylamine. Dopamine were analysed by high performance liquid chromatography with electrochemical detection. The chromatographic system consisted of a LDC Constametric III pump, a cation exchange column (Nucleosil 10 SA, 25 cm $\times$ 4.6 cm) and a BAS amperometric detector model LC4b/CC4. The mobile phase was a citrate-acetate buffer, pH 5.2 as previously described [16]. The flow rate was 2.5 ml/min and the catecholamine concentration were calculated from peak height ratios (sample/internal standard). Specific radioimmunoassay techniques were used to analyse ANP as previously described [17] and plasma insulin [18]. Plasma renin activity was analysed with a commercial kit (Phadebas angiotensin-I test, OY Medix, Helsinki, Finland). Serum and urinary concentrations of lithium were measured by atomic absorption (Modell IL 750) according to the method previously described by Amidsen [19] with the exception that proteins was precipitated with HNO₃. To correct for the precipitation of protein serum lithium concentrations was multiplied by a factor of 0.965. Determinations of blood glucose, haematocrit, Na and K were carried out in the Department of Clinical Chemistry, Huddinge Hospital, using routine methods.

Statistical analysis

Unless stated otherwise, all data are given as means $\pm$ SEM. Non-parametric statistics were applied. The Mann-Whitney U test was used to test differences between diabetic patients and control subjects. The Friedman two-way analysis of ranks and the Wilcoxon matched-pairs signed-ranks test were applied to test changes from basal values. Correlations between variables were tested with Spearman's rank correlation. The null hypothesis was rejected with p -values less than 0.05.

Results

Mean plasma blood glucose levels did not differ between the two groups at the start of the NaCl infusion (diabetes: 4.7 ± 0.4 mmol/l; control 4.0 ± 0.1 mmol/l) and were kept constant throughout the study in both groups. There was no glucose detectable in urine at any time point during the study period in either group. In the diabetic group mean plasma insulin levels (μ U/ml) were 26.9 ± 4.9 in the basal period, 22.7 ± 5.0 after NaCl and 18.1 ± 3.8 2 h after the NaCl load. The corresponding plasma insulin levels in the control group were 6.4 ± 0.9 , 3.9 ± 0.3 and 4.4 ± 0.4 μ U/ml, respectively. There was a significant and comparable fall in haematocrit in response to NaCl in both groups. Urine flow rate was 7.2 ± 0.4 ml/min in the control group and 7.9 ± 0.5 ml/min in the diabetic group.

The haemodynamic parameters are presented in Table 1. In the control subjects glomerular filtration rate (GFR) (estimated by inulin clearance) decreased by approximately 12% ($p < 0.05$) following NaCl infusion, whereas GFR remained constant in the diabetic subjects. In both groups there was a transient fall in filtration fraction. The plasma renin activity did not differ significantly between the two groups in the basal state or following NaCl infusion (Table 1). Similarly there were no differences between the two groups with regard to plasma levels of ANP in the basal state. However, while plasma levels of ANP levels increased significantly in the control group following NaCl infusion, ANP levels in the diabetic group did not change (Table 1).

Table 1. Renal haemodynamic parameters in the basal and the 1st to 4th hour clearance periods in control and diabetic subjects

Clearance period	Inulin clearance (ml/min)		PAH-clearance (ml/min)		Filtration fraction (%)		Renin (ug·l ⁻¹ ·h ⁻¹)		ANP (pmol/l)	
	Control subjects	Diabetic patients	Control subjects	Diabetic patients	Control subjects	Diabetic patients	Control subjects	Diabetic patients	Control subjects	Diabetic patients
Basal	122 ± 7	119 ± 10	644 ± 19	638 ± 42	18.9 ± 0.9	18.9 ± 1.0	0.9 ± 0.2	1.4 ± 0.4	10.5 ± 0.5	10.7 ± 0.8
1st hour	113 ± 4 ^a	113 ± 11	628 ± 8	650 ± 50	18.0 ± 0.8	17.6 ± 0.9 ^a	–	–	–	–
2nd hour	108 ± 4 ^a	119 ± 10	643 ± 11	659 ± 53	16.9 ± 0.5 ^b	18.2 ± 0.9	0.4 ± 0.05 ^a	0.6 ± 0.2 ^a	16.4 ± 3.7 ^b	12.0 ± 1.6
3rd hour	107 ± 4 ^b	114 ± 14	597 ± 11	588 ± 45 ^a	17.9 ± 0.7 ^a	19.0 ± 1.2	–	–	–	–
4th hour	112 ± 5	112 ± 12	596 ± 10	577 ± 45 ^a	18.8 ± 0.8	19.5 ± 1.4	0.4 ± 0.04 ^a	0.5 ± 0.1 ^a	13.5 ± 1.8	12.5 ± 1.9

Mean ± SEM indicated

^a *p* < 0.05 vs basal; ^b *p* < 0.01 vs basal

PAH = para-amino hippuric acid;

ANP = atrial natriuretic peptide

Table 2. The renal handling of sodium and dopamine in the basal and the 1st to 4th hour clearance periods in control and diabetic subjects

Clearance period	Urinary sodium (mmol/h)		Proximal reabsorption (%)		Distal reabsorption (%)		Urinary dopamine (nmol/h)	
	Control subjects	Diabetic patients	Control subjects	Diabetic patients	Control subjects	Diabetic patients	Control subjects	Diabetic patients
Basal	11.5 ± 1.5	17.0 ± 3.1	73.9 ± 1.7	70.1 ± 2.0	95.3 ± 0.5	93.9 ± 1.0	63.1 ± 9.1	81.4 ± 5.5
1st hour	16.2 ± 2.4 ^b	17.0 ± 3.1	71.8 ± 1.5 ^a	71.7 ± 1.3	94.1 ± 0.7	93.2 ± 1.2 ^a	70.9 ± 12.3	74.5 ± 5.9
2nd hour	16.6 ± 1.8 ^b	20.9 ± 2.5 ^a	69.6 ± 1.6 ^b	68.8 ± 1.7	94.0 ± 0.7 ^a	92.6 ± 1.2 ^a	71.2 ± 8.0 ^b	83.3 ± 5.6
3rd hour	19.1 ± 1.9 ^b	24.4 ± 1.8 ^a	67.8 ± 2.0 ^b	67.6 ± 1.4	93.2 ± 0.6 ^b	91.1 ± 1.2 ^a	73.3 ± 9.0 ^b	83.9 ± 5.7
4th hour	22.1 ± 2.3 ^b	25.1 ± 2.0 ^a	69.6 ± 2.0 ^a	68.0 ± 2.2	91.4 ± 1.4 ^b	90.5 ± 1.1 ^a	69.1 ± 9.8 ^a	83.1 ± 4.8

Mean ± SEM indicated

^a *p* < 0.05 vs basal; ^b *p* < 0.01 vs basal

Data on the intrarenal handling of sodium and the urinary output of sodium and dopamine in the basal state and in response to NaCl infusion are presented in Table 2. Basal urinary sodium excretion tended to be higher in the diabetic patients than in the control subjects, although the difference did not attain statistical significance. During the 2-h NaCl infusion sodium excretion increased by 46 ± 8% in the control group (*p* < 0.01), whereas in the diabetic group a delayed and less marked (19 ± 7%) increase was observed. Accordingly, the increase in sodium excretion was lower in the diabetic group (Fig. 1).

One and two hours after termination of the NaCl infusion the cumulative sodium excretion above baseline was still less in the diabetic group. Neither proximal nor distal fractional reabsorption of sodium differed significantly between the diabetic and control groups in the basal state. However, in the control group the fractional proximal tubular reabsorption of sodium (assessed by *C_{Li}*), decreased immediately following NaCl infusion (*p* < 0.01), whereas there was no significant decrease in the diabetic group. In both groups there were statistically significant and comparable falls in distal tubular handling of sodium. Baseline urinary excretion of dopamine tended to be greater in the diabetic patients than in the control subjects, although the difference was not statistically significant. There was a marked increase in urinary dopamine excretion during and following NaCl infusion in the control group (Table 2). In contrast, there was no change in dopamine excretion in the diabetic group. The mean urinary dopamine excretion above baseline was significantly greater in the control group (8.4 ± 2.1 nmol/h) than in the diabetic group (–2.2 ± 2.1 nmol/h; *p* < 0.01). The ratio between urinary excretion of sodium and urinary dopamine (U-Na/U-DA) in the basal state did not differ significantly

between the diabetic patients and control subjects (0.21 ± 0.04 and 0.25 ± 0.06, respectively). In both groups the ratio increased in a similar way following NaCl infusion, and the ratio did not differ significantly between the two groups at any time point. Whether calculated for the whole group or for the control and diabetic groups separately, there was no significant correlation between basal sodium excretion and change in sodium or dopamine excretion following NaCl infusion. There was no significant correlation between the changes in plasma ANP levels and the changes in urinary dopamine output, respectively. Neither was there any significant correlation between plasma insulin levels and urinary dopamine or sodium output at any time point.

Discussion

Although impaired efficiency in excreting sodium in response to an intravenous sodium load in patients with Type 1 diabetes has been demonstrated in several studies [3–5], the mechanism(s) underlying this abnormality are not well understood. In keeping with recent results by Trevisan et al. [4], the current findings demonstrate that the delayed and blunted acute natriuretic response to NaCl-infusion in diabetic patients is mainly due to impaired inhibition of proximal tubular sodium reabsorption. The results of this study also show for the first time that in diabetic patients the urinary dopamine response to NaCl-infusion is impaired. In the control subjects NaCl-infusion resulted in a prompt increase in urinary sodium excretion which was paralleled by a corresponding increase in urine dopamine output. In contrast, in the diabetic patients the increase in sodium excretion was markedly delayed and no

Fig. 1. The cumulative sodium excretion (mmol/h) above baseline in control subjects (□) and patients with diabetes mellitus (■). Vertical bars indicate SEM. * $p < 0.05$ and ** $p = 0.01$

significant increase in urinary dopamine output was observed. The ratio between urinary excretion of sodium and urine output of dopamine was comparable in the diabetic patients and the control subjects both in the basal state and following NaCl-infusion. This finding, in keeping with results recently presented by Patrick et al. [20], suggests that the relationship between dopamine and sodium excretion is normal in the basal state in diabetic patients and that the blunted acute natriuretic response to NaCl-infusion was associated with impaired dopamine mobilization rather than with attenuated renal tubular sensitivity to dopamine. Taken together these observations suggest that the delayed sodium excretion in Type 1 diabetes may, at least partly, be secondary to attenuated intrarenal dopamine formation and, consequently, impaired inhibition of $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity in the proximal tubule.

To the best of our knowledge, the dopamine response to intravenous or oral salt loading has not previously been studied in diabetic patients. The mechanism(s) causing the attenuated dopamine response to NaCl-infusion in the diabetic patients are not clear. All subjects were normoglycaemic at the start of NaCl-infusion and blood glucose remained constant throughout the 4 h observation period. Consequently hyperglycaemia cannot explain the abnormal mobilization of dopamine in the diabetic patients. Baseline output of dopamine and sodium tended to be higher in the diabetic group as compared with the control group. Although the differences between the two groups did not reach statistical significance the data may suggest a higher dietary sodium intake in the diabetic group. Conceivably, higher dietary sodium intake might then have impaired the ability to acutely increase dopamine formation and sodium excretion in response to NaCl-infusion in a comparable way to the control subjects. However, the lack of any correlation between basal sodium or dopamine excretion with either the changes in urinary output of sodium or dopamine excretion following NaCl-infusion renders this possibility unlikely. It has been reported that lithium carbonate used

for the assessment of C_{Li} reduces cumulative sodium excretion by up to 40% [21]. Whether lithium carbonate influences dopamine excretion is not known. Whilst such an effect cannot be excluded, it is unlikely that an effect of lithium contributed to the difference in dopamine response, since both groups received the same amount of lithium.

Since circulating insulin levels were higher in the diabetic patients than in the control subjects, a suppressive effect of insulin on renal dopamine formation is another possible explanation for the attenuated dopamine formation and sodium excretion in the diabetic group. Indeed, several studies have demonstrated that insulin exerts an antinatriuretic effect in the kidney [4, 7–9]. However, the antinatriuretic site of action of insulin within the kidney is controversial. By micropuncture technique De Fronzo et al. [7] located the acute antinatriuretic effect of insulin mainly to the distal tubule. Using the euglycaemic insulin clamp technique and lithium clearance determination, Skøtt et al. [8] studied the effect of insulin on renal sodium handling in healthy subjects. Their results demonstrate that physiological elevation of plasma insulin levels causes sodium retention by stimulating distal tubular reabsorption of sodium. However, in conflict with these results, observations by Trevisan et al. [4] imply that hyperinsulinaemia leads to increased proximal sodium reabsorption. In addition the results showed that hyperinsulinaemia causes attenuated inhibition of proximal tubular sodium reabsorption in response to volume expansion by NaCl. Brimble et al. [22], however, did not observe an antinatriuretic effect of hyperinsulinaemia in subjects in whom central blood volume was increased during water immersion. In view of these results, although conflicting, we cannot exclude the possibility that in our diabetic patients hyperinsulinaemia may have contributed to the impaired acute natriuretic response to NaCl-infusion. If this were the case, since insulin may stimulate [23] and dopamine inhibits [12] tubular $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity, one might speculate that the stimulatory action of insulin on sodium reabsorption in the proximal tubule may, at least partly, be mediated by inhibition of dopamine formation. However, further studies are needed to test the validity of this hypothesis.

Acute volume expansion is a powerful stimulus for the release of ANP from the atria [24] and it has been documented that ANP contributes to the natriuretic response to volume expansion [25, 26]. In the present study NaCl administration resulted in significantly increased plasma ANP levels in the control subjects, whereas no significant changes were observed in the diabetic patients. Sodium handling and plasma ANP concentrations in response to NaCl-infusion were also recently investigated in normotensive patients with Type 1 diabetes by Trevisan et al. [4]. In agreement with our findings they observed an attenuated ANP response in diabetic subjects. Conceivably therefore, the impaired ANP response may also have contributed to the attenuated sodium excretion in the diabetic patients. Many of the physiological effects of ANP such as natriuresis, diuresis, vasodilation and inhibition of the renin-aldosterone system are shared by dopamine and it has been suggested that the renal effect of ANP may, at least partly, be mediated by dopamine [27]. In rats dop-

amine receptor antagonists has been shown to attenuate the natriuresis and diuresis stimulated by exogenous ANP [27–30]. In man, however, inhibition of dopamine production by inhibition of dopa-decarboxylase using carbidopa does not seem to affect the renal natriuretic response to ANP. This implies that in humans the renal effects of ANP are not mediated by dopamine [31]. The lack of any correlation between the rise in ANP and urinary output of dopamine in the present study also argues against ANP acting through intrarenal dopamine to cause natriuresis.

In summary, this study confirms previous results showing that patients with Type 1 diabetes have a blunted excretion of sodium in response to an acute NaCl-load. This abnormality seems mainly to be due to impaired inhibition of proximal tubular sodium reabsorption, which may, at least partly, be the result of defective intrarenal dopamine mobilisation.

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