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Vasopressin contributes to hyperfiltration, albuminuria, and renal hypertrophy in diabetes mellitus: Study in vasopressin-deficient Brattleboro rats

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ABSTRACT **Diabetic nephropathy represents a major complication of diabetes mellitus (DM), and the origin of this complication is poorly understood. Vasopressin (VP), which is elevated in type I and type II DM, has been shown to increase glomerular filtration rate in normal rats and to contribute to progression of chronic renal failure in 5/6 nephrectomized rats. The present study was thus designed to evaluate whether VP contributes to the renal disorders of DM. Renal function was compared in Brattleboro rats with diabetes insipidus (DI) lacking VP and in normal Long-Evans (LE) rats, with or without streptozotocin-induced DM. Blood and urine were collected after 2 and 4 weeks of DM, and creatinine clearance, urinary glucose and albumin excretion, and kidney weight were measured. Plasma glucose increased 3-fold in DM rats of both strains, but glucose excretion was ≈40% lower in DI-DM than in LE-DM, suggesting less intense metabolic disorders. Creatinine clearance increased significantly in LE-DM ($P < 0.01$) but failed to increase in DI-DM. Urinary albumin excretion more than doubled in LE-DM but rose by only 34% in DI-DM rats ($P < 0.05$). Kidney hypertrophy was also less intense in DI-DM than in LE-DM ($P < 0.001$). These results suggest that VP plays a critical role in diabetic hyperfiltration and albuminuria induced by DM. This hormone thus seems to be an additional risk factor for diabetic nephropathy and, thus, a potential target for prevention and/or therapeutic intervention.**

One of the major complications of diabetes mellitus (DM) is a progressive nephropathy that develops in about one-third of patients within 10–20 years after the onset of the disease and leads in most cases to end stage renal failure (1). This represents a major problem of public health because a large fraction of dialysis requirements is attributable to DM nephropathy. Although a number of studies have already been devoted to this problem, the factors contributing to diabetic nephropathy are not yet fully identified.

A characteristic feature observed in diabetic patients is an elevation of plasma vasopressin (VP), well documented in both type I and type II DM (2–5). This elevation also occurs in animal models of DM, whether experimental or genetically determined (6, 7). Several studies have investigated the possible factors responsible for this increase in VP secretion (3, 6, 8, 9). But they did not succeed in identifying the responsible stimulus for this increase. They revealed a resetting of the osmostat in diabetics but concluded that hyperglycemia was not responsible for this resetting because increasing plasma glucose and osmolality by intravenous infusion of hypertonic dextrose produced no increase in plasma vasopressin in diabetics or in healthy controls (8).

Little attention has been given to the possible functional consequences of the rise in plasma VP. To our knowledge, the possible contribution of VP to the renal complications of DM has never been investigated in spite of several previous findings suggesting that this hormone represents a risk factor for progression of renal failure: (*i*) An increase in water intake resulting in a fall in endogenous VP level was shown to slow the progression of chronic renal failure induced by 5/6 nephrectomy in rats (10). (*ii*) In normal rats, VP was found to induce a distinct hyperfiltration (11–13), an increase in albumin excretion (14), and a marked hypertrophy of the kidney (15). (*iii*) VP was shown to participate in the protein-induced glomerular hyperfiltration (15, 16), a factor known to promote albumin leakage through the glomerular filter, and glomerulosclerosis (17).

The major effect of VP on the kidney is to favor water reabsorption in the collecting duct, an effect dependent on its binding to V2 receptors. The effects of VP on glomerular filtration rate (GFR), albuminuria, and kidney hypertrophy are thought to result, indirectly, from its antidiuretic activity and the ensuing alterations in tubulo-glomerular feedback control of glomerular haemodynamics (12, 15, 18). In addition to its renal effects, VP also may influence vascular resistance through V1a receptors, expressed in smooth muscle cells. Less often mentioned, V1a receptors are also abundantly expressed in the liver (19–21), and VP was shown to stimulate glycogenolysis, gluconeogenesis, and ureagenesis in isolated perfused liver or hepatocyte suspensions, in the same way as does glucagon (22–27).

Because of the known influence of VP on renal function and liver metabolism, it is possible to assume that this hormone might contribute to perturbations in renal and metabolic complications observed in DM. The present study was designed to evaluate this possibility. We took advantage of Brattleboro rats, which cannot secrete VP because of a deletion in the corresponding gene (28), resulting in a recessive form of central diabetes insipidus (29, 30). We studied plasma composition and renal function in homozygous Brattleboro rats and in control Long-Evans rats (from which the Brattleboro strain originated) 2 and 4 weeks after induction of type I DM by streptozotocin administration. Because VP is mainly concerned with the control of water excretion, special atten-

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Abbreviations: VP, vasopressin (antidiuretic hormone); BW, body weight; Cont, control rats (nondiabetic); DI, diabetes insipidus (Brattleboro rats with diabetes insipidus); DM, Diabetes mellitus (Type I); LE, Long-Evans rats; T^cH_2O, solute-free water reabsorption; U_{osm}, urine osmolality; P_{osm}, plasma osmolality; V, urine flow rate; GFR, glomerular filtration rate.
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tion was given to fluid intake and output and to solute-free water handling. Factors that are known to be abnormal in early type I DM also were assessed, including glucose and urea handling (resulting from liver metabolism), creatinine clearance (an index of glomerular filtration rate), and renal mass. Because microalbuminuria is recognized as an early sign of diabetic nephropathy, we also evaluated urinary albumin excretion.

The results of this study suggest that VP is essential for the development of diabetic nephropathy because Brattleboro rats with DM exhibited no or markedly reduced hyperfiltration, albuminuria, and renal hypertrophy. VP thus appears to be a risk factor in this frequent complication of DM and a future possible therapeutic target for its prevention.¶

MATERIALS AND METHODS

Animals. Experiments were carried out in 16 male homozygous Brattleboro rats with hereditary central diabetes insipidus (DI) bred in our laboratory (Institut National de la Santé et de la Recherche Médicale, Unité 90, Necker Hospital), and in 16 male Long-Evans (LE) rats bought from R. Janvier (Le-Gesnet-Saint-Isle, Mayenne, France). All rats weighed 250–280 g at the beginning of the experiment. They were housed in individual metabolic cages and were offered regular powdered food (M25C, Extralabo, Provins, France) and tap water ad libitum during the whole experiment.

Experimental Protocol. After 1 week of adaptation to metabolic cages, urine was collected for two consecutive 24-hour periods to evaluate basal urine flow rate (V) and osmolality. Metabolic cages were siliconized to minimize urine losses. Rats of each strain then were divided into two groups of eight rats each, with similar mean body weight (BW), osmolar excretion, and urine concentrating activity. DM was induced in one subgroup of each strain by a single i.p. injection of streptozotocin (Sigma), 65 mg/kg BW in 0.1 M sodium citrate buffer. The eight other rats of each strain served as controls (Cont) and received buffer only. Two days later, DM was confirmed by the presence of glucose in urine.

Rats then were studied during the last 2 days of the second and fourth weeks after induction of DM. Two consecutive 24-hour urine collections were performed, and BW and daily water and food intake were measured. On week two, immediately after completion of urine collection, a blood sample (400 μl) was collected from a jugular vein in a heparanized tube, under brief ether anesthesia. On week four, after completion of urine collection, rats were anesthetized with 50 mg/kg BW i.p. sodium pentobarbital (Sanofi, Libourne, France). A blood sample was taken, and kidneys and liver were removed and weighed.

Analysis. Urine osmolality (U_{osm}) and plasma osmolality (P_{osm}) were measured with a microosmometer (Roebling, Berlin, Germany). Concentration of urea, glucose, and creatinine was measured with an automatic analyzer (Hitachi 717, Tokyo). Urine VP concentration was measured by radioimmunoassay (Vasopressin RIA, Nichols Institute, San Juan Capistrano, CA) and urine albumin concentration by radial immunodiffusion using a rat antialbumin antibody (Nordic, Tilburg, The Netherlands). The minimum concentration detectable was 3 mg/100 ml. Urine from LE-DM rats and from all DI rats had to be concentrated 5- to 8-fold with a Speedvac concentrator (CVC 100H, Savant) for measurement of albumin. The exact concentration factor was determined by the difference in weight before and after partial evaporation. Daily excretion of the different urinary solutes, VP, and albumin, as well as creatinine clearance, an index of GFR, and solute-free water reabsorption (T^cH_2O) were calculated according to standard formulas.

Statistics. Results are expressed as means ± SEM. All urine data are means of the two successive 24-hour urine collections for each rat. Data was analyzed by two-way ANOVA (rat strain and streptozotocin treatment), followed by Fisher's post hoc test. A P value ≤ 0.05 was considered statistically significant.

RESULTS

Main results observed 2 weeks after induction of DM are shown in Table 1. One rat of the DI-Cont group that ate much less than other rats of the same group and lost weight during the first week was excluded from the study. Rats with DM did not grow (LE) or even lost weight (DI), although both strains increased their food intake to the same extent compared with their respective controls (+38% and +40%, no interaction in ANOVA, Table 1). Urinary excretion of VP was markedly increased in LE-DM, amounting to 19.7 ± 1.6 ng/day, versus only 3.3 ± 0.2 ng/day in LE-Cont ($P < 0.001$). VP was undetectable in urine of DI rats.

In LE rats, as expected, DM induced an increase in water intake and V (+94 and +76 ml/day, respectively) and an almost 2-fold decrease in U_{osm} (Table 1). However, because of the >3-fold elevation in osmolar excretion, T^cH_2O was also markedly increased (+226 ml/day). Control DI rats exhibited very high V, hypotonic urine, and increased plasma osmolality

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Table 1. Different variables observed 2 weeks after injection of streptozotocin or vehicle

		Long-Evans			Brattleboro			ANOVA		
		Cont, $n = 8$	DM, $n = 8$	DM/Cont	Cont, $n = 7$	DM, $n = 8$	DM/Cont	S	D	I
BW	g	320 ± 3	264 ± 5***	0.83	286 ± 9	228 ± 6***	0.80	c	c	
BW gain	g	55 ± 3	−1 ± 3***	−0.01	11 ± 6	−20 ± 6***	−1.86	c	c	a
Food intake	ml/day	21 ± 1	29 ± 1***	1.38	20 ± 1	28 ± 1***	1.40		c	
Water intake	ml/day	27 ± 1	121 ± 7***	4.48	203 ± 11	371 ± 29***	1.83	c	c	a
Urine flow rate	ml/day	16 ± 1	92 ± 6***	5.75	156 ± 11	309 ± 23***	1.98	c	c	b
Urine osmolality	mOsm/kg H_2O	2337 ± 97	1443 ± 34***	0.62	191 ± 13	342 ± 15***	1.79	c	c	c
Osm. exc.	mmol/day	36 ± 1	132 ± 7***	3.67	29 ± 1	104 ± 5***	3.57	c	c	a
$T^c H_2O$	ml/day	78 ± 2	304 ± 16***	3.90	−66 ± 11	−4 ± 16***	0.06	c	c	c
Extr. water loss	ml/day	12 ± 1	29 ± 3***	2.50	47 ± 4	72 ± 10***	1.55	c	c	
Plasma osmolality	mOsm/kg H_2O	313 ± 1	336 ± 4***	1.07	328 ± 4	354 ± 5***	1.08	c	c	
P_{osm}–P_{glu}	mmol/liter	302 ± 1	306 ± 4	1.01	319 ± 4	321 ± 4	1.01	c		

Values are means ± SEM. BW gain, body weight gain between days 0 and 14; Osm. exc., osmolar excretion; Extr. water loss, extrarenal water losses; P_{osm}, plasma osmolality; P_{glu}, plasma glucose concentration. ANOVA: S, strain effect; D, diabetes effect; I, interaction; a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$. Fisher's post hoc test: ***, $P < 0.001$ vs. Cont in each strain.

(Table 1). As in LE rats, DM induced, in DI rats, an increase in water intake and V (+168 and +153 ml/day, respectively). These increases were larger, in absolute terms, than those observed in LE rats but were smaller relative to their respective controls (Table 1). Osmolar excretion was increased by DM in DI rats in the same proportion as in LE rats, and the excretion of free water (which amounted to 66 ml/day in DI-Cont) was almost abolished. Extrarenal water loss was increased to the same extent in LE and DI rats. DM induced a similar increase in plasma osmolality in rats with or without VP (no interaction in ANOVA). This increase was almost entirely attributable to glucose (Table 1).

LE rats with DM exhibited a significantly higher creatinine clearance than control rats (+22%, $P < 0.05$, and +53%, $P < 0.01$, at 2 and 4 weeks, respectively), indicating a progressive increase in GFR. In contrast, in rats without VP, DM did not induce any significant change in creatinine clearance (Table 2). In LE rats, albumin excretion was 2-fold higher in DM than in controls at 2 weeks, and 3.3-fold higher at 4 weeks. The rise in albumin excretion was much less intense in DI rats with DM (Table 2).

Both LE-DM and DI-DM rats exhibited marked hyperglycemia and glycosuria (Fig. 1). However, glucose excretion was ≈40% lower in DI than in LE rats ($P < 0.05$) whereas hyperglycemia was modestly higher in DI ($P < 0.02$). Glucose represented ≈48% of total urinary solutes in LE-DM rats and was markedly concentrated in urine with respect to plasma (25-fold). In DI-DM rats, glucose represented only 38% of total solutes and was much less concentrated (Table 3).

The lower glucose excretion observed in DI rats is not attributable to greater maximum reabsorptive capacity because DI-DM reabsorbed the same amount of glucose as did LE-DM (Table 3). Thus, because plasma glucose and glucose reabsorption were similar, the difference in glucose excretion was mainly accounted for by the difference in GFR. Excretion of urea in DM rats of both strains exceeded that in their respective controls by ≈10 mmol/day (31.2 ± 1.2 vs. 21.5 ± 0.5 mmol/day in LE, and 27.5 ± 1.6 vs. 17.6 ± 0.5 mmol/day in DI). Because gluconeogenesis and ureagenesis are closely associated in the liver and because the excretion of these two solutes is elevated by DM, we looked for a possible correlation between their respective excretions in DM rats. A significant positive correlation was indeed found in LE-DM rats ($r = 0.921$, $P < 0.001$) but not in DI-DM rats ($r = 0.253$, nonsignificant) (data not shown).

As shown in Fig. 2*A*, the increases in V and T^cH_2O observed in LE-DM rats were both positively correlated with the rise in osmolar excretion. In contrast, no correlation between free water excretion and osmolar load existed in DI-DM rats ($r = 0.039$) (data not shown). In LE-DM, glucose was the solute that accounted for the largest part of free water reabsorption, and the correlation of T^cH_2O with glucose excretion was almost as good as that with total osmoles ($r = 0.905$, $P < 0.001$) (data not shown). It may be assumed that the rise in VP secretion observed in LE-DM rats efficiently increased water reabsorption in collecting ducts because a significant correlation was observed between urinary VP excretion and T^cH_2O (Fig. 2*B*). The correlation of VP excretion with glucose excretion was even more significant ($r = 0.894$, $P = 0.001$) (data not shown).

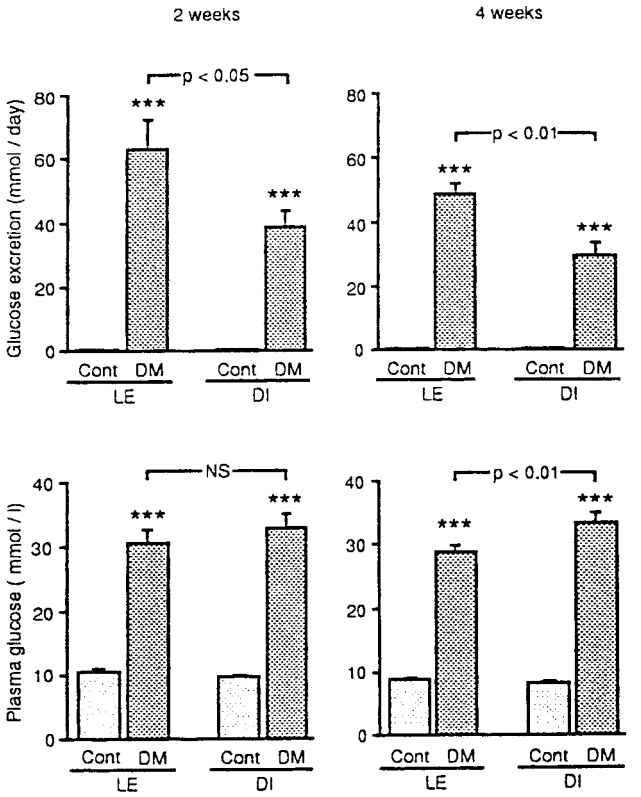

FIG. 1. Glucose excretion (top) and plasma glucose concentration (bottom) in Cont and diabetic (DM) rats with and without VP (LE and DI, respectively), 2 and 4 weeks after induction of DM. ANOVA followed by Fisher's post hoc test: ***, $P < 0.001$ DM vs. Cont in each strain.

Regarding organ weight (Table 4), DM in LE rats induced a significant hypertrophy of liver and kidney relative to BW with, however, a much higher magnitude for the kidney (+80%) than for the liver (+30%). In DI rats, the two organs were significantly less hypertrophied than in LE rats (+29% and +15%, respectively).

DISCUSSION

VP secretion is known to be increased in DM, and previous studies suggest that this hormone influences renal haemodynamics and urinary albumin excretion. Understanding the consequences of this elevation in VP may thus be important in the prevention of the renal complications of diabetes. The present experiment evaluated the possible contribution of this hormone to several disturbances observed in DM. The main results show that, in the absence of VP, the glomerular hyperfiltration and rise in albumin excretion typical of DM were absent or largely blunted. Glycosuria was less severe and hypertrophy of the kidney and liver less intense in Brattleboro

Table 2. Creatinine clearance and albumin excretion observed 2 and 4 weeks after injection of streptozotocin or vehicle

		Long-Evans			Brattleboro			ANOVA		
		Cont, $n = 8$	DM, $n = 8$	DM/Cont	Cont, $n = 7$	DM, $n = 8$	DM/Cont	S	D	I
Creatinine clearance, ml/min	2 weeks	2.50 ± 0.14	3.05 ± 0.14*	1.22	2.29 ± 0.14	2.36 ± 0.21	1.03	c	a	
	4 weeks	2.08 ± 0.08	3.19 ± 0.09**	1.53	1.94 ± 0.16	1.67 ± 0.24	0.86	c	b	c
Albumin excretion, mg/day	2 weeks	1.08 ± 0.03	2.21 ± 0.16***	2.05	1.21 ± 0.33	1.62 ± 0.13***	1.34		c	a
	4 weeks	0.74 ± 0.04	2.45 ± 0.19***	3.30	1.57 ± 0.28	1.78 ± 0.20	1.13		c	c

Values are means ± SEM. ANOVA: S, strain effect; D, diabetes effect; I, interaction; a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$. Fisher's post hoc test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ vs. Cont in each strain.

Table 3. Glucose handling 2 weeks after injection of streptozotocin (DM) or vehicle (Cont)

		Long-Evans			Brattleboro			ANOVA		
		Cont, $n = 8$	DM, $n = 8$	DM/Cont	Cont, $n = 7$	DM, $n = 8$	DM/Cont	S	D	I
Plasma glucose	mmol/liter	10.5 ± 0.1	30.6 ± 2.0***	2.91	9.8 ± 0.1	32.8 ± 2.1***	3.35		c	
Urine glucose	mmol/liter	1.5 ± 0.1	764.0 ± 12.0***		0.0 ± 0.0	149.0 ± 7.0***		c	c	c
U/P		0.1	25.0		-	4.5				
Glucose filtered	mmol/day	38 ± 1	135 ± 8***	3.53	32 ± 2	108 ± 9***	3.34	a	c	
Glucose reabsorbed	mmol/day	38 ± 1	72 ± 8***	1.88	32 ± 2	70 ± 10***	2.15		c	
Glucose excreted	mmol/day	0 ± 0	63 ± 9***		0 ± 0	39 ± 5***		a	c	a

Values are means ± SEM. ANOVA: S, strain effect; D, diabetes effect; I, interaction; a, $P < 0.05$; c, $P < 0.001$. Fisher's post hoc test: DM vs. Cont: ***, $P < 0.001$ vs. Cont in each strain.

than in LE controls. These results strongly suggest that VP is involved in the onset and/or early phase of the renal complications of DM.

The streptozotocin model of DM mimics type I diabetes. This model is known to exhibit an increased VP secretion (6, 7), a finding observed in LE rats in this study as judged by their urinary VP excretion. Control LE and Brattleboro rats share the same genetic background because the Brattleboro strain originated from a LE colony (29). Consequently, there is no reason to think that pancreatic β cells of Brattleboro rats could be less sensitive to the action of streptozotocin than those of LE rats. The differences in the intensity of diabetic symptoms or complications observed between the two strains are thus most likely attributable, directly or indirectly, to the lack of VP in Brattleboro rats.

In this study, basal glycemia in LE-Cont and DI-Cont is ≈2-fold above values usually reported for normal rats. This high value is not attributable to erroneous measurements because Wistar rats studied at the same period in our laboratory (for other experiments) exhibited normal glycemia (5.52 ± 0.24 mmol/liter). Urinary glucose excretion was also higher in LE-Cont rats than in normal Wistar rats (22.2 ± 0.7 vs. 14.7 ± 2.1 μmol/day, $P < 0.01$). Interestingly, a strain of rats exhibiting spontaneous type II diabetes has been isolated from a LE colony (31). These observations suggest that "normal" LE rats are probably prone to developing DM and already exhibit some degree of hyperglycemia and glycosuria. To our knowledge, only one previous study evaluated the influence of VP in DM by comparing Brattleboro DI rats and LE rats. In this study, focused mainly on cardiovascular function, glycemia in control LE rats was 7.3 mmol/l. Glycosuria and renal function were not evaluated (32).

Diabetic rats usually grow less than normal rats, or even lose weight, thus leading to different BW after some weeks of diabetes. This was indeed the case in the present study. To take these differences into account, we could have factored all results by BW, but this would have introduced another bias because the factors under study do not progress linearly with BW. We reasoned that metabolic and excretory functions are most probably quantitatively related to food intake. Because control rats of the two strains had similar food intakes per day, and because DM induced similar increases in food intake in both strains, we considered that it was not appropriate to normalize data per unit BW.

Because VP is elevated in DM, the possibility that its receptors could be desensitized has been considered in several studies. They revealed that V_{1a} receptor density is down-regulated in liver and kidney without any change in affinity for the hormone (33, 34). Similar findings also have been reported for V_{1a} receptors in platelets of human subjects with DM (35). In contrast, no change was observed in V_2 receptor density or affinity in kidney (33). Thus, it may be assumed that the rise in endogenous VP occurring in DM will affect renal function (V_2 receptors mainly) more than hepatic function (V_{1a} receptors) because of this different pattern of down-regulation.

Effects of Vasopressin on Renal Function in DM. LE-DM rats exhibited a much lower U_{osm} than LE-Cont (an almost 900 mOsm/kg H_2O difference). As a whole, urinary osmoles were concentrated 4.29-fold above plasma osmolality in LE-DM rats versus 7.47 in LE-Cont (Table 1). Glucose, the most abundant urinary osmole in DM, was concentrated 25-fold more in urine than in plasma in LE-DM rats (versus only 4.5-fold in the absence of VP in DI-DM rats). Thus, in spite of the "osmotic diuresis" this solute is considered to induce, it is nevertheless quite dramatically concentrated in the urine.

The major effect of VP on the kidney is to promote water reabsorption in the collecting ducts, an effect mediated by V_2 receptors and resulting in the production of hyperosmotic urine. The antidiuretic action of VP is often evaluated by considering the maximal osmolality achieved in urine. However, this parameter does not take into account the amount of osmoles that are raised to that osmolality. The calculation of the amount of solute-free water reabsorbed by the kidney to achieve the observed U_{osm} provides a better estimate of the true VP-dependent concentrating effort of the kidney (i.e., the "osmotic work"). It is well established that both the urinary concentrating ability (in the presence of VP) and the diluting ability (in the absence of VP) decrease with increasing osmolar load (36). A diminished U_{osm} observed in the face of a marked increase in osmolar load does not necessarily mean that the kidney reabsorbs less water. Actually, the amount of water reabsorbed under the influence of VP may be increased, but not enough to maintain the same osmolality. This is what occurs in LE-DM rats. Their osmolar excretion increased 3.7-fold. Although their U_{osm} was reduced compared with that of LE-Cont, the 3.9-fold rise in T^cH_2O shows that VP enabled the reabsorption of a much larger amount of free water in DM than in Cont rats. Moreover, a close correlation between T^cH_2O and the load of excreted solutes was present in LE-DM. Although V increased with the severity of DM, the amount of water saved under the influence of VP also increased in proportion to the rise in osmolar excretion (Fig. 2). If VP had not increased in DM rats, the fall in U_{osm} and the rise in V (thus the loss of water) would have been even greater. Thus, the rise

Table 4. Kidney and liver weight 4 weeks after injection of streptozotocin or vehicle

	Long-Evans			Brattleboro			ANOVA		
	Cont, $n = 8$	DM, $n = 8$	DM/Cont	Cont, $n = 7$	DM, $n = 8$	DM/Cont	S	D	I
Kidney weight	1.82 ± 0.03	3.33 ± 0.17***	1.83	2.05 ± 0.15	2.67 ± 0.09***	1.30		c	c
Liver weight	10.15 ± 0.14	12.88 ± 0.26***	1.27	10.13 ± 0.26	11.66 ± 0.42***	1.15	a	c	a

Values are means ± SEM in g/300 g body weight. ANOVA: S, strain effect; D, diabetes effect; I, interaction; a, $P < 0.05$; c, $P < 0.001$. Fisher's post hoc test: ***, $P < 0.001$ vs. Cont in each strain.

in VP observed in DM, and the fact that V_2 receptor density is not down-regulated, may be considered as appropriate adaptations that limit water requirements for excreting a markedly elevated solute load (37) (see note added in proof).

In the absence of VP, an increase in osmolar load is known to reduce the diluting ability of the kidney so that urine tends to become isoosmotic to plasma (36). This is indeed the case in DI-DM rats (Table 1), and this failure to dilute urine explains the lack of correlation between free water reabsorption and solute excretion in DM rats of this strain.

If the elevation of VP in DM is a positive adaptation in the short term, it may turn out to have adverse consequences in the long term for the following reasons. Hyperfiltration occurring in the early phase of DM (38–40) is known to lead to a delayed progressive deterioration of renal function (1). Now, chronic elevation in plasma VP, or in its selective V2 agonist dDVP, has been shown to induce distinct glomerular hyperfiltration (11, 12, 41–43), rise in albumin excretion (suggesting an alteration of the glomerular filter) (14), and renal hypertrophy (15). In rats with 5/6 nephrectomy, a reduction in VP level, brought about by a chronic elevation in water intake, slowed down the progression of chronic renal failure (10). As discussed elsewhere, these effects probably depend, indirectly, on the tubular effects of VP, which secondarily influence the tubuloglomerular feedback control of GFR (12, 18). In the present experiment, rats without VP did not exhibit hyperfiltration and had a lesser kidney hypertrophy and less intense albuminuria than rats with VP. Blood pressure was unfortunately not measured in this study. Whether rats with streptozotocin-induced DM exhibit an increase in blood pressure remains controversial (44). However, we do not think that the hypertensive effects of vasopressin (mediated by V1 receptors) could be responsible for the hyperfiltration and increased albuminuria of LE-DM because dDAVP, a non-pressor-selective V2 agonist, is able to induce hyperfiltration in normal rats (12). In addition, we have shown that chronic infusion of dDAVP for 1 week in normal rats increases their urinary albumin excretion (14) and that chronic infusion of a selective V2 receptor antagonist for 1 week in DM rats prevents the rise in albumin excretion observed in untreated rats studied in parallel (45). On the whole, these results suggest that VP contributes to hyperfiltration and albuminuria of DM and that this hormone could participate in the induction of diabetic nephropathy.

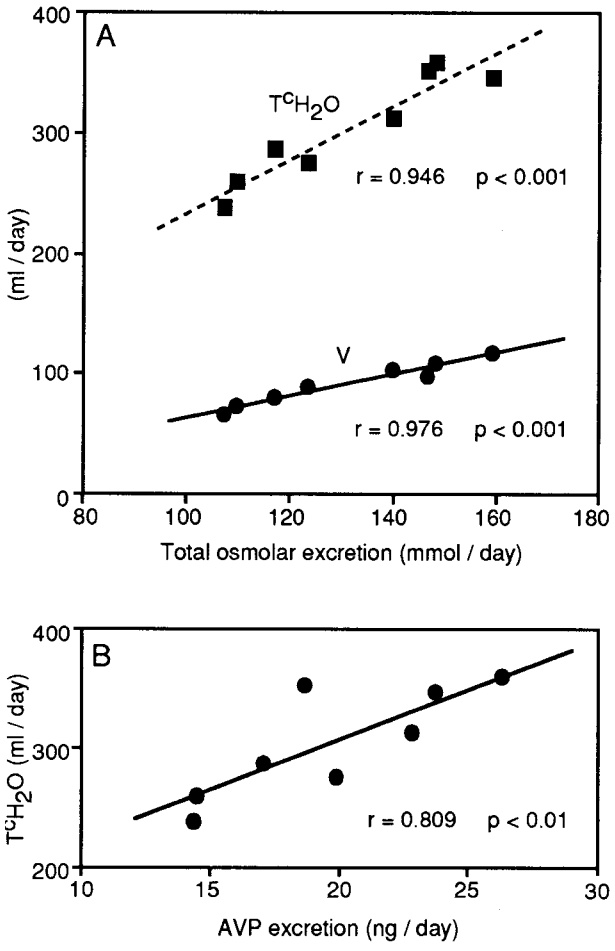

FIG. 2. Relationship between V or T^CH_2O and total osmolar excretion (*A*) and relationship between free water reabsorption and VP excretion (*B*) in LE-DM rats 2 weeks after induction of DM. Linear regressions and correlation coefficients are shown.

Effects of Vasopressin on Hepatic Function in DM. The lower level of glucose excretion in DI-DM than in LE-DM rats, taken in isolation, suggests a lesser intensity of metabolic perturbations attributable to DM in rats lacking VP. However, the similar rise in glycemia in both strains observed here, as well as in the study by Tomlinson *et al.* (32), does not seem to support this conclusion. How could these apparently discordant results be explained? Let us consider the factors that determine the concentration of glucose in blood. Glycemia is the result of an equilibrium between glucose production on the one hand and glucose consumption plus excretion on the other hand. A similar plasma glucose concentration in the two DM groups does not necessarily mean that they both behaved equally in response to streptozotocin but could result from opposite and quantitatively equivalent changes in glucose production and catabolism and/or excretion.

The higher excretion of glucose in LE-DM rats may thus result either from a higher level of glucose production or from a lower glucose breakdown in these rats than in DM rats lacking VP. An increased VP-dependent production of glucose in LE rats is unlikely because the rise in VP that should stimulate glucose (and urea) synthesis should be compensated for by the selective desensitization of liver V_{1a} receptors reported in DM rats (33, 34). It seems thus more likely that glucose breakdown was higher in DI than in LE rats. Such a difference in glucose catabolism also could account for the fact that glucose but not urea excretion was lower in DI-DM than in LE-DM rats, although these two components are formed simultaneously in proportional amounts in the liver. It also could explain why DI rats with DM lost weight, although their food intake increased as much as that of LE-DM rats.

What could bring DI rats to have a higher glucose breakdown than LE rats? This could possibly be attributable to a higher body temperature. To our knowledge, absolute measurements of body temperature in LE and DI rats are not available, but VP is known to play a role in the control of basal body temperature (46–48) and to be antipyretic (49–51). Moreover, heat dissipation is impaired in humans with DI (52). Because they lack VP, Brattleboro rats could exhibit an increased metabolism and thus may catabolize a greater fraction of their endogenously produced glucose than do LE rats. In the aggregate, the similar rise in food intake, plasma glucose, and urea excretion in LE-DM and DI-DM rats suggests that the metabolic perturbations induced by streptozotocin administration are equivalent in both strains.

In summary, the results presented in this study suggest that the rise in VP that occurs in DM contributes to limit the rise in urine output accompanying a markedly increased solute excretion. In addition to this *a priori* beneficial effect, our results suggest that the rise in VP also could play a role in glomerular hyperfiltration, albuminuria, and renal hypertrophy that occur in the early phase of the disease and are known to lead to a progressive deterioration of renal function. Consequently, this hormone, and, more specifically, its actions on renal V_2 receptors (as deduced from previous findings), could

represent a novel risk factor for diabetic nephropathy. Further studies taking advantage of newly designed non-peptide-selective V_2 receptor antagonists (53–55) could bring useful additional information with regard to this possibility and could possibly represent a new therapeutic strategy for the prevention of diabetic nephropathy.

Note Added in Proof. Recently, Ahloulay *et al.* presented evidence that the rise in vasopressin observed in DM contributes to increased urinary concentrating activity and thus to limited water requirements induced by the metabolic derangements of DM (56).

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