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Renal vascular reactivity to vasopressin in rats with diabetes mellitus

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Abstract

We evaluated how renal vascular reactivity to vasopressin changes when nitric oxide (NO) synthesis varies, as has been reported to occur in the course of insulin-dependent diabetes mellitus. Renal vasoconstrictor responses to vasopressin were obtained in young and older Sprague–Dawley control rats (3 and 10 months old) and in age-matched diabetic rats that had been treated with streptozotocin (60 mg/kg i.v.) at the age of 2 months. In young rats, vasopressin (3–1000 ng/kg/min i.v.) induced in vivo a dose-dependent decrease in renal blood flow, which was diminished in streptozotocin diabetic rats ($P < 0.05$). Similarly, in in vitro perfused kidneys, the concentration–response curve for vasopressin (0.03–10 nM) was shifted 3-fold to the right in kidneys isolated from young diabetic rats ($P < 0.05$). This shift was abolished in the presence of an inhibitor of nitric oxide synthesis, N^G-nitro-L-arginine (100 μM), in the perfusate. In 10-month-old rats, the in vivo renal vasoconstrictor dose–response curve to vasopressin was shifted 10-fold to the left as compared to that for young rats ($P < 0.001$). This shift was similar in both control and diabetic rats.

In conclusion, the present study documented the existence of hyporesponsiveness to vasopressin in the early stage of diabetes, possibly related to nitric oxide overproduction. In contrast, renal vascular hyperreactivity to vasopressin occurs with aging, whether the rats are diabetic or not.

Keywords: Vasopressin; Renal vascular reactivity; Diabetes; Age; Nitric oxide (NO)

1. Introduction

Nitric oxide (NO) release from endothelium plays an important role in the regulation of vascular tone, in the inhibition of both platelet and leukocyte aggregation as well as the adhesion process (Moncada et al., 1991; Kubes et al., 1991). These properties suggest that the level of NO production by the endothelium may play a pivotal role in the regulation of vascular disease.

In diabetes mellitus, biphasic changes in NO disposition have been suggested to occur, an initially increased production, followed later during the disease by an impaired production. Indeed, during the first weeks (early stage) of streptozotocin-induced diabetes in rats, characteristic glomerular hyperfiltration has been observed, which was abolished by NO synthase inhibitors (Bank and Aynedjian, 1993; Tolins et al., 1993). Furthermore, endothelium-dependent relaxation increases in mesenteric arteries (Heygate et al., 1996) from diabetic rats and angiotensin II-elicited vasoconstriction decreases in isolated perfused kidneys from diabetic rats (Sarrubbi et al., 1989). In contrast, after several months of diabetes (late stage), decreased endothelium-dependent relaxation is a common feature in animals with experimental diabetes (Oyama et al., 1986; Meraji et al., 1987; Feletou et al., 1994) and in diabetic patients (Johnstone et al., 1993; Kawagishi et al., 1999). In agreement with a defect in endogenous NO disposition, noradrenaline-induced vasoconstriction was reported to be increased (Taylor et al., 1994) although no change was found in another study (Mayhan, 1998).

Diabetes mellitus has also been shown to modify several components of the vasopressinergic system. Plasma

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vasopressin levels are increased during the course of the disease (Zerbe et al., 1979; Trinder et al., 1994; Phillips et al., 1995) and the number of vasopressin V_1 receptors is decreased in the liver and the kidney (Trinder et al., 1994; Phillips et al., 1995). Downregulation of vasopressin V_1 receptors has also been reported in cultured rat aortic vascular smooth muscle cells during exposure to high glucose (Williams et al., 1992). Vasopressin-elicited renal vasoconstriction seems to be decreased (Sarrubbi et al., 1989) although the response in mesenteric arteries was left unchanged (Hebden et al., 1988).

We previously reported that endogenous NO is able to blunt the vasopressin-induced renal vasoconstriction both in vivo in the anaesthetized rat (Loichot et al., 2000) and in vitro in the isolated perfused rat kidney (Barthelmebs et al., 1996, 1997). The aim of the present study was therefore to evaluate the renal vascular reactivity to vasopressin in early- and late-stage insulin-dependent diabetes mellitus in rats, with particular attention to its modulation by NO.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (7–8 weeks old, 200–250 g; Iffa Credo breeding, l'Arbresle, France) were used. The animals were housed in a room at 20 °C with a 12-h light/dark cycle (light on at 6:00 am) and allowed free access to tap water and standard food unless otherwise stated (AO4 pellets, UAR, Villemoisson/Orge, France). The rats stayed in our animal facility for at least 1 week before experiments were started. Insulin-dependent diabetes mellitus was induced by a single intravenous injection of streptozotocin (60 mg/kg i.v.). Control rats received streptozotocin solvent (citrate buffer, pH = 4). Plasma glucose levels were determined in tail blood using Glucostix® (Department AMES Bayer Diagnostic, Puteaux, France). The rats received insulin everyday (Ultratard® HM ge 40, Novo Nordisk, Boulogne Billancourt, France). The dose (2–6 units/day) was individually adjusted every week to maintain nonfasting glycemia at about 300–400 mg/dl. The experiments were performed according to the guidelines of the European Community and the French Government concerning the use of animals.

2.2. Measurements in conscious rats

One month after induction of diabetes, diabetic and control rats were placed in individual metabolic cages (Iffa Credo) with free access to distilled water and food. After a 24-h period to allow the rats to adapt to the cages, urine was collected over a first 24-h period, with blood sampling from the tail vein at the end of this period. Urine was then subsequently collected over a second 24-h period during which the diabetic rats had restricted access to food, which was matched with the food intake of the control rats. Blood samples were again collected from the tail vein at the end of the second urine collection period. Urinary volume was measured as well as water and food intake. Antibiotics/antimycotics were added to the urine collection vial for the measurement of nitrites/nitrates (NO_x), the stable metabolic products of NO (Green et al., 1982). NO_x levels were measured in urine and plasma with the Griess reaction. Sodium was measured in urine by indirect potentiometry with ion-selective electrodes (EL-Ise, Beckman, Gagny, France). Vasopressin was measured by radioimmunoassay (Bühlmann Laboratories, Allschwil, Suisse) in an aliquot of urine preserved on boric acid (10 mg/ml urine). Creatinine was measured in urine and plasma with Jaffe's method, allowing calculation of endogenous creatinine clearance.

2.3. In vivo studies

Sprague–Dawley rats were anaesthetized by an i.p. injection of sodium thiobutabarbitone (100 mg/kg), and placed on a temperature-controlled table to maintain body temperature to 37 °C and prepared for the experiment. Briefly, following cannulation of the trachea, a catheter was inserted into the left femoral artery for mean arterial blood pressure monitoring via a strain-gauge transducer (Statham P23 Db transducer, Statham Instruments, Hato Rey, Porto Rico) and for blood sampling. Another catheter was inserted into the jugular vein for i.v. infusions. After a subcostal incision, an electromagnetic blood flow probe (internal diameter 0.6 mm; Skalar electromagnetic blood flowmeter, Delft, The Netherlands) was placed around the left renal artery and the renal blood flow was continuously monitored (Philips PM 8222 Recorder, Philips, Bobigny, France). To compensate for fluid loss during surgery, the rats were supplemented intravenously with 2 ml 6% bovine serum albumin in Tyrode's solution.

After a 30-min recovery period, basal parameters (mean arterial blood pressure, renal blood flow) were recorded before i.v. infusion of increasing doses of vasopressin (10–900 ng/kg/min). Each dose was infused until the maximum response was obtained in about 10 min. Infusion was then stopped and recovery was allowed before infusion of the next dose. Vasopressin-induced changes in renal blood flow and in mean arterial blood pressure were expressed as the percentage change from the preinfusion value of each parameter. At the end of the experiment, a blood sample (100 μl) was collected for measurement of glycated haemoglobin (HbA_{1C}) with an affinity chromatographic method (Glycotest II®, Touzart et Matignon, Gérardmer, France).

2.4. In vitro studies in the isolated perfused rat kidney

Under sodium pentobarbital anesthesia (45 mg/kg i.p.), the right kidney was prepared with special care to avoid

ischemia. After perfusion via the mesenteric artery was started, the kidney was excised and in vitro perfused in a closed circuit, as described previously (Barthelmebs et al., 1990). The perfusion medium was a prewarmed (37 °C), oxygenated (95% O_2/5% CO_2) Tyrode's solution of the following composition (mM): NaCl 137; KCl 2.7; $CaCl_2$ 1.8; $MgCl_2$ 1.1; NaH_2PO_4 0.42; $NaHCO_3$ 12; glucose 10; pH was adjusted to 7.4. The medium was supplemented with Ficoll 400 (4.7%) to restore viscosity (viscosity relative to water, $\eta = 2.3$). Following a 60-min equilibration period, the perfusion flow rate was adjusted to 8 ml/min and kept constant thereafter (Gilson Minipuls 3, Bioblock, Illkirch, France). Perfusion pressure was measured at the aorto-renal crossing and continuously monitored (Statham P23 Db transducer) and recorded (Philips PM 8222 recorder). Renal vascular resistance was calculated as the ratio of perfusion pressure/perfusion flow rate.

Increasing concentrations of vasopressin (0.03–10 nM) were infused into the perfusate near the kidney. Each concentration was infused until a steady-state response was obtained. Recovery was allowed before infusion of the next concentration. One concentration–response curve was made with each kidney preparation. Responses were compared with those elicited in the same kidney by a supramaximal concentration of noradrenaline (10 μM) given at the end of the experiment. The vasopressin concentration–response curve was also evaluated in the presence of N^G-nitro-L-arginine (L-NNA, 100 μM). Vasoconstriction was expressed as increase in renal vascular resistance. The maximum response (E_{max}) and the concentration inducing a half-maximum effect (EC_{50}) were derived from individual agonist curve data by a nonlinear curve fit (GraphPad Prism, GraphPad Software, San Diego, USA) using the following equation: $E = E_{max}/(1 + (EC_{50}/C))$, with concentration C and effect E.

2.5. Drugs

[Arg^8]vasopressin (Biogenesis, Stinsford, UK); bovine serum albumin, fraction V (Euromedex, Souffelweyersheim, France); Ficoll 400 (Ficoll® 400, Amersham Pharmacia Fine Chemicals, Uppsala, Sweden); N^G-nitro-L-arginine, noradrenaline hydrochloride, streptozotocin (Sigma, St. Quentin-Fallavier, France); sodium pentobarbital (Nembutal®, Sanofi Santé Animale, Libourne, France); sodium thiobutabarbitone (Inactin®, Byk Gulden, Konstanz, Germany). All other chemicals were of pro analysis quality from Merck (Darmstadt, Germany). Vasopressin was prepared as stock solution (1 mg/ml in distilled water) and stored in fractions at −20 °C. To avoid adsorption, the materials were silicone treated (Aquasil, Interchim, Montluçon, France).

2.6. Statistical analysis

The results are expressed as means ± S.E.M. Differences were tested for statistical significance with Student's *t*-test or with the nonparametric Mann–Whitney test when necessary. Dose– and concentration–response curves for vasopressin were analysed by two-way analysis of variance (ANOVA) for repeated measurements. A P value < 0.05 was considered significant. All statistical calculations were run with GraphPad Prism (GraphPad Software) or SigmaStat (SPSS, Chicago, USA).

3. Results

3.1. Effects of early diabetes on renal functions in conscious rats

The data are presented in Table 1. One month after induction of diabetes, the diabetic rats presented with a delay in growth as compared with controls. They displayed typical hyperphagia and polydipsia. Increased urinary sodium excretion accompanied polyuria which persisted even in the diabetic rats pair-fed with the control rats. As expected, the creatinine clearance was significantly increased. Urinary excretion of vasopressin (ng/day/100 g) was increased 8-fold in the diabetic rats. A 5-fold increase persisted when urinary excretion was normalized for creatinine clearance. Urinary excretion of NO_x (μmol/day/100 g) was increased 3.7-fold in diabetic rats. This increase was partially linked to glomerular hyperfiltration since only a 1.7-fold increase remained after normalization for creatinine clearance. The increase disappeared completely in food-restricted diabetic rats, a group which also had no change in plasma NO_x as compared with the controls (14 ± 3 μmol/l, $n = 15$ vs. 20 ± 2 μmol/l, $n = 13$, NS). Glycated haemoglobin (HbA_{1C}) more than doubled in the diabetic rats ($11.4 \pm 0.7\%$, $n = 15$ vs. $4.3 \pm 0.1\%$, $n = 13$, $P < 0.001$).

3.2. Effects of early diabetes on renal vascular reactivity in vivo

Basal renal blood flow (8.2 ± 0.4 ml/min, $n = 15$ vs. 7.8 ± 0.5 ml/min, $n = 15$, NS) and mean arterial blood pressure (118 ± 3 mm Hg, $n = 15$ vs. 125 ± 2 mm Hg, $n = 15$, NS) were similar in anesthetized diabetic and control rats. Vasopressin elicited a dose-dependent decrease in renal blood flow (Fig. 1). In the control rats, vasopressin-induced renal vasoconstriction became evident at a dose of 30 ng/kg/min and reached a maximum at 300 ng/kg/min. In the diabetic rats, vasoconstriction was significantly blunted (Fig. 1, $P < 0.05$). Vasoconstriction now became evident only at a dose of 100 ng/kg/min and reached its maximum at 1000 ng/kg/min. Vasopressin elicited a dose-dependent increase in mean arterial blood pressure (Fig. 1), with the same characteristics as for renal blood flow, namely, a diminished response in the diabetic group vs. that of the control group ($P < 0.05$).

Table 1
Effects of 1-month streptozotocin-induced diabetes on renal functions measured in conscious rats

Experimental group (n)	Body weight (g)	Food intake (g/day/100 g)	Water intake (ml/day/100 g)	Diuresis (ml/day/100 g)	UV_{Na} (mmol/day/100 g)	C_{creat} (ml/min/100 g)	UV_{AVP}		UV_{NO_x}	
							ng/day/100 g	pg/ml C_{creat}	µmol/day/100 g	nmol/ml C_{creat}
Free access to food										
Control (19)	350±6	7.2±0.1	9.5±0.4	3.1±0.2	0.53±0.02	0.24±0.02	0.7±0.1	1.47±0.28	2.26±0.16[a]	7.1±0.9[a]
Diabetic (18)	319±5	12.3±0.5	42.2±4.9	38.1±5.0	1.26±0.10	0.49±0.03	5.8±0.6	7.84±0.77	8.42±0.90[a]	12.0±1.0[a]
Student's t-test or Mann–Whitney test	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.01
Pair-feeding in diabetics										
Control (13)	352±3	5.4±0.1	7.9±0.4	2.8±0.3	0.35±0.03	0.20±0.02	/	/	1.20±0.12	4.6±0.7
Diabetic (15)	308±8	5.4±0.1	27.1±3.3	23.5±3.2	0.53±0.04	0.35±0.04	/	/	1.41±0.21	3.6±0.8
Student's t-test or Mann–Whitney test	<0.001	NS	<0.001	<0.001	<0.01	<0.01	/	/	NS	NS

Results are expressed as means±S.E.M. The number of rats per group is given in brackets (n). Statistical analysis was performed with Student's t-test or the nonparametric Mann–Whitney test when necessary. UV_{Na}, natriuresis; C_{creat}, creatinine clearance; UV_{AVP}, vasopressin urinary excretion; UV_{NO_x}, nitrites/nitrates (NO_x) urinary excretion; /, not measured; NS, not significant.
[a]Only measured in 11 rats/group. Intake and excretion data are normalized for 100 g of rat body weight.

Fig. 1. In vivo effects of vasopressin (i.v.) on renal blood flow and mean arterial blood pressure, in anesthetized control rats (△, $n=15$) and in streptozotocin diabetic rats (□, $n=15$) at an early stage of diabetes (1 month). Results are given as means±S.E.M. of the percentage changes from the corresponding preinfusion values. Statistical analysis by two-way ANOVA with repeated measurements revealed a significant difference between dose–response curves in diabetic and control rats ($P<0.05$).

3.3. Effects of early diabetes on renal vascular reactivity in vitro

The left kidney weight was higher in diabetic rats than in the controls (1.52 ± 0.08 g, $n=12$ vs. 1.29 ± 0.04 g, $n=10$, $P<0.05$), in agreement with the well-documented renal hypertrophy in diabetes. In the isolated perfused kidneys, perfusion pressure (47 ± 2 mm Hg, $n=12$ vs. 49 ± 2 mm Hg, $n=10$, NS) and basal renal vascular resistance (5.8 ± 0.2 mm Hg/ml/min, $n=12$ vs. 6.1 ± 0.2 mm Hg/ml/min, $n=10$, NS) did not differ between the two groups. As observed in vivo, vasopressin induced a concentration-dependent renal vasoconstriction (Fig. 2A). Responses to vasopressin were significantly lower in kidneys from diabetic rats, with a significant shift of the concentration–response curve to the right ($P<0.05$), but without change in the maximum effect. The maximum vasoconstrictor response induced by noradrenaline (10 µM) in the same kidneys was not modified by diabetes (25.8 ± 2.7 mm Hg/ml/min increase in renal vascular resistance, $n=6$ vs. 26.7 ± 2.3 mm Hg/ml/min increase in controls, $n=8$, NS). Addition of L-NNA (100 µM) to the perfusate produced a small increase in basal renal vascular resistance, which was similar in diabetic and in control rats

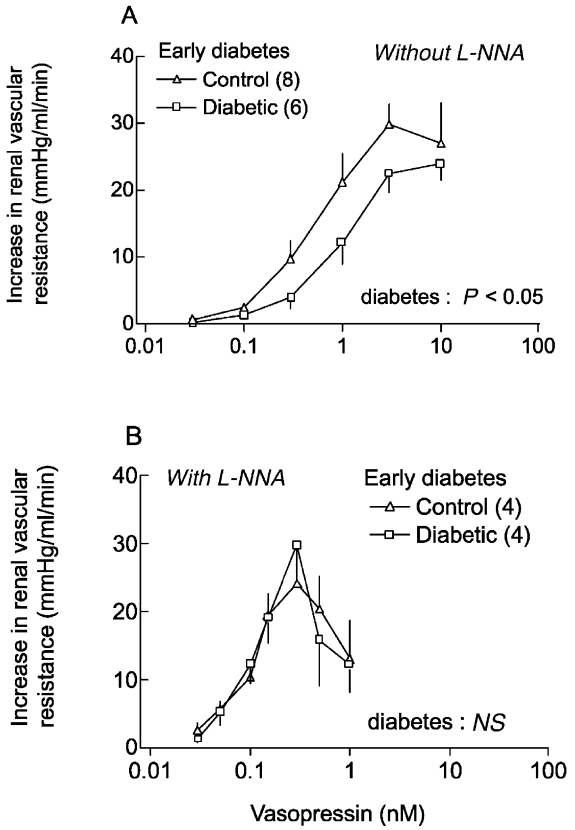

Fig. 2. In vitro vasopressin-induced concentration-dependent vasoconstriction in perfused kidneys isolated from control rats (△, $n = 8$) or from 1-month streptozotocin diabetic rats (□, $n = 6$). Concentration–response curves were made with separate kidneys in the absence (A) and presence (B) of N^{ω}-nitro-L-arginine (L-NNA, 100 μM). Data (means ± S.E.M.) are expressed as increases in renal vascular resistance. Statistical analysis by two-way ANOVA with repeated measurements revealed a significant difference between dose–response curves in diabetic and control rats ($P < 0.05$) in the absence of L-NNA. This difference did not persist in the presence of L-NNA.

(5.5 ± 2.7 mm Hg/ml/min increase in basal renal vascular resistance, $n = 4$ vs. 4.4 ± 1.5 mm Hg/ml/min increase, $n = 4$, NS). After NO synthase inhibition, the vasoconstrictor concentration–response curves for vasopressin were shifted to the left in both diabetic and control rats (Fig. 2B) and more so in diabetic rats, so that no difference remained between the two concentration–response curves in kidneys from diabetics and controls. L-NNA did not affect the vasopressin-induced maximum vasoconstrictor response, whether in diabetic or in control rats.

3.4. Effects of late diabetes on renal functions in conscious rats

In a late stage of diabetes mellitus, 8 months after its induction with streptozotocin, the diabetic rats continued to display several features already observed in the early stage of the disease (1 month), such as reduced growth, hyperphagia, polydipsia, polyuria and increased excretion of

Table 2
Effects of 8-month streptozotocin-induced diabetes on renal functions measured in conscious rats

Experimental group (n)	Body weight (g)	Food intake (g/day/100 g)	Water intake (ml/day/100 g)	Diuresis (ml/day/100 g)	UV_{Na} (mmol/day/100 g)	C_{creat} (ml/min/100 g)	UV_{AVP} ng/day/100 g	UV_{AVP} pg/ml C_{creat}
Free access to food								
Control (8)	629 ± 26	3.4 ± 0.3	4.6 ± 0.5	2.3 ± 0.3	0.30 ± 0.02	0.37 ± 0.06	0.70 ± 0.20	1.60 ± 0.63
Diabetic (7)	503 ± 11	6.5 ± 0.6	23.7 ± 4.8	20.8 ± 4.8	0.67 ± 0.10	0.38 ± 0.02	4.10 ± 1.57	7.73 ± 3.06
Student's t-test or Mann–Whitney test	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	NS	< 0.001	< 0.01

Results are expressed as means ± S.E.M. The number of rats per group is given in brackets (n). Statistical analysis was performed with Student's t-test or the nonparametric Mann–Whitney test when necessary. UV_{Na}, natriuresis; C_{creat}, creatinine clearance; UV_{AVP}, vasopressin urinary excretion; NS, not significant. Intake and excretion data are normalized for 100 g of rat body weight.

sodium (Table 2). The increase in urinary vasopressin excretion also persisted at a similar level. Creatinine clearance, however, was no longer increased, but no sign of renal failure appeared at this stage of the diabetes. Glycated haemoglobin (HbA_{1C}) remained high in diabetic rats ($12.3 \pm 1.8\%$, $n = 7$ vs. $5.4 \pm 0.4\%$ in controls, $n = 8$, $P < 0.001$), confirming the persistence of unbalanced glycemia.

3.5. Effects of late diabetes on renal vascular reactivity in vivo

Basal renal blood flow (7.3 ± 0.6 ml/min, $n = 7$ vs. 7.2 ± 0.5 ml/min, $n = 8$, NS) and mean arterial blood pressure (103 ± 5 mm Hg, $n = 7$ vs. 112 ± 3 mm Hg, $n = 8$, NS) were not different in anaesthetized diabetic and control rats. Vasopressin elicited a dose-dependent decrease in renal blood flow with a concomitant increase in mean arterial blood pressure (Fig. 3, closed symbols). The dose–response curves for vasopressin were shifted to the left in both late diabetic and late control rats as compared to those from younger rats (see below). Responses also tended to be lower in the late diabetic rats as compared to late control rats, but statistical significance was not reached.

3.6. Effects of aging on renal vascular reactivity in vivo

Vasopressin-induced changes in renal blood flow and mean arterial blood pressure measured in 3-month and 10-month-old anesthetized rats were very different (Fig. 3). Renal vasoconstriction ($P < 0.001$) and mean arterial blood pressure responses ($P < 0.05$) were markedly greater in the 10-month-old rats, whether control or diabetic. Indeed, 30 ng/kg/min vasopressin, which only elicited minor effects on the renal circulation in young rats, induced near-maximal vasoconstriction in older rats. The maximum vasoconstrictor response to vasopressin, however, was not changed with aging.

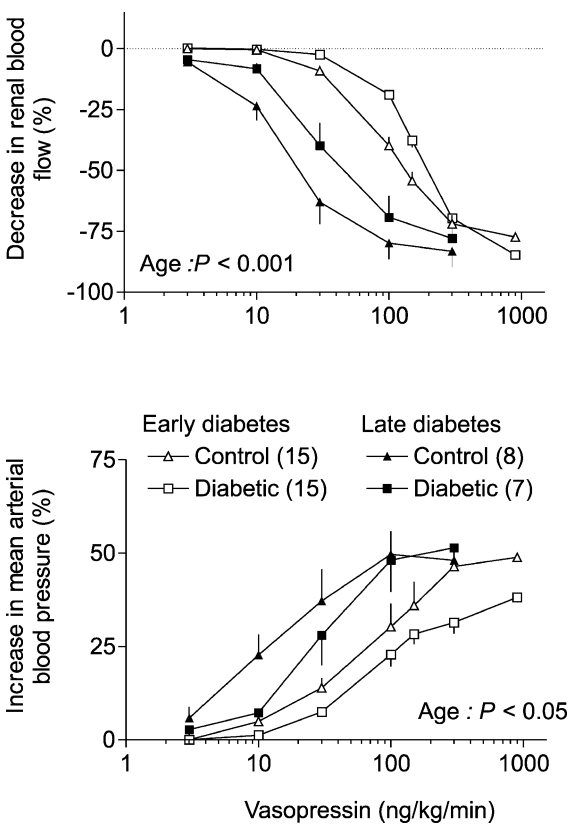

Fig. 3. In vivo effects of vasopressin (i.v.) on renal blood flow and mean arterial blood pressure, in anesthetized streptozotocin diabetic rats (ν, $n = 7$) at a late stage of diabetes (7 months) and in age-matched (10 months old) control rats (σ, $n = 8$). Data from the early stage of diabetes (1 month; □, $n = 15$) with age-matched (3 months old) controls (△, $n = 15$) are indicated for comparison. Results are given as means ± S.E.M. of the percentage changes from the corresponding preinfusion values. Statistical analysis by two-way ANOVA with repeated measurements revealed a significant difference with age ($P < 0.001$ or $P < 0.05$) but no significant difference regarding diabetes, in the late stage of streptozotocin-induced diabetes.

4. Discussion

The present study demonstrated a decrease in sensitivity to vasopressin-induced renal vasoconstriction and in the blood pressure response in streptozotocin diabetic rats, at an early stage of the disease (1 month), when responses are compared to responses in age-matched controls (3 months old). In older rats (10 months old), diabetic or not, the sensitivity to vasopressin-induced vascular responses was markedly increased.

The vasopressin-induced renal vasoconstrictor response was reduced in early diabetes, both when evaluated in vivo, by the decrease in renal blood flow in anaesthetized rats, or in vitro, by the increase in renal vascular resistance in constant flow-perfused kidneys. This result is consistent with those of other studies in perfused kidneys isolated from diabetics rats (Sarrubbi et al., 1989). Hyporeactivity to vasopressin was, however, usually not observed when isolated artery rings (aorta, mesenteric, renal or intrarenal) from streptozotocin or spontaneously diabetic rats (Bio-Bred rats) were challenged in vitro (Hadoke et al., 1998; Torffvit and Edvisson, 1997; Van Buren et al., 1998). Hyporeactivity to vasopressin does not seem to be specific for renal arteries, inasmuch as we also observed a decrease in the pressor response to vasopressin in vivo and since hyporeactivity was also reported for the cutaneous circulation (Lawrence and Brain, 1992). Hyporeactivity also does not seem to be specific for vasopressin since similar results have been reported with other vasoconstrictors in early diabetes (Sarrubbi et al., 1989; Wilkes et al., 1992). However, it seems clear that hyporeactivity to vasoconstrictors needs perfused vessels, as was the case in the isolated kidney and also in vivo, where vessels are blood perfused. We chose to perform our study in kidneys perfused with a Tyrode's solution supplemented with Ficoll 400, so that its

viscosity relative to water was raised up to 2.3. This perfusate allows the modulation of vasopressin-induced vasoconstriction to be observed as shear stress, mainly via the release of endothelial NO (Barthelmebs et al., 1997). Similar modulation by NO of the renal vasoconstrictor response to vasopressin has also been reported for the rat in vivo (Loichot et al., 2000).

Early diabetes mellitus in patients is characterized by glomerular hyperfiltration, which is also found in rats 2–6 weeks after induction of diabetes with streptozotocin (Bank and Aynedjian, 1993; Tolins et al., 1993). Glomerular hyperfiltration also occurred in our study as shown by the increase in endogenous creatinine clearance. Abnormal renal hemodynamics in diabetes have been linked to increased endogenous NO production or release. Indeed, (1) increased endothelium-dependent relaxation in response to acetylcholine has been reported in renal blood vessels from early diabetic rats (Bhardwaj and Moore, 1988), (2) elevated levels of NO_x have been found in arterial plasma and urine of pair-fed diabetic vs. control rats (Bank and Aynedjian, 1993; Tolins et al., 1993), (3) inhibition of NO synthase abolished glomerular hyperfiltration in rats while it decreased urinary NO_x levels (Bank and Aynedjian, 1993; Tolins et al., 1993) and finally (4) endothelial NO synthase mRNA and protein were increased in the renal cortex as soon as 1 week after streptozotocin induction of diabetes (Choi et al., 1997). We evaluated the contribution of NO in the early renal hyporeactivity of the isolated perfused rat kidney to vasopressin. Since the concentration–response curves for vasopressin became similar in diabetic and control rats after NO synthase inhibition, our results are consistent with an increased modulation by NO of vasopressin-elicited renal vasoconstriction in early diabetes. However, we did not find a change in urinary excretion and plasma levels of NO_x in the same rats. To limit artefacts in diabetic rats, NO_x measurements were performed with pair-feeding so as to limit NO_x intake due to polyphagia, and with distilled water as drinking fluid to exclude changes in NO_x intake linked to polydipsia. NO_x excretion was also corrected for changes in creatinine clearance to eliminate effects linked to increased NO_x filtration due to glomerular hyperfiltration. Fifty percent of the NO_x intake was shown to be excreted in urine (Green et al., 1981). Furthermore, inhibition of proximal tubular reabsorption that increases diuresis and natriuresis, also enhanced urinary excretion of NO_x that are partly reabsorbed proximally (Süto et al., 1995). The urinary excretion of NO_x therefore does not seem to be a good marker for endogenous vascular NO production in diabetic rats. On the other hand, plasma NO_x levels might also not necessarily match NO diffusing from the endothelial cells to adjacent basolateral smooth muscle cells where the modulation of vascular tone occurs.

Another possible explanation for the hyporeactivity of the kidney to vasopressin-elicited vasoconstriction could be a decrease in abundance and/or sensitivity of vasopressin V_1 receptors. Increased plasma vasopressin has been found repeatedly in diabetic patients (Zerbe et al., 1979) as well as in rats with streptozotocin-induced diabetes (Trinder et al., 1994; Phillips et al., 1995). In agreement with these data, we measured an increased urinary excretion of vasopressin normalized for creatinine clearance. This parameter reflects the mean circulating level of the peptide since vasopressin is freely filtered by the glomerulus, not secreted and weakly metabolized in the tubule (Lauson, 1967). The increase in plasma vasopressin occurring in diabetes has been found associated with a 40–75% decrease in vasopressin V_1 receptor density in kidney and liver (Trinder et al., 1994) but with no change in receptor affinity. What occurs in vessels is not known. However, a decrease in receptor density did not seem to have contributed to the renal vascular hyporeactivity to vasopressin in our study since the vasoconstrictor response curves to the peptide in the isolated kidney were perfectly superimposable after NO synthase inhibition. In biological systems, responses are often maximal with activation of only a small fraction of available receptors. The existence of vasopressin V_1 spare receptors in the renal vasculature must, however, be confirmed by further investigations.

In older rats (10 months old), we observed a marked overall increase in the vascular sensitivity to vasopressin, whether we considered the dose-dependent decrease in renal blood flow or the increase in blood pressure. The dose–response curves for vasopressin were shifted to the left by a factor of 10. This effect seems primarily due to aging since it was also present in control rats. Age-matched diabetic rats showed similar hyperreactivity to vasopressin without any effect specific for diabetes. Aging, as well as the late stage of diabetes mellitus, are characterized by endothelial dysfunction, resulting in decreased NO availability. Multiple mechanisms seem to contribute to this dysfunction, such as a decrease in endothelial NO production as well as an increase in NO metabolism or quenching. Direct measurements using a porphyrinic NO microsensor showed a decrease in endothelial NO release with aging (Tschudi et al., 1996). Endothelial NO synthase (mRNA or protein) was, however, found to be increased (Cernadas et al., 1998) or decreased (Barton et al., 1997) with aging and was increased in diabetes (Rösen et al., 1995). Quenching of NO is possible through enhanced superoxide anion levels or alteration in antioxidant defense (Laight et al., 2000). Superoxide dismutase, a superoxide anion scavenger, has indeed been shown to reverse the alteration in acetylcholine-induced relaxation in isolated arteries from diabetic (Pieper et al., 1996) or old rats (Rodríquez-Martínez et al., 1998). Hyperglycemia, which also activates protein kinase C, might thus alter NO production in diabetic rats (King et al., 1997) or through decreasing NO synthase activity as shown in endothelial cells in culture (Ohara et al., 1995). Furthermore, advanced glycated end products contribute to NO quenching (Bucala et al., 1991) and to downregulation of endothelial NO

synthase expression in aortic endothelial cells in culture (Rojas et al., 2000). A correlation between glycated haemoglobin and alteration in endothelium-dependent relaxation has been reported (Ródriguez-Mañas et al., 1998).

In addition to endothelial dysfunction related to NO, other mechanisms could also be involved in the vascular hyperreactivity to vasopressin in older and diabetic rats. Endothelium-dependent relaxation linked to endothelium-dependent hyperpolarization factor was reported to be decreased in isolated arteries from diabetic (Fukao et al., 1997; Kamata et al., 2000) or old rats (Fujii et al., 1993), although these effects were not found in other studies (Alvarez de Sotomayor et al., 1999; Endo et al., 1995). Endothelium-dependent vasodilation could, finally, be blunted by an increased release of vasoconstrictor prostaglandins. Indeed, acetylcholine-induced relaxation was enhanced by indomethacin in isolated arteries from old rats (Matz et al., 2000). Likewise, a thromboxane A_2 receptor antagonist restored the endothelium-dependent relaxation in isolated renal arteries from diabetic rats (Dai et al., 1993). Clearly, further investigations are necessary to analyse the mechanisms involved in the vascular hyperreactivity to vasopressin with aging and/or late diabetes. In particular, the density of vasopressin V_1 receptors should be addressed.

In conclusion, our results document vasopressin renal vascular hyporeactivity in the early stage of diabetes mellitus. An increase in the modulation by NO of the vasoconstriction elicited by the peptide seems to explain this hyporeactivity. In contrast, enhanced renal vasoconstriction in response to vasopressin appears with aging, which did not seem to worsen with diabetes. Both the mechanism of this vascular hyperreactivity and its pathophysiological significance remain to be elucidated.

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