Original Article

Reduction of glomerular hyperfiltration by dopamine D₂-like receptor blockade in experimental diabetes mellitus

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Abstract

Background. Dopamine D₂-like receptors are involved in the physiological response of renal haemodynamics to amino-acid infusion. The present study was performed to investigate whether domperidone, a D₂-like receptor antagonist, modulates the pathological hyperfiltration in experimental diabetes mellitus.

Methods. Renal function was studied in anaesthetized rats 2 weeks after induction of moderate diabetes mellitus by streptozotocin, and in non-diabetic controls. Rats in both groups continuously received domperidone or vehicle via drinking water. Following infusion of Ringer's saline for measurement of baseline values, an i.v. amino-acid load was applied to investigate the renal functional reserve.

Results. In vehicle-treated diabetic rats baseline glomerular filtration rate and renal plasma flow were significantly higher compared with controls (1.10 ± 0.04 vs 0.83 ± 0.02 (P < 0.004) and 4.83 ± 0.26 vs 3.32 ± 0.24 ml/min/100 g body weight (bw) (P < 0.001) respectively). Domperidone completely normalized glomerular filtration rate and renal plasma flow in diabetic rats to values observed in vehicle-treated controls (0.81 ± 0.07 (P = 0.740) and 3.35 ± 0.30 ml/min/100 g bw (P = 0.889) respectively). In the clearance experiments, arterial blood pressure, urinary flow rate and sodium excretion did not significantly differ when comparing the four groups. However, in conscious rats, urinary flow rate and sodium excretion were significantly higher in diabetic rats compared with non-diabetic controls. In both non-diabetic groups, amino-acid infusion induced a significant glomerular hyperfiltration that was completely absent in diabetic rats, and restored by domperidone treatment. In conscious vehicle-treated diabetic rats urinary albumin excretion was enhanced (449.0 ± 47.7 vs 185.7 ± 18.1 µg/24 h in non-diabetic rats (P < 0.001)) and significantly lowered in diabetic rats by domperidone treatment (109.8 ± 15.4 µg/24 h (P < 0.001)).

Conclusion. The data suggest that dopaminergic mechanisms are involved in the changes in renal haemodynamics during early experimental diabetes mellitus in rats.

Keywords: D₂-like receptors; diabetes mellitus; dopamine; nephropathy; rats; renal haemodynamics

Introduction

Some 40% of patients with type I diabetes mellitus develop progressive nephropathy. Elevated glomerular filtration rate (GFR) is observed in 25-40% of insulin-dependent diabetics in the early state of the disease. Since this glomerular hyperfiltration has been said to contribute to the progression of diabetic nephropathy [1] numerous studies focused on the underlying mechanisms of this renal haemodynamic disorder. Beside poor metabolic control [2], abnormalities in circulating concentrations or tissue levels of vasoactive hormones such as angiotensin II, catecholamines, and prostaglandins may contribute to the diabetic changes in renal haemodynamics [3,4]. On the glomerular level, diabetic hyperfiltration has been ascribed to an increase in glomerular capillary pressure and in glomerular plasma flow rate as the result of a predominant vasodilatation of the afferent arterioles [5].

Glomerular hyperfiltration has also been described as a functional response of the kidney to ingestion of an oral protein load or to intravenous infusion of amino acids [6]. Beside other mechanisms, dopamine receptor activation might contribute to this physiological amino acid-induced hyperfiltration since it was completely abolished by dopamine D₂-like receptor antagonists [7]. The present study was performed to test whether blockade of D₂-like receptors might also influence pathological changes in renal haemodynamics. For this purpose, rats in the early stage of experimental diabetes mellitus were subchronically treated with domperidone, a peripherally acting selective antagonist of D₂-like receptors, and renal...
function was studied in both metabolic cage and clearance experiments. Since it has been reported that the renal functional reserve is significantly reduced in diabetic nephropathy [8], GFR and renal plasma flow were challenged by infusion of mixed amino acids.

**Subjects and methods**

All animal experimentation described was conducted in accordance with the German Law for Animal Protection. Male Sprague-Dawley rats (Charles River, Sulzfeld, Germany) weighing 180–200 g were used. Diabetes mellitus was induced by an intraperitoneal injection of 60 mg/kg streptozotocin (Sigma Chemicals, Deisenhofen, Germany) dissolved in sodium citrate buffer (pH 4.0). Control rats were injected with vehicle. Rats were included in the diabetic groups if blood glucose levels measured 24 h after streptozotocin injection in capillary tail blood samples were within the range of 250–350 mg/dl. By using this protocol, insulin substitution was not required throughout the study. Rats had free access to a regular rat chow (Altromin 1320®, Altromin, Lage, Germany). After onset of diabetes the animals were divided into two groups, one of which was continued on tap water (DM-VHC, n=10) whereas in the other group the dopamine D₂-like receptor antagonist domperidone (Biotrend, Cologne, Germany) was administered via the drinking water (DM-DOM, n=10). Accordingly, non-diabetic control rats received either drug free tap water (CTR-VHC, n=10) or the domperidone-containing drinking water (CTR-DOM, n=8). In all animals, the domperidone dosage was adjusted to 8 mg/day according to the daily fluid consumption. On day 12 of the treatment periods, rats were placed in metabolic cages for 24 h to measure food and fluid intake, urinary volume, and excretion rate of albumin and electrolytes.

**Measurement of renal haemodynamics**

Fourteen to 16 days after induction of diabetes mellitus, rats were anaesthetized with an intraperitoneal injection of thiopental sodium (80 mg/kg; Trapanal®, Byk Gulden, Constance, Germany) and placed on a heated table (RT®, Effenberger, Munich, Germany), which was thermoregulated to maintain the rectal temperature at 37.1°C. A tracheostomy was performed to facilitate spontaneous breathing. Two catheters were inserted into the right jugular vein for infusion of Ringer's saline solution (in millimolar concentrations: 111 NaCl, 30 NaHCO₃, 4.7 KCl). The left carotid artery was cannulated for drawing of blood samples and continuous recording of blood pressure. A catheter, inserted deeply in the bladder, served for urine collection. After surgical preparation, Ringer solution was infused via the first i.v. catheter at a rate of 6.6 ml/h over 30 min, followed by a sustained infusion of 1 ml/h/100 g bw. Through the second catheter [¹H]-insulin (1.2 μCi/ml) and [¹⁴C]-para-aminohippuric acid (PAH; 1.0 μCi/ml) dissolved in Ringer solution were infused at a rate of 0.6 ml/h throughout the entire experiment for assessment of GFR and renal plasma flow (RPF) respectively. The animals were allowed to recover from surgical procedures for 60 min before the measurements were started.

Two clearance periods of 20 min each were carried out by infusion of Ringer solution to observe baseline values. Thereafter, Ringer infusion was switched to an amino acid solution (10%) and, after 10 min, another two clearance periods were performed. The composition of the amino-acid solution (g/l) was: 3.8 l-isoleucine, 6.6 l-leucine, 9.3 l-lysine, 2.8 l-methionine, 4.1 l-phenylalanine, 4.6 l-threonine, 1.2 l-tryptophan, 4.1 l-valine, 9.2 l-arginine, 4.4 l-histidine, 7.7 aminoacetic acid, 14.3 l-alanine, 9.2 l-proline, 0.7 l-cysteine, 9.9 l-glutamic acid, 4.6 l-ornithinyl-l-aspartate, 5.9 l-serine, 0.5 l-tyrosine, dissolved in Ringer solution. Blood samples (180 μl) were drawn at the midpoint of each clearance period. After termination of the last clearance period, samples of peripheral arterial (P₈) and renal venous blood (P₉) were drawn simultaneously to estimate renal PAH extraction (E_PAH) from the [¹⁴C]PAH-activity according to the formula:

\[ E_{PAH} = \left( \frac{P_9}{P_8} \right) \cdot P_A \]

GFR and RPF were determined as the renal clearance of inulin and PAH respectively. RPF was corrected for the PAH extraction. Renal blood flow (RBF), renal vascular resistance (RVR), and renal filtration fraction (FF) were calculated by the following equations:

\[ RBF = \frac{P_8}{P_9} \cdot (1 - Hct) \]

\[ RVR = \frac{P_9}{P_8} \cdot MAP/RBF \]

\[ FF = \frac{GFR}{RPF} \]

After termination of the experiments both kidneys were removed, decapsulated, and dried at 70°C for 16 h.

**Analytical methods**

Urine volume and kidney weight were measured gravimetrically. Blood samples were centrifuged, and the hematocrit was assessed. Urinary and plasma concentrations of electrolytes were determined by flame photometry (Elex 6361®, Eppendorf, Hamburg, Germany), the [¹H]-insulin and [¹⁴C]PAH radioactivity was measured by liquid-phase scintillation counting (2550 TR®, Canberra Packard, Frankfurt, Germany). Blood glucose levels were determined by a glucometer (Reflolux®, Boehringer Mannheim, Germany). Urinary dopamine was measured by high-pressure liquid chromatography (HPLC) with electrochemical detection (Sykam, Gielching, Germany) as described previously [9]. In brief, dihydroxybenzylamine (DHBA) was added to the urine sample as internal standard (ISTD). After pH was adjusted to 8.6, neutral aluminium oxide was added. Following this absorption step, the samples were washed twice with bidistilled water and finally eluted with phosphoric acid. The eluate was applied onto the reversed-phase HPLC system. The mobile phase consisted of a citrate buffer, octane sulphonic acid (sodium salt), methanol, and acetonitrile in bidistilled water. ISTD-corrected recovery of dopamine added to the urine averaged 96 to 104%. Urinary albumin concentrations were determined using a rat specific enzyme-linked immunosorbenent assay kit (Celltrend, Luckenwalde, Germany).

**Statistical analysis**

Values of clearance periods at baseline and during amino acid infusion were calculated individually and summarized per group as means ± SEM. Within-group comparison of the experimental periods and baseline were performed by the two-sided paired Student's t-test. For intergroup comparison, analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons was carried out. Urinary
albumin excretion was log-transformed before calculation. P values of less than 0.05 were considered to be statistically significant.

Results

Metabolic cage experiments

Results of the experiments in conscious rats are shown in Table 1. Weight gain over 2 weeks was approximately 30% in diabetic rats and 45% in control animals. Diabetic rats receiving tap water (DM-VHC) or domperidone (DM-DOM) exhibited a significantly higher fluid and food intake, blood glucose concentration, urinary flow rate (UV), and excretion of sodium ($U_{Na}V$) compared with the respective control groups (CTR-VHC or CTR-DOM). As observed in CTR-DOM, domperidone treatment per se did not elicit a significant effect on bw, fluid or food intake, blood glucose, or excretory function of the kidney. Comparing non-diabetic rats (CTR-VHC) with animals of the DM-VHC group, a significant increase in urinary albumin excretion ($U_{AB}V$) was observed which was completely abolished when diabetic rats were treated with domperidone (DM-DOM; Table 1).

Clearance experiments

Comparison of domperidone-treated non-diabetic rats (CTR-DOM) vs vehicle-treated non-diabetic rats (CTR-VHC). At baseline, GFR, RPF, filtration fraction (FF) and renal vascular resistance (RVR) were not significantly different in CTR-VHC compared with CTR-DOM (Figures 1 and 2). In both groups, baseline UV, $U_{Na}V$ and urinary dopamine excretion ($U_{DA}V$) were similar, indicating that domperidone itself did not elicit a significant effect on renal haemodynamics or excretory function of the kidney (Table 3). Basal systemic parameters, i.e. mean arterial blood pressure (MAP), sodium and potassium plasma concentrations, and heart rate (HR) were not significantly different as well (Tables 2 and 3). In both groups, amino acid infusion resulted in a significant increase in GFR and RPF (26–34%), indicating presence of renal functional reserve (Figure 1). Because of these renal haemodynamic changes, FF was not affected while RVR was significantly reduced by 15–23% in both groups. The effect of amino acids on UV, $U_{Na}V$, and $U_{DA}V$ were similar and did not markedly change systemic parameters (MAP, sodium and potassium plasma concentrations, and HR) in CTR-VHC and CTR-DOM (Tables 2 and 3). In both groups, kidney dry weight was $0.72 \pm 0.02$ g respectively.

Comparison of vehicle-treated diabetic rats (DM-VHC) vs vehicle-treated non-diabetic rats (CTR-VHC). In DM-VHC compared with CTR-VHC, baseline GFR and RPF were significantly increased by 33 and 46% respectively, (Figure 1). Accordingly, a slight decrease in FF and a markedly lower RVR (−33%) was observed in DM-VHC (Figure 2). Regarding UV, $U_{Na}V$, and $U_{DA}V$, no significant differences between the groups was found. Additionally, basal MAP, sodium and potassium plasma concentrations were similar, while HR was significantly decreased by 17% in DM-VHC (Tables 2 and 3). In DM-VHC, amino-acid infusion resulted in a marginal increase in GFR and RPF by some 8% over baseline, indicating that the renal functional reserve was significantly diminished compared with CTR-VHC (Figure 1). No change in FF was observed during amino-acid infusion in both groups, whereas RVR was significantly reduced by 15% in CTR-VHC but not affected in DM-VHC (Figure 2). The increase in UV, $U_{Na}V$, and $U_{DA}V$ by amino acids was more pronounced in CTR-VHC compared with DM-VHC (Table 3). In both groups, amino acid infusion did not affect MAP, HR, or plasma sodium or potassium concentrations (Tables 2 and 3).

Comparison of domperidone-treated diabetic rats (DM-DOM) vs vehicle-treated diabetic rats (DM-VHC). At baseline, the diabetes-induced glomerular hyperfiltration and renal hyperperfusion as shown in DM-VHC was completely attenuated by domperidone treatment (DM-DOM, Figure 1). In both groups, FF was not different at baseline while RVR was significantly decreased by 30% in DM-VHC compared with DM-DOM (Figure 2). Similar baseline UV, $U_{Na}V$, and $U_{DA}V$ and systemic parameters were observed in both groups (Tables 2 and 3). Amino-acid infusion resulted in a significant increase in GFR and RPF (45%) in DM-DOM which was associated with a significant decrease in RVR by 28%, while FF was not affected (Figures 1 and 2). In contrast, amino acids did not markedly change renal

Table 1. Data of metabolic cage experiments in controls (CTR) and in animals with streptozotocin-induced diabetes mellitus (DM), receiving domperidone (DOM) or vehicle (VHC) for 2 weeks

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Fluid intake (ml/24 h)</th>
<th>Food intake (g/24 h)</th>
<th>Blood glucose (mg/dl)</th>
<th>UV (ml/24 h)</th>
<th>$U_{Na}V$ (mmol/24 h)</th>
<th>$U_{AB}V$ (mg/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTR-VHC</td>
<td>293 ± 5</td>
<td>38 ± 2</td>
<td>31 ± 2</td>
<td>91 ± 8</td>
<td>14 ± 1</td>
<td>2.32 ± 0.46</td>
<td>185.7 ± 18.1</td>
</tr>
<tr>
<td>CTR-DOM</td>
<td>300 ± 6</td>
<td>42 ± 1</td>
<td>33 ± 2</td>
<td>98 ± 7</td>
<td>11 ± 1</td>
<td>1.63 ± 0.15</td>
<td>177.9 ± 37.5</td>
</tr>
<tr>
<td>DM-VHC</td>
<td>251 ± 6a</td>
<td>179 ± 27a</td>
<td>51 ± 2a</td>
<td>387 ± 25a</td>
<td>168 ± 14a</td>
<td>4.09 ± 0.33a</td>
<td>449.0 ± 47.7a</td>
</tr>
<tr>
<td>DM-DOM</td>
<td>272 ± 9b</td>
<td>188 ± 26b</td>
<td>51 ± 3b</td>
<td>401 ± 24b</td>
<td>176 ± 18b</td>
<td>3.91 ± 0.25b</td>
<td>109.8 ± 15.4bc</td>
</tr>
</tbody>
</table>

Values are means ± SEM. *P < 0.05 vs respective CTR group; **P < 0.05 vs respective VHC group; ***P < 0.05 vs CTR-DOM group.
Dopamine D$_2$-like receptors and diabetic hyperfiltration

Haemodynamics in DM-VHC. The effect of amino-acid infusion on UV, U$_{Na}$V, and U$_{DA}$V was similar in both groups (Table 3). Compared with baseline, infusion of amino acids did not alter MAP and HR (Tables 2 and 3). In both diabetic groups, kidney dry weight was approximately 1.00 $\pm$ 0.04 g respectively, indicating a significant increase by some 40% in comparison to non-diabetic rats.

Comparison of domperidone-treated diabetic rats (DM-DOM) vs vehicle-treated non-diabetic rats (CTR-VHC). In both groups, baseline GFR and RPF were not significantly different (Figure 1), suggesting that domperidone attenuated the diabetes-induced increase in renal haemodynamics. As a consequence, basal FF and RVR were similar in CTR-VHC and DM-DOM (Figure 2). At baseline, UV, U$_{Na}$V, and U$_{DA}$V were slightly but not significantly reduced in DM-DOM compared with CTR-VHC (Table 3). Similar basal MAP, sodium and potassium plasma concentrations, were observed in both groups, while HR was significantly lower (9%) in DM-DOM compared with CTR-VHC (Tables 2 and 3). In the DM-DOM group, infusion of amino acids induced a significant increase in GFR and RPF (45%), indicating that domperidone treatment in diabetic rats restored the renal functional reserve (Figure 1). No changes in FF were observed during

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**Fig. 1.** Effect of domperidone (DOM) on glomerular filtration rate (GFR; upper panel) and renal plasma flow (RPF; lower panel) in control (CTR) and diabetic rats (DM). Values are depicted at baseline (hatched bars) and in response to infusion of amino acids (open bars). Data are expressed as means $\pm$ SEM; n = 8–10 per group. VHC, application of vehicle. *P < 0.05 vs baseline. $^{b}$P < 0.05 vs CTR-VHC at baseline. $^{c}$P < 0.05 vs DM-VHC at baseline.

**Fig. 2.** Effect of domperidone (DOM) on renal vascular resistance (RVR; upper panel) and filtration fraction (FF; lower panel) in control (CTR) and diabetic rats (DM). Values are depicted at baseline (hatched bars) and in response to infusion of amino acids (open bars). Data are expressed as means $\pm$ SEM; n = 8–10 per group. VHC, application of vehicle. *P < 0.05 vs baseline. $^{b}$P < 0.05 vs CTR-VHC at baseline. $^{c}$P < 0.05 vs DM-VHC at baseline.

**Table 2.** Systemic haemodynamics and haematocrit in anaesthetized control rats (CTR) and animals with streptozotocin-induced diabetes mellitus (DM), receiving domperidone (DOM) or vehicle (VHC) for 2 weeks

<table>
<thead>
<tr>
<th>Groups</th>
<th>Periods</th>
<th>MAP (mmHg)</th>
<th>HR (min)</th>
<th>Hct (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTR-VHC</td>
<td>Baseline</td>
<td>108 $\pm$ 3</td>
<td>386 $\pm$ 7</td>
<td>44 $\pm$ 1</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>Amino acids</td>
<td>115 $\pm$ 3</td>
<td>383 $\pm$ 9</td>
<td>43 $\pm$ 1$^{a}$</td>
</tr>
<tr>
<td>CTR-DOM</td>
<td>Baseline</td>
<td>110 $\pm$ 1</td>
<td>384 $\pm$ 8</td>
<td>43 $\pm$ 1$^{a}$</td>
</tr>
<tr>
<td>(n = 8)</td>
<td>Amino acids</td>
<td>107 $\pm$ 3</td>
<td>389 $\pm$ 9</td>
<td>43 $\pm$ 1$^{a}$</td>
</tr>
<tr>
<td>DM-VHC</td>
<td>Baseline</td>
<td>107 $\pm$ 2</td>
<td>321 $\pm$ 10$^{a}$</td>
<td>44 $\pm$ 1</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>Amino acids</td>
<td>109 $\pm$ 2</td>
<td>319 $\pm$ 10$^{a}$</td>
<td>43 $\pm$ 1$^{a}$</td>
</tr>
<tr>
<td>DM-DOM</td>
<td>Baseline</td>
<td>106 $\pm$ 3</td>
<td>354 $\pm$ 7$^{b}$</td>
<td>46 $\pm$ 1</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>Amino acids</td>
<td>107 $\pm$ 3</td>
<td>353 $\pm$ 9</td>
<td>46 $\pm$ 1</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SEM. $^{a}$P < 0.05 vs respective CTR group; $^{b}$P < 0.05 vs respective VHC group; $^{c}$P < 0.05 vs baseline.
infusion of amino acids, whereas RVR was significantly reduced in both groups. The effect of amino acids on UV, $U_{NaV}$, and $U_{DAV}$ was more pronounced in CTR-VHC compared with DM-DOM (Table 3), while systemic parameters were not altered in both groups (Tables 2 and 3).

**Discussion**

The present investigation was performed to study the effect of pharmacological blockade of dopamine $D_2$-like receptors on glomerular hyperfiltration in an animal model of diabetes mellitus. Intraperitoneal administration of streptozotocin in rats led to a significant metabolic disorder expressed by hyperglycaemia, polyuria and hyperphagia. The selected dose of streptozotocin induced a moderate hyperglycaemia, indicating that a submaximal impairment of insulin secretion was achieved. Earlier, Hostetter et al. [5] described the induction of a more severe hyperglycaemia by the same dose of streptozotocin in rats. However, in that study, older animals were used and the drug was administered by the i.v. route. The normal behaviour and the significant weight gain of the diabetic animals in the present experiments confirmed that the physical conditions were satisfying and that insulin substitution was not required. Glomerular hyperfiltration, renal hyperperfusion, and albuminuria were observed as early as 2 weeks after induction of diabetes. Signs of nephropathy during the initial phase of experimental diabetes have been reported also by others [5,10].

In the present experiments, subchronic administration of the dopamine $D_2$-like receptor antagonist domperidone did not affect renal or systemic haemodynamics in normoglycaemic controls but completely abolished the diabetes-induced changes in renal haemodynamics and urinary albumin excretion without affecting systemic blood pressure. Therefore, a subchronic dopamine $D_2$-like inhibition appears to influence beneficially the course of early diabetic nephropathy. However, since only a short duration of experimental diabetes mellitus was employed in the present study, it remains difficult to discriminate whether the abolished albuminuria indicates a beneficial effect of domperidone treatment on glomerular injury or whether it represents the pure changes in glomerular haemodynamics. This context has to be clarified by long-term studies with additional histological assessment of the pathological changes in the structure of the glomerulus.

The present experiments extend the observation that dopamine receptors of the $D_2$-like family are involved in the physiological amino acid-induced increase in GFR in rats [7,11,12] and humans [13] to a pathophysiological model, i.e. the hyperfiltration in rats with experimental diabetes mellitus. However, the mechanisms of how endogenous dopamine, most probably via $D_2$-like receptors, modulates renal haemodynamics still has to be clarified. The dopamine, which is synthesized by the epithelial cells of the proximal tubules and released into the tubular lumen, does not appear to affect renal function to a significant degree [9]. Therefore, one would rather expect that dopamine exerts its functional effects via release into the renal interstitium, as suggested recently by Wang et al. [14]. Since these experiments were performed in rats without metabolic disorder or amino acid challenge a conclusion from those data for the dopaminergic effects observed in the present study cannot be drawn.

A potential site of action at which endogenous dopamine might influence renal haemodynamics arises from the results of a recent micropuncture study. In normal rats, pharmacological activation of dopamine $D_3$ receptors, another member of the $D_2$-like family, induced an increase in GFR that might have been due to a post-glomerular vasoconstriction [15]. Since, however, in the present study the normalization of the diabetic hyperfiltration was not accompanied by a decrease in filtration fraction, a specific action of domperidone on the tone of the post-glomerular capillaries does not appear to be the mechanism of the observed effects in diabetic rats.

From the parallel observations that pharmacological modulation of $D_2$-like receptors affects both physiological increase in GFR due to amino acids and pathophysiological diabetes-induced hyperfiltration, one might speculate that these changes in renal haemodynamics might be mediated by the same mechanisms. However, in healthy humans the increase in GFR

<table>
<thead>
<tr>
<th>Groups</th>
<th>Periods</th>
<th>$Na_{Plasma}$ (mmol/l)</th>
<th>$K_{Plasma}$ (mmol/l)</th>
<th>UV (µl/min)</th>
<th>$U_{NaV}$ (µmol/min)</th>
<th>FE$_{Na}$ (%)</th>
<th>$U_{DAV}$ (pmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTR-VHC</td>
<td>Baseline</td>
<td>140 ± 2</td>
<td>4.0 ± 0.2</td>
<td>13.9 ± 2.9</td>
<td>0.72 ± 0.19</td>
<td>0.53 ± 0.16</td>
<td>4.61 ± 0.35</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>Amino acids</td>
<td>140 ± 2</td>
<td>3.7 ± 0.2</td>
<td>24.6 ± 2.8*</td>
<td>2.98 ± 0.35*</td>
<td>1.94 ± 0.25*</td>
<td>9.29 ± 0.91*</td>
</tr>
<tr>
<td>CTR-DOM</td>
<td>Baseline</td>
<td>138 ± 2</td>
<td>4.3 ± 0.1</td>
<td>13.9 ± 4.8</td>
<td>0.76 ± 0.30</td>
<td>0.64 ± 0.23</td>
<td>6.71 ± 0.78</td>
</tr>
<tr>
<td>(n = 8)</td>
<td>Amino acids</td>
<td>137 ± 1</td>
<td>4.1 ± 0.1</td>
<td>27.6 ± 4.6*</td>
<td>3.23 ± 0.83*</td>
<td>2.25 ± 0.66*</td>
<td>9.62 ± 0.97*</td>
</tr>
<tr>
<td>DM-VHC</td>
<td>Baseline</td>
<td>141 ± 1</td>
<td>4.0 ± 0.2</td>
<td>12.6 ± 2.9</td>
<td>1.32 ± 0.39</td>
<td>0.91 ± 0.28</td>
<td>5.82 ± 0.47</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>Amino acids</td>
<td>141 ± 1</td>
<td>3.9 ± 0.2</td>
<td>14.7 ± 2.0</td>
<td>1.96 ± 0.39</td>
<td>1.18 ± 0.23*</td>
<td>8.94 ± 0.81*</td>
</tr>
<tr>
<td>DM-DOM</td>
<td>Baseline</td>
<td>139 ± 2</td>
<td>4.2 ± 0.1</td>
<td>8.0 ± 1.8</td>
<td>0.49 ± 0.21</td>
<td>0.38 ± 0.13</td>
<td>5.47 ± 0.46</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>Amino acids</td>
<td>139 ± 2</td>
<td>3.9 ± 0.2</td>
<td>11.7 ± 1.0*</td>
<td>1.12 ± 0.27*</td>
<td>0.73 ± 0.19*</td>
<td>10.75 ± 0.99*</td>
</tr>
</tbody>
</table>

Renal excretion values are expressed per 100 g body weight (except for FE$_{Na}$). Values are means ± SEM. *P < 0.05 vs respective CTR group; †P < 0.05 vs respective CTR group; ‡P < 0.05 vs baseline.
after infusion of exogenous dopamine and amino acids were said to depend on different mechanisms [16]. In addition, in type 1 diabetic patients, the renal haemodynamic response to dopamine was preserved but that to amino acids was diminished [17]. The authors concluded that dopamine appears to be less important in mediating the amino acid-induced glomerular hyperfiltration in humans. However, care has to be taken when comparing those observations with the results of the present animal experiments. In the clinical studies, exogenous dopamine was employed which, due to different plasma concentrations and due to additional systemic haemodynamic actions, might affect renal function in a different way from endogenous dopamine. Renal functional reserve, i.e. the ability of the GFR to increase in response to exogenous stimuli, is attenuated in diabetic nephropathy [8].

In the present study, domperidone, beside normalizing the diabetic hyperfiltration, also restored the renal reserve filtration capacity induced by amino-acid infusion. In a previous study in normal rats, domperidone, applied intravenously and at a higher dose, decreased the amino acid-induced hyperfiltration [11]. On the one hand, this might reflect a pure dose-dependency of the action of domperidone on glomerular function. On the other hand, this potential dissociation might also be due to different mechanisms modulating the amino acid-induced hyperfiltration and the GFR increase in diabetic rats as suggested for man. Nonetheless, the present data indicate that chronic administration of domperidone in a dose not suppressing acute changes in GFR might still be able, by normalizing basal renal vascular resistance, to preserve the response of renal haemodynamics to acute stimuli. The discrepancies outlined above, however, make it difficult to extrapolate to the human situation the obvious beneficial effects of domperidone treatment on renal haemodynamics in diabetic rats.

At variance with the present data, attenuation of diabetic hyperfiltration has been reported in response to dopamine precursors, which was ascribed to D₁ receptor stimulation [18]. However, essential differences in the experimental setting, e.g. higher hyperglycaemia, insulin substitution, a different time course, and additional arterial hypertension of the animals confound the comparison of these data with the present study. In addition, in this previous study, the dopamine pro-drugs were administered in doses large enough to raise the peripheral concentrations of dopamine extensively and therefore might have led to unspecific adrenergic effects in the systemic vasculature.

It might be argued that in the present study the higher fluid turnover of diabetic rats in the conscious state compared with the anaesthetized state might result in an extracellular volume contraction during the clearance experiments which might have confounded the observations. However, baseline haematocrit as a parameter of the intravascular volume state was not significantly different in the four study groups. Similarly, there was no volume or sodium retention in the diabetic animals during the clearance experiments, which one would expect if the extracellular volume were contracted. Therefore, all groups appeared to be well balanced with respect to volume and sodium excretion, suggesting that the attenuation of the diabetes-induced hyperfiltration by domperidone treatment did not result from unspecified changes in volume balance. Since dopamine D₂-like receptors have been localized in the renal vasculature, in the cortical tubules, and the medulla [19,20] this receptor might influence, beside haemodynamics, also excretory function of the kidney. Indeed, some decrease in urinary sodium excretion was observed in conscious and anaesthetized rats during administration of domperidone. However, these changes were not observed consistently when comparing the diabetic and the control groups. Thus, a significant influence of domperidone on tubular sodium handling cannot be concluded from the present data.

To summarize, pharmacological D₂-like receptor blockade by domperidone attenuates hyperfiltration and restores renal reserve filtration capacity in experimental diabetes mellitus. These data suggest that dopaminergic effects may contribute to the glomerular malfunction in early diabetic nephropathy. Whether an increased tubular synthesis of dopamine is involved in the pathophysiological changes of the kidney and whether these data observed in diabetic rats can be extended to the human situation has to be clarified.

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