Adenosine-induced renal vasoconstriction in diabetes mellitus rats: role of nitric oxide

Axel C. Pflueger, Hartmut Osswald, and Franklyn G. Knox

Adenosine-induced renal vasoconstriction in diabetes mellitus rats: role of nitric oxide. Am. J. Physiol. 276 (Renal Physiol. 45): F340–F346, 1999.—In rats with streptozotocin (STZ)-induced diabetes, the renal vasoconstrictor effect of adenosine is enhanced. We investigated the role of nitric oxide (NO) in the renal vascular response to exogenous and endogenous adenosine in control and STZ diabetic rats. Exogenous adenosine (0.01–100 nmol) injected into the abdominal aorta decreased renal blood flow (RBF) in a dose-dependent manner to a much greater extent in STZ rats than in control rats (P < 0.001). Inhibition of NO synthesis with L-nitro-l-arginine (L-NNA, 30 μmol/kg iv) and with renal perfusion pressure controlled potentiated the adenosine-induced renal vasoconstriction to a significantly greater extent in control rats than in STZ rats. In control rats, L-NNA shifted the dose-response curve of exogenous adenosine-induced RBF reductions to the left by a factor of 32 [half-maximal effective dose (ED50), from 5.5 to 0.17 nmol adenosine, n = 6] and in STZ rats only by a factor of 4.6 (ED50, from 0.32 to 0.07 nmol adenosine, n = 6). The renal response to endogenous adenosine was assessed by the magnitude of the postocclusive reduction of RBF (POR) after a 30-s renal artery occlusion. POR was markedly enhanced in STZ rats (–67.8 ± 3.8%, P < 0.001) compared with control rats (–38.8 ± 4.3%). L-NNA markedly enhanced POR in control rats but did not increase POR in STZ rats. These findings demonstrate a greater potentiation of the adenosine-induced renal vasoconstriction in the presence of L-NNA infusion in control rats compared with STZ rats. We conclude that the increased vasoconstrictor sensitivity of the diabetic renal vasculature to adenosine is caused by a defective NO-dependent renal vasodilation of the afferent arteriole in diabetic rats.

The renal vascular response to adenosine is unique in that adenosine induces vasoconstriction of the afferent glomerular arteriole via adenosine A1 receptors (26, 30), in contrast to other vascular beds in which adenosine acts as a vasodilator. A number of factors and conditions modify the renal vasoconstrictor response to adenosine including sodium intake, renal arterial perfusion pressure, ureteral obstruction, prostaglandins, nitric oxide (NO), and diabetes mellitus (15, 26, 29, 30, 32). In a previous study, we demonstrated an increased sensitivity of the renal vasculature to endogenous and exogenous adenosine in streptozotocin (STZ)-induced diabetic rats (32). The effects of adenosine on the renal vasculature in that study were mediated via adenosine A1 receptors, since they could be inhibited in a dose-dependent manner by the selective adenosine A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (25, 32).

There is considerable evidence indicating that endothelial-dependent vasodilation mediated by NO is impaired in humans and animals in the early phase of diabetes mellitus (5, 10, 11, 13, 23). Extrarenal NO-dependent vasodilation is impaired in both large and small arteries obtained from diabetic animals (5, 11, 13, 23). The mechanisms responsible for diabetes-induced endothelial-dependent vascular dysfunction have been explored most extensively in the aorta obtained from diabetic animals (13, 23). In the kidney of animals with insulin-dependent diabetes mellitus (IDDM), several studies report alterations of NO generation (2, 7, 9, 39, 40). Both increases (2, 39) and decreases (7, 9, 40) of NO synthesis have been reported in the diabetic kidney. Furthermore, increasing experimental evidence shows that endothelial-derived NO-mediated vasodilation in renal vessels is impaired in diabetic animals (8, 10, 28, 40), but the overall role of NO-dependent renal vasodilation remains controversial (34).

In clinical settings, patients with diabetes mellitus and impaired renal function have a higher incidence of contrast media-induced acute renal failure (35, 41), a pathophysiological phenomenon, which is proposed to be mediated by adenosine-induced renal vasoconstriction (3). The higher susceptibility of the diabetic renal vasculature to contrast media-induced (35, 41) and adenosine-induced renal vasoconstriction (32) could be in part due to a diminished NO-dependent renal vasodilation in diabetes mellitus. Therefore, in the present study, we determined whether the increased sensitivity of the diabetic renal vasculature to adenosine is caused by a reduced endothelial NO-dependent renal vasodilation in STZ-induced diabetes mellitus in rats. The results showed a greater adenosine-induced renal vasoconstriction in STZ compared with control rats. The adenosine-induced renal vasoconstriction was potentiated by inhibition of NO synthesis to a much greater extent in control rats than in STZ rats.

Materials and Methods

The renal vascular response to adenosine on renal blood flow (RBF) was studied during the inhibition of NO synthesis by N-nitro-l-arginine (L-NNA) with increased and controlled renal perfusion pressure in nondiabetic control and STZ-diabetic rats. The response to exogenous adenosine was

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determined by injecting adenosine via a catheter placed into the abdominal aorta, which reduced RBF in a dose-dependent manner. The vascular response to endogenous adenosine was assessed by quantification of the postocclusive reduction of RBF (POR), a phenomenon shown to be mediated by adenosine A1 receptors (32).

Animal Preparation

Experiments were performed in nonfasted male Sprague-Dawley rats; the animals had free access to a regular rat pellet diet and tap water until the morning of the experiments. The rats were anesthetized with thiopental intraperitoneally (Trapanal, Byk-Gulden) with a dose of 100 mg/kg in control rats and 80 mg/kg in diabetic rats. The animals were placed on a heated table to maintain body temperature at 37°C via a servocontrolled rectal thermometer. Respiration was spontaneous through an endotracheal tube. Two catheters were inserted into the right jugular vein. One catheter served for continuous infusion of isotonic saline (0.85 g/dl) at a rate of 2 ml/h, and the other catheter served for vehicle or drug administration with a continuous infusion of isotonic saline (0.85 g/dl) at a rate of 1 ml/h. A polyethylene catheter was advanced through the left carotid artery into the abdominal aorta. The tip of the catheter was positioned 2–3 mm above the renal artery and connected to a microinjection device for manually time-controlled (0.3–0.5 s) 30-µl bolus injection of adenosine. Care was exercised in maintaining the tip of the catheter precisely at its position. In previous experiments, the authors have shown (unpublished data) that by positioning the tip of the catheter 2–3 mm above the renal artery, the highest effective dose of adenosine administered by this technique effectively enters the renal microcirculation. Positioning the tip of the catheter further above or closer to the renal artery branch showed a lesser effect of the adenosine-induced reduction of RBF. Arterial blood pressure was continuously monitored via a right femoral artery catheter connected to a Statham pressure transducer. The heart rate was derived from the pressure tracing connected to a thermoprinter (model WK-280 R; Foehr Medical Instruments). The left kidney was exposed by a subcostal flank incision and placed in a Lucite holder. After careful preparation of the kidney hilus, a flow probe was fitted around the renal artery and connected to an electromagnetic flowmeter (model 501; Carolina Medical Electronics) for continuous monitoring of RBF. Calibration of zero flow was confirmed by occluding the renal artery branch to the flow probe. The abdominal cavity was covered by a sheet of Parafilm (Laboratory Film, American National Can) to prevent evaporation of fluid. Urine from the left kidney was collected via a uretreal catheter, and the bladder was cannulated for free drainage of urine from the right kidney. The rats were allowed to stabilize following the surgical procedure for 60 min.

Experimental Insulin-Dependent Diabetes Mellitus

The animal model of IDDM was achieved by an intraperitoneal injection of 50 mg/kg STZ (Sigma) dissolved in sodium citrate buffer (pH 4.2). Tail blood samples taken 3 days after STZ injection were collected on the day of the experiment provided measurements of blood glucose levels. Animals with a blood glucose below 250 mg/dl were not included in the experimental series. The experiments started 4–5 wk after STZ administration, and nondiabetic age-matched rats served as controls. The 50 mg/kg STZ model of IDDM was chosen to reduce malnourishment, catabolic state, weight loss, and hyperphagia, since the administered dose of STZ does not completely destroy all β-islet cells of the rat pancreas (37) and a remaining small basal insulin production is achieved with this IDDM model. Furthermore, moderate hyperglycemia was stable until the day of the experiment (blood glucose values: 3 days after STZ, 329 ± 31 mg/dl; on the day of the experiment, 368 ± 29.3 mg/dl).

The progression of diabetic nephropathy is attributed basically to hyperglycemia and insulin deficiency (10); hence, the diabetic model of the current study without insulin treatment may represent a more progressive state of the development of diabetic nephropathy than comparable models with insulin treatment. Consequently, the presented findings may be restricted to STZ-induced diabetes mellitus without insulin treatment.

Exogenous Adenosine

Exogenous adenosine (Serva), dissolved in 0.85 g/dl NaCl, was given in sequentially increasing doses, starting from 0.01 nmol and increasing to 100 nmol, with 3–5 min between doses. Adenosine was administered via the aortic catheter in a bolus of 30 µl over 0.3–0.5 s. Adenosine bolus injections (0.01 up to 10 nmol) did not affect arterial blood pressure; higher doses (30–100 nmol) of adenosine bolus injections slightly decreased arterial blood pressure, however, only after the RBF response was almost complete. Vehicle bolus injections with isotonic saline did not result in any change of RBF. The reduction of RBF (∆RBF) due to adenosine injections was assessed as the difference of minimal postinjection RBF (RBFmin) to baseline RBF (RBFbaseline) as a percentage of the preinjection RBF (RBFbaseline).

\[
\Delta RBF = \frac{(RBF_{baseline} - RBF_{min})}{RBF_{baseline}} \quad (1)
\]

The percentage of RBF reduction (∆RBF, %) in response to adenosine injections (0.01–100 nmol) was plotted in graphs as dose-response curves. RBF measurements were performed by the means of an electromagnetic flowmeter fitted around the left renal artery in a pulsatile fashion, since RBF changes occurred within the time range of the 0.1 s. These RBF changes cannot be observed when the pulsatile recordings are dampened. In the pulsatile RBF measurement, RBF pulse curves interfere with the precise measurement of very small RBF reductions. The pulsatile amplitude of the baseline RBF tracing usually ranges from 17–20% of basal RBF; hence, the minimal precise detectable RBF reduction with the pulsatile RBF registration should be greater than 17–20% ∆RBF. Under conditions in which the minimal dose of adenosine (e.g., 0.01 nmol) did not reach the minimal detectable RBF reduction (at least 20%), the dose-response curve was extrapolated to calculate the half-maximal effective dose (ED50).

Endogenous Adenosine

The renal response to endogenous adenosine was assessed by the POR as described previously (32). In brief, POR was determined by release of two to three occlusions (30 s) of the renal artery. During renal artery occlusion, renal adenosine generation increases by hydrolysis of ATP throughout the kidney (30, 31). The accumulated renal adenosine stimulates A1 receptors on the afferent arteriole and causes renal vasoconstriction, which is apparent after release of the renal artery occlusion in the POR (see Fig. 2) (30–32). As shown previously (32), the POR after release of a renal artery clamp is mediated by adenosine A1 receptor stimulation and can be inhibited in a dose-dependent manner with the highly selective adenosine A1 receptor antagonist DPCPX (26, 32). The extent of the POR was determined via the ratio of minimal RBF after the occlusion to basal RBF as expressed in Eq. 1. A recovery period of 8–10 min followed each occlusion.
Flow in STZ-induced diabetic rats

Table 2. Hemodynamic parameters and measurements of postocclusive reduction of renal blood flow in nondiabetic control rats

<table>
<thead>
<tr>
<th>Control Group</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>UV L-Kidney, ml/h</th>
<th>RBF baseline, ml/min</th>
<th>∆RBF, ml/min</th>
<th>∆RBF, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>103 ± 5.5</td>
<td>373 ± 14</td>
<td>1.5 ± 0.4</td>
<td>4.5 ± 0.2</td>
<td>−18 ± 0.2</td>
<td>−38.8 ± 4.3</td>
</tr>
<tr>
<td>L-NNA</td>
<td>137 ± 4.5</td>
<td>342 ± 29</td>
<td>0.9 ± 0.2</td>
<td>2.6 ± 0.1</td>
<td>−16 ± 0.1</td>
<td>−60.5 ± 2.4</td>
</tr>
<tr>
<td>L-NNA + AC</td>
<td>101 ± 2.6</td>
<td>351 ± 4.2</td>
<td>0.8 ± 0.2</td>
<td>1.8 ± 0.1</td>
<td>−11 ± 0.1</td>
<td>−62.1 ± 2.2</td>
</tr>
<tr>
<td>Group 1: V vs. N</td>
<td>P &lt; 0.001</td>
<td>NS</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>NS</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Group 1: V vs. N + AC</td>
<td>NS</td>
<td>NS</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>NS</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of animals. Postocclusive reduction (POR) of renal blood flow (RBF) was measured with vehicle (V), N-nitro-L-arginine (L-NNA) with increased renal perfusion pressure (N), and L-NNA with controlled renal perfusion pressure (L-NNA + AC i.e., N + AC) in nondiabetic control rats. NS, not significant. MAP, mean arterial pressure; HR, heart rate; UV L-kidney, left kidney urine flow rate; RBF, ml/min; ∆RBF, change in RBF.

Experimental Protocol

The experiments were started after the 60-min recovery period following surgery. The renal response to exogenous adenosine was assessed with intra-aortic single injections of adenosine, and thereafter the renal response to endogenous adenosine was determined with the POR. The renal responses to exogenous and endogenous adenosine were assessed during three experimental periods; between each experimental period, an equilibration period of 20 min was allowed for stabilization. At the end of the experiment, the animals were killed by an overdose of thiopental, and the left kidney was removed and weighed.

Experimental Groups

Group 1 (n = 6): Effect of L-NNA on renal vascular response to adenosine in control rats. In the first experimental period, the renal response to exogenous and endogenous adenosine was assessed with an isotonic normal saline vehicle. In the second part, L-NNA (30 µmol/kg, Sigma) was intravenously infused in 1 ml of isotonic normal saline over 15 min; thereafter, the renal response to exogenous and endogenous adenosine was determined. In the third part, an aortic clamp (L-NNA + AC) was placed around the abdominal aorta above the branch of the renal artery to adjust L-NNA-induced increases in renal perfusion pressure to normotension (90–110 mmHg; L-NNA + AC), and then renal responses to adenosine were repeated.

Group 2 (n = 6): Effect of L-NNA on renal vascular response to endogenous adenosine in STZ rats. The protocol of this group was identical to group 1, but with STZ rats.

Group 3 (n = 5): Effect of time and vehicle on renal vascular response to adenosine in control rats. In all three experimental periods, an isotonic normal saline vehicle was infused (1% body wt/min), and the renal response to exogenous and endogenous adenosine was assessed.

Group 4 (n = 5): Effect of time and vehicle on renal vascular response to endogenous adenosine in STZ rats. The protocol of this group was identical to group 3, but with STZ rats.

Table 2. Hemodynamic parameters and measurements of postocclusive reduction of renal blood flow in STZ-induced diabetic rats

<table>
<thead>
<tr>
<th>STZ Group</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>UV L-Kidney, ml/h</th>
<th>RBF baseline, ml/min</th>
<th>∆RBF, ml/min</th>
<th>∆RBF, %</th>
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</thead>
<tbody>
<tr>
<td>Group 2 (n = 6)</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>94 ± 5.3</td>
<td>366 ± 7.4</td>
<td>1.5 ± 0.2</td>
<td>4.3 ± 0.3</td>
<td>−2.8 ± 0.2</td>
<td>−67.8 ± 3.8</td>
</tr>
<tr>
<td>L-NNA</td>
<td>133 ± 2.1</td>
<td>352 ± 7</td>
<td>0.9 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>−1 ± 0.1</td>
<td>−78.2 ± 4.2</td>
</tr>
<tr>
<td>L-NNA + AC</td>
<td>101 ± 2.4</td>
<td>348 ± 10</td>
<td>0.8 ± 0.2</td>
<td>1.9 ± 0.1</td>
<td>−1 ± 0.1</td>
<td>−68.4 ± 4.4</td>
</tr>
<tr>
<td>Group 2: V vs. N</td>
<td>P &lt; 0.001</td>
<td>NS</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Group 2: V vs. N + AC</td>
<td>NS</td>
<td>NS</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.01</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = number of animals. POR values were measured with vehicle, L-NNA with increased renal perfusion pressure, and L-NNA with controlled renal perfusion pressure (L-NNA + AC) in streptozotocin (STZ)-induced diabetic rats.

Analytic Methods

Blood glucose levels were measured with a blood glucose meter (One Touch, Lifescan) 3 days after STZ injection and on the day of the experiment after the equilibration period.

Statistical Analysis

Data are expressed as means ± SE. Two-factor ANOVA with repeated measures on one, and unpaired Student’s t-test were performed as appropriate. Significance was considered with P < 0.05.

RESULTS

Exogenous Adenosine

There was no significant differences between control, STZ, and time-vehicle control rats with respect to baseline values for mean arterial blood pressure, heart rate, RBF, and hematocrit (Tables 1–3). In STZ-diabetic rats, the adenosine-induced reduction of RBF was markedly potentiated compared with control rats. Adenosine, 1 nmol, injected into the abdominal aorta decreased RBF by Δ−24.3 ± 0.6% in control rats and by Δ−68.7 ± 5.5% in STZ rats (Fig. 1, A and B). The dose-response curve of RBF reduction by single injections of adenosine during vehicle infusion was shifted to the left by a factor of 20 in STZ rats compared with the dose-response curve of control rats. The calculated ED50 of adenosine during vehicle infusion was 5.5 nmol in control rats and 0.3 nmol in STZ rats. The left shift of the dose-response curve of STZ rats compared with control rats indicates that 20-fold higher doses of adenosine in control rats is required to decrease RBF to the same extent compared with STZ rats. Therefore the sensitivity of adenosine-induced RBF reduction is increased in STZ rats. When the RBF response to adenosine was markedly potentiated compared with control rats.

Table 1. Hemodynamic parameters and measurements of postocclusive reduction of renal blood flow in nondiabetic control rats
sine was expressed as maximal effective dose ($ED_{Max}$), the $ED_{Max}$ in control rats was 30-fold higher (100 nmol) than the $ED_{Max}$ of STZ rats (3 nmol; Fig. 1, A and B, and Fig. 3).

L-NNA Increased Mean Arterial Pressure in Control and STZ Rats

Two periods of L-NNA infusion were studied: L-NNA with increased renal perfusion pressure (L-NNA), and when renal perfusion pressure was controlled normotensive (90–110 mmHg) by an aortic clamp above the renal artery (L-NNA + AC).

L-NNA with Increased Renal Perfusion Pressure

In the L-NNA-infused rats with increased renal perfusion pressure, the dose-response curve of control rats during vehicle infusion was shifted to the left by a factor of 3 ($ED_{50}$ from 5.5 to 1.73 nmol adenosine; Fig. 1A). Also in STZ rats, L-NNA shifted the dose-response curve of vehicle infusion to the left by a factor of 3 ($ED_{50}$ from 0.3 to 0.09 nmol adenosine; Fig. 1B).

L-NNA with Controlled Renal Perfusion Pressure

In control rats, L-NNA + AC increased the adenosine-induced renal vasoconstriction to a greater extent than with L-NNA alone. Compared with vehicle infusion, L-NNA + AC shifted the dose-response curve of control rats to the left by a factor of 32 ($ED_{50}$ from 5.5 to 0.17 nmol adenosine). However, L-NNA + AC did not significantly change the renal response to exogenous adenosine in STZ rats ($ED_{50}$ = 0.07 nmol adenosine) when compared with L-NNA with increased renal perfusion pressure ($ED_{50}$ = 0.09 nmol adenosine). The dose-response curve of STZ rats during vehicle infusion was shifted to the left with L-NNA + AC by a factor of 4.6 ($ED_{50}$ from 0.32 to 0.07 nmol adenosine). The left shift of the dose-response curves from vehicle infusion to L-NNA + AC was significantly higher ($P < 0.001$) in control (factor of 32) rats compared with STZ rats (factor of 4.6). The $ED_{50}$ of the dose-response curve of L-NNA + AC in control rats (0.17 nmol) was significantly different ($P < 0.001$) from the $ED_{50}$ of dose-response curve of L-NNA + AC in STZ rats (0.07 nmol). The Hill coefficients of all dose-response curves were not significantly different from 1.

Endogenous Adenosine

The renal vascular response to endogenous adenosine, assessed by POR, was significantly enhanced in STZ rats ($Δ = 67.8 \pm 3.8\%$) compared with control rats ($Δ = 38.8 \pm 4.3\%$, unpaired $P < 0.001$; Tables 1 and 2, and Figs. 2 and 3). In control rats, L-NNA with increased (L-NNA) and with controlled renal perfusion pressure (L-NNA + AC) increased the POR by $+55.9 \pm 4.7\%$ ($P < 0.01$) and $+60.1 \pm 5.1\%$ ($P < 0.001$), respectively (Fig. 3; Table 1), whereas in STZ rats L-NNA did not significantly increase the POR (Fig. 3; Table 2). The L-NNA-induced potentiation of POR was significantly different ($P < 0.01$) between the two groups, control and STZ rats (Tables 1 and 2).

Table 3. Summary of data characterizing the nondiabetic control and STZ-diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Nondiabetic</th>
<th>STZ Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Age, wk</td>
<td>12 ± 1</td>
<td>14.5 ± 1*</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>321.2 ± 7.1</td>
<td>356.8 ± 12.3*</td>
</tr>
<tr>
<td>Kidney wt, g</td>
<td>1.93 ± 0.2</td>
<td>2.45 ± 0.2*</td>
</tr>
<tr>
<td>Kidney wt, g/100 g body wt</td>
<td>0.598 ± 0.02</td>
<td>0.651 ± 0.1</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>46.8 ± 0.5</td>
<td>47.5 ± 0.6</td>
</tr>
<tr>
<td>Blood glucose, mg/dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 days after STZ injection</td>
<td>94.6 ± 5.1</td>
<td>318.7 ± 12.3†</td>
</tr>
<tr>
<td>Day of experiment</td>
<td>98.5 ± 4.8</td>
<td>356.1 ± 26.7†</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05. †P < 0.01, comparison between nondiabetic control and STZ-diabetic animals.
The present findings show that the adenosine-induced renal vasoconstriction was markedly enhanced in STZ rats compared with control rats. Adenosine, 1 nmol, injected into the abdominal aorta caused markedly greater reduction of RBF in STZ rats compared with control rats. Furthermore, after a 30-s renal artery occlusion, the effect of endogenously released adenosine caused a markedly greater reduction of RBF in STZ rats compared with control rats, as demonstrated by the enhanced POR in STZ rats. During renal ischemia, e.g., renal artery occlusion, adenosine generation is increased by hydrolysis of ATP to adenosine throughout the kidney (30, 31). The accumulated adenosine stimulates A1 receptors on the afferent arteriole and causes renal vasoconstriction as manifested in the POR (30–32). The diabetic kidney has been shown to have a much higher susceptibility to ischemic periods, e.g., renal artery occlusion, which eventually lead to diabetic nephropathy (27, 32). Since the POR is an adenosine A1 receptor-mediated phenomenon in the diabetic renal vasculature (32), adenosine A1 receptor stimulation of the afferent arteriole may be an important factor that is responsible for the increased sensitivity of the diabetic kidney to adenosine. An increased adenosine A1 receptor density could potentially contribute to the increased sensitivity of the diabetic renal vasculature to adenosine. Adenosine A1 receptor density was found to be slightly elevated in glomeruli of STZ rats (1). However, this almost 2-fold increase in receptor density was not significant, and probably does not account for the 20- to 30-fold increased sensitivity of the diabetic vasculature to adenosine. Thus other factors including diminished NO-dependent vasodilator capacity of the diabetic renal vasculature to counteract the renal vasoconstrictor action of adenosine may account for the present findings. We therefore investigated the RBF response to adenosine in the presence of NO synthesis inhibition with increased and normal renal perfusion pressure in STZ diabetic rats.

Attenuated Effect of NO Inhibition on Adenosine-Induced Renal Vasoconstriction in STZ-Diabetic Rats

In the present studies, the inhibition of NO synthesis markedly potentiated the adenosine-induced renal vasoconstriction in control rats but not in STZ diabetic rats. L-NNA resulted in increased systemic blood pressure, increased renal perfusion pressure, and decreased RBF. During inhibition of NO synthesis, RBF decreases due to intrarenal vasoconstriction and increased renal vascular resistance (2, 10, 24, 39). The increased renal perfusion pressure influenced the renal vascular response to adenosine during inhibition of NO synthesis in control rats but not in STZ rats. L-NNA in the presence of controlled renal perfusion pressure caused a much greater potentiation of the adenosine-induced renal vasoconstriction (left shift of dose-response curve by a factor of 32) than L-NNA in the presence of increased perfusion pressure (left shift of dose-response curve by a factor of 3) in control rats. In STZ rats, however, adenosine caused the same degree of renal vasoconstriction in the presence of L-NNA both with and without increased renal perfusion pressure. The adenosine-induced renal vasoconstriction was increased with L-NNA and controlled renal perfusion pressure to a much greater extent in control rats than in STZ rats. Furthermore, 0.3 nmol adenosine injected into the abdominal aorta caused a similar reduction of RBF in control rats during NO synthesis inhibition.
with controlled renal perfusion pressure as in STZ rats without NO synthesis inhibition.

Taken together, inhibition of NO synthesis increased the adenosine-induced renal vasoconstriction markedly in control rats but not in STZ rats. The adenosine-induced renal vasoconstriction is primarily a response due to A₁ receptor stimulation of the afferent arterioles, whereas inhibition of NO synthesis causes renal vasoconstriction throughout the kidney in cortical and medullary capillaries (24). Hence, the observation that NO synthesis inhibition caused a similar reduction of total RBF in both groups may be due to renal vasconstriction of the postglomerular renal vasculature. In contrast, the NO-dependent vasodilatation of afferent arterioles in STZ rats is attenuated since NO synthesis inhibition did not increase the adenosine-induced renal vasoconstriction in STZ rats compared with control rats. Therefore, a diminished or defective NO-dependent renal vasodilatation of the afferent arterioles in STZ-diabetic rats could account for 1) the increased sensitivity of the diabetic renal vasculature to the vasoconstrictor potency of adenosine and 2) the attenuated effects of NO inhibition on the adenosine-induced renal vasoconstriction.

Dysfunction of NO-Dependent Vasodilation in Afferent Arterioles of Diabetic Renal Vasculature

Although numerous studies report increased NO generation in the urine and plasma of diabetic rats indicating an increased NO generation in diabetes (2, 39), the NO-dependent vasodilatation of the extrarenal vasculature is diminished in animals with diabetes mellitus (10, 13, 33, 38). This apparent paradox might be resolved by considering an uncoupling of NO production from cGMP-dependent vasodilatation in diabetes mellitus. Therefore, increased NO generation may not correspond with cGMP-coupled renal vasodilatation. Numerous factors may be responsible for the dysfunction of the NO-dependent renal vasodilatation in diabetes mellitus. These factors include receptor and postreceptor dysfunction, decrease in transport of NO, increased destruction of NO, decreased responsiveness to NO, increased production of NO antagonists including free radicals, and quenching of NO by advanced glycosylation end products (10, 34). Advanced glycosylation end products inactivate NO (4, 17), and suppressed superoxide dismutase activity reduces the tonic influence of NO on renal arterioles during the early stage of diabetes mellitus (28). Furthermore, vasoconstrictor mediators, such as endothelin-1, are found at increased levels in diabetes mellitus (14). These vasoconstrictor mediators may inhibit NO generation (19) in diabetes and thus decrease the vasodilator capacity of the diabetic vasculature.

In agreement with our findings, Dai et al. (8) found an impaired vasodilatation in response to acetylcholine in renal arteries of diabetic rats which increased with the duration of the diabetic state at 6 and 16 wk following STZ injections. In another study (21), the administration of the NO donor glyceryl trinitrate did not increase renal plasma flow in STZ rats compared with the control rats. In this study, STZ rats had a markedly increased urinary NO₂/NO₃ excretion compared with the nondiabetic rats despite the reduced responsiveness to the NO donor. Furthermore, a reduced capacity to generate NO-dependent cGMP has been found in glomeruli of STZ rats (7, 9, 40). In addition, functional defects of the afferent arterioles (6), a diminished myogenic responsiveness (16), and insulin deficiency (6, 18, 20, 36) may attenuate the responsiveness of the diabetic renal vasculature to NO.

In summary, the present findings show that the diabetic renal vasculature has an increased sensitivity to the adenosine-induced renal vasoconstriction. The adenosine-induced renal vasoconstriction is increased by inhibition of NO synthesis in control rats but to a much lesser extent in diabetic STZ rats. The observations demonstrate that the afferent arterioles in the diabetic renal vasculature have a diminished NO-dependent vasodilatation to counteract adenosine-induced renal vasoconstriction.

The present observations are of clinical relevance since adenosine has been proposed as a pathophysiological factor in the development of acute renal failure induced by contrast media (3). Contrast media-induced acute renal failure has a higher incidence in diabetic patients with impaired renal function (35, 41). In these situations, renal adenosine is thought to be released during renal ischemic conditions with subsequent effects on the vascular and tubular system of the kidney. Therefore, in diabetes mellitus, a defective NO-dependent renal vasodilatation of the afferent arterioles may contribute to the adenosine-mediated vasoconstriction leading to contrast media-induced renal failure. In these situations, adenosine receptor antagonists may decrease adenosine-induced renal vasoconstriction and improve the outcome (12).

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Address for reprint requests: F. G. Knox, Dept. of Medicine and Physiology and Biophysics, Guggenheim 9, Mayo Clinic, Rochester, MN 55905 (E-mail: Pflueger.Axel@mayo.edu).

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