Effect of renal interstitial adenosine infusion on phosphate excretion in diabetes mellitus rats

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Pflueger, Axel C., Theresa J. Berndt, and Franklyn G. Knox. Effect of renal interstitial adenosine infusion on phosphate excretion in diabetes mellitus rats. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R1228–R1235, 1998.—We previously demonstrated an increased sensitivity of the renal vasculature to adenosine (ADO) mediated via ADO A1 receptors in streptozotocin (STZ) diabetic rats. Because ADO stimulates Pi reabsorption in the proximal tubule, the present study was performed to determine whether the sensitivity of the renal tubular system to the antiphosphaturic effect of ADO is enhanced in STZ rats. Clearance studies were performed, and ADO was infused into the renal interstitium via implanted matrices in STZ- and control (Con) rats to mimic the effects of endogenous ADO. Renal phosphate excretion was significantly increased in STZ rats (0.75 ± 0.05 µmol/24 h) compared with Con rats (0.35 ± 0.08 µmol/24 h), and fractional phosphate excretion (FEPi) tended to be higher in STZ rats (34.8 ± 4.1%) than in Con rats (26.7 ± 2.2%). Renal interstitial ADO infusion (5 µmol/h) was significantly more antiphosphaturic in STZ rats (FEPi decreased by 6.9 ± 1.36%; P < 0.05) than in Con rats (FEPi decreased by 2.9 ± 1.6%; P > 0.05), in which ADO only tended to decrease FEPi. To determine the role of ADO A1 receptors on Pi excretion, the selective ADO A1 receptor blocker 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) was infused into the renal interstitium. DPCPX increased FEPi by 4.3 ± 1.2% (P < 0.05) in the presence and 7.1 ± 3.9% (P < 0.05) in the absence of ADO infusion in Con rats but had no effect on FEPi in STZ rats. In conclusion, STZ-diabetes mellitus enhances the antiphosphaturic effect of ADO by mechanisms unrelated to ADO A1 receptor stimulation.

Renal hemodynamics; experimental insulin-dependent diabetes mellitus; adenosine A1 receptor blockade; phosphate reabsorption; phosphaturia

Adenosine (ADO) generated in the kidney and other tissues by ATP metabolism modulates a variety of renal functions, including renal blood flow, renin secretion, glomerular filtration rate (GFR), erythropoietin release, and sodium and phosphate excretions. The effects on tubule transport are mediated by both renal hemodynamic and direct tubular mechanisms. ADO has been reported to modulate tubular phosphate transport via ADO receptor-related and -unrelated mechanisms (21). The primary ADO receptor types identified in the kidney are ADO A1 and ADO A2 receptors. ADO A1 receptor activation inhibits adenylyl cyclase activity and cAMP accumulation via G-coupled protein, and ADO A2 receptor activation stimulates adenylyl cyclase and increases cAMP accumulation (30). ADO A1 and A2 receptors are found throughout the nephron (9, 19, 28, 38, 43). Recent data suggest that endogenous ADO stimulates sodium phosphate cotransport in proximal tubule cells in animals (10, 12) and humans (5) primarily via ADO A1 receptor stimulation, which reduces cAMP-induced inhibition of sodium-coupled phosphate transport. Phosphate handling is altered in diabetes mellitus (14), and in a previous study we found an increased sensitivity of the renal vasculature to ADO in streptozotocin (STZ)-induced diabetic rats (35). The observed ADO effects on the renal vasculature of diabetic rats in that study were mediated via ADO A1 receptors because they could be reversed by the infusion of the highly selective ADO A1 receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX).

Because the renal vasculature of the diabetic rat has an increased sensitivity to exogenous and endogenous ADO, we determined whether the tubular sensitivity to ADO on phosphate transport is enhanced in diabetic kidneys. Therefore, we determined whether interstitial ADO decreases urinary excretion of phosphate and cAMP and whether ADO A1 receptors are involved in phosphate reabsorption in STZ-diabetic and control (Con) rats. To mimic endogenous ADO metabolism, ADO was infused into the renal interstitium via implanted matrices in rats. To determine the role of ADO A1 receptors in tubular phosphate transport in STZ rats, the selective ADO A1 receptor antagonist DPCPX was also infused into the renal interstitium in these experiments. Previous studies have used implanted polyethylene matrices to infuse ADO into the renal interstitial space (34) and renal interstitial concentrations have been estimated by microdialysis (6). It has been shown that ADO infusion into the interstitium produces distribution of ADO throughout the renal interstitium without systemic effects (34).

Material and Methods

All animals used in this study were male Sprague-Dawley rats purchased from Harlan Sprague Dawley (Indianapolis, IN). Rats had free access to a regular rat pellet diet (0.28% NaCl) and tap water. On the day of the matrix implantation, the range of body weights was 225 to 250 g. Experiments were performed on age-matched animals 2–3 wk after the matrix implantation in Con and STZ rats. STZ was injected 1 wk after the matrix implantation in the STZ groups.

Matrix Implantation

The model of renal interstitial infusion via matrices was used to prevent potential systemic effects and endocrine influences of the intrarenally administered substances. The implanted matrix was used for renal interstitial infusion of a 0.9% normal saline vehicle, ADO, and the selective ADO A1 receptor antagonist DPCPX (Research Biochemicals International, Natick, MA). Matrices were made from polyethylene tubing (PE-50) and polyethylene material with 70-µm pores (Bel-Art Product, Pequannock, N J ) and implanted in the left
kidney as previously described (34). Male Sprague-Dawley rats (225–250 g) were anesthetized with an intramuscular injection of 1 ml/kg body wt of a solution with equal volumes of 20 mg/ml xylazine (Miles Laboratories, Shawnee, KS) and 100 mg/ml ketamine hydrochloride (Parke-Davis, Morris Plains, N.J.). The left kidney was exposed with a medial abdominal incision. A conical pipette tip was inserted to a depth of 7 mm into the ventral side of the kidney at the corticomedullary level. The pipette was removed, and the matrix was placed into the kidney and flushed with heparinized (10 IU in 1 ml) normal saline. A mesh (Mersilene RM-53, Ethicon) was placed over the matrix insertion to improve sealing, and the open end of the matrix tubing was ligated.

After irrigation of the abdominal cavity with normal saline, the muscular fascia was closed with interrupted 3–0-coated Vicryl stitches. The skin was closed with a running 4–0 silk suture, and 100,000 units of penicillin G procaine (Streptacin; Phoenix Pharmaceutical, St. Joseph, MO) were injected intramuscularly. In the acute experiments, the renal interstitial infusion rate was 500 µl/h, because renal interstitial infusions of normal saline (0.9% NaCl) at a rate of 600 µl/h have been shown not to increase renal interstitial hydrostatic pressure (26, 34).

Experimental Insulin-Dependent Diabetes Mellitus

The animal model of insulin-dependent diabetes mellitus was achieved by an intraperitoneal injection of 60 mg/kg STZ (Sigma) dissolved in sodium citrate buffer (pH 4.2). Three days after STZ injection, blood glucose levels were measured in tail blood samples. Animals with a blood glucose below 200 mg/dl were not included in the experimental series. The experiments started 2–3 wk after STZ administration without insulin treatment, and nondiabetic age-matched rats served as controls.

Animal Preparation

Age-matched Con rats were anesthetized with 100 mg/kg ip Inactin (thiobutabarbitol, Byk-Gulden), and STZ rats were anesthetized with 80 mg/kg ip Inactin. The animals were placed on a heated table, and body temperature was measured via a rectal thermometer and maintained at 37°C. After tracheostomy, spontaneous respiration was supported through a ventilated (1–2/min oxygen) endotracheal tube. Polyethylene catheters (PE-50) were placed into the right jugular vein for intravenous infusion and into the left carotid artery for continuous blood pressure tracing and blood collection for clearance studies. The heart rate was derived from the blood pressure tracing. Intravenous infusion of 2 ml·100 g body wt⁻¹·h⁻¹ of a solution of 2.5% inulin and 5% albumin in 0.9% normal saline was given for 1 h and then continued without albumin. In previous studies (32), it was shown that an infusion of fluid at 2% body wt/h did not affect phosphate excretion. The left kidney was exposed by an abdominal midline incision, and the matrix tubing was flushed with heparinized saline. Then, 0.9% normal saline was directly infused (500 µl/h) into the renal interstitium via the implanted matrix throughout the equilibration period and the first clearance period. The tubing leading from the implanted matrix was connected to a syringe for renal interstitial infusion, and nondiabetic age-matched rats served as controls.

Clearance Studies

In all clearance periods, renal interstitial infusion started with a bolus infusion (100 µl over 5–8 min) followed by a continuous infusion (500 µl/h). Vehicle was infused into renal matrix in the first clearance period, followed by the second clearance period with ADO infusion (5 µmol/h), and the third clearance period with the combined administration of ADO and the highly selective ADOₐ₁ receptor antagonist DPCPX (29), as a 10 µg/kg DPCPX bolus and 5 µmol/h ADO continuous infusion. In preliminary experiments, the ADO dose of 5 µmol/h (80 nmol/min) was determined to have no effects on GFR or renal blood flow, which is consistent with the findings of Pawlowska et al. (34). The distribution of the fluid infused via the matrix throughout the renal interstitium was confirmed by renal interstitial infusion of lissamine green dye at the end of the experiment. Because DPCPX was shown to inhibit ADO-induced renal vascular effects in a dose of 100 µg/kg iv given systemically (35), DPCPX infusion via the matrix was administered in an equivalent dose of 10 µg/kg.

Experimental Protocol

Two to three weeks after the matrix implantation, the experiments were performed in Con and STZ rats. After animal preparation, animals were allowed to stabilize for 60 min. Three clearance periods (30 min each) were collected in each experiment. A control vehicle clearance period was taken during renal interstitial infusion of normal saline (8 µl/min).

Then, ADO (Sigma) was infused (as a bolus of 25 µmol in 100 µl over 5–8 min and as a continuous infusion of 5 µmol/h at 500 µl/h) into the renal interstitium in groups 3 and 4, and a second clearance was taken. Alternatively, vehicle was infused (as a bolus in 10 µl over 5–8 min and as a continuous infusion at 500 µl/h) into the renal interstitium in groups 5 and 6, respectively. In clearance period 3, DPCPX (dissolved in 10% EtOH normal saline with pH 8–9) was infused (as a bolus of 10 µg/kg in 100 µl over 5 min), followed by a continuous infusion of vehicle at 500 µl/h in groups 5 and 6 and by a continuous ADO infusion of 5 µmol/h at 500 µl/h in groups 3 and 4. Urine samples were collected from both kidneys and analyzed for potassium, sodium, phosphate, and CAMP. After each clearance period, 0.5 ml blood was withdrawn from the left carotid artery for analysis of plasma electrolytes and insulin. At the end of the experiments, animals were killed by an overdose of Inactin.

Group 1: Metabolic balance studies of excretions of sodium, potassium, and phosphate in Con rats. Con rats (n = 6) were housed singly in metabolic cages for 48 h. Animals had free access to a regular pelleted diet and drinking water, and urine was collected after 24 h to measure urine volume, sodium, and phosphate excretions. During the first 24 h, the rats were allowed to stabilize, and urine samples were collected on the second day. Blood glucose was obtained from tail blood samples before the experiment.

Group 2: Metabolic balance studies of excretions of sodium, potassium, and phosphate in STZ rats. STZ rats (n = 6) were housed singly in metabolic cages for 48 h 2–3 wk after STZ injection. Animals had free access to a regular pelleted diet and drinking water. Urine was collected after 24 h to measure urine volume and sodium and phosphate excretions. During the first 24 h the rats were allowed to stabilize, and urine was collected on the second day. Blood glucose was obtained from tail blood samples before the experiment.

Group 3: Effect of renal interstitial ADO infusion and renal interstitial ADOₐ₁ receptor blockade (DPCPX) on renal function in Con rats. One hour after initiation of the intravenous inulin infusion and renal interstitial infusion of vehicle,
the first 30-min control clearance was taken, and urine samples from the left and right kidney were collected. During the second clearance period ADO was infused (bolus and continuous infusion, see above) into the renal interstitium, and the clearance period started 5 min after the ADO bolus injection. The protocol of the third clearance period was the same as the second; however, in group 3 (n = 6) DPCPX was added as a bolus infusion over 5 min.

Group 4: Effect of renal interstitial ADO infusion and renal interstitial ADO A1 receptor blockade (DPCPX) on renal function in STZ rats. The experimental protocol was the same as in group 3, but in STZ rats (n = 6).

Group 5: Effect of renal interstitial normal saline infusion and renal interstitial ADO A1 receptor blockade (DPCPX) on renal function in Con rats. The experimental protocol was the same as in group 3; however, in group 5 (n = 5), 0.9% NaCl was infused instead of ADO as a vehicle control.

Group 6: Effect of renal interstitial normal saline infusion and renal interstitial ADO A1 receptor blockade (DPCPX) on renal function in STZ rats. The experimental protocol was the same as in group 5, but in STZ rats (n = 5).

Group 7: Time vehicle experiments: effect of renal interstitial normal saline infusion and time on renal function in Con rats. The experimental protocol was the same as in group 5; however, in group 7 (n = 4), vehicle was infused throughout all three clearance periods.

Group 8: Time vehicle experiments: effect of renal interstitial normal saline infusion and time on renal function in STZ rats. The experimental protocol was the same as in group 7 but in STZ rats (n = 4).

Analytic Methods

Blood glucose levels were measured with a blood glucose meter (OneTouch, Lifescan) 3 days after STZ injection and on the day of the experiment after the equilibration period. GFR was calculated based on the clearance of inulin. Inulin and phosphate concentrations in plasma and urine were measured by the Anthrone (22) and Chen (11) methods, respectively. Sodium and potassium concentrations in plasma and urine were measured by using a flame photometer (IL943 Flame Photometer, Instrumentation Laboratory). Urinary cAMP was measured using a radioimmunoassay kit (18), (Rianen Assay System, DuPont). An ink printer (15–6327–57 Gould Instruments) was used for continuous arterial blood pressure recording via a Statham transducer.

Statistical Analysis

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

RESULTS

In Con rats of group 1 (body wt: 364 ± 5.2 g), the urine volume was 14 ± 1.5 ml/24 h, urinary phosphate excretion was 0.35 ± 0.08 μmol/24 h, and urinary sodium excretion was 1.73 ± 0.05 μmol/24 h. The blood glucose levels were 71 ± 3.2 mg/dl. In STZ rats of group 2 (body wt: 318 ± 6.4 g), the urine volume (72.3 ± 9.1 ml/24 h) was significantly higher than in Con rats (P < 0.001). Urinary phosphate excretion in this group (0.75 ± 0.05 μmol/24 h) was significantly higher compared with Con rats (P < 0.001), as was urinary sodium excretion (3.73 ± 0.08 μmol/24 h) compared with Con rats (P < 0.001). The blood glucose levels were 298 ± 12 mg/dl.

In Con rats of group 3 (body wt: 376 ± 3.4 g; left kidney wt: 2.3 ± 0.07 g) renal interstitial ADO infusion tended to decrease fractional phosphate excretion (FEPi) of the left kidney (by 2.90 ± 1.6%, P > 0.05) when calculated from the individual values and decreased urinary cAMP by 21% (from 61.2 ± 4 to 48.3 ± 4.1 pmol·ml⁻¹·min⁻¹; P < 0.05). ADO had no effect on fractional excretion of sodium (FENa) [change (Δ) = +0.44 ± 0.53%, P > 0.05], whereas the combined infusion of ADO and DPCPX increased FEPi (Δ = +4.1 ± 1.1%, P < 0.05) and FENa (Δ = +1.7 ± 0.7, P < 0.05) and tended to increase urinary cAMP by 21% (from 48.3 ± 4.1 to 58.6 ± 5.9 pmol·ml⁻¹·min⁻¹; P > 0.05) in comparison to the intrarenal infusion of ADO alone (Fig. 1 and Table 1). The hematocrits did not change during all three clearance periods (46 ± 0.5 vs. 46.3 ± 0.3 vs. 46.2 ± 0.3). The blood glucose was 66 ± 1.8 mg/dl and stable throughout the experiment.

In STZ rats of group 4 (body wt: 326 ± 3.7 g; left kidney wt: 2.4 ± 0.05 g), GFR was not significantly different compared with Con rats. The hyperfiltration state reported by others in the STZ-induced diabetic rat model involves several factors, including insulin treatment, volume of infusion rate, and the amount of time after STZ injection. In STZ rats without insulin treatment and 2 wk after STZ injection (25, 44), hyperfiltration did not occur and GFRs were lower in STZ rats without insulin treatment and severe hyperglycemia compared with Con rats (25). In the current study, animals were studied 2–3 wk after STZ injection and did not receive insulin treatment and therefore these factors may account for the lack of hyperfiltration. In STZ rats of group 4, baseline FEPi (34.8 ± 4.1%) tended to be higher (P = 0.05) compared with Con rats of group 3 (FEPi = 26.7 ± 2.2%) and was significantly higher (P < 0.05) in the diabetic rats of group 6 (FEPi = 39.8 ± 2.7%) when compared with the Con rats of group 5 (FEPi = 28.3 ± 3.4%). In STZ rats of group 4, renal interstitial...
Table 1. Renal response to renal interstitial infusion of ADO and the ADO A₁ receptor antagonist DPCPX in control and diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>UV, µl/min</th>
<th>GFR, ml/min</th>
<th>Pₐ, mM</th>
<th>FEPᵢ, %</th>
<th>FENₐ, %</th>
<th>cAMP, pmol·ml⁻¹·min⁻¹</th>
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</thead>
<tbody>
<tr>
<td>Con Vehicle</td>
<td>128 ± 4.6</td>
<td>323 ± 9</td>
<td>60.7 ± 8.4</td>
<td>1.2 ± 0.1</td>
<td>2.8 ± 0.1</td>
<td>26.7 ± 2.2</td>
<td>3.9 ± 0.5</td>
<td>61.2 ± 4.0</td>
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<td>ADO</td>
<td>129 ± 6.3</td>
<td>343 ± 7</td>
<td>69.5 ± 9.6</td>
<td>1.1 ± 0.4</td>
<td>2.8 ± 0.1</td>
<td>23.8 ± 1.5</td>
<td>4.6 ± 0.2</td>
<td>48.3 ± 4.1⁷</td>
</tr>
<tr>
<td>ADO + DPCPX</td>
<td>128 ± 4.8</td>
<td>375 ± 10</td>
<td>69.7 ± 8.5</td>
<td>1.3 ± 0.1</td>
<td>2.4 ± 0.1‡</td>
<td>30.8 ± 2.0</td>
<td>5.6 ± 0.5</td>
<td>58.6 ± 5.9</td>
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<tr>
<td>STZ Vehicle</td>
<td>123 ± 4.6</td>
<td>336 ± 10</td>
<td>43.2 ± 10.9</td>
<td>1.0 ± 0.1</td>
<td>2.2 ± 0.1‡</td>
<td>34.8 ± 4.1</td>
<td>4.0 ± 1.4</td>
<td>124.6 ± 12I⁷</td>
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<tr>
<td>ADO</td>
<td>123 ± 3.3</td>
<td>333 ± 9</td>
<td>49.7 ± 7.9</td>
<td>0.9 ± 0.1</td>
<td>2.3 ± 0.1‡</td>
<td>28.1 ± 3.7</td>
<td>4.9 ± 0.9</td>
<td>97.3 ± 11I⁷</td>
</tr>
<tr>
<td>ADO + DPCPX</td>
<td>125 ± 4.0</td>
<td>349 ± 13</td>
<td>51.0 ± 8.9</td>
<td>0.9 ± 0.1</td>
<td>2.1 ± 0.1†</td>
<td>26.6 ± 4.1</td>
<td>5.9 ± 1.1</td>
<td>107.2 ± 11I⁷</td>
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</table>

Values are means ± SE of nondiabetic control rats (Con, group 3, n = 6) with vehicle, adenosine (ADO), and ADO and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and streptozotocin (STZ)-induced diabetic rats (group 4, n = 6) with vehicle, ADO, and ADO and DPCPX. MAP, mean arterial pressure; HR, heart rate; UV, urine volume; GFR, glomerular filtration rate; Pₐ, plasma phosphate concentration; FEPᵢ, fractional phosphate excretion; FENₐ, fractional sodium excretion. Comparisons within each group with vehicle are expressed as *P < 0.05, †P < 0.01, and ‡P < 0.001. Comparisons between the 2 groups, Con and STZ, are expressed as *P < 0.05, †P < 0.01, and ‡P < 0.001.

In time vehicle controls with renal interstitial infusion of DPCPX to block ADO A₁ receptors significantly decreased FE Pᵢ, of the left kidney (by 6.95 ± 1.4%, P < 0.05) and urinary cAMP by 22% (from 124.6 ± 12 to 97.3 ± 11 pmol·ml⁻¹·min⁻¹; P < 0.05) but did not change FE Nₐ (Δ = 1.6 ± 0.3, P > 0.05). The ADO-induced decrease in FE Pᵢ, in STZ rats (−6.95 ± 1.4%) was significantly greater (P < 0.05) compared with the ADO-induced decrease of FE Pᵢ, in Con rats (−2.90 ± 1.6%) when calculated from the individual values of each group. The percent changes of FE Pᵢ, of the mean values by ADO in STZ rats and Con rats, −19.3% (from 34.8 ± 4.2 to 28.2 ± 2.7) and −10.9% (from 26.7 ± 2.2 to 23.8 ± 1.5), respectively, were significantly different (P < 0.05). The combined administration of ADO and DPCPX in STZ rats did not increase but rather decreased FE Pᵢ, (−8.3 ± 2.2%, P < 0.05) compared with vehicle conditions of the same group, and significantly increased FE Nₐ (by 2.2 ± 0.9%, P < 0.05; see Table 1). The hematocrits did not change during all three clearance periods (46.8 ± 0.8 vs. 46.4 ± 0.9 vs. 46.2 ± 0.8). The blood glucose was 263 ± 4.4 mg/dl and stable throughout the experiment. In STZ rats of group 8 (body wt: 322.8 ± 16.2 g; left kidney wt: 2.2 ± 0.1 g; right kidney wt: 2.2 ± 0.2 g), renal interstitial infusion of vehicle and time did not change renal function with respect to GFR, urine volume, FE Pᵢ, and FE Nₐ (see Tables 3 and 4). The hematocrits did not change during all three clearance periods (45.5 ± 0.2 vs. 46.3 ± 0.3 vs. 45.3 ± 0.3). The blood glucose was 80.4 ± 4.4 mg/dl and stable throughout the experiment. In time vehicle STZ rats of group 4 (body wt: 322.8 ± 16.2 g; left kidney wt: 2.7 ± 1.2 g; right kidney wt: 2.7 ± 1.2 g), renal interstitial infusion of vehicle and time did not change renal function with respect to GFR, urine volume, FE Pᵢ, and FE Nₐ (see Tables 3 and 4). The hematocrits did not change during all three clearance periods (46.0 ± 0.2 vs. 45.8 ± 0.2 vs. 45.6 ± 0.3). The blood glucose was 373 ± 5.9 mg/dl and was stable throughout the experiment.

In the contralateral (right) kidneys of Con rats, GFRs tended to be higher than the GFRs of the right kidneys of STZ rats (see Tables 5 and 6). Baseline FE Pᵢ, of the right kidney tended to be higher in STZ rats of group 4 and was significantly higher in STZ rats of group 6. In Con rats, interstitial ADO infusion of the left kidney by 0.1 g, renal interstitial infusion of vehicle and time did not change renal function with respect to GFR, urine volume, FE Pᵢ, and FE Nₐ (see Tables 3 and 4). The hematocrits did not change during all three clearance periods (45.5 ± 0.2 vs. 46.3 ± 0.3 vs. 45.3 ± 0.3). The blood glucose was 80.4 ± 4.4 mg/dl and stable throughout the experiment.
had no effects on phosphate excretion of the right kidney. The coadministration of ADO and the ADO A₁ receptor antagonist DPCPX into the renal interstitium of the left kidney tended to increase phosphate excretion, and when DPCPX was given alone, it significantly increased phosphate excretion of the contralateral kidney (from 24.7 ± 4.5 to 34.5 ± 3.1%, P < 0.01). In STZ rats, ADO infusion into the renal interstitium of the left kidney tended to decrease phosphate excretion of the contralateral (right) kidney (from 31.0 ± 4.1 to 25.0 ± 3.8%, P > 0.05), whereas infusion of the ADO A₁ receptor antagonist DPCPX into the left renal interstitium did not change phosphate excretion of the contralateral kidney in STZ rats.

**DISCUSSION**

AD O and Renal Phosphate Excretion in Con Rats

In the present study we demonstrated that the renal interstitial infusion of an ADO A₁ receptor antagonist (DPCPX) increases phosphate and cAMP excretions in control rats. Conversely, the renal interstitial infusion of ADO tended to decrease phosphate excretion and also concomitantly decreased cAMP excretion. These observations are consistent with the hypothesis that in normal conditions, stimulation of ADO A₁ receptors inhibits cAMP generation and increases phosphate transport by proximal tubule cells. Cai et al. (10) reported that ADO A₁ receptor blockade inhibits phosphate transport in rat renal proximal tubule cells and increases cAMP production. Similar results were found on opossum kidney cells in which ADO A₁ receptor blockade inhibited sodium-phosphate cotransport (13). Conversely, the ADO analog [R]-(-)N⁶-phenylisopropyl-adenosine significantly decreased sodium-phosphate cotransport (13).

In STZ rats, ADO infusion significantly decreased phosphate transport and stimulated the phosphate uptake in opossum kidney epithelial cells (12). In addition, in humans the administration of the selective ADO A₁ receptor antagonist FK-453 significantly enhanced urinary phosphate excretion without altering GFR (5). Furthermore, dipyridamole, a well-known inhibitor of ADO uptake in many cell types including renal tubular cells (17) was reported to abolish the phosphaturia in humans with idiopathic phosphate leak (31). Thus endogenous ADO stimulates phosphate reabsorption, at least in part, by cAMP inhibition via activation of ADO A₁ receptors.

AD O and Phosphate Excretion in STZ-Diabetic Rats

In STZ rats, ADO infusion significantly decreased phosphate and cAMP excretions. This ADO-induced decrease of phosphate excretion was significantly greater in STZ rats compared with Con rats, whereas the ADO-induced decrease in cAMP excretion was similar in both groups. Thus the proximal tubule of STZ rats exhibits a higher sensitivity to ADO-induced changes on phosphate transport than nondiabetic Con animals. It is important to note that in diabetic rats the ADO-induced decrease in phosphate excretion was not reversed by the ADO A₁ receptor antagonist (DPCPX) either in the presence or absence of ADO infusion. Interestingly, the renal response of the contralateral

Table 2. Renal response to renal interstitial infusion of vehicle and the ADO A₁ receptor antagonist DPCPX in control and diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>UV, µl/min</th>
<th>GFR, ml/min</th>
<th>P₀, mM</th>
<th>FE₀, %</th>
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<td>Vehicle</td>
<td>130 ± 5.5</td>
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<td>57.4 ± 3.1</td>
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<td>28.3 ± 3.4</td>
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<tr>
<td>Vehicle</td>
<td>130 ± 5.7</td>
<td>352 ± 6</td>
<td>55.0 ± 5.1</td>
<td>1.3 ± 0.2</td>
<td>2.9 ± 0.2</td>
<td>28.7 ± 3.2</td>
<td>3.4 ± 0.5</td>
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<td>DPCPX</td>
<td>127 ± 6.0</td>
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<td>67.8 ± 13.8</td>
<td>1.5 ± 0.2</td>
<td>2.5 ± 0.1</td>
<td>35.4 ± 4.4a</td>
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<td>STZ</td>
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<td>Vehicle</td>
<td>119 ± 2.5</td>
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<td>46.8 ± 12.4</td>
<td>0.9 ± 0.1</td>
<td>2.5 ± 0.1*</td>
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<td>Vehicle</td>
<td>125 ± 3.9</td>
<td>330 ± 10</td>
<td>45.2 ± 13.3</td>
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<tr>
<td>DPCPX</td>
<td>122 ± 3.4</td>
<td>349 ± 6**</td>
<td>57.2 ± 14.6</td>
<td>0.9 ± 0.1*</td>
<td>2.4 ± 0.1</td>
<td>40.2 ± 5.5</td>
<td>9.2 ± 2.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE of nondiabetic control rats (group 5, n = 5) with vehicle and DPCPX and STZ-induced diabetic rats (group 6, n = 5) with vehicle and DPCPX. Comparisons within each group with vehicle are expressed as *P < 0.05 and P < 0.01. Comparisons between the 2 groups, Con and STZ, are expressed as *P < 0.05.

Table 3. Time vehicle control experiments: renal response to renal interstitial infusion of normal saline vehicle (0.9% NaCl) in control and diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>UV, µl/min</th>
<th>GFR, ml/min</th>
<th>P₀, mM</th>
<th>FE₀, %</th>
<th>cAMP, pmol·ml⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>120 ± 4.8</td>
<td>360 ± 7.3</td>
<td>44.8 ± 10.3</td>
<td>1.5 ± 0.1</td>
<td>2.6 ± 0.08</td>
<td>28.1 ± 6.5</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>Vehicle</td>
<td>119 ± 6.6</td>
<td>353 ± 4.4</td>
<td>41.8 ± 10.8</td>
<td>1.6 ± 0.3</td>
<td>2.6 ± 0.05</td>
<td>31.2 ± 5.7</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>Vehicle</td>
<td>119 ± 7.2</td>
<td>354 ± 8.3</td>
<td>45.6 ± 10.3</td>
<td>1.5 ± 0.03</td>
<td>2.5 ± 0.07</td>
<td>31.5 ± 2.7</td>
<td>2.6 ± 0.7</td>
</tr>
<tr>
<td>STZ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>113 ± 5.7</td>
<td>359 ± 3.1</td>
<td>40.8 ± 3.9</td>
<td>1.6 ± 0.3</td>
<td>2.4 ± 0.3</td>
<td>31 ± 1.6</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>Vehicle</td>
<td>109 ± 4.7</td>
<td>365 ± 6.5</td>
<td>40.8 ± 7.1</td>
<td>1.6 ± 0.3</td>
<td>2.3 ± 0.2a</td>
<td>32.2 ± 3.3</td>
<td>2.6 ± 0.7</td>
</tr>
<tr>
<td>Vehicle</td>
<td>105 ± 6.9</td>
<td>363 ± 6.6</td>
<td>40.6 ± 1.7</td>
<td>1.4 ± 0.2</td>
<td>2.1 ± 0.08a</td>
<td>33.2 ± 9.5</td>
<td>2.5 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE of nondiabetic control rats (group 7, n = 4) with normal saline vehicle and STZ-induced diabetic rats (group 8, n = 4) with normal saline vehicle. Comparisons between the 2 groups, Con and STZ, are expressed as *P < 0.05.
(right) kidney to infusion of ADO and ADO A1 receptor antagonist into the renal interstitium of the left kidney showed similar changes of renal function with respect to GFR, phosphate excretion, and sodium excretion in Con and STZ rats. ADO A1 receptor blockade was only phosphaturic in the contralateral kidney of Con rats but not STZ rats. These ADO-related changes of phosphate excretion of the contralateral kidney could be attributed to ADO-mediated renal reflexes (27, 41). Alternatively, absorption and recirculation of ADO and the ADO A1 receptor antagonist cannot be ruled out. However, given the short plasma half-life time of ADO, the ADO A1 receptor antagonist cannot be ruled out. Furthermore, because sodium excretion, but not phosphate excretion, was significantly increased by infusion of the ADO A1 receptor antagonist, it is likely that phosphate reabsorption by the proximal tubule of STZ rats is stimulated by ADO but not via ADO A1 receptors.

Increased Sensitivity of the Diabetic Renal Tubular System to ADO due to ADO Receptor-Unrelated Mechanisms

ADO may bypass the receptor level of tubular cells via membrane vesicles (42) and interfere intracellularly with cAMP and protein kinase A and C on phosphate reabsorption in diabetic rats. Thus, ADO-induced increase of phosphate reabsorption would not be reversible by ADO A1 receptor blockade in STZ rats as shown in our experiments. Friedlander et al. (20) suggested an ADO receptor-independent mechanism in which ADO influences phosphate transport in cultured opossum kidney cells. They propose that luminal cAMP degradation into ADO followed by cellular ADO uptake is a mechanism by which ADO intracellularly alters phosphate transport. Metabolism of intracellular ADO may be reduced in diabetes mellitus, and less ADO is metabolized to ATP and cAMP, which reduces the cAMP-induced inhibition of sodium phosphate cotransport. Furthermore, ADO is reported to stimulate the production of inositol phosphates and to elevate calcium concentration intracellularly independent of ADO receptor stimulation (40). Therefore, alterations of ADO metabolism in diabetes mellitus could potentially contribute to the enhanced ADO-induced phosphate transport in STZ rats due to these ADO receptor-unrelated mechanisms. In that case, a reduced catabolism and/or reuptake of ADO in the renal tubules of STZ rats may cause a prolonged and increased tubular response to endogenous ADO in the diabetic kidney. Increased ADO accumulation may subsequently alter cAMP-dependent sodium phosphate transport. In fact, enhanced

Table 4. Time vehicle control experiments: renal response of the contralateral (right) kidney to renal interstitial infusion of normal saline vehicle (0.9% NaCl) in the left kidney in control and diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>UV, µl/min</th>
<th>GFR, ml/min</th>
<th>FE, %</th>
<th>FE Na, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>37.6 ± 4.7</td>
<td>1.5 ± 0.2</td>
<td>19.9 ± 3.4</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>STZ</td>
<td>31.2 ± 6.3</td>
<td>1.3 ± 0.3</td>
<td>23.4 ± 3.8</td>
<td>1.3 ± 0.06</td>
</tr>
<tr>
<td>Vehicle</td>
<td>34.8 ± 2.8</td>
<td>1.3 ± 0.3</td>
<td>24.3 ± 1.2</td>
<td>2 ± 0.06</td>
</tr>
</tbody>
</table>

Table 5. Renal response of the contralateral (right) kidney to renal interstitial infusion of ADO and the ADO A1 receptor antagonist DPCPX in the left kidney in control and diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>UV, µl/min</th>
<th>GFR, ml/min</th>
<th>FE, %</th>
<th>FE Na, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>56.6 ± 9.1</td>
<td>1.3 ± 0.1</td>
<td>22.6 ± 4.8</td>
<td>3.8 ± 0.8</td>
</tr>
<tr>
<td>ADO</td>
<td>60.2 ± 10.0</td>
<td>1.0 ± 0.06</td>
<td>22.0 ± 4.2</td>
<td>4.6 ± 0.8</td>
</tr>
<tr>
<td>ADO + DPCPX</td>
<td>66.4 ± 12.4</td>
<td>1.3 ± 0.1</td>
<td>25.8 ± 3.9</td>
<td>5.1 ± 0.8</td>
</tr>
<tr>
<td>STZ</td>
<td>29.0 ± 4.1</td>
<td>1.1 ± 0.1</td>
<td>31.0 ± 4.1</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>ADO</td>
<td>34.4 ± 7.1</td>
<td>0.9 ± 0.2</td>
<td>25.0 ± 3.8</td>
<td>3.4 ± 0.8</td>
</tr>
<tr>
<td>ADO + DPCPX</td>
<td>33.8 ± 8.4</td>
<td>0.8 ± 0.1</td>
<td>24.7 ± 3.4</td>
<td>5.0 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE of nondiabetic control rats (group 3, n = 6) with vehicle, ADO, and ADO and DPCPX. Comparisons within each group with the vehicle are expressed as *P < 0.05. Comparisons between the 2 groups, Con and STZ, are expressed as **P < 0.05.

Table 6. Renal response of the contralateral (right) kidney to renal interstitial infusion of vehicle and the ADO A1 receptor antagonist DPCPX in the left kidney in control and diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>UV, µl/min</th>
<th>GFR, ml/min</th>
<th>FE, %</th>
<th>FE Na, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>29.0 ± 5.7</td>
<td>1.1 ± 0.1</td>
<td>22.6 ± 4.1</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>DPCPX</td>
<td>37.4 ± 4.9</td>
<td>1.4 ± 0.3</td>
<td>34.5 ± 3.1</td>
<td>4.4 ± 0.8</td>
</tr>
<tr>
<td>STZ</td>
<td>32.8 ± 4.3</td>
<td>0.9 ± 0.1</td>
<td>39.4 ± 6.4</td>
<td>3.1 ± 0.8</td>
</tr>
<tr>
<td>Vehicle</td>
<td>36.8 ± 9.2</td>
<td>0.9 ± 0.1</td>
<td>38.0 ± 5.7</td>
<td>4.1 ± 1.2</td>
</tr>
<tr>
<td>DPCPX</td>
<td>52.8 ± 13.7</td>
<td>0.9 ± 0.1</td>
<td>35.1 ± 2.9</td>
<td>7.1 ± 2.1</td>
</tr>
</tbody>
</table>

Values are means ± SE of nondiabetic control rats (group 5, n = 5) with vehicle and DPCPX and STZ-induced diabetic rats (group 6, n = 5) with vehicle and DPCPX. Comparisons within each group with vehicle are expressed as *P < 0.05 and **P < 0.01. Comparisons between the 2 groups, Con and STZ, are expressed as *P < 0.05.
ADO generation was found in diabetic kidneys of STZ rats (3), and Morrison et al. (33) reported an increased ADO sensitivity in the hippocampus of STZ rats because of a loss of nucleoside-uptake processes. These changes of ADO metabolism in diabetes mellitus could be due to several factors, including glycosylation of membrane proteins, alterations of second messengers, and mediator generation.

On the other hand, an increased tubular sensitivity to ADO in diabetic rats could provide a compensatory mechanism for the phosphate leak observed in diabetes mellitus. In the present study, basal phosphate excretion was higher in STZ rats compared with Con rats. Because daily food intake is increased by 20–30% in STZ rats, it could be hypothesized that increased nutritional phosphate load is responsible for increased urinary phosphate excretion. However, a 20–30% increase in food intake only partially accounts for a twofold elevation of daily phosphate excretion in STZ rats. Our findings of a renal phosphate leak in the early onset of STZ rats are in agreement with clinical studies (4, 16). Consistent with these studies, renal mechanisms seem to be the determining factor for an increased urinary phosphate excretion in STZ rats. Renal phosphate leak in STZ rats without insulin treatment is most likely due to insulin deficiency because insulin stimulates the sodium-phosphate symport in brush-border membranes of the proximal tubule (1, 2, 14, 15, 23, 24). Stimulation of the sodium-phosphate cotransport by insulin in the proximal tubule occurs most likely by inhibition of cAMP accumulation, which in turn reduces cAMP-induced inhibition of sodium-phosphate cotransport. Thus insulin deficiency increases cAMP generation, which decreases sodium phosphate reabsorption leading to an increased phosphate excretion. Furthermore, high glucose concentrations activate G proteins, which stimulate adenyly cyclase and cAMP generation (36). Increased cAMP generation will ultimately decrease sodium-phosphate cotransport. Therefore, glucose and hyperglycemia in STZ rats may account for increased cAMP accumulation and excretion in the diabetic kidney with a subsequent cAMP-dependent increase of phosphate excretion. Indeed, we have found markedly increased excretion of cAMP in STZ compared with Con rats. In addition, because glucose also inhibits phosphate uptake and enhances phosphate efflux in canine proximal tubule cells (8), it is likely that insulin deficiency and hyperglycemia are the primary underlying factors for increased renal phosphate excretion in STZ rats and an increased sensitivity of the proximal tubule to ADO on phosphate transport may provide a compensatory mechanism for limiting the renal phosphate leak in diabetes mellitus.

In conclusion, diabetic rats have an increased sensitivity to ADO-induced increases in phosphate reabsorption; however, this is not reversed by infusion of an ADO $A_1$ receptor antagonist.

**Perspectives**

The present observations are of clinical relevance because ADO has been proposed as a pathophysiologic factor in the development of acute renal failure such as that induced by contrast media (7). Contrast media-induced acute renal failure has a higher incidence in diabetic patients with impaired renal function (37). In these situations, renal ADO is thought to be released by renal ischemia having effects on the vascular and tubular system of the kidney. Infusing ADO into the renal interstitium of diabetic rats creates a situation comparable to the diffusion of ADO out of cells that occurs during renal ischemia. Therefore, it has been hypothesized that the renal action of ADO as a pathophysiological factor in contrast media-induced renal failure is enhanced in diabetes mellitus. The present findings demonstrated an increased sensitivity to the ADO-induced increase in phosphate reabsorption in STZ rats. These observations provide new insights in the tubular phosphate transport in diabetes mellitus and may contribute to the pathophysiological mechanisms that occur in renal dysfunction in diabetic patients.

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