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ORIGINAL ARTICLE

Activation of adenosine receptors improves renal antioxidant status in diabetic Wistar but not SHR rats

DANIELA PATINHA[1,2], JOANA AFONSO[1], TERESA SOUSA[1], MANUELA MORATO[1,2,3] & ANTÓNIO ALBINO-TEIXEIRA[1,2]

[1]*Departamento de Farmacologia e Terapêutica, Faculdade de Medicina, Universidade do Porto, Porto, Portugal,* [2]*Neurofarmacologia, Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal, and* [3]*Laboratório de Farmacologia, Departamento de Ciências do Medicamento, Faculdade de Farmácia, REQUIMTE, Universidade do Porto, Porto, Portugal*

Abstract

Background. Diabetes and hypertension independently contribute to renal injury, and the major mechanisms involved are increased reactive oxygen species (ROS) bioavailability and renin-angiotensin system (RAS) activation. We investigated the role of adenosine in controlling ROS production and RAS activation associated with renal dysfunction in hypertension and diabetes.

Methods. Fourteen days after induction of diabetes with streptozotocin in 12-week-old male Wistar and spontaneously hypertensive (SHR) rats, animals were treated during 7 days with 2-chloroadenosine (CADO group, 5 mg/kg/d), a stable analogue of adenosine, or underwent a sham operation procedure. At the end of the study (day 21), intra-arterial systolic blood pressure (SBP) was measured, and 24-h urine and plasma samples and renal tissue were collected.

Results. CADO treatment decreased the plasma glucose concentration and glucose and protein excretion by more than 30% in both strains. CADO treatment decreased SBP in diabetic SHR rats (143 ± 8 versus 114 ± 4 mmHg, $p < 0.05$), but not in diabetic Wistar rats. The hypotensive effect of CADO was associated to a ~70% increase in plasma angiotensinogen (AGT) concentration and a ~50% decrease in urinary AGT excretion. CADO also caused a decrease in medullary and cortical hydrogen peroxide production of about 40%, which was associated with a proportional increase in glutathione peroxidase (GPx) activity in diabetic Wistar but not in diabetic SHR animals.

Conclusions. These results suggest that activation of adenosine receptors improves renal antioxidant capacity in diabetic Wistar but not SHR rats, although it improves glucose metabolism in both strains. Furthermore, activation of adenosine receptors does not seem to be directly influencing AGT production.

Key words: *Adenosine, angiotensinogen, hydrogen peroxide, hyperglycemia, kidney, systolic blood pressure*

Introduction

Diabetes and hypertension often coexist and increase the risk of chronic kidney disease and cardiovascular complications (1). Increased oxidative stress and renin-angiotensin system (RAS) activation in the kidney are two important contributors to the initiation and progression of diabetic and hypertensive renal damage (2,3). Therefore, the control of these pathophysiological mechanisms should be required for renoprotection in both diseases.

Adenosine, an important regulatory nucleoside known for its cytoprotective role in tissues under stress, has been shown to improve glucose homeostasis and insulin signalling/secretion (4-7). Besides influencing glucose metabolism, adenosine also protects the kidney under hyperglycemic conditions. Adenosine exerts its effects through activation of

Correspondence: Professor Manuela Morato, PhD, Departamento de Farmacologia e Terapêutica, Faculdade de Medicina do Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal. Fax: +351 22 551 36 43. E-mail: mmorato@med.up.pt

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four G-protein coupled receptors: the high-affinity A_1 subtype, the moderate-affinity A_{2A} and A_3 subtypes, and the low-affinity A_{2B} subtype (8). Both A_1 and A_{2A} receptors appear to be renoprotective in diabetic rats, as evidenced by the fact that diabetic nephropathy is more pronounced in adenosine A_1 (9-11) and A_{2A} (12) receptor knockout mice. Also, in streptozotocin (STZ)-diabetic rats, systemic administration of selective adenosine A_{2A} receptor agonists (12) or daily intraperitoneal (i.p.) treatment with a non-selective agonist of adenosine receptors (13) improves renal function by reducing inflammation (12,13).

The renoprotection afforded by adenosine may also involve the control of oxidative stress and RAS activation in the kidney. Adenosine confers increased resistance to oxidative damage. Oxidative stress enhances adenosine concentration in the kidney (14), and activation of adenosine receptors has been reported to improve the antioxidant capacity, by increasing the activity of hydrogen peroxide (H_2O_2)-metabolizing enzymes in the heart (15) and in endothelial cells (16). In STZ-induced diabetes there is increased renal oxidative stress (17), the concentration of adenosine is increased in the renal glomeruli (18), and the distribution of adenosine receptors is considerably altered (19), probably reflecting an adaptive defence mechanism. Noteworthy, increasing adenosine extracellular concentrations prevent diabetes-induced alterations of kidney function in rats (20).

The ability of adenosine to modulate the RAS has also been shown in several studies. Adenosine A_1 receptor activation restrains RAS activity (21,22). Accordingly, we have previously demonstrated that the non-selective antagonism of adenosine receptors causes hypertension (23) accompanied by increased systemic RAS activity (24,25) and augmented reactive oxygen species (ROS) production (26). In renal microcirculation, adenosine and the RAS also co-operate in the regulation of renal hemodynamics by controlling the renal vascular tone through a unifying pathway that requires both adenosine A_1 and angiotensin II AT_1 receptors (27). Renal RAS is increased in diabetic and hypertensive nephropathy (3,28-32), contributing to impaired renal hemodynamics, proteinuria, and fibrogenesis (3). Angiotensin II also exacerbates renal redox dysfunction since it stimulates the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-derived production of ROS within the kidney (33,34). Thus, the putative attenuation of renal RAS activation by adenosine would expectedly improve renal function.

Despite the evidence for adenosine to modulate oxidative stress and the RAS, the associated mechanisms have not been explored in hypertensive diabetic nephropathy. We tested the hypothesis that adenosine ameliorates renal dysfunction associated to hyperglycemia and hypertension by attenuating the production of ROS, namely H_2O_2, in the renal cortex and/or medulla, and by decreasing renal RAS activation. To assess renal RAS activation, urinary angiotensinogen (AGT) was quantified, since it is described as a marker of renal RAS activation in animal models (35-37) and in humans (38-40).

Materials and methods

All chemicals were from Sigma Aldrich (St. Louis, MO, USA) and of the highest grade available if not otherwise stated.

Animals, diabetes induction, and treatment

Animals had free access to water and food (SAFE—Scientific Animal Food and Engineering, Épinay-sur-Orge, France; Na^+: 2.5 g/kg; K^+: 6.7 g/kg; protein: 120 g/kg) and were housed under controlled conditions of temperature (22°C), humidity (60%), and 12 h/12 h light-dark cycle. All experiments were performed in accordance with the European Union guidelines for the protection of animals used for scientific purposes (Directive 86/609/EEC and Decision 1999/575/EC) and approved by the local Institutional Committee. On day 0, male Wistar rats (11–12 weeks) and male spontaneously hypertensive rats (SHR, 12 weeks) (Charles River, Barcelona, Spain) were intraperitoneally injected with STZ (65 mg/kg). After 48 h, blood glucose concentrations were determined using an auto-analyser (Abbott Diabetes Care Ltd, Santa Clara, CA, USA), and animals with blood glucose concentrations above 300 mg/dL were considered diabetic. Two weeks after induction of diabetes animals were randomly assigned into four groups of 12 animals each (two groups of each strain). Animals were anesthetized with pentobarbital sodium (50 mg/kg; i.p.), and osmotic mini-pumps (Alzet model 2ML1; Alza, Palo Alto, CA, USA) were intraperitoneally implanted to allow continuous (10 μL/h) infusion of 2-chloroadenosine (CADO, 5 mg/kg/d) in one of the groups of each strain (from now on referred to as diabetic-Wistar+CADO and diabetic-SHR+CADO animals). The remaining diabetic animals underwent a sham operation procedure (and are from now on referred to as diabetic-Wistar and diabetic-SHR animals). A schematic representation of the experimental protocol is presented in Figure 1. CADO is a stable analogue of adenosine that is not a substrate for adenosine deaminase nor for cell uptake, acting on the extracellular domains of adenosine receptors (41) with high affinity for adenosine A_1 and A_{2A} receptors and, much less, for A_3 and A_{2B} receptors (8).

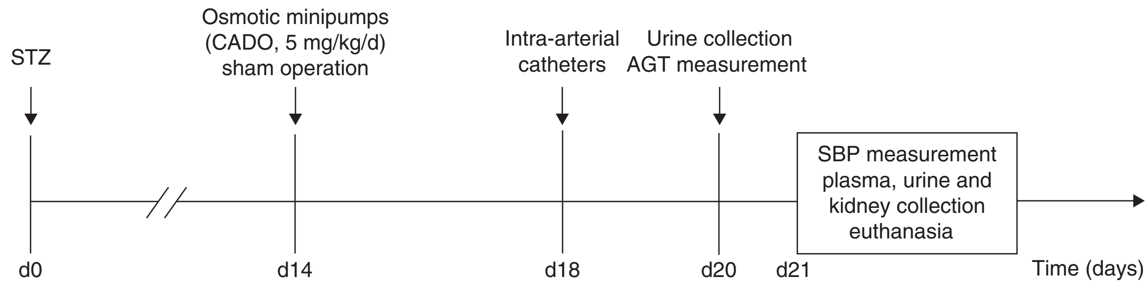

Figure 1. Schematic representation of the experimental protocol.

Blood pressure measurements

On day 18, half of the animals in each group were anaesthetized as described above, and an intra-arterial polyethylene catheter (PE10 connected to PE50; Bilaney, Dusseldorf, Germany) was placed on the aorta via the left femoral artery, passed subcutaneously using a trocar, and externalized at the back of the neck, where it was secured to the skin of the animal. Catheters were daily flushed with saline heparinized solution (0.1%) to prevent clotting. At the end of the study (day 21), the catheter was connected to a pressure transducer (B. Braun, Bethlehem, PA, USA) coupled with a polygraph (Letica Polygraph 6006; Letica, Barcelona, Spain) to measure intra-arterial systolic blood pressure (SBP) in conscious, unrestrained animals.

Sample collection

At the end of the study (day 21), 24-hour urine samples were collected in 0.6 mL of distilled water containing 50 μg of pepstatin A, 10 mg of sodium azide, 300 nmol of enalaprilat, and 125 mmol of ethylenediamine tetra-acetic acid (EDTA), for AGT determination (37). Additional ordinary 24-hour urine samples were collected for further measurements. Animals were then anaesthetized, as previously described, and blood samples were harvested from the left ventricle to heparinized or EDTA ice-cold tubes. After centrifugation of blood samples (3000 rpm, 15 min, 4°C), plasma was collected. Also, the left kidney was perfused with saline, via aorta, excised, and total cortex and medulla isolated and snap-frozen in liquid nitrogen. All samples were stored at -80°C until assayed.

Metabolic parameters and renal function

Glucose and creatinine concentrations were determined in urine and plasma samples by a glucose oxidase method and the colorimetric Jaffé method, respectively. Total urinary protein concentration was determined using pyrogallol red. Na^+ concentration was measured using ion-selective electrodes. These assays were performed using a Cobas Mira Plus analyser (ABX Diagnostics, Geneva, Switzerland). Because high glucose concentrations interfere with the creatinine Jaffé method, creatinine concentration values were corrected using the method previously reported by Moreira-Rodrigues and co-workers (42). Glomerular filtration rate (GFR) was calculated using the formula $GFR = [U_{creatinine}] \times V / [P_{creatinine}]$, where $[U_{creatinine}]$ and $[P_{creatinine}]$ denote the corrected creatinine concentration in the urine and plasma samples, respectively, and V denotes the urine flow rate (in mL/min). Although creatinine clearance gives a poor estimation of GFR, it is a non-invasive method that can provide information about the variations following treatments. Fractional Na^+ excretion (FE_{Na}) was calculated using the formula $[U_{Na}] \times [P_{creatinine}] / [P_{Na}] \times [Ucreatinine]$, where $[P_{Na}]$ and $[U_{Na}]$ represent plasma and urinary Na^+ concentrations.

AGT quantification

Quantification of AGT was performed using a commercial ELISA kit, according to the protocol provided by the manufacturer (Rat Total Angiotensinogen Assay Kit; Immuno-Biological Laboratories Co. Hamburg, Germany).

Urinary and renal oxidative status parameters

Quantification of urinary thiobarbituric acid reactive substances (TBARS) was performed as previously reported by Sousa et al. (26). Urinary 8-isoprostane quantification was performed using a commercial kit according to the protocol provided by the manufacturer (Urinary Isoprostane ELISA Kit; Oxford Biomedical Research Inc., Oxford, MI, USA). Renal H_2O_2 production was quantified in cortex and medulla samples that were incubated for 60 min at 37°C in 1 mL of oxygenated KREBS-HEPES media. The media were then used for determination of H_2O_2 production using a commercial kit according to the protocol provided by the manufacturer (Amplex Red Hydrogen Peroxide Assay kit; Molecular Probes,

Table I. Metabolic and renal function parameters of diabetic-Wistar and SHR rats treated or not with CADO.

	Plasma glucose (mg/dL)	BW (g)	Food intake (g/24h)	Water intake (mL/24h)	U-excretion (mL/24h)	U-glucose (g/kg/24h)	U-proteins (mg/kg/24h)	GFR (mL/min)	FE_{Na} (%)
Diabetic-Wistar	590.5 ± 42.9	223.9 ± 7.5	37.8 ± 1.7	180.1 ± 15.4	197.7 ± 12.5	30.0 ± 2.4	197.0 ± 41.0	2.8 ± 0.6	0.57±0.06
Diabetic-Wistar+CADO	389.3 ± 23.0	237.4 ± 7.6	33.1 ± 1.8	162.9 ± 11.1	144.8 ± 10.2	18.5 ± 1.1	115.7 ± 9.4	2.8 ± 0.3	0.55±0.55
P value	<0.05	NS	NS	NS	<0.05	<0.05	<0.05	NS	NS
Diabetic-SHR	382.4 ± 44.4	176.8 ± 9.1	25.3 ± 1.3	125.7 ± 9.1	105.5 ± 9.7	23.4 ± 2.4	197.5 ± 24.2	2.0 ± 0.3	0.80±0.12
Diabetic-SHR+CADO	232.2 ± 51.0	195.5 ± 6.8	24.7 ± 1.0	114.9 ± 6.4	95.9 ± 5.3	13.2 ± 0.9	83.7 ± 12.6	1.6 ± 0.1	0.80±0.03
P value	<0.05	NS	NS	NS	NS	<0.05	<0.05	NS	NS

Results expressed as mean ± SEM, n = 9–12 for all parameters (except GFR, n = 6–8).
BW = body weight; FE_{Na} = fractional excretion of Na^+; GFR = glomerular filtration rate; NS = not significant; U = urine.

Eugene, OR, USA). The activities of catalase and glutathione peroxidase (GPx) were determined in the supernatant of centrifuged (10 min, 15,700 *g*, 4°C) homogenized kidney samples (cold phosphate buffer, 50 mM, 7.4, containing Triton 0.1% (v/v)). Catalase activity was quantified by monitoring H_2O_2 decay at 240 nm, at 25°C during 40 s (26). One unit of catalase was defined as the amount of enzyme that decomposes 1 μmol of H_2O_2 per min. Results are expressed as units per mg of protein (extinction coefficient of 0.0394 mM/cm). GPx activity was assayed spectrophotometrically by following NADPH oxidation at 340 nm when glutathione is regenerated by glutathione reductase (26). Results are expressed as nmol of oxidized NADPH per min per mg of protein (extinction coefficient of 6.22 mM/cm).

Statistical analysis

Statistical analysis was performed using unpaired Student's *t* test. Values are presented as means ± SEM. *P* values of less than 0.05 were considered statistically significant.

Results

Metabolic parameters and renal function

CADO treatment lowered the plasma glucose concentration (Wistar 590.5 ± 42.9 versus 389.3 ± 23.0 mg/dL, and SHR 382.4 ± 44.4 versus 232.2 ± 51.0 mg/dL, $p < 0.05$), glucosuria (Wistar 30.0 ± 2.4 versus 18.5 ± 1.1 g/kg/24h, and SHR 23.4 ± 2.4 versus 13.2 ± 0.9 g/kg/24h, $p < 0.05$), and proteinuria (Wistar 197.0 ± 41.0 versus 115.7 ± 9.4 mg/kg/24h, and SHR 197.5 ± 24.2 versus 83.7 ± 12.6 mg/kg/24h, $p < 0.05$) in both strains (Table I). CADO also reduced urine excretion but only in diabetic-Wistar animals (Wistar 197.7 ± 12.5 versus 144.8 ± 10.2 mL/24h, $p < 0.05$; SHR 105.5 ± 9.7 versus 95.9 ± 5.3 mL/24h, NS) and caused no alteration in body weight, food intake, water intake, GFR, and FE_{Na} in either strain (Table I).

Blood pressure measurements

CADO treatment did not alter SBP in diabetic-Wistar animals (122 ± 6 versus 115 ± 8 mmHg, NS) but caused a reduction of SBP (≈ 20 mmHg) in diabetic-SHR (143 ± 8 versus 114 ± 4 mmHg, $p < 0.05$) (Figure 2).

Plasma and urinary AGT

CADO treatment did not alter plasma or urinary AGT in diabetic-Wistar animals (plasma 1.6 ± 0.1 versus

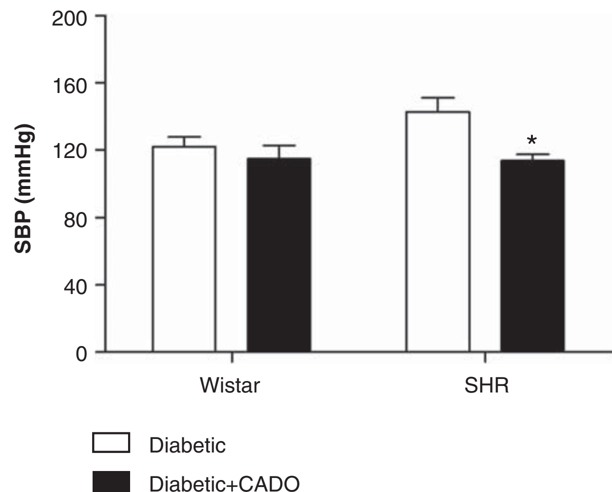

Figure 2. Systolic blood pressure (SBP; mmHg; n = 4–6) of diabetic-Wistar and SHR rats treated (■) or not (□) with 2-chloroadenosine (CADO). Results expressed as Mean±SEM
*p <0.05 versus corresponding diabetic group.

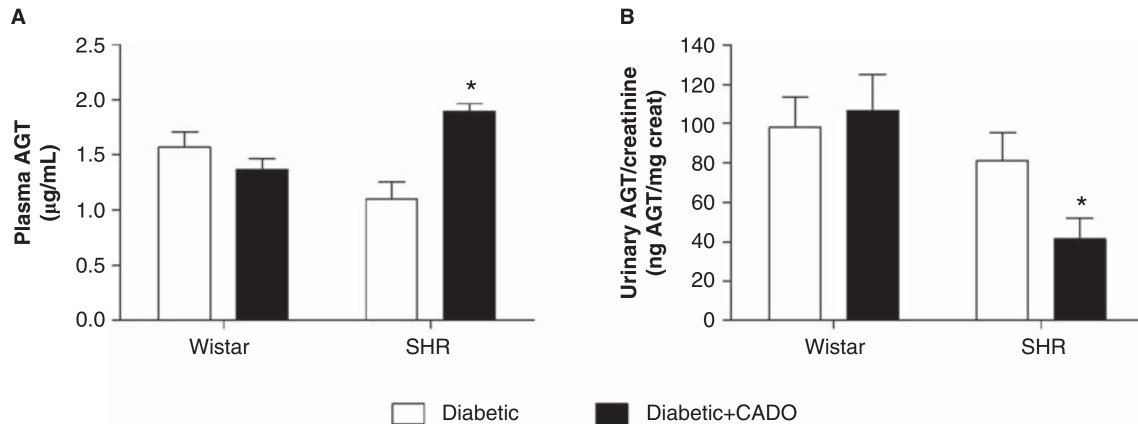

Figure 3. Angiotensinogen (AGT) in diabetic-Wistar and SHR rats treated (■) or not (□) with 2-chloroadenosine (CADO). A: plasma AGT concentration (μg/mL; $n = 6$–7); B: urinary AGT excretion (ngAGT/mg Creat; $n = 9$–11). Results expressed as Mean±SEM. *p <0.05 versus corresponding diabetic group.

1.4 ± 0.1 μg/mL; urine 98.1 ± 15.5 versus 106.7 ± 18.3 ngAGT/mg Creat; NS), but in diabetic-SHR animals the same treatment evoked an increase in plasma AGT and a marked reduction in urinary AGT (plasma 1.1 ± 0.2 versus 1.9 ± 0.1 μg/mL; urine 81.2 ± 14.3 versus 41.2 ± 10.6 ngAGT/mg Creat; $p < 0.05$) (Figure 3).

Urinary and renal oxidative status

In diabetic-Wistar animals, CADO treatment lowered renal medullary and cortical H_2O_2 production (medulla 1.11 ± 0.10 versus 0.57 ± 0.09 nmol/mgprot; cortex 0.30 ± 0.03 versus 0.17 ± 0.02 nmol/mgprot; $p < 0.05$) and increased GPx activity (medulla 119.6 ±

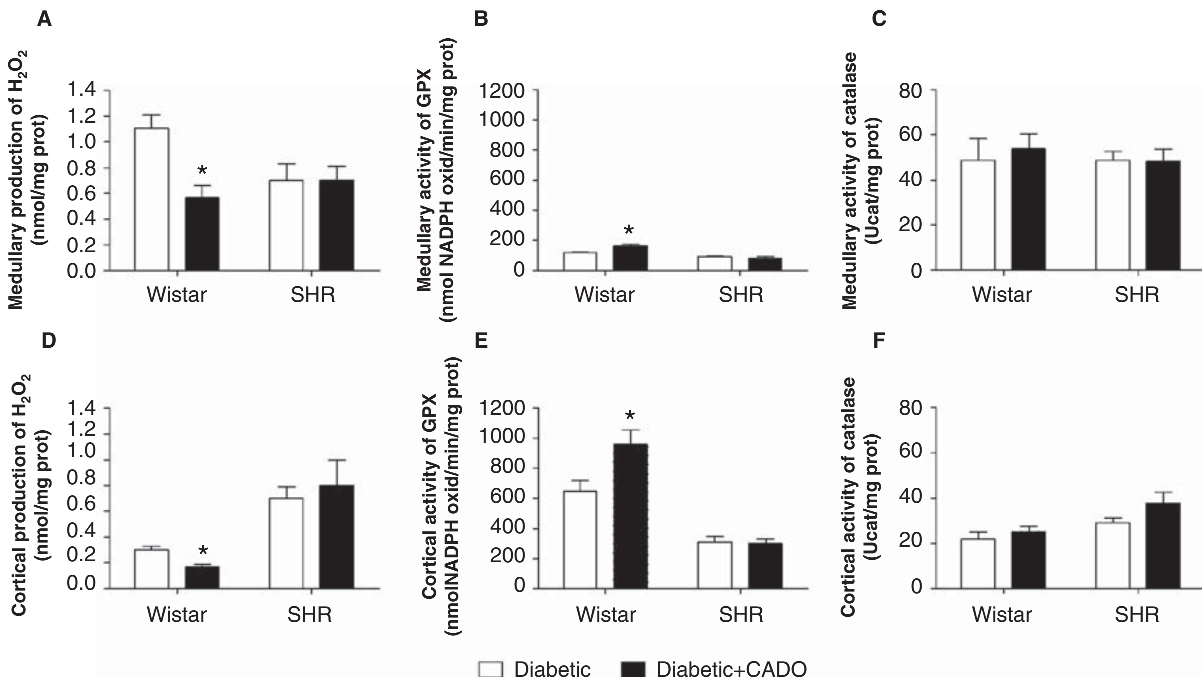

Figure 4. Production of hydrogen peroxide (H_2O_2) and activity of H_2O_2-neutralizing enzymes in the kidney of diabetic-Wistar and SHR rats treated (■) or not (□) with 2-chloroadenosine (CADO). A: renal medullary H_2O_2 production ($n = 6$–8; nmol/mgprot); B: renal medullary glutathione peroxidase (GPx) activity ($n = 5$–6; nmolNADPH/min/mgprot); C: renal medullary catalase activity ($n = 5$–8; Ucat/mgprot); D: renal cortical H_2O_2 production ($n = 5$–7; nmol/mgprot); E: renal cortical GPx activity ($n = 5$–6; nmolNADPH/min/mgprot); F: renal cortical catalase activity ($n = 5$–8 Ucat/mgprot). Results expressed as Mean±SEM. *p <0.05 versus corresponding diabetic group.

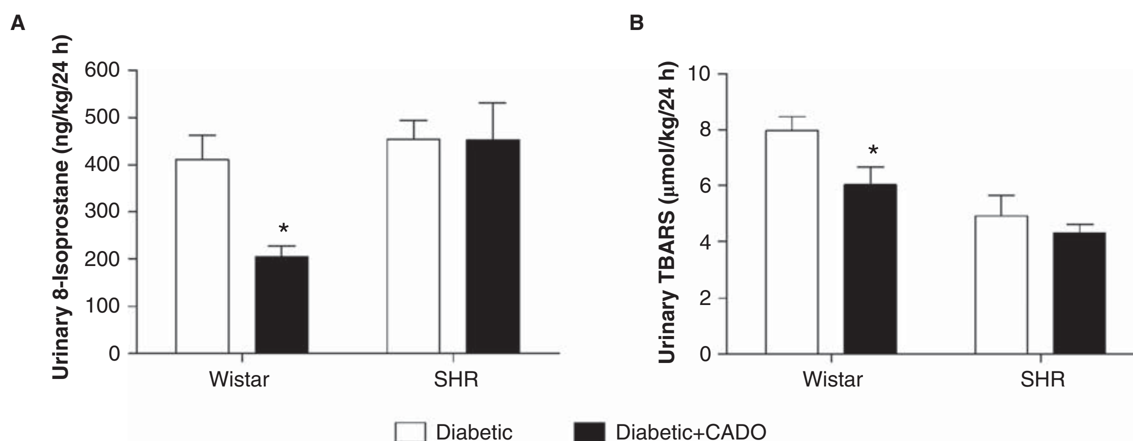

Figure 5. Urinary markers of oxidative stress in diabetic-Wistar and SHR rats treated (■) or not (□) with 2-chloroadenosine (CADO). A: 8-isoprostane (ng/kg/24h; $n = 8–12$); B: thiobarbituric acid reactive substances (TBARS) (µmol/kg/24h; $n = 10–12$). Results expressed as Mean±SEM. *p <0.05 versus corresponding diabetic group.

5.2 versus 163.8 ± 11.6 nmolNADPH/min/mgprot; cortex 648.9 ± 71.4 versus 960.6 ± 92.2 nmolNADPH/min/mgprot; $p < 0.05$), without changing catalase activity, while in diabetic-SHR animals it did not affect H_2O_2 production or its metabolism by GPx and catalase (Figure 4). CADO administration lowered the urinary excretion of 8-isoprostane (Wistar 409.9 ± 52.7 versus 204.9 ± 22.3 ng/kg/24h, $p < 0.05$; and SHR 454.7 ± 38.8 versus 453.8 ± 77.7 ng/kg/24h, NS) and TBARS (Wistar 8.0 ± 0.5 versus 6.0 ± 0.6 µmol/kg/24h, $p < 0.05$; SHR 4.9 ± 0.8 versus 4.3 ± 0.3 µmol/kg/24h, NS) only in diabetic-Wistar animals (Figure 5).

Discussion

Our results show that the mechanisms whereby CADO may protect the kidney in diabetic nephropathy are considerably different between Wistar and SHR animals, despite the similar improvement of glucose metabolism observed in both strains. In normotensive diabetics, CADO ameliorated renal dysfunction by attenuating renal ROS production, without changing renal RAS activity or SBP. In hypertensive diabetic animals, CADO did not improve the renal redox status but significantly reduced SBP, which appeared to cause compensatory changes in systemic and renal RAS activity.

The mitigation of renal redox dysfunction elicited by the activation of adenosine receptors with CADO appears to be due to an enhancement of the antioxidant defence. Diabetic-Wistar rats treated with CADO had lower renal H_2O_2 production associated with enhanced renal GPx activity than those not treated with CADO. Likewise, urinary 8-isoprostanes and TBARS were lower in diabetic-Wistar animals treated with CADO. Several authors had previously described that adenosine confers increased protection against tissue injury. Adenosine has been shown to decrease total renal concentrations of TBARS in diabetic animals (13) and to protect cells from H_2O_2-induced oxidative injury (16,43). This cytoprotective effect has been associated with an increase in the antioxidant capacity, namely by stimulating the activities of GPx (15,16) and catalase (15). GPx are selenium-containing enzymes that play an important role in antioxidant defence by degrading H_2O_2 and lipid hydroperoxides. Therefore, the rise of renal GPx activity explains the reduction in both renal H_2O_2 levels and urinary biomarkers of lipid peroxidation observed in diabetic-Wistar animals treated with CADO. Importantly, GPx activity is dependent on glutathione availability. The glutathione antioxidant system has been reported to be impaired in vascular smooth cells (44) and kidneys (45) of SHR rats, therefore contributing to the increased oxidative stress associated to the pathogenesis of renal injury and hypertension in this strain. The importance of the glutathione antioxidant system in the regulation of blood pressure has also been underlined in some studies. For example, the administration of a glutathione synthesis inhibitor, buthionine sulfoximine, significantly increases oxidative stress and blood pressure in rats (46). Furthermore, in elderly people, antihypertensive therapy improves the glutathione antioxidant system (47). Our study also points to a deficient glutathione antioxidant system in diabetic-SHR animals that probably limits the protective antioxidant response to CADO treatment.

Besides exerting antioxidant effects, adenosine may also be renoprotective by attenuating RAS activity.

Adenosine is a known physiological modulator of renin release (23-25,48). In the present study, plasma concentrations and urinary excretion of AGT were used as markers of systemic and renal RAS, respectively, reflecting the angiotensin II concentration as formerly suggested by others (35-40). Even though CADO treatment altered plasma and urinary AGT in diabetic-SHR, the fact that it also markedly reduced SBP in this strain suggests that RAS changes were mainly dependent on blood pressure levels. Accordingly, in diabetic-Wistar animals neither SBP nor RAS activity were altered by CADO treatment. Acute activation of adenosine receptors causes hypotension in both Wistar and SHR rats, but the SHR rats are much more sensitive to the hemodynamic effects of adenosine (49). In fact, a six-fold higher dose was needed to induce similar SBP reduction in Wistar compared with SHR rats (49). Although we used CADO instead of adenosine, it is possible that the dose tested was enough to induce hypotension in diabetic-SHR but not in diabetic-Wistar rats. The decrease in SBP induced by CADO treatment in diabetic-SHR animals was associated with a marked increase in plasma AGT concentration. These results are probably not a direct consequence of activation of adenosine receptors by CADO, but the result of the stimulation of compensatory mechanisms, namely activation of the systemic RAS through increased AGT synthesis, to prevent further decrease in SBP. Indeed, we recently observed (28) a decrease in plasma AGT concentration following angiotensin II infusion but a marked rise in plasma AGT concentration after polyethylene glycol-catalase-induced decrease in SBP in angiotensin II-hypertensive rats. In the current study, the increased systemic AGT observed in diabetic-SHR rats treated with CADO was associated to a decrease in urinary excretion of AGT, which is in line with the hypothesis that systemic and renal AGT may be synchronized and influence SBP (unpublished data and (50)).

CADO treatment lowered the plasma glucose concentration and glucose excretion both in normotensive and hypertensive diabetic animals, reflecting adenosine's beneficial effects in glucose homeostasis and insulin action. In fact, in diabetic rats, stimulation of adenosine A_1 receptors has been associated with increased glucose uptake (4) and insulin sensitivity (5), thus decreasing the plasma glucose concentration. Accordingly, the adenosine A_1 receptor knockout mice develop glucose intolerance in parallel with insulin resistance (51), while this is not observed in mice overexpressing adenosine A_1 receptors in the adipose tissue and fed a high-fat diet (5). In addition, the activation of adenosine A_{2A} and A_{2B} receptors increases beta-cell proliferation and function (7) and pancreatic insulin content (6), respectively. Thus, the effect of CADO treatment probably reflects non-insulin and insulin-dependent mechanisms that result from the activation of adenosine A_1 and A_2 receptors.

CADO treatment was also associated to reduced proteinuria, both in diabetic-Wistar and SHR animals. In diabetic-Wistar rats, this might be explained by the increased expression of A_{2A} receptors in the renal cortex (19), whose activation improves glomerular structure by mediating anti-inflammatory effects (12,13) and protecting the filtration barrier (52). Here, we show for the first time that activation of adenosine receptors also improves protein handling in diabetic-SHR rats, probably through the same mechanisms that operate in diabetic-Wistar rats. CADO treatment did not alter GFR or FE_{Na} in diabetic-Wistar or SHR rats. We have previously shown that although endogenous adenosine adjusts renal vascular tonus and Na^+ reabsorption, it does not influence GFR in diabetics (27). In euglycemic animals, blockade of adenosine A_1 or A_2 receptors is known to increase or decrease natriuresis, respectively (27,53,54). However, in diabetic animals, the natriuretic effect of activation of adenosine A_2 receptors is absent (27). Thus, in diabetic animals treated with CADO we would expect a predominant anti-natriuretic effect mediated by A_1 receptors. The unchanged FE_{Na} following adenosine receptor activation in diabetes has previously been reported (55). Although further studies need to be performed to elucidate this, one can speculate that it may be due to the saturation of the Na^+ transport along the tubule and/or to the marked osmotic diuresis.

In conclusion, our study indicates that adenosine receptor activation ameliorates renal function in both normotensive and hypertensive diabetic animals. This renoprotection is mediated by an increase of renal antioxidant capacity in normotensive animals, by a reduction of SBP in hypertensive rats, and by the improvement in glucose metabolism and reduction of proteinuria in both strains. We found no evidence for a direct regulation of the RAS by adenosine receptor activation in diabetes. Nevertheless, the hypotensive effect of CADO in hypertensive diabetic animals appears to trigger a compensatory increase in systemic AGT paralleled by a marked decrease in renal AGT which highlights, once more, the differential regulation of these systems.

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