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Short communication

Renal vasculature reactivity to agonist of P2X7 receptor is increased in streptozotocin-induced diabetes

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ABSTRACT

Background: Diabetic nephropathy is characterized by the dysfunction of renal microvasculature. The involvement of the P2X7 receptor, being a part of the purinergic system, is presumable in this process. The aim of our study was to investigate the P2X7 receptor-mediated renal microvasculature response and renal metabolism of extracellular adenine nucleotides in diabetic rats.

Methods: Study was performed on streptozotocin-induced diabetic Wistar rats. The vascular response to BzATP, an agonist of the P2X7 receptor, was monitored based on the changes of cortical blood flow (CBF), glomerular filtration rate (GFR) and glomerular inulin space (GIS). The renal interstitial fluid (RIF) was probed by microdialysis technique and concentrations of ATP and adenosine were measured. Activity on NTDPase and 5′-nucleotidases was measured on renal membranes.

Results: Diabetic kidneys were characterized by decreased ATP RIF and increased adenosine RIF concentrations with accompanied enhancement of NTDPase and 5′-nucleotidase activities. BzATP induced a more pronounced increase of CBF and decrease of GFR and GIS in diabetes rats. These effects were abolished by A438079, an antagonist of the P2X7 receptor.

Conclusions: It is possible that increased P2X7 receptor reactivity may be involved in the pathogenesis of diabetic nephropathy.

Introduction

Diabetes may lead to the development of diabetic nephropathy (DN) which is the most common cause of end-stage renal disease. DN is characterized, on organ level, by the dysfunction of renal microvasculature and the reduction of the glomerular filtration rate and, on cellular level, by changes in membrane receptors expression/activity. Receptor P2X7 is a member of P2X family (P2Xs1-7) of multimeric ligand-gated ion channels solely activated by extracellular ATP. It possesses characteristics of a low-conductance channel allowing the passage of small cations (Ca^{2+}, Na^{+}, K^{+}), across the membrane and a large reversible pore channel permeant to hydrophilic molecules of a molecular mass up to 900 Da [1]. P2X7 receptor protein expression in normal kidney tissue is low or undetectable, but is increased in diabetes and hypertension arterials [2]. It has been also shown that fibroblasts isolated from diabetic patients are characterized by enhanced cell response to BzATP-induced stimulation of P2X7 [3]. ATP is released constitutively and may be induced by physical, *e.g.*, shear stress, biochemical, *e.g.*, bradykinin, and pharmacological, *e.g.*, nebivolol stimulations [4]. Importantly, the release of ATP from renal cells is increased under hyperglycemia condition [5]. Once released, ATP may act in an autocrine or paracrine manner using connexin hemichannels [6].

It has been previously reported that diabetes also increases the activity of enzymes metabolizing adenine nucleotides in serum [7], may lead to increased nucleotide degradation and subsequent elevation of adenosine concentration. ATP and adenosine are known vasoactive compounds which affect renal microcirculation through P2 and P1 receptors [8]. Thus, the aim of our present study was to investigate the renal metabolism of extracellular adenine nucleotides and renal microvasculature response to BzATP action in diabetic rats.

Materials and methods

The animals

The studies were performed on male diabetic (streptozotocin 65 mg/kg, *ip*) and age-matched control Wistar rats (200–250 g).

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Experiments were performed after 12 days on animals with glucose concentration greater than 17 mM (Accu-Check Go, Roche, Basel, Switzerland). Procedures were approved by the local Bioethics Commission at the Medical University of Gdańsk.

Renal microdialysis

In vivo renal microdialysis was used to probe the renal interstitial fluid (RIF) of anesthetized rats (Inactin, 100 mg/kg, *ip*) and ATP was assayed using luciferine/luciferase assay (CLS II Roche Diagnostics, Basel, Switzerland) and adenosine by chemiluminescent method [9]. ATP and adenosine concentrations in RIF were estimated according to the method of zero net flux [10].

Renal membranes NTPDase and 5′-nucleotidase assay

The membranes were isolated using a discontinuous Percoll gradient [11]. Nucleoside triphosphate diposphohydrolase (EC 3.6.1.5, NTPDases) activity was determined in buffer (mM): 0.15 $CaCl_2$, 0.1 EDTA, 10 glucose, 225 sucrose, 45 Tris–HCl and pH 8.0 with 1.0 mM ATP or 1.0 mM ADP as substrates. The activity of 5′-nucleotidase (EC 3.1.3.5) was determined in buffer (mM): 1 $MgSO_4$, 100 Tris–HCl buffer and pH 7.5 with 2.0 mM AMP as a substrate.

Inorganic phosphate concentration was measured in supernatant by modified Gomori method using ammonium molybdate as the colorimetric reagent and KH_2PO_4 as standard [15]. NTPDase and 5′-nucleotidase specific activities are reported as nmol Pi released/min/mg protein [12].

Determination of glomerular inulin space (GIS)

Glomerular inulin space (GIS) was measured according to the previously described method [13]. Isolated glomeruli (2000/200 μl) were suspended in ice-cold PBS with 0.2 μCi [^{3}H]-inulin, placed in a shaking water bath (1.7 Hz, 37 °C) and incubated with 1 μM or 10 μM BzATP, agonist of P2X7 receptor for 2 min with or without 10 μM A438079, an antagonist of the P2X7 receptor. Reactions were terminated by the centrifugation (5000 × *g*, 5 s) of glomeruli suspension through ice-cold silicone oil. Glomerular pellet was resuspended in 0.3% Triton X-100. The radioactivity of samples (pellet and supernatant) was measured in a liquid scintillation counter. GIS of a single glomerulus was calculated as follows: GIS = pellet radioactivity/(supernatant radioactivity × number of glomeruli in pellet). Each GIS determination was carried out in triplicate samples.

Laser Doppler renal flowmetry

The polyethylene catheters (PE-50) were inserted into the femoral vein and artery of anesthetized (Inactin, 100 mg/kg, *ip*) and tracheostomized rats. The urinary bladder was catheterized. The animals received intravenous infusions of 150 mM NaCl (bolus 200 μl, sustained 45 μl/min) containing 5 μCi/ml [^{3}H]-inulin and 3% bovine albumin. The left kidney was exposed and placed in a Lucite holder. Blood perfusion of the superficial renal cortex (CBF) was measured as laser-Doppler fluxes using a Periflux 4001 system (Perimed AB, Jarfalla, Sweden) and CBF probe placed on the kidney surface. After surgery recovery, the first three collection 10-min periods were used as control period. Then, P2X7 receptor ligands (agonist: 3′-O-(4-benzoyl)benzoyl adenosine 5′-triphosphate, BzATP and selective antagonist: A438079 were intravenously infused (experimental period). The animals were divided into two groups receiving: (i) BzATP: (0.2 μmol/kg + 2 nmol/kg/min, low dose) or (1.0 μmol/kg + 10 nmol/kg/min, high dose), (ii) A438079 (40 μmol/kg + 400 nmol/kg/min) for 30 min and then BzATP (0.2 μmol/kg + 2 nmol/kg/min). On completion of the experiment, all the animals were killed by an anesthetic overdose.

Statistical analysis

The statistical significance of the differences between the groups was analyzed using One-way ANOVA with Sigma Plot 11.0 software, p values <0.05 were considered statistically significant.

Materials

Inactin, 3′-O-(4-benzoyl)benzoyl adenosine 5′-triphosphate, A438079 hydrochloride hydrate streptozotocin, and scintillation cocktail UltimaGold were purchased from Sigma–Aldrich Co. (Saint Louis, MO, USA) and the heparin from Polfa Warszawa S.A. (Warszawa, Poland). [^{3}H]-inulin was obtained from Perkin–Elmer (Waltham, MA, USA). All other agents were purchased from Avantor Performance Materials Poland S.A. (Gliwice, Poland).

Results

As presented in Fig. 1, ATP concentration of renal interstitial fluid (RIF) in diabetic rats was lower by about 39% compared with normal rats (1.34 ± 0.21 *vs.* 2.19 ± 0.18 nM, $p < 0.05$), and was accompanied by a 2.4-fold higher concentration of adenosine RIF (237 ± 40 *vs.* 98 ± 28 nM, $p < 0.05$).

The changes of ATP and adenosine concentrations in the RIF of diabetic rats might be the result of changes in the NTPDase and 5′-nucleotidase activities. As presented in Fig. 2, the renal membranes isolated from normal rats hydrolyze both ATP and ADP, however, the rate of ATP hydrolysis was 1.6-fold greater than the rate of ADP hydrolysis (205 ± 19 *vs.* 130 ± 7 nmol Pi/min/mg protein, $p < 0.05$). In diabetic rats, rates of ATP and ADP hydrolysis were increased: ATP hydrolysis by about 44% (295 ± 25 *vs.* 205 ± 19 nmol Pi/min/mg protein, $p < 0.05$) and ADP hydrolysis by about 52% (198 ± 22 *vs.* 130 ± 7 nmol Pi/min/mg protein, $p < 0.05$). The changes in NTDPase activity in diabetic rats were accompanied with twofold increase activity of 5′-nucleotidase (203 ± 15 *vs.* 101 ± 10 nmol Pi/min/mg protein, $p < 0.05$).

As presented in Fig. 3, infusion of BzATP (0.2 μmol/kg + 2 nmol/kg/min) evoked an enhancement of CBF by 12% in the diabetic rats

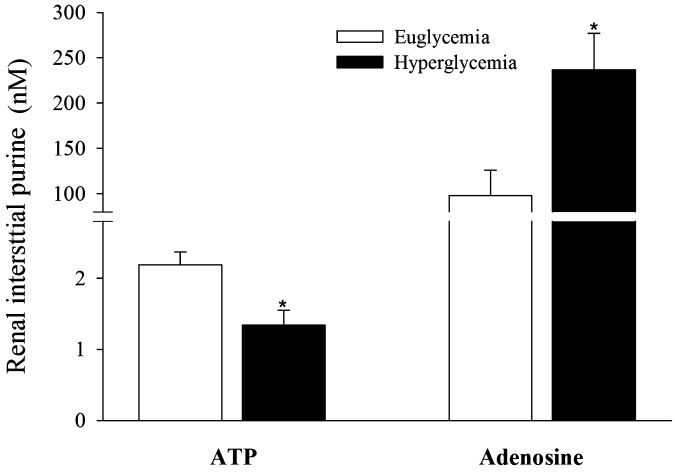

Fig. 1. ATP and adenosine concentrations in renal interstitial fluid of the cortex in euglycemic and hyperglycemic rats. A microdialysis probe was implanted into anesthetized rats' kidneys and perfused at various concentrations of ATP (0, 5, 10, 15 nM) or adenosine (0, 50, 100, 150, 300 nM). Concentrations of ATP and adenosine were estimated according to the zero net flux method. The results are expressed as means ± SE, $n = 5$. $^*p < 0.05$.