Insulin restores expression of adenosine kinase in streptozotocin-induced diabetes mellitus rats

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Received 25 October 2001; accepted 1 March 2002

Abstract

The activity of adenosine kinase is significantly impaired in tissues of diabetic rat. Changes in the activity of adenosine kinase were accompanied by alterations in its mRNA and protein level. These changes depended on insulin level and were not related to glucose level. During the first 7 h after insulin treatment the level of adenosine kinase mRNA, protein and enzymatic activity in kidneys, liver and heart returned to normal values. The observed relation between insulin and adenosine kinase expression level may suggest that insulin increases the rate of adenosine kinase gene transcription. Decreased activity of adenosine kinase was associated with elevated level of adenosine in diabetic tissues. On the 10th day after the STZ treatment there was a 3.5 and 2-fold increase in adenosine content in heart and liver, respectively. On the other hand, in diabetic kidney adenosine level was elevated only by 20%. Administration of insulin to diabetic rats resulted in a drop of adenosine to the level seen in normal heart and liver whereas, in kidneys the adenosine content was 50% lower than that observed under normal conditions. The time-dependent course of changes in adenosine level was different from that observed for adenosine kinase activity, what may suggest that other factors, possibly nucleoside transporters are also important for controlling adenosine level in the cell.

Key words: diabetes, adenosine kinase, insulin, adenosine

Introduction

Insulin-dependent diabetes mellitus is associated with the multiple defects in various tissues [1–3]. Altered myocardial metabolism in diabetic animals results in impaired ventricular performance with decreased cardiac output [4, 5]. In humans, an increase in basement membrane thickness of the microvasculature is the common feature in Type I diabetes [6]. Alteration of renal vasoconstrictive mechanism is considered as a possible factor for glomerular hyperfiltration and intrarenal hypertension. It has been postulated that glomerular hyperfiltration in early diabetes may result in glomerular damage, leading to diabetic nephropathy [7–9]. Furthermore, diabetic neuropathy encompasses a variety of abnormalities affecting both somatic and autonomic peripheral nerve function [10, 11]. The progressive loss of the ability of pancreatic β-cells to release insulin together with the preserved sensitivity of tissues to this hormone is the major feature of Type I diabetes. There is considerable evidence that some molecules dramatically influence the sensitivity of various tissues to insulin [12–19]. It has been demonstrated that adenosine beside its many physiological functions can also in great extent modify insulin action on muscle, adipocytes and liver [14–19].

The majority of basal adenosine production during normoxia is derived from the action of S-adenosylhomocysteine hydrolase [20, 21]. However, during enhanced oxygen demand or metabolic load, the increased amounts of adenosine are formed almost exclusively from AMP by means of 5′-nucleotidase (5′-NT) [22–24]. In the cell, generated adenosine could be deaminated to inosine by adenosine deaminase (ADA), phosphorylated to AMP by adenosine kinase (AK), or transported into extracellular fluid where it exerts its effect by coupling to A1, A2 or A3 receptors [25, 26].
It has been reported that the responsiveness of various tissues including heart [27], platelets [28], hippocampus [29], renal vasculature [30, 31] and smooth muscle [32] to adenosine in diabetes is altered. We have previously showed that the adenosine concentration is increased in renal venous plasma of diabetic rats and that the activity and expression level of adenosine kinase is lowered in tissues of diabetic rats [33, 34]. In the present study, we have measured the adenosine concentration in diabetic tissues and investigated the effect of insulin on activity and expression level of adenosine kinase.

Materials and methods

Pefabloc SC, xanthine oxidase, adenosine deaminase, peroxidase and nucleoside phosphorylase were from Boehringer Mannheim GmbH Biochemica (Mannheim, Germany). Thiotubarbitral sodium (Inactin), luminol, leupeptin, adenosine, ATP, AMP, alkaline phosphatase-conjugated goat anti-rabbit IgG, 5-bromo-4-chloro-3-indoyl phosphate, streptozotocin and Nitro Blue Tetrazolium were obtained from Sigma-Aldrich Sp. z o.o. (Poznan, Poland). Glucose Hexokinase Reagent Set was from Pointe Scientific, Inc. (Lincoln Park, MI, USA). Transfer Membrane was from Millipore Corp. (Bedford, MA, USA). DE81 ion exchange filters were obtained from Whatman (Maidstone, UK). U-[14C] adenosine (20 MBq/µmol) was from Amersham (Buckinghamshire, UK). All used primers were from Integrated Technologies, Inc. (Coralville, IA, USA). Moloney murine leukemia virus reverse transcriptase (MMLV-RT) was from Epicentre Technologies (Madison, WI, USA). Tth DNA polymerase, Tfl DNA polymerase, and RNasin were from Promega (Madison, WI, USA). All other reagents were of analytical grade. Male Wistar rats (200–240 g) fed Altromin C 1000 diet (Altromin GmbH, Lage, Germany) were used for all experiments. All animals had access to food and water ad libitum.

Experimental diabetes

Diabetes was induced by a single intravenous injection of 75 mg/kg body weight streptozotocin (STZ). STZ was dissolved in 10 mM citrate buffer, pH 4.5. Control rats (hereafter referred to as normal rats) were injected with citrate instead of STZ. On the 1st, 5th, 10th day after STZ injection and on the day of the experiment, blood glucose levels were measured from tail blood. Only rats with the glucose level of 20–30 mM were used for further experiments. On the day of the experiment randomly selected rats were anesthetized with pentobarbital (40 mg/kg of body wt), and the tissues of interest were removed. The half of each tissue was immediately frozen in liquid nitrogen. The second half was placed in ice-cold saline for cytosol preparation.

Infusion experiments

The experiments were performed on STZ-induced diabetic rats. Rats were anesthetized with Inactin (100 mg/kg). The animals were placed on a heated table, and body temperature was maintained at 37°C. The left femoral vein was canulated for continuous infusion of isotonic saline or 5% glucose at the rate of 2.5 ml/h. One catheter was inserted into the right femoral vein for withdrawal of blood samples. The rats were allowed to stabilize from the surgical procedure for 60 min. One group of rats (INS) obtained an injection (intramuscularly) of 5 units/kg of insulin (long-acting), and the second group of rats (DIA) obtained citrate instead of insulin. The INS rats were infused with 5% glucose, and the DIA rats were infused with isotonic saline. Blood samples (50 µL) were collected at 30 min intervals for glucose measurements. After 6 h of infusion, tissues were removed and placed into liquid nitrogen.

Preparation of tissue cytosol

The appropriate tissue was homogenized in three volumes of 50 mM Hepes KOH, pH 7.2, 100 mM KCl, 1 mM EDTA, 0.2 mM Pefabloc SC, 5 µM leupeptin, in glass homogenizer with a power-driven Teflon pestle. The homogenate was centrifuged at 100 000 × g for 1 h. The resulting supernatant (in 0.1 ml portions) was stored at –20°C as the cytosolic fraction.

Preparation of tissue extracts for adenosine measurement

Rats were anesthetized and the tissue of interest was Freeze-clamped in aluminum tongs precooled in liquid nitrogen, removed and placed in liquid nitrogen. Frozen tissues were weighed and then thoroughly pulverized in frozen 5% perchloric acid. Tissues were thawed during constant grinding, centrifuged to remove cellular debris, and neutralized with K2CO3 to pH 7.0. Perchlorate crystals were removed by centrifugation. Tissues were extracted to the final concentration of 100 mg wet wt/ml extract.

Adenosine kinase assay

The activity of adenosine kinase was assayed at 25°C by the radiochemical method [34, 35]. All enzyme assays were done.