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Original research article

Experimental diabetes mellitus type 1 increases hippocampal content of kynurenic acid in rats

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ABSTRACT

Background: Diabetes mellitus (DM) is frequently associated with peripheral and central complications and has recently emerged as a risk factor for cognitive impairment and dementia. Kynurenic acid (KYNA), a unique tryptophan derivative, displays pleiotropic effects including blockade of ionotropic glutamate and α7 nicotinic receptors. Here, the influence of experimental diabetes on KYNA synthesis was studied in rat brain.

Methods: DM was induced by i.p. administration of streptozotocin (STZ). Five weeks later, KYNA content and the activity of semi-purified kynurenine aminotransferases (KATs) were measured in frontal cortex, hippocampus and striatum of diabetic and insulin-treated rats, using HPLC-based methods.

Results: Hippocampal but not cortical or striatal KYNA concentration was considerably increased during DM, either untreated or treated with insulin (220% and 170% of CTR, respectively). The activity of kynurenine aminotransferase I (KAT I) was not affected by DM in all of the studied structures. KAT II activity was moderately increased in cortex (145% of CTR) and hippocampus (126% of CTR), but not in striatum of diabetic animals. Insulin treatment normalized cortical but not hippocampal KAT II activity.

Conclusions: A novel factor potentially implicated in diabetic hippocampal dysfunction has been identified. Observed increase of KYNA level may stem from the activation of endogenous neuroprotection, however, it may also have negative impact on cognition.

Introduction

The prevalence of diabetes mellitus (DM) is rapidly increasing in modern societies and currently affects 5–7% of the population worldwide [1,2]. The concept of diabetes has widened during past decades due to recognition of its broad range and several mechanisms involved in pathogenesis. With hyperglycemia as a common feature, diabetes is currently categorized into four major types: DM type 1, DM type 2, other types of DM and gestational diabetes. Insufficient production or compromised action of insulin leads to increased blood glucose level and triggers complex panel of metabolic disturbances, accompanied by inflammation, oxidative stress and vascular damage [2,3]. Adequate control of disease with the use of insulin and novel oral hypoglycemic agents is nowadays easier to achieve. However, at the later stage of disorder, various complications frequently affect multiple tissues and organs, including central and peripheral nervous system [3].

DM frequently coincides with depression and has been associated with higher risk of dementia and cognitive decline, however, the underlying mechanisms remain poorly defined and seem to be multifactorial [4–6]. Emerging data suggest the involvement of glucose toxicity, insulin resistance, mitochondrial dysfunction, inflammatory processes or impaired neurogenesis [4,5]. Furthermore, a number of abnormalities in neurotransmitter systems including glutamate-mediated transmission have been linked with diabetic deficit of cognition [7]. Cortical and hippocampal alterations in glutamate level and disturbed expression and

Abbreviations: AMPA, α-amino-isoxazolepropionate; CNS, central nervous system; DM, diabetes mellitus; ERK 1/2, extracellular signal-regulated kinases; GFAP, glial fibrillary acidic protein; GSK-3β, glycogen synthase kinase-3β; HPLC, high pressure liquid chromatography; IDO, indoleamine 2,3-dioxygenase; INS, insulin; KAT, kynurenine aminotransferase; KMO, kynurenine 3-monooxygenase; KYNA, kynurenic acid; NMDA, N-methyl-D-aspartate; STZ, streptozotocin.

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functioning of N-methyl-D-aspartate (NMDA) and α-amino-isoxazolepropionate (AMPA) receptors were reported in animals with experimentally induced DM [8–11].

Kynurenic acid (KYNA) is a unique endogenous tryptophan derivative with pleiotropic activities [12,13]. Initially recognized as a broad-spectrum endogenous antagonist of ionotropic glutamate receptors, KYNA was later discovered to target also α7 nicotinic receptors, and to act as a G protein-coupled GPR35 receptor ligand and an agonist of human aryl hydrocarbon receptors [13,14]. In the brain, KYNA is formed primarily within glial cells along kynurenine pathway, in the reaction catalyzed by kynurenine aminotransferases (KATs) I-IV converting its immediate precursor, L-kynurenine [14,15]. Among tryptophan metabolites, only KYNA displays neuroprotective properties; therefore its deficiency was implicated in the development of neuronal loss under pathological conditions [12,13]. On the other hand, increased KYNA levels, via blockade of glutamate-mediated neurotransmission may exert negative impact on memory and cognitive processes [16].

We have previously shown that production of KYNA is stimulated in cortical slices and glial cultures under conditions resembling hyperglycemic ketosis and by ketone body, β-hydroxybutyrate, in a protein kinase A-dependent way [17]. The aim of this study was to assess the effect of experimental DM type 1, untreated or treated with insulin, on KYNA levels and the activity of its biosynthetic enzymes, KAT I and II *in vivo*, in rat brain.

Experimental procedure

Animals

Experiments were performed on male Wistar rats (220–250 g). Animals were housed under standard laboratory conditions (20 °C environmental temperature; food and water available ad libitum). Experimental procedures have been approved by the Local Ethical Committee in Lublin and are in agreement with European Communities Council Directive on the use of animals in experimental studies.

Diabetes mellitus (DM)

DM type 1 was induced by single administration of streptozotocin (STZ), in the dose of 60 mg/kg *i.p*, diluted in 0.05 M citrate buffer, pH 4.2. The injection volume was 1 ml/100 g of animal body weight. Experimental groups included 12 animals each. Glucosuria, confirmed by semiquantitative method using Ketodiastix (Bayer) test straps one week after injection of STZ, was the criterion of diagnosing DM. Administration of insulin was started after confirmation of glucosuria. NPH insulin was given *sc* once daily, in the substitution dose of 9 IU/kg [18], sufficient to prevent glucosuria. DM group received 0.9% NaCl instead of insulin. Control group (CTR) was given appropriate volume of 0.05 M citrate buffer instead of STZ and, subsequently, injected daily with 0.9% NaCl. Treatment was conducted for four weeks. Diabetic animals (DM and DM + INS) were monitored daily for body weight and urine glucose.

Substances

STZ and L-kynurenine sulphate salt were obtained from Sigma–Aldrich (St. Louis, USA). NPH insulin (Gensulin N) was received from Bioton and 0.9% NaCl was acquired from Polpharma. All the high pressure liquid chromatography (HPLC) reagents were purchased from J.T. Baker Laboratory Chemicals (Holland). Other reagents were obtained from POCH (Gliwice, Poland).

KYNA levels in the brain structures

35 days after administration of STZ, rats were decapitated and their brains were quickly removed from the skull. Hippocampi, striata, and frontal cortices were quickly dissected on ice and stored separately (−72 °C) until further analyses. On the day of analysis, randomly chosen single tissue structures from either right or left hemisphere were homogenized 1:10 (weight/volume) in distilled water (Bandelin Sonopuls, Germany), at ice-cold bath (4 °C). The obtained homogenate was centrifuged (13,600 × RCF, 5 min, 4 °C), acidified with 0.1 ml of 1 N HCl and 14 μl of 50% trichloroacetic acid and centrifuged again (conditions as above). Supernatants were applied to the cation-exchange columns (Dowex 50 W, H^+ form), which were prewashed with 1 ml of water and 1 ml of 0.1 N HCl. Columns were subsequently washed with 1 ml of 0.1 N HCl and 1 ml of water. KYNA was eluted with 2.5 ml of water.

Determination of activity of kynurenine aminotransferases I and II (KAT I and II)

KAT I and KAT II activities were assayed with a method described by Guidetti et al. [15]. Briefly, rats cortices, hippocampi and striata were homogenized 1:10 (weight:volume) in dialysis buffer (40 ml of Tris–acetate buffer, pH 8.0, 40 mg of pyridoxal-5′-phosphate, 3.12 ml of 2-mercaptoethanol, 3960 ml of distilled water). Homogenate was centrifuged (13,600 × RCF, 10 min, +4 °C). Supernatants were placed separately in cellulose membranes (Dialysis tubing, Sigma) and dialyzed against 4 l of dialyzing buffer (composed as above) for 15 h, at 4 °C. The dialyzates (100 μl) were incubated (37 °C, 20 h) in the reaction mixture containing (final concentrations): 2 μM L-kynurenine, 1 mM pyruvate, 70 μM pyridoxal-5′-phosphate, 150 mM Tris–acetate buffer, pH 7.0 or 9.5, for KAT II or KAT I, respectively. Glutamine (2 mM), the inhibitor of KAT I, was added to samples assayed for KAT II activity. After incubation, samples were rapidly chilled to 4 °C, acidified with 1 ml of 0.1 N HCl and 14 μl of 50% trichloroacetic acid, and centrifuged (13,600 × RCF, 5 min, +4 °C). The obtained supernatants were treated as described above.

Blank samples were prepared from heat-inactivated dialysate (98 °C, 15 min).

Quantification of KYNA

Eluted KYNA was subjected to the HPLC and quantified fluorimetrically (Varian HPLC system; ESA catecholamine HR-80.3 μm, C_{18} reverse-phase column), as previously described [19]. The mobile phase (pH 6.2) contained 250 mM zinc acetate, 50 mM sodium acetate and 4% acetonitrile. Retention time of KYNA was 4.5–5.0 min with 1 ml/min mobile phase flow. Each chromatographic assay was preceded by the measurements of standardized concentrations of KYNA (0.2, 0.4, 0.6, 0.8 and 1.0 pmol) in order to obtain calibration curve.

Statistical analyses

The statistical analyses were performed using one-way analysis of variance (ANOVA test), with the adjustment of p value by the Bonferroni method. Data are presented as the mean values ± SD.

Results

Serum glucose level reached 176 mg/dl (123% of CTR; ns) in DM + INS group and 585 mg/dl (409% of CTR; $p < 0.001$) in DM group.