Orally administered tryptophan and experimental type 2 diabetes

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Abstract

There is a link between diabetes and oxidative stress. Hyperglycaemia leads to free radical generation and alterations of endogenous antioxidants. Our aim is to study the effect of orally administered L-tryptophan (TRP), the melatonin precursor, an endogenous antioxidant, on circulating levels of glycaemia, insulin and melatonin, and on the superoxide dismutase and catalase antioxidant systems in non-diabetic (ND) and type 2 diabetic (n5-STZ) male Wistar rats.

At 19:30 every day for 15 days, TRP (125 mg/kg body weight) was administered orally. At 09:00 every two days the glycaemia was measured and every day the intake of food and water was recorded. At the beginning and end of treatment (at 09:00; 21:00; 02:00) plasma insulin and melatonin levels were measured, and (at 09:00) the enzymatic activities of catalase and superoxide dismutase (SOD) in erythrocytes were also measured. Glycaemia values were greater \( (p < 0.01) \) in n5-STZ rats than in ND rats, while insulin levels were lower \( (p < 0.05) \) at all times studied and these parameters were not altered by the TRP administration. Melatonin levels at 02:00 were lower in n5-STZ than in ND rats \( (p < 0.05) \). The TRP administration did not modify the circulating melatonin levels in ND rats, but raised \( (p < 0.01) \) the levels at 02:00 in the treated n5-STZ group. In ND rats after TRP administration there was a decline in catalase activity \( (p < 0.05) \), while in n5-STZ rats there was a rise \( (p < 0.01) \) at the end of treatment. However, there were no significant changes in SOD activity. There was increased food intake (g/day) in the treated n5-STZ group \( (p < 0.01) \). In conclusion, the oral administration of TRP did not modify glycaemia or insulinaemia levels, but raised melatonin levels in diabetic rats at 02:00, lowered catalase activity in ND rats but raised it in n5-STZ rats, and increased food intake in n5-STZ rats. (Mol Cell Biochem xxx: 1–5, 2004)

Key words: catalase, food intake, glycaemia, insulin, L-tryptophan, melatonin, superoxide dismutase, type 2 diabetes

Introduction

There is a link between diabetes and oxidative stress [1, 2]. Hyperglycaemia appears to play a major role in free radical production [3, 4]. During oxidative stress, endogenous mechanisms, enzymes and antioxidant molecules are deployed to destroy reactive oxygen species and reduce the harmful effects. In normal conditions, these mechanisms are sufficient to counteract free radical production [5], but in diabetes an enhanced oxidative stress has been observed as indicated by increased free radical production [6] and diminished antioxidant status [7], although there have been contradictory results for this last aspect [8]. However, agents with antioxidant activity might offer additional benefits to the diabetic patient and could be useful in preventing or delaying the development of diabetic complications [9].

Melatonin is considered to be an efficient direct and indirect antioxidant. It detoxifies the highly reactive hydroxyl radical, neutralizes other toxic species, and stimulates several antioxidative enzymes [10]. After melatonin administration (intraperitoneal or subcutaneous) in type 1 and 2 diabetic rats, many studies have observed a decrease in lipid peroxidation, glycaemia and protein glycosilation [11], increased antioxidant status [12], and lower hyperinsulinemia values in type 2 diabetic rats [13]. Tryptophan is the precursor of serotonin and melatonin. There is described a decrease of...
the free fraction of L-tryptophan (TRP) in plasma in children with insulin-dependent diabetes [14] and in diabetic rats [15]. Oral administration of TRP to rats and chicks causes a rapid elevation of circulating melatonin [16]. In non-insulin-dependent diabetic patients, administration of 5-hydroxytryptophan causes decreased carbohydrate intake and weight loss [17].

Our aim is to study the effect of orally administered TRP, the melatonin precursor, on circulating levels of glucose, insulin and melatonin, and on the superoxide dismutase and catalase antioxidant systems in non-diabetic (ND) and type 2 diabetic (neonatal diabetes model: n5-STZ) male Wistar rats.

Materials and methods

Experimental animals

We used male Wistar rats, fed a standard commercial diet (maintenance diet Letica, Panlab S.L., Barcelona, Spain; 61.41% w/w carbohydrate, 3.96% fibre, 15.06% protein, 2.66% fat). The rats had free access to water and food. They were housed in the animalarium of the University of Extremadura at room temperature (24 ± 2 °C), with lighting from 08:00 to 20:00 hours. The animals were cared for in accordance with the principles of the Guide for Care and Use of Experimental Animals.

Induction of experimental diabetes

We generated a model of experimental type 2 diabetes, known as the n5-STZ model, induced by the administration of streptozotocin (STZ; Sigma-Aldrich Química S.A., Alcobendas, Madrid, Spain) during the neonatal period, as described by Portha et al. [18]. The n5-STZ model was obtained by a single dose of STZ (80 mg/kg body weight) dissolved in citrate buffer (0.1 mol/l) at pH 4.5, administered intraperitoneally on day 5 after birth. The ND control rats received only the citrate buffer, intraperitoneally.

At the age of 2.5 months, the body weight and glycaemia were measured and an oral glucose tolerance test was performed (0.5 g/kg body weight). Only those diabetic animals presenting a clear oral glucose intolerance were selected to form part of the diabetic groups in this study.

Study of oral administration of L-tryptophan

In the ND and in the n5-STZ rats, TRP (125 mg/kg body weight) dissolved in 0.9% NaCl was administered via a gastric cannula, with the animal awake at 19:30 every day during 15 days. Likewise, NaCl was administered to their respective control groups (ND-NaCl and n5-STZ-NaCl).

Blood samples were obtained from a cut made at the tip of the animal’s tail. Blood was collected into heparinized Eppendorff tubes, and the plasma was separated by centrifugation. The erythrocytes were washed and haemolysed with distilled water. The samples were frozen and stored at −70 °C until assays.

At 09:00 every two days the glycaemia was measured using a glucometer and reactive strips (Glucocard Memory; A. Menarini Diagnostics, San Adrián del Besós), and every day the intake of food and water was recorded. At the beginning and the end of treatment (at 09:00; 21:00 and 02:00) plasma insulin and melatonin levels were measured by radioimmunoassay (DRG's Instrument GmbH, Marburg, Germany), and at (09:00) the enzymatic activities of catalase (CAT, mg/g haemoglobin) and superoxide dismutase (SOD, IU/g haemoglobin) in erythrocytes were measured by spectrophotometry. Catalase was measured using Aebi's technique [19] and superoxide dismutase using a commercial kit (RANSOD, Laboratorios Randox S.L., Barcelona, Spain).

The results are expressed as mean ± S.E.M. (n = 6 for each group). The Wilcoxon test was applied for paired samples and the Mann-Whitney U test for unpaired samples.

Results

As shown in Fig. 1, glycaemia values (mmol/l) were greater (p < 0.01) in the n5-STZ rats (day 0: 18.74 ± 2.32 and day 15: 22.01 ± 2.99) than in the ND rats (5.16 ± 0.15 and 5.49 ± 0.32, respectively), while insulin levels (Fig. 2) were lower (p < 0.05) at the times studied. These parameters