Life Sciences 85 (2009) 526–533

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Pineal melatonin synthesis is decreased in type 2 diabetic Goto–Kakizaki rats

Thomas Frese [a], Andreas Gunter Bach [a,*], Eckhard Mühlbauer [b], Klaus Pönicke [c], Hans-Jürgen Brömme [d], André Welp [a], Elmar Peschke [a]

[a] Institute of Anatomy and Cell Biology, Martin Luther University Halle-Wittenberg, Grosse Steinstrasse 52, 06097 Halle, Germany
[b] Saxon Academy of Sciences Leipzig, Karl Tauchnitz-Str. 1, 04107 Leipzig, Germany
[c] Institute of Pharmacology and Toxicology, Martin Luther University Halle-Wittenberg, Magdeburger Str. 4, 06097 Halle, Germany
[d] Institute of Pathophysiology, Martin Luther University Halle-Wittenberg, Ernst-Grube-Str. 40, 06097 Halle, Germany

ARTICLE INFO

Article history:
Received 9 March 2009
Accepted 10 August 2009

Keywords:
Pineal gland
Melatonin synthesis
Type 2 diabetes
Goto–Kakizaki
Noradrenaline

ABSTRACT

Aims: It is not well understood why the amplitude of melatonin rhythms is reduced in diabetic animals and humans. This paper addresses the differences in the pineal melatonin synthesis of type 2 diabetic Goto–Kakizaki (GK) rats compared to non-diabetic Wistar rats (8 and 50 weeks old).
Main methods: Plasma melatonin concentrations and the pineal content of melatonin and its precursors (tryptophan, 5-hydroxytryptophan, serotonin, and N-acetylserotonin) were quantified at the middle of the day and night. Additionally, the expression of melatonin synthesizing enzymes, pineal noradrenaline content, and pineal protein content were considered, and the melatonin secreting capacity of pineal glands was studied in vitro.
Key findings: The pineal glands of diabetic GK rats have a different expression pattern of melatonin synthesizing enzymes. The amount of all precursors of melatonin is reduced in pineal glands of diabetic GK rats. The pineal glands of diabetic GK rats contain less noradrenaline, indicating a reduced stimulation of nighttime melatonin synthesis. The pineal glands of diabetic GK rats produce less melatonin in reaction to noradrenaline in vivo and in vitro. The pineal glands of diabetic GK rats contain less protein, probably a consequence of diabetic neuropathy.
Significance: This is the first time that melatonin synthesis is examined in a type 2 diabetic rat model. The present data unveiled several reasons for a reduced melatonin secretion in diabetic animals and presents an important link in the interaction between melatonin and insulin.

Introduction

Overload of pancreatic β-cells leads to β-cell exhaustion and finally to the development of diabetes. Reduced β-cell mass is evident in type 2 diabetes, and apoptosis is implicated in this process (Araki et al. 2003). Melatonin decreases plasma insulin levels in both humans (Boden et al. 1996) and rats (Peschke et al. 1997; Rasmussen et al. 1999; Wolden-Hanson et al. 2000; Peschke et al. 2000; Picinato et al. 2002; Nishida et al. 2003). The hormone uses a number of signaling pathways including cAMP (Peschke et al. 2000), cGMP (Stumpf et al. 2008; Stumpf et al. 2009) and inositol-1,4,5-triphosphate (Bach et al. 2005) as second messengers to influence the insulin secretion of pancreatic β-cells (Peschke 2008) and therefore helps to prevent an overstimulation and exhaustion of these cells.

Pinealectomy leads to hyperglycemia and a diabetes-like metabolic state in Wistar (WR) rats (Diaz and Blazquez 1986; Mellado et al. 1989; Rodriguez et al. 1989). Other studies have demonstrated that long-term melatonin administration to non-insulin-dependent diabetic Otsuka Long–Evans Tokushima Fatty (OLETF) rats resulted in reduced hyperinsulinemia, decreased insulin resistance, and a normalised blood plasma lipid constellation (Nishida et al. 2002). Studies on type 2 diabetic patients led to similar results (Hussain et al. 2006; Kadhim et al. 2006). Recent finding of the melatonin MT2-receptor in human pancreatic islets underlines a possible link between circadian rhythm regulation and glucose homeostasis through the melatonin signaling pathway (Peschke et al. 2007; Bouatia-Naji et al. 2009).

However, under diabetic conditions in humans and animals plasma melatonin concentrations are decreased (O'Brien et al. 1986; Tutuncu et al. 2005; Peschke et al. 2006; Peschke et al. 2007). Considering the proposed antidiabetic properties of melatonin, it is especially detrimental that this hormone is decreased in a disease in which it could potentially provide relief. To date very few studies about the influence of diabetic situations on the pineal gland exist (Garcia et al. 2008) and it is largely unclear why the melatonin plasma concentration is reduced under diabetic conditions.

Noradrenaline is the main stimulus of pineal melatonin synthesis (Simonneaux and Ribelayga 2003). Therefore, diabetes-induced

* Corresponding author.
E-mail address: andreas.bach@medizin.uni-halle.de (A.G. Bach).

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doi:10.1016/j.lfs.2009.08.004

changes of the noradrenergic system in general and particularly changes in pineal noradrenaline content may be integral in the reduced melatonin concentrations under diabetic conditions.

The present paper reports on an investigation of pineal melatonin synthesis in diabetic Goto–Kakizaki (GK) rats versus that in Wistar rats as non-diabetic controls. Groups of 8-week-old and 50-week-old animals were examined. Diurnal-dependent changes were considered. GK rats are a non-obese strain that was established by Goto and Kakizaki (Goto et al. 1976; Goto and Kakizaki 1981; Goto et al. 1988) as a polygenic model of type 2 diabetes (Frese et al. 2007). The strain was created through selective inbreeding of Wistar rats with abnormal glucose tolerance repeated over several generations. In vitro studies demonstrated impaired glucose-stimulated insulin secretion of the pancreatic β-cells in young GK rats, as early as newborn (Frese et al. 2007). Middle-aged animals presented the metabolic disturbances associated with diabetes due to reduced pancreatic β-cell content, reduced β-cell insulin content, and impaired glucose sensing (Frese et al. 2007). GK rat is one of the best characterized animal models used for studying diabetic complications. To our knowledge, no similar investigation has been reported on other common type 2 diabetic rat models like Zucker fatty rat, Zucker diabetic fatty rat, SHR/N-cp rat, JCR/LA-cp rat, OLETF rat, Torri rat, and Cohen diabetic rat. Therefore, this is the first time that melatonin synthesis is examined in a type 2 diabetic rat model.

Materials and methods

Overview

The precursors of pineal melatonin (tryptophan, 5-hydroxytryptophan, serotonin, and N-acetylserotonin), as well as melatonin itself, were quantified in a single sample. This was achieved by dividing pineal lysate into multiple fractions that were subsequently processed by multiple detection techniques. Concentrations of pineal melatonin precursors were detected by high-performance liquid chromatography (HPLC). Pineal and plasma melatonin concentrations were estimated with a melatonin radioimmunoassay (RIA).

Noradrenaline in the pineal glands of non-diabetic and diabetic animals was detected by HPLC. In addition, body weight, pineal weight, pineal protein content, plasma glucose, and mRNA amounts of the pineal enzymes that are involved in melatonin synthesis, i.e. tryptophan hydroxylase (TPH), aromatic amino-acid decarboxylase (AAADC), arylalkylamine-N-acetyltransferase (AA-NAT) and hydroxyindole-O-methyltransferase (HIOMT), were determined.

Animals, tissue sampling, and blood glucose determination

Male Wistar rats (outbred, Schönwalde, Germany) and diabetic Goto–Kakizaki (GK) rats (inbred, Taconic M&B, Ry, Denmark) (Goto and Kakizaki 1981; Ling et al. 1998) were maintained in groups of three animals per cage and subjected to a light regime of L:D = 12:12, light on at 06:00 h. Rats were fed a standard diet (Altromin 1324, Altromin, Lage, Germany) ad libitum. At the age of 8 weeks and at the age of 50 weeks animals were sacrificed in the middle of the light (12:00) or dark (00:00) period while under deep anesthesia (10 animals per group, in 50-week-old GK rats 5 animals per group).

Tissue sampling was carried out under red light during the dark phase. Blood was taken from the right heart ventricle to determine blood glucose and melatonin plasma concentrations. Blood glucose was analysed with the MediSense® Precision® Xtra glucometer (Abbott Diagnostics, Wiesbaden, Germany). The animals were treated at all times according to European animal welfare regulations.

Preparation of pineal samples and protein determination

Pineal glands were stored immediately after excision at 4 °C in 100 µl acidic antioxidant solution (4% perchloric acid, 2 mM ethylene diamine tetraacetic acid, 1% ascorbic acid, 0.1% cysteine) per gland in 1.5 ml Eppendorf tubes. At this stage samples were frozen at −80 °C until further preparation.

Thawed samples were disrupted by sonification on ice with a SH70G sonotrode connected to a Bandelin Sonoplus (BANDELIN Electronic GmbH & Co. KG, Berlin, Germany) at 90% cycle, 50% power for 10 s. The homogenate was centrifuged at 20,000×g and 4 °C for 20 min. The supernatant containing melatonin and its precursors was divided into fractions for melatonin RIA and for HPLC determination of precursors. The fractions were frozen at −80 °C until measurement. Analyte recoveries were very similar for melatonin and its precursors, ranging from 95% to 105%, with a mean recovery of 99%.

The pellet was resuspended in 100 µl PBS (phosphate-buffered saline; Biochrom AG, Berlin, Germany) and was used for a Bradford protein assay (BioRad Laboratories GmbH, München, Germany) in a 96-well plate.

HPLC determination of noradrenaline in pineal samples

Pineal lysate was injected into a pre-column system packed with phenylboronic acid-modified silica (Recipe, München, Germany) with phosphate buffer as the mobile phase (0.2 M diammonium hydrogen phosphate, 10 mM EDTA, 100 mg/l sodium azide, pH 8.7, at a flow rate of 0.5 ml/min). After 5 min, the bound catecholamines were eluted by acidification with phosphate buffer at pH 3.0 (0.1 M sodium dihydrogen phosphate, 5 mM octanesulfonic acid and 100 mg/l sodium azide to methanol 89:11 v/v, at a flow rate of 1 ml/min), and separated on a LiChrospher RP-18e column (E. Merck, Darmstadt, Germany). The separated catecholamines were oxidised in a post-column derivatization system by addition at 0.3 ml/min of 2 mM potassium hexacyanoferrate-III solution (containing 0.1 M sodium dihydrogen phosphate and 0.2 M disodium hydrogen phosphate) followed by addition at 0.3 ml/min of 1.5 mM ascorbic acid (containing 1 mM dl-homocysteine) to reduce excess potassium hexacyanoferrate-III.

The resulting aminochromes were transformed to trihydroxyindoles by addition at 0.3 ml/min of 5 M NaOH and were quantified by fluorescence detection (excitation 405 nm, emission 520 nm).

HPLC determinations in pineal samples and in medium supernatant

An Agilent Series 1100 HPLC system (Agilent Technologies Deutschland GmbH, Böblingen, Germany) was used. The system consists of an isocratic pump, automatic sample injector, and spectrofluorometric detector. The wavelengths for extinction and emission were 285 nm and 345 nm, respectively. Data acquisition was performed using the manufacturer's software. The analytical column was a LiChroCART 125-4 Superspher 60 RP-select B (Merck, MZ-Analysentechnik GmbH, Mainz, Germany), protected by a LiChroCART 10-2 Superspher 60 RP-select B guard column. The mobile phase was methanol–phosphate buffer (0.1 M, pH 4.6 25:75 v/v, at a flow rate of 0.6 ml/min).

The pineal homogenate was thawed, mixed, and neutralised with 10% NaOH-solution; 10 µl of the sample was injected into the HPLC system. Pure solutions of 10 µM tryptophan, 4 µM 5-hydroxytryptophan, 4 µM serotonin, and 4 µM N-acetylserotonin in the mobile phase were used for peak identification and for calculation of concentrations in pineal samples. All measurements were performed in duplicate, with repeated measuring of empty controls (pure mobile phase) and pure controls (substances in mobile phase) to monitor the performance of the HPLC system.

For determination of melatonin in medium supernatant, the analytical column was an EC 125/2 NUCLEODUR 100-3 C18 ec (Macherey Nagel GmbH & Co. KG, Düren, Germany), protected by a CC 8/3 NUCLEODUR 100-3 C18 ec guard column. The mobile phase was acetonitrile–phosphate buffer (15 mM, pH 6.8 23:77 v/v, at a flow rate