Diabetes induces changes in melatonin concentrations in peripheral tissues of rat

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Abstract

OBJECTIVE: Exogenous melatonin was found to protect target organs under conditions of *diabetes mellitus*, however, concentrations of the hormone in peripheral tissues have not been determined. Therefore the aim of the present study was to measure the daily profile of melatonin levels in the pineal gland, plasma, pancreas, kidney, spleen, duodenum and colon of control and diabetic rats.

MATERIAL & METHODS: Diabetes was induced by a single injection of streptozotocin (STZ, 65 mg/kg of body weight) and samples were collected over a 24 hr cycle on day 17 after STZ treatment. Melatonin and corticosterone levels were measured directly in plasma and after extraction in the pineal gland and peripheral organs (pancreas, kidney, spleen, duodenum and colon).

RESULTS: A significant daily rhythm of melatonin concentrations was found not only in the pineal gland and plasma but also in the pancreas, kidney, spleen and duodenum. The daily pattern of melatonin levels in the colon was arrhythmic without a characteristic night-time increase of hormone concentration. Experimentally induced diabetes resulted in lower melatonin levels in the pancreas, kidney and duodenum as compared to control. No differences between STZ-treated and control rats were found in the spleen and colon. Plasma corticosterone levels were enhanced in diabetic rats in comparison with controls and the daily profile was not rhythmic.

CONCLUSION: Our data suggest that the lower amplitude of melatonin rhythm in target organs induced by experimental diabetes can contribute to desynchronization of daily rhythms and can lower the antioxidative capacity of tissues.

ORIGINAL ARTICLE

INTRODUCTION

In addition to many pathophysiological events diabetes is frequently associated with abnormalities in daily rhythms of physiological and behavioral processes. Desynchronization of circadian rhythms may further deteriorate the health status of diabetic patients.

Circadian rhythms are controlled by circadian clocks localized in the brain – the suprachiasmatic nucleus (SCN) of the hypothalamus and in cells of peripheral organs [38]. Peripheral oscillators are regulated by the central SCN pacemaker and are entrainable also by endocrine and metabolic signals. The nature of these pathways is not fully understood but alterations in communication between central and peripheral clocks may result in metabolic and behavioral disturbances.

The endocrine system exhibits pronounced daily rhythmicity and represents an important route interconnecting the central and peripheral oscillators. Daily rhythms of many hormones are disturbed in diabetic patients [34] or rodents [45] with induced diabetes.

Corticosterone and melatonin are frequently used as markers of functioning of the circadian system. Changes in corticosterone rhythmicity were reported in relation to diabetes [30,16]. Altered corticosterone rhythmicity in streptozotocin (STZ) induced diabetes is accompanied with impaired stress responsiveness and basal hyperactivation of the diabetic hypothalamo–pituitary–adrenocortical axis induced by decreased glucocorticoid negative feedback sensitivity [16].

Melatonin, a main secretor product of the pineal gland, is a component of circadian organization that can serve as an endogenous Zeitgeber for peripheral oscillators. Diabetes induced by alloxan or STZ reduced the nocturnal pineal melatonin content in the Siberian hamster [14]. The same treatment was not effective in rats, which are probably less sensitive than hamsters to alterations in plasma insulin levels [15]. However, recent studies demonstrated decreased nocturnal serum melatonin concentrations and increased melatonin receptor status in the pancreas of diabetic Goto Kakizaki rats [32].

Exogenous melatonin was reported to possess protective effects against STZ induced pancreatic beta cell damage [2], against renal injury [7] and vascular reactivity [40,39] of STZ–treated diabetic rats. A functional interrelationship was suggested to exist between beta cells of the endocrine pancreas and the pineal gland [31]. Insulin injection was reported to increase the activity of the rate-limiting enzyme of melatonin biosynthesis, pineal arylalkylamine-N-acetyltransferase (AA-NAT) and the serum melatonin level [21]. However, other studies showed that insulin inactivated AA-NAT [26].

Since melatonin may play a physiological role in diabetes and data about a daily profile of this hormone in diabetic rodents are not conclusive, in the present study we measured the daily profile of this hormone in STZ-treated diabetic rats. Although melatonin can penetrate across biological membranes, its concentrations in target organs have not been determined in diabetic individuals and it is not clear to what extent the tissue levels reflect pineal or plasma concentrations.

Therefore the aim of our study was to measure the daily profile of melatonin in the pineal gland and plasma, as well as in peripheral organs, like the pancreas, kidney, gastrointestinal tract (duodenum and distal colon) and spleen of control and STZ-treated diabetic rats. To monitor changes in the circadian system of diabetic rats, we determined also the daily corticosterone plasma rhythm.

MATERIALS AND METHODS

Animals

Male Wistar rats were used in the experiment (Breeding Facility Dobra Voda, Slovakia). The animals were housed in a temperature-controlled room (21 °C ± 2 °C) with a light:dark regimen 12:12 (light on at 7.00 hr) and access to water and food *ad libitum*. The experiment was performed according to the protocol approved by the Ethical Committee for the Care and Use of Laboratory Animals at Comenius University Bratislava, in accordance with the published ethical principles and standards [44]. After two weeks of adaptation to our experimental facility, the animals were randomly assigned into two experimental groups (36 control rats and 35 rats with experimentally induced diabetes).

Induction of diabetes

Diabetes was induced by *intraperitoneal* injection of STZ (SERVA, Germany) in the dose of 65 mg/kg of body weight. The drug was dissolved in 0.1 M citrate buffer (pH 4.5). The volume of the applied substance was calculated for each animal according to individual body weight (maximal volume 1 ml per rat). The control rats received citrate buffer only. The level of glucose and development of diabetes was monitored by diagnostic test strips for urine glucose analysis (Gluko Phan, Pliva-Lachema, Czech Republic) one day after STZ administration.

Sample collection

On day 17 after STZ treatment the animals were killed after carbon dioxide anesthesia. Decapitation was performed over a 24 hr period in 4 hr intervals (6 animals per group per time point), beginning at 13.00 hr (Zeitgeber time (ZT) – 6 hr). During the dark-time sampling dim red light was used. Blood was collected into heparinized tubes, spined and plasma was stored at –18 °C until assay. Pineal glands and samples of pancreas, kidney, spleen duodenum and distal colon were excised and stored at –18°C until melatonin extraction.

Assays

Plasma glucose levels were measured by a commercially available kit for enzymatic determination of glucose in plasma (BIO-LA-Test, Pliva-Lachema, Czech Republic).

Melatonin concentrations were measured by radioimmunoassay (RIA) directly in plasma and after methanol and chloroform extraction in pineal glands and other tissues, respectively. Pineal glands were homogenized in 0.3 ml of methanol. After methanol evaporation the residues were dissolved in 0.1 M Tricine buffer (pH=5.5) (Sigma, USA) and stored at –18 °C until RIA. Tissue samples (*cca*. 100 mg of wet tissue) were homogenized in 0.8 ml of distilled water and extracted with chloroform. After centrifugation, water and lipid phases were aspirated and chloroform was evaporated under vacuum. The residues were dissolved in Tricine buffer and stored at –18 °C until assay. Efficiency of extraction was tested by adding ^{3}H-melatonin (2 500 DPM) into each sample and was about 60%. Melatonin RIA [10] was previously validated in our lab for rats [48]. We used sheep melatonin antiserum (G/S/704-6483, Stockgrand Ltd., Guildford, UK) and ^{3}H-labeled melatonin (specific activity of 3.07 TBq/mmol; Amersham Biosciences, UK).

Corticosterone concentrations in plasma were determined using commercially available RIA kit (DRG Instruments, Germany) according to the manufacturer's instructions.

Statistical analysis

Data for each group and time point are given as mean ± standard error of mean (S.E.M). The rhythmic pattern of data over the 24 hr cycle was statistically verified by cosinor analysis [27,21]. The results are given as mesor (the time series mean), amplitude (one-half the peak-trough difference expressed herein relative to the mesor) and acrophase (peak time referenced to the time of lights on in the animal facility). Statistical differences between two experimental groups were determined by unpaired Student t-test.

RESULTS

Development of diabetes after STZ treatment was confirmed by measuring glucose levels in urine during the experiment and in blood at the end of the experiment. As expected, glucose content in urine was increased after STZ treatment and plasma glucose levels in STZ-treated rats were higher ($p>0.001$) than in control rats (21.1 ± 2.8 mmol/l *vs.* 7.7 ± 0.3 mmol/l). Initially body weight was approximately the same in both groups (253 g ± 2 g), but 15 days after treatment it was decreased in diabetic rats in comparison with control animals (247 g ± 5 g *vs.* 295 g ± 4 g).

Melatonin and corticosterone concentrations in plasma exhibited the expected rhythmic pattern in control rats (Figure 1A, B; Table 1). Acrophases of both

Table 1. Melatonin rhythm assessed by cosinor analysis with 24 hr period in the pineal gland, plasma and selected peripheral tissues.

	Tissue [unites]	Group	Mesor	Amplitude	Acrophase [hr:min]	p-value
Melatonin	**Pineal gland** [pg/pineal gland]	**STZ**	452	313	20:14	0.001
		Control	807	746	19:43	0.001
	Plasma [pg/ml]	**STZ**	92	95	19:57	0.001
		Control	105	111	19:37	0.001
	Kidney [pg/g]	**STZ**	111	100	19:34	0.001
		Control	187	161	19:25	0.001
	Pancreas [pg/g]	**STZ**	98	44	19:33	0.001
		Control	126	72	18:56	0.001
	Spleen [pg/g]	**STZ**	57	29	19:54	0.001
		Control	66	24	18:28	0.01
	Duodenum [pg/g]	**STZ**	93	26	21:32	0.05
		Control	111	66	19:58	0.001
	Distal colon [pg/g]	**STZ**	133	/	/	ns
		Control	146	/	/	ns
Corticosterone	**Plasma** [ng/ml]	**STZ**	87	/	/	ns
		Control	20	18	12:39	0.001

The values of acrophases are given in Zeitgeber time (ZT 0 = dark to light transition). Abbreviations: ns - non significant; STZ-streptozotocin-treated rats.

rhythms were in antiphase. A substantial increase in corticosterone concentrations was found after diabetes induction (Figure 1B). Circulating melatonin concentration was reduced at the beginning of the dark period in diabetic rats in comparison with controls (Figure 1A; Table 1).

In addition to plasma, a distinct rhythmic profile of melatonin with higher hormone concentrations during the dark-time than during the light-time was found in the pineal gland, kidney, pancreas, spleen and duodenum of both control and STZ-treated rats (Table 1). The animals with experimental diabetes exhibited diminished melatonin production in the pineal gland in comparison with control animals (Figure 2A). Mesor (452 pg/pineal gland *vs.* 807 pg/pineal gland) and amplitude of the rhythm (313 pg/pineal gland *vs.* 746 pg/pineal gland) were substantially lower in STZ-treated rats in comparison with control rats (Table 1).

Reduced melatonin concentrations were determined in the kidney (Figure 2B), pancreas (Figure 2C) and duodenum (Figure 2E) of diabetic rats in comparison with controls. The decline was apparent in both mesor and amplitude of the melatonin rhythm (Table 1). Melatonin concentrations in the spleen displayed a daily rhythmic pattern (Figure 2D) and the values were the lowest of all tissues assayed. There were no significant differences between amplitude and mesor of melatonin rhythm between control and diabetic rats in this organ.

A strictly different pattern of melatonin concentrations in comparison with any other tissue analyzed was found in the distal colon (Figure 2F). Hormone concentrations in this organ did not exhibit rhythmic changes over the 24hr period and no significant differences were determined between control and diabetic rats (Table 1).

DISCUSSION

As expected, a circadian rhythm in melatonin concentrations was found in the pineal gland and plasma in both diabetic and control animals. A pronounced melatonin rhythm was detected in pancreatic tissue both under physiological conditions and after induction of diabetes. These data represent the first reported 24 hr profile of melatonin in this tissue.

The origin of melatonin in the pancreas has not yet been unequivocally established. Local melatonin biosynthesis is conceivable in this tissue. High expression of a key enzyme of melatonin synthesizing pathway was determined in the pancreas [17]. However, even in the case of local biosynthesis of melatonin in the pancreas, the local hormone production does not change the typical circadian profile of melatonin levels characterized by high hormone concentrations during the dark part of the day in the nocturnal rat. A similar profile, characterized by about 4-times higher night-time melatonin level as compared to the day-time level has been demonstrated in the pancreas of the diurnal chicken [11]. Thus the daily profile of melatonin concentration in the pancreas reflects rather actual environmental conditions (light/dark cycle) than feeding rhythmicity. The daily profiles of plasma glucose that reflect the feeding regimen are in antiphase in diurnal birds [12] and nocturnal rats [6].

Several research groups have suggested a mutual relationship between the pineal gland synthesizing melatonin and pancreatic β-cells producing insulin. Pinealectomy resulted in hyperglycemia [9,22] and prolonged melatonin administration decreased plasma insulin and leptin concentrations [35,46]. Suppressive

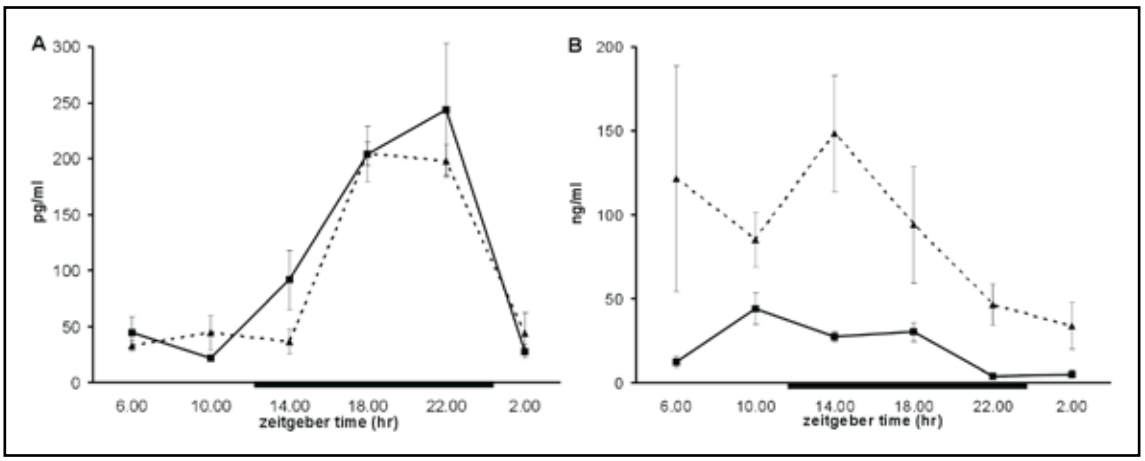

Figure 1. Circadian profile of melatonin (A) and corticosterone (B) levels in plasma of control and streptozotocin (STZ)-treated rats. The control group is represented by squares and solid line, STZ-treated group by triangles and broken line. Data are given as mean ± S.E.M. of each group (n=6, STZ-treated group at ZT 6.00 hr n=5). Black bar at the bottom of the graph represents the dark part of the day.

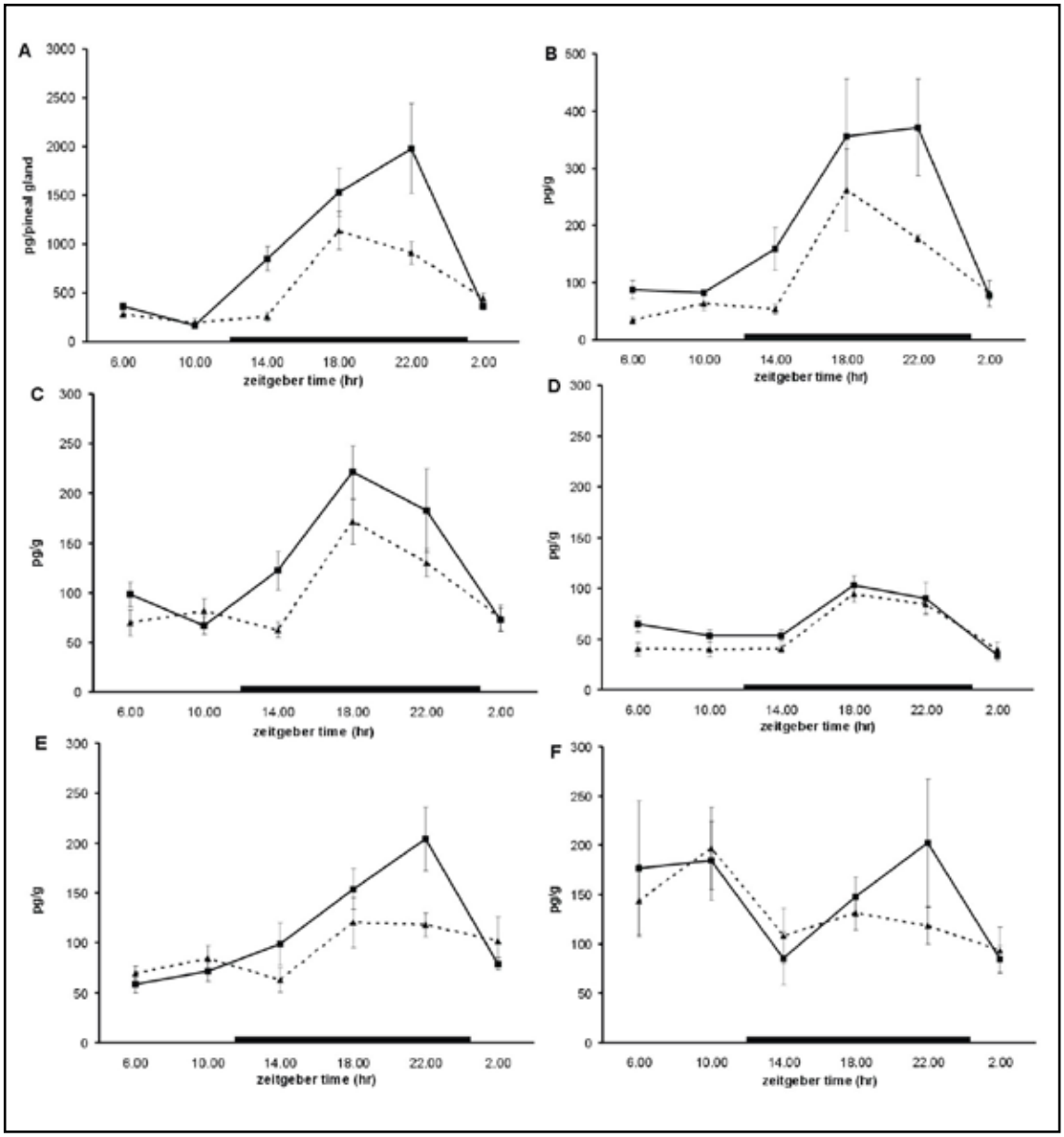

Figure 2. Circadian profile of melatonin levels in pineal gland (A), kidney (B), pancreas (C), spleen (D), duodenum (E) and distal colon (F) of control and streptozotocin (STZ)-treated rats. The control group is represented by squares and solid line, STZ-treated group by triangles and broken line. Data are given as mean ± S.E.M. of each group (n=6, STZ-treated group at ZT 6.00 hr n=5). Black bar at the bottom of the graph represents the dark part of the day.

effects of melatonin on insulin secretion were documented in spontaneously diabetic obese rats Otsuka Long-Evens Tokushima strain [29,28]. Melatonin declined hyperinsulinemia in these rats and improved the metabolism of lipids. Moreover, spontaneously diabetic rats exhibited decreased night-time melatonin plasma concentrations in comparison with control animals. Diminished plasma melatonin concentrations during night-time were recorded also in diabetic patients [32].

Our results are in accord with data reporting lowered night-time melatonin levels in diabetic patients and animals. However, we found a more apparent melatonin decline in the pineal gland and several tissues than in plasma. A mutual interrelationship between the pancreas and pineal gland in diabetes induced by STZ can be assumed. The duration of diabetes might be an important factor to observe declined melatonin levels since no differences were found in acute diabetes in rats [15,13].

Diminished melatonin concentrations in diabetic individuals may result from declined melatonin synthesis or higher metabolic turnover. Lower biosynthesis of melatonin is supported by the findings of lower activity of AA-NAT in spontaneously diabetic rats [32]. Lower dark-time melatonin concentrations in different organs

in our experiment suggest a role of increased degradation or utilization of this compound in diabetic individuals.

Lower levels of melatonin in the pancreas may influence some functions of this organ in diabetes. Thus, peripheral oscillators present in the pancreas might be disturbed [24]. We determined changed expression of clock genes in the liver and heart of diabetic rats [13], but the pancreas has not been studied so far in this respect. In addition to weakened oscillation capacity, reduced melatonin concentrations may contribute to lower protection of organs against reactive oxygen compounds [36].

The kidney is another organ expressing lower night-time melatonin levels in diabetic rats as compared with controls. A significant rhythm was observed both in control and diabetic rats, and the amplitude of the rhythm was declined in diabetic animals. Diabetic nephropathies are the most frequent complications of diabetes. Oxidation stress, induced by hyperglycemia, has been implicated in the development of diabetic nephropathies [20]. Night-time melatonin concentrations in patients with chronic renal failure were depressed [18]. Different antioxidants may protect the kidney against development of nephropathies [25] and recent studies demonstrated protective effects of melatonin [1,7]. Lower melatonin content in STZ-treated rats may contribute to lower antioxidant status of the diabetic kidney. Moreover, lower amplitude of intra-renal melatonin rhythm may result in lower synchronization of peripheral oscillators in the kidney [33]. Such oscillators were observed in the kidney [42] but there are no data about their activity in diabetes.

Circadian rhythm in melatonin concentrations was found in the spleen of both diabetic and control animals in our experiment, but neither the amplitude nor the acrophase of the rhythms differ. In comparison with other organs, the melatonin content in the spleen was considerably lower than in the pancreas and kidney. Several studies demonstrated the effect of melatonin on the immune system [8] but concentrations of the hormone have not been measured in the spleen before the present study. Melatonin may interact with the immune system on many levels and its central effects on cytokine synthesis have been suggested [3].

Melatonin was identified in the gastrointestinal tract (GIT) using a variety of methods including immunocytochemical, chromatographic and radioimmunoassay methods [4]. However, a daily pattern of melatonin concentration in the GIT of mammals has not been unequivocally established. Bubenik *et al.* [5] did not detect a rhythmic pattern of melatonin in the gut, while recent data reported a significant difference between day and night melatonin values in the duodenum [41,43]. Our present results showed a distinct circadian profile of this hormone in the duodenum. High levels were measured during the dark and low during the light part of the day. In parallel to other organs, also in the duodenum the melatonin content was significantly diminished in STZ-treated rats.

In the colon we failed to find any rhythmic pattern of melatonin levels and there were no clear differences between light-time and dark-time concentrations, nor between control and diabetic groups of animals. The absence of daily profile in the colon is of interest since it suggests a special function and/or metabolism of melatonin in this part of gut [23]. A protective role of melatonin in the colon was demonstrated in different independent studies, but the mechanism has not been elucidated yet [37].

Our previous study demonstrated a disturbed profile of selected clock gene expression in peripheral organs of rats during the acute phase of diabetes after STZ administration [13]. These data are in accord with other studies focusing on clock gene expression in diabetic rats [47]. The decreased amplitude of the melatonin rhythm in the organs studied in the present experiment may contribute to desynchronization of clock gene expression in peripheral organs of diabetic rats. Moreover, a lower content of melatonin in these tissues can contribute to a lower antioxidant status observed in diabetes.

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