

Effect of melatonin on the functioning of glutathione system in the liver of alloxan diabetic rats in lighting conditions around the clock

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Abstract. Insertion of melatonin for 7 days helped to reduce 1,7 times basal glucose level in the group of animals with overt diabetes. Activity of glucose-6-phosphate dehydrogenase, glutathione peroxidase and glutathione reductase in the liver of rats with overt diabetes was on 47%, 33%, and 35% respectively lower than in control rats that were under artificial equinox. In rat liver with overt and latent diabetes occurred reduction of reduced glutathione content on 25% and 41% respectively compared with those of control. Insertion of melatonin to diabetic rats helped in normalization of parameters.

Keywords: melatonin, alloxan diabetes, glutathione system, liver, rats.

Introduction. Melatonin is a lipophilic hormone produced by the Pineal gland during the night [3, 15]. It may act as a paracrine, intracrine and autocrine agent expressing an overall homeostatic function and pleiotropic effect. It is responsible for carrying out the following functions by controlling other hormones: 1) regulation of circadian sleep-wake cycle; 2) controls sex drive and reproduction by inhibiting release of GnTH (Gonadotrophic Hormone); 3) controls body weight and energy balance; 4) controls appetite; 5) controls metabolic function; 6) controls balance; 7) controls muscular coordination; 8) controls immune system when it is affected by bacterial and viral diseases, affected by chemical pollutants and in the presence of excessive radical activity; 9) antioxidant effects; 10) may reduce damage caused by types of Parkinson's disease; 11) prevent cardiac arrhythmia; 12) increase longevity; 13) prevents damage to DNA by some carcinogens. Melatonin production is inhibited when there is an increase in the light received by the retina while production is stimulated when there is a decrease in the light received by the retina (darkness stimulates production) [16]. Hence, during evenings, as the light received by the retina reduces melatonin production sets in, this evening onset is called the dim-light melatonin onset (DLMO). Exposure to light inhibits the enzyme N-acetyltransferase, the enzyme which converts Serotonin to Melatonin, hence reducing melatonin production. Being exposed to bright lights in the evening or too little light during the day can disrupt the body's normal melatonin cycles. For example, jet lag, shift work, and poor vision can disrupt melatonin cycles [4]. Most functions of melatonin are produced through activation of melatonin receptors, while other functions are carried out due to its pervasive and powerful antioxidant, with a particular role in protection of nuclear and mitochondrial DNA [5].

Alloxan and streptozotocin are toxic glucose analogues that preferentially accumulate in pancreatic beta cells via the GLUT2 glucose transporter. In the presence of intracellular thiols, especially glutathione, alloxan generates reactive oxygen species (ROS) in a cyclic redox reaction with its reduction product, dialuric acid. Autoxidation of dialuric acid generates superoxide radicals, hydrogen peroxide and, in a final iron-catalysed reaction step, hydroxyl radicals. These hydroxyl radicals are ultimately responsible for the death of the beta cells,

which have a particularly low antioxidative defence capacity, and the ensuing state of insulin-dependent 'alloxan diabetes'. As a thiol reagent, alloxan also selectively inhibits glucose-induced insulin secretion through its ability to inhibit the beta cell glucose sensor glucokinase. Following its uptake into the beta cells, streptozotocin is split into its glucose and methylnitrosourea moiety. Owing to its alkylating properties, the latter modifies biological macromolecules, fragments DNA and destroys the beta cells, causing a state of insulin-dependent diabetes. The targeting of mitochondrial DNA, thereby impairing the signalling function of beta cell mitochondrial metabolism, also explains how streptozotocin is able to inhibit glucose-induced insulin secretion [11].

Melatonin influences insulin secretion both in vivo and in vitro. (i) The effects are MT(1)- and MT(2)-receptor-mediated. (ii) They are specific, high-affinity, pertussis-toxin-sensitive, G(i)-protein-coupled, leading to inhibition of the cAMP-pathway and decrease of insulin release. Furthermore, melatonin inhibits the cGMP-pathway, possibly mediated by MT(2) receptors. In this way, melatonin likely inhibits insulin release. A third system, the IP(3)-pathway, is mediated by G(q)-proteins, phospholipase C and IP(3), which mobilize Ca(2+) from intracellular stores, with a resultant increase in insulin. (iii) Insulin secretion in vivo, as well as from isolated islets, exhibits a circadian rhythm. This rhythm, which is apparently generated within the islets, is influenced by melatonin, which induces a phase shift in insulin secretion. (iv) Observation of the circadian expression of clock genes in the pancreas could possibly be an indication of the generation of circadian rhythms in the pancreatic islets themselves. (v) Melatonin influences diabetes and associated metabolic disturbances. The diabetogens, alloxan and streptozotocin, lead to selective destruction of beta-cells through their accumulation in these cells, where they induce the generation of ROS. Beta-cells are very susceptible to oxidative stress because they possess only low-antioxidative capacity. Results suggest that melatonin in pharmacological doses provides protection against ROS. (vi) Finally, melatonin levels in plasma, as well as the arylalkylamine-N-acetyltransferase (AANAT) activity, are lower in diabetic than in nondiabetic rats and humans. In contrast, in the pineal gland, the AANAT mRNA is increased and the insulin

receptor mRNA is decreased, which indicates a close interrelationship between insulin and melatonin.

It acts as an antioxidant, neutralizing harmful oxidative radicals, and it is capable of activating certain antioxidant enzymes [3]. It is a powerful antioxidant that easily crosses the cell membranes and blood-brain barrier [10, 17]. It acts as a direct scavenger of OH⁻, O₂, and NO.

Oxidative stress plays a pivotal role in the development of diabetes complications, both microvascular and cardiovascular [1]. The increase in glycoxidation and lipoxidation products in plasma and tissue proteins suggests that oxidative stress is increased in diabetes [6, 9].

Exogenous melatonin normalizes impaired due alloxan diabetes and tetrachlormethane hepatitis glucose-6-phosphatase activity in rat liver [19].

It has been ascertained that an alloxan monohydrate administration to rats results in a significant elevation of the level of basal glycemia in the blood, and an increase of the activities of lactate dehydrogenase and glucose-6-phosphatase in the liver, however a decrease of the glycogen content and the activity glucose-6-phosphate dehydrogenase was in a direct dependence on the presence of hyperglycemia. The established changes of the indices of the carbohydrate metabolism in animals with alloxan diabetes turned out to be more marked under the conditions of permanent lighting than with equinox or permanent darkness. With a 7-day introduction of a higher dose of melatonin an improvement of the state of carbohydrate metabolism was marked and that was accompanied with a normalization of the indices under study, apart from the activities of glucose-6-phosphatase in the liver which is normalized in case of a 42-day administration that was also characterized by a normalization of the level of glycosylated hemoglobin in the rats blood [18].

The aim was to determine the influence of melatonin on basal levels of glucose, reduced glutathione (GSH), activity of glucose-6-phosphate dehydrogenase (G6PD), glutathione peroxidase (GPx) and glutathione reductase (GR) in the liver of alloxan diabetic rats under conditions of constant light.

Material and methods. The experiments were carried out on 60 sexually mature male albino, not thoroughbred rats with the body mass – 0,18-0,20 kg. Alloxan diabetes was evoked via injecting the rats with a 5% solution of alloxan monohydrate intraperitoneally in a dose of 170 mg/kg following a 24 hour period of fasting [11]. The melatonin preparation was used in the research (the manufacturer – “Sigma”, USA). The animals were divided into 6 subgroups: 1) rats (the control group) that were under artificial equinox (Light:Darkness=12:12); 2) rats that were under conditions of constant light (L:D=24:0); 3) alloxan diabetic rats (L:D=24:0); 4) alloxan diabetic animals which were introduced the melatonin preparation intraperitoneally in a dose of 10 mg/kg at 8 a. m. daily during 7 days starting with a 5-th 24 hour period after the injection of alloxan (L:D=24:0); 5) alloxan diabetic rats with latent (basal glycemia < 6,9 mmol/l) diabetes (L:D=24:0); 6) rats with latent diabetes which were introduced the melatonin preparation intraperitoneally in a dose of 10 mg/kg at 8 a. m. daily during 7 days starting with a 5-th 24 hour period after the injection of alloxan (L:D=24:0). Blood was taken from

the tail vein evaluate the BG level with the use of One Touch Ultra (LifeScan, USA). On the third day the death of a part (50%) of the alloxan diabetic animals was observed. Rats were sacrificed at the twelfth day of the experiment accordance with the ethical treatment of animals. Liver tissue immediately after decapitation took the cold and refrigerated prepared homogenates in 50 mM Tris-HCl buffer (pH=7.4). Determinations of GSH, activity G6PD, GPx and GR were by standard methods [8]. Statistical analysis of results was conducted by Student’s test. Sufficient level considered probability differences $r \leq 0,05$.

Results. Staying animals in lighting conditions (figure 1) around the clock throughout the week was accompanied by a tendency to increase in basal blood glucose by 10% from baseline this indicator on the 4 th day of the experiment. Insertion of melatonin for 7 days helped to reduce (but not normalization as in the previous series of experiments under conditions of constant darkness and equinox) [7, 18] 1,7 times compared with the baseline, basal glucose level in the group of animals with overt diabetes, indicating its hypoglycemic action but less pronounced.

The prevalence of diabetes has exponentially increased in recent decades due to environmental factors such as nocturnal lifestyle and aging, both of which influence the amount of melatonin produced in the pineal gland. [14].

Activity (table 1) of G6PD, GPx and GR in the liver of rats with overt diabetes was on 47%, 33%, and 35% respectively lower than in control rats that were under artificial equinox.

Probable reduction of melatonin synthesis and secretion under conditions of constant illumination coupled with reduced sensitivity to insulin, reduces the activity (table 1) of G6PD in control rats and rats with diabetes. Under these conditions there was no typical increase in activity of G6PD in the group of animals with latent diabetes [18], but rather there was a decline of this indicator compared with those of control rats, provided equinox.

In the liver of rats with latent diabetes activities of G6PD, GPx and GR were on 22%, 15% and 20% respectively lower than in control animals. In rat liver with overt and latent diabetes occurred reduction of G-SH on 25% and 41% respectively compared with those of control. We know [3] that pinealectomy, same as its hypofunction caused by permanent lighting, leading to decreased synthesis and secretion of melatonin, which causes insulin resistance and reduce the gene expression of glucose transporter GLUT 4, 2, 1. It is logical that the activity of G6PD is reduced under conditions of constant illumination during diabetes mellitus, whether an administration of melatonin leads to increased its activity.

Under the influence of melatonin increase (on 20% than in control) activity of G6PD in the liver of rats may be due to the increasing number of substrate for G6PD (stimulating the flow of glucose into cells and its phosphorylation) and direct action [14].

Insertion of melatonin to diabetic rats helped in normalization of parameters that we studied. According to our investigations the introduction of melatonin intraperitoneally in a dose of 10 mg/kg at 8 a. m. daily during 7 days to alloxan diabetic rats under

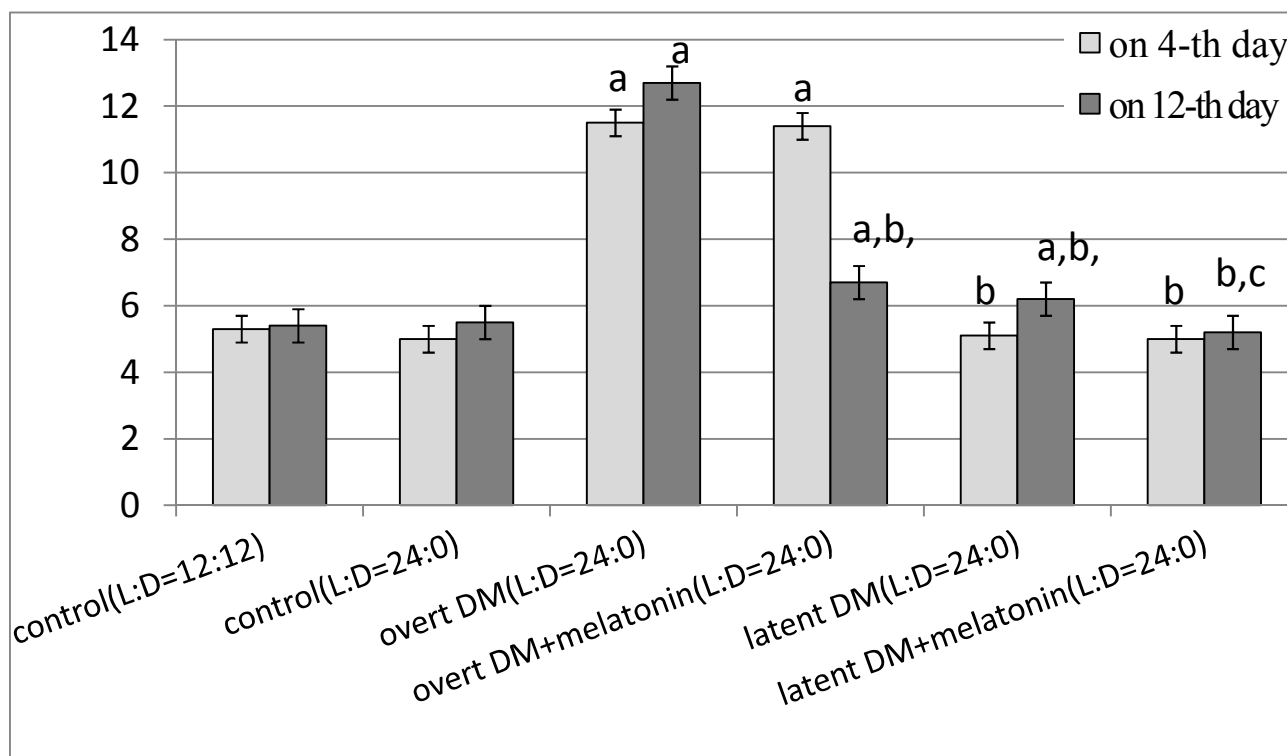


Fig. 1. The level of basal glycemia (mmol/l) in blood of rats, (n=6, $\bar{x} \pm Sx$): 1. a, b, c - changes are reliable ($p \leq 0,05$). 2. a - concerning intact rats ; b - concerning rats with overt diabetes; c – concerning rats with latent diabetes; d – concerning indices on 4-th day.

Table 1. Influence of melatonin on the indices of glutathione system in the liver of alloxan diabetic rats ($\bar{x} \pm Sx$, n=6)

Indexes	Glucose-6-phosphate dehydrogenase, nmol / min×mg	Glutathione reductase, nmol / min×mg	G-SH, mkmol/g tissue	Glutathione peroxidase, nmol / min×mg
Control group (L:D=12:12)	6,5±0,18	4,4±0,22	7,0±0,42	155,8±12,4
Control group (L:D=24:0)	5,8±0,25 ^a	4,3±0,25	6,9±0,45	152,5±11,1
Overt diabetes (L:D = 24:0)	3,4±0,46 ^a	2,9±0,27 ^a	4,1±0,30 ^a	104,4±12,0 ^a
Overt diabetes+melatonin (L:D=24:0)	6,6±0,55 ^b	4,7±0,32 ^b	6,7±0,41 ^b	150,2±10,3 ^b
Latent diabetes (L:D=24:0)	5,0±0,52 ^{a,b}	3,5±0,30 ^{a,b}	5,3±0,40 ^{a,b}	132,4±12,1
Latent diabetes + melatonin (L:D=24:0)	7,7±0,26 ^{a,b,c}	4,5±0,26 ^{b,c}	7,1±0,40 ^{b,c}	160,0±9,0 ^b

1. a, b, c - changes are reliable ($p \leq 0,05$).
2. a - concerning intact rats ;
b - concerning rats with overt diabetes;
c – concerning rats with latent diabetes.

conditions of constant light is conducive to a decrease in them of the level of fasting glucose, as well as – a stabilization of the indices of the body’s antioxidant defense (glucose-6-phosphate dehydrogenase, glutathione peroxidase, glutathione reductase and reduced glutathione in liver) disturbed under the conditions of an absolute deficit of insulin.

An elevated oxidative status in the aging organism may be involved in the development of non-insulin dependent

diabetes mellitus (NIDDM). Melatonin, a potent antioxidant agent, is essential for glucose homeostasis and regulation. It was determined [13] the influence of melatonin supplementation on the oxidative stress parameters in elderly NIDDM patients. The malondialdehyde (MDA) concentration, Cu-Zn superoxide dismutase (SOD-1) activity in erythrocytes, the level of nitrate/nitrite in plasma and morning melatonin concentration and oxidase activity of ceruloplasmin (Cp)

in serum in 15 elderly NIDDM patients at baseline and after the 30 days of melatonin supplementation (5 mg daily) in comparison with levels in 15 healthy elderly volunteers were determined. A significant increase of MDA level and decrease of SOD-1 activity and melatonin concentration were observed in NIDDM patients. Cp oxidase activity and nitrate/nitrite level were similar in both examined groups. Melatonin administration in NIDDM patients resulted in a significant increase in the morning melatonin concentration and SOD-1 activity, and a reduction in the MDA level and Cp oxidase activity. Statistically significant alterations in nitrate/nitrite levels were not observed. These results indicate an improvement of antioxidative defense after melatonin supplementation in the NIDDM individuals and suggest melatonin

supplementation as an additional treatment for the control of diabetic complications [13].

It was detected, that melatonin stimulates glucose transport to skeletal muscle cells via insulin receptor substrate-1/phosphoinositide 3-kinase (IRS-1/PI-3-kinase) pathway, which implies, at the molecular level, its role in glucose homeostasis and possibly in diabetes. Additionally, exposure to light at night and aging, both of which lower endogenous melatonin levels may contribute to the incidence and/or development of diabetes [12, 14].

Conclusion. Under conditions of permanent light exogenous melatonin activates antioxidant glutathione dependent enzymes in the liver of alloxan diabetic rats that ultimately provides increased content of G-SH – one of the main endogenous antioxidant.

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Влияние мелатонина на функционирование глутатионовой системы в печени крыс с аллоксановым диабетом в условиях круглосуточного освещения

И. Н. Яремий, А. Ю. Кушнир, К. А. Харченко

Аннотация. Введение мелатонина в течение 7 дней способствовало уменьшению в 1,7 раза базального уровня глюкозы в группе животных с явным диабетом. Активность глюкозо-6-фосфатдегидрогеназы, глутатионпероксидазы и глутатионредуктазы в печени крыс с явным диабетом была на 47%, 33% и 35% соответственно ниже, чем у контрольных крыс, которые были при искусственном равноденствия. В печени крыс с явным и скрытым диабетом произошло снижение содержания глутатиона восстановленного на 25% и 41% соответственно по сравнению с соответствующим контролем. Введение мелатонина диабетическим крысам помогло в нормализации показателей.