Effect of Prolonged Melatonin Administration on Metabolic Parameters and Organ Weights In Young Male and Female Sprague-Dawley Rats

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Abstract

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The question of the introduction of melatonin as a drug remains still open. Especially the long term melatonin administration have to be analysed and discussed. The aim of present study was to analyze the effect of low doses of melatonin (4 μg/ml of tap water) administered for 70 days, daily from 15.00 to 08.00 h (from 08.00 to15.00 h animals were drinking tap water) on selected metabolic parameters in male and female Sprague-Dawley rats. In addition to concentration/content of triacylglycerols, phospholipids, cholesterol, malondialdehyde, glucose and glycogen in the serum and tissues, serum corticosterone and insulin, weights of selected organs, periovarial and epididymal fat and body weight were recordered. Melatonin was not administered to control groups. Oral glucose tolerance tests were carried out before melatonin administration and 9 weeks thereafter.

Male and female rats aged 5 weeks were adapted to standard vivarium conditions and artificial light regimen L:D-12:12 h. The animals were fed MP diet containing 2.5 % fat at least and drank tap water or melatonin solution *ad libitum*. The rats were weighed twice a week and food and water intake was recorded. After 10 weeks they were sacrificed, organs and tissues were weighed and the aforementioned metabolic parameters were determined in the serum, liver, heart muscle and bone marrow (femur).

Melatonin administration decreased significantly the serum triacylglycerol concentration and liver glycogen content in male rats, and it increased liver the phospholipid content in females. Melatonin did not acutely modify the values of other metabolic parameters. Prolonged melatonin administration significantly increased the weight of heart muscle and periovarial fat in females, in males significantly reduced the weight of adrenals, liver, heart muscle and epididymal fat as well as their body weight from day 19 to the end of experiment. Body weight in MEL-drinking females was similar to that in controls. Water and food intake in MEL-drinking males did not differ from controls, in females was temporarily increased in week 6.

Prolonged melatonin administration did not significantly influence the glycemia level. The oral glucose tolerance test curves were normal before melatonin administration; one abnormal curve (out of 9 individuals) in the female group and 2 abnormal curves in the male group (out of 9 individuals) were recorded 9 weeks after melatonin administration. Prolonged melatonin treatment resulted in sexual differences in body weight, epididymal and periovarial fat, and heart muscle weight.

Melatonin, metabolic and hormone responses, body and organ weights

Although pineal gland is the main site of melatonin (MEL) synthesis in mammals its synthesis has been proved in the retina, extraorbital lacrimal glands, Harder's glands, bone marrow cells and in many other tissues, too. The MEL synthesis in pineal gland is under light control and is distinctly circadian: MEL levels are low during the light and rise during the night. The rhythm of MEL synthesis is under control of hypothalamic suprachiasmatic nucleus and is synchronised by circadian light:dark rhythm. The length of MEL pulse in the dark transfers the information on the length of light and dark part of the day and on the season

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in the organism, so it functions as a daily (for all animal species) and annual (for photoperiodic species with seasonal reproduction) calendar (Yu and Reiter 1993; Vijayalaxmi et al. 2002).

The MEL effects are mediated through specific highly affinitive receptors localized in the plasmatic membrane and are associated with GTP-binding proteins (ML1, ML2) or nuclear receptors (orphan ROR-RZR receptors); in some cases MEL can act without receptors, too (Dubocovich 1988; Becker -André et al. 1994).

A significant factor of endogenous MEL availability is the age. In all species studied so far the aging is associated with progressive reduction of circadian MEL synthesis in pineal gland. Equally the onset of many degenerative and proliferative diseases is associated with aging; what remains unclear is whether the increase of these diseases is related to reduced antioxidative protection potentially provided by MEL (Reiter 1992).

MEL participates in many important physiological functions including thermoregulation (Saarela and Reiter 1994), neuroimmunomodulation in rodents as well in humans (Maestroni 1993), displays analgetic effects (Ebadi et al. 1998), decreases both the incidence and growth of spontaneous and induced tumors in animals (Hill and Blask 1988), acts as an efficient antioxidant *in vitro* (Tan et al. 1993a) as well as *in vivo* (Tan et al. 1993b), and is a potential cardioprotective agent (Lagneux et al. 2000).

Although MEL is known to affect body weight, adiposity and food intake in seasonal animals (Wade and Bartness 1984), the mechanism of MEL impact on energetic metabolism in mammals is not well known so far. As the liver functions as the center of the intermediary metabolism it is more than possible that metabolic effects of MEL interfere with the liver metabolism. It has been proved that MEL also effects the kidney metabolism, urine production (Richardson et al. 1992), and blood pressure (Kawashima et al. 1987).

The role of epiphysis and MEL in glucose metabolism has been explored. In male Wistar rats pinealectomy increased night glucose levels and its average daily concentration but did not influence the average daily insulin concentration in comparison with controls. MEL administration to pinealectomized animals adjusted the average daily glucose concentration but did not adjust its 24-h rhythm (Fleur et al. 2001). MEL is assumed to act directly on target cells e.g. hepatocytes and pancreatic b-cells (Acuna-Castroviejo et al. 1994; Peschke et al. 2000) containing MEL-binding elements but it is possible that MEL and epiphysis affects glucose metabolism through suprachiasmatic hypothalamic nucleus activity modulation (Fleur et al. 2001). In rats pinealectomy decreased liver and muscle glycogenesis (Milcu et al. 1971), increased plasma glucose and glucagon concentration and decreased plasma insulin concentration (Diaz and Blazquez 1986). Lima et al. (1998) recorded glucose intolerance and impairment of secretion and effects of insulin after pinealectomy. The effects of melatonin administration to laboratory animals are controversial. In some studies MEL did not influence insulin secretion and glucose metabolism (Frankel and Strandberg 1991; Bizot-Espiard et al. 1998), in others either its inhibitory (Bailey et al. 1974) or stimulatory effects on the aforementioned processes (Iizuka 1996) were recorded.

We regard the cholesterol decreasing effect of MEL to be of great importance. Ayoama et al. (1988) have found that long-term MEL administration significantly decreased the plasma cholesterol and prevented fat liver in genetically hypercholesterolemic rats. In rats MEL prevented hyperlipemia caused by glucocorticoids administration (Ayoama et al. 1987) or by high cholesterol food feeding (Mori et al. 1989) but did not prevent hypercholesterolemia in old rats (Vaughan et al. 1982).

The aim of our study was to contribute to current knowledge on effects of prolonged MEL administration on certain metabolic parameters and organ weights in Sprague-Dawley rats of both sexes.

Materials and Methods

Male and female Sprague-Dawley rats (SD) (obtained from Central vivarium, Faculty of Medicine, P. J. Šafárik University, Košice, Slovak Republic) aged 36-37 days, weighing 115-140 g were used in the experiment. The animals were kept under standard vivarium conditions (temperature 23 ± 2 °C, relative humidity 60-70%), the light:dark regimen with 12 h of light and 12 h of darkness, LD 12:12, with lights on at 07:00 h, with intensity 150 lux per cage (TESLA, fluorescent lamps, 40W). The rats were fed MP diet containing 2.5 % fat at least (Top-Dovo, Dobrá Voda, Slovak Republic), drank tap water and MEL solution, respectively *ad libitum*. Three to five animals were housed per cage.

MEL (Sigma, Deisenhofen, Germany) was administered in tap water in a concentration of 4 μg/ml discontinuously for the period of 2.5 months, daily from 15.00 to 08.00 h (from 08.00 to 15.00 h the animals were drinking tap water). Ten mg of MEL were dissolved in 0.2 ml of 30% ethanol and mixed up with tap water to concentration of 4 μg/ml. The solution of MEL was freshly prepared three times a week. The bottles with MEL solution were covered with a dark foil. The drinking water of control groups contained 0.01% ethanol.

The animals were divided into 4 groups:

1. MEL-drinking females (MEL-females), n (the number of animals) = 9
2. control females (intact animals, INT-females), n = 6
3. MEL-drinking males (MEL-males), n = 9
4. control males (intact animals, INT-males), n = 6

The rats were weighed twice a week; in 6th and 10th week of experiment their food and water intake was observed. After 10 weeks following overnight fasting the animals were sacrificed by quick decapitation between 08.00-11.00 h; liver, heart muscle, spleen, thymus, adrenal glands and white fat (periovarial and epididymal) were weighed. The following metabolic parameters were analyzed in the serum, liver, heart muscle and bone marrow (femur): concentrations of glucose, triacylglycerols, cholesterol, phospholipids, corticosterone and insulin were determined in the serum from mixed blood. In the liver, the concentrations/contents of glycogen, triacylglycerol, cholesterol, phospholipids and malondialdehyde were measured. The concentration of triacylglycerols, phospholipids and malondialdehyde was determined in the bone marrow. In the heart muscle, glycogen concentration/content was measured. Phospholipids were measured from lipid phosphorus according to Bartlett (1959), cholesterol according to Zlatkis et al. (1953), glycogen according to Roe and Dailey (1966), malondialdehyde was measured in reaction with thiobarbituric acid (Satch 1978), for triacylglycerol and glucose measurement commercial sets of Lachema (Brno, Czech Republic) were used, insulin was determined by RIA with the use of commercial set of Linco Research (St. Charles, MO, USA), corticosterone was measured using fluorimetry according to Guillemin et al. (1958).

Results were evaluated by one-way analysis of variance and Kruskal-Wallis test. The criterion for the choice of relevant test was the value of Bartletts´ number. Data are presented as means standard error of the mean (S.E.M.) and significant differences between groups as: * * * for $p \leq 0.001$; * * for $p \leq 0.01$; * for $p \leq 0.05$.

Oral glucose tolerance tests (OGTTs) were carried out before MEL application and 9 weeks after its administration as follows: 1 g of glucose per 1 kg body weight in 20% glucose solution was administered intragastrically to animals following overnight fasting. Twenty-five μl of the blood were collected from rat tail vein before glucose administration and 15, 30, 60 and 120 minutes after glucose load. Blood glucose concentration was determined enzymatically using commercial sets of Lachema (Brno, Czech Republic) and presented as graphs. The glycemic curve was considered abnormal when its value in minute 120 exceeded the initial value by 20% (fasting) and more. The experiment was carried out from May to July.

Results

MEL administration significantly decreased the concentration of serum triacylglycerols and liver glycogen content in male rats and increased liver phospholipid content in females. The effect of MEL on other metabolic parameters observed in the serum and tissues was not significant (Table 1). MEL significantly increased the absolute and relative weights of the heart muscle in female rats. In males, MEL significantly reduced absolute weights of adrenal glands, liver and heart muscle but did not alter the weights of thymus and spleen when compared to intact rats (Table 2). Prolonged administration of MEL significantly influenced the weight of white fat. In female rats, absolute and relative weights of periovarial fat were markedly increased. On the contrary, absolute and relative weights of epididymal fat in males were reduced (Table 2). MEL significantly reduced the body weight of male rats from day 19 to the end of the experiment (70 days) (except for day 28 and 34 of the experiment) with more prominent difference between groups on day 62 and 66. Body weight of MEL-drinking females was similar to that of controls (Fig. 1). MEL increased transitorily food and water intake in females (it was on the border of significance) in week 6 of the experiment.

Table 1
The effect of 2.5-months long MEL administration on selected metabolic parameters

	INT-female rats n = 6	MEL-female rats n = 9	INT-male rats n = 6	MEL-male rats n = 9
Serum				
GLU (mmol/l)	5.42 ± 0.23	5.78 ± 0.34	4.03 ± 0.43	4.20 ± 0.16
TAG (mmol/l)	0.26 ± 0.04	0.32 ± 0.03	0.50 ± 0.10	0.22 ± 0.03 * *
CH (mmol/l)	0.87 ± 0.12	0.98 ± 0.05	0.74 ± 0.03	0.79 ± 0.03
PL (mmol/l)	1.60 ± 0.28	1.80 ± 0.14	0.99 ± 0.06	0.99 ± 0.17
CTS (pmol/ml)	289.00 ± 36.86	317.90 ± 85.89	286.10 ± 49.29	332.36 ± 68.03
INS (ng/ml)	0.20 ± 0.02	0.16 ± 0.03	0.14 ± 0.02	0.14 ± 0.02
Liver				
GLY (m mol/g)	2.89 ± 0.73	2.76 ± 0.24	3.68 ± 0.45	2.75 ± 0.31
GLY (m mol)	20.65 ± 4.04	18.41 ± 1.46	37.20 ± 3.80	24.80 ± 2.79 *
TAG (m mol/g)	27.55 ± 2.82	36.82 ± 4.17	19.37 ± 4.77	22.16 ± 1.62
TAG (m mol)	173.48 ± 20.70	244.68 ± 23.84	202.32 ± 51.81	203.08 ± 20.79
CH (m mol/g)	16.33 ± 0.15	16.56 ± 0.67	16.42 ± 0.98	17.27 ± 0.30
CH (m mol)	100.95 ± 2.89	109.23 ± 3.47	168.30 ± 12.32	156.53 ± 6.52
PL (m mol/g)	46.62 ± 1.00	52.57 ± 2.50	46.43 ± 2.57	48.97 ± 0.92
PL (m mol)	285.37 ± 9.09	349.66 ± 22.65 *	475.78 ± 30.75	444.24 ± 18.24
MDA (nmol/g)	20.34 ± 1.47	20.90 ± 3.32	20.08 ± 1.82	23.69 ± 3.48
MDA (nmol)	127.09 ± 11.62	138.64 ± 21.14	207.73 ± 23.41	220.25 ± 35.34
Bone Marrow				
TAG (m mol/g)	16.73 ± 1.27	27.91±4.49	51.86 ± 10.94	29.40 ± 7.38
PL (m mol/g)	13.41 ± 2.03	14.58±0.76	12.87 ± 1.16	13.18 ± 1.30
MDA (nmol/g)	71.45 ± 9.75	53.37±11.79	22.78 ± 2.07	26.09 ± 1.63
Heart Muscle				
GLY (m mol/g)	5.55 ± 1.59	5.52 ± 0.73	8.01 ± 0.98	6.85 ± 0.98
GLY (m mol)	3.47 ± 0.93	3.86 ± 0.55	8.61 ± 0.88	6.43 ± 1.09

Data in Table 1 are expressed as means ± SEM, significant differences between groups are designated as: * * for $p \leq 0.01$; * for $p \leq 0.05$ (MEL-group vs INT-group). Abbreviations: INT-intact, MEL-melatonin, GLU-glucose, GLY-glycogen, TAG-triacylglycerols, CH-cholesterol, PL-phospholipids, MDA-malondialdehyde, CTS-corticosterone, INS-insulin, n-number of animals.

In week 10, differences in food and water intake between groups were not significant. Food and water intake in MEL-drinking males did not significantly differ from the intact ones (Table 3). The average daily MEL intake was 119.36 m g in females and 161.60 m g in males. Before MEL administration, OGTT curves had a physiological course in males and females. One abnormal curve (out of 9 individuals) in females and 2 abnormal curves in males (out of 9 individuals) were recorded 9 weeks after MEL administration (Fig. 3). All curves in control males and females had a physiological course (Fig. 2).

Discussion

There are rather few studies dealing with metabolic effects of exogenous MEL in young rats. Summarising the results of our experiment, only small metabolic changes after 2.5 months of MEL administration to young SD rats could be found. As to carbohydrate metabolism, the liver glycogen content in males was decreased probably due to increased

Table 2
The effect of 2.5-months long MEL administration on weights of selected organs and tissues

	INT-female rats n = 6	MEL-female rats N = 9	INT-male rats n = 6	MEL-male rats n = 9
Liver				
absolute (g)	6.18 ± 0.14	6.63 ± 0.21	10.23 ± 0.28	9.09 ± 0.39 *
relative (%)	2.96 ± 0.04	3.09 ± 0.12	2.74 ± 0.06	2.82 ± 0.07
Heart Muscle				
absolute (mg)	622.50 ± 21.39	690.56 ± 14.78 *	1090.83 ± 38.50	916.89 ± 27.01 * *
relative (%)	0.30 ± 0.003	0.32 ± 0.009 *	0.29 ± 0.007	0.29 ± 0.006
Spleen				
absolute (mg)	461.67 ± 22.19	468.33 ± 20.58	690.17 ± 35.96	653.33 ± 22.22
relative (%)	0.20 ± 0.01	0.22 ± 0.01	0.19 ± 0.008	0.20 ± 0.006
Thymus				
absolute (mg)	246.00 ± 28.71	284.89 ± 19.57	291.17 ± 14.90	241.67 ± 22.52
relative (%)	0.11 ± 0.02	0.13 ± 0.009	0.08 ± 0.003	0.07 ± 0.007
Adrenals				
absolute (mg)	56.00 ± 2.45	53.33 ± 1.20	49.20 ± 2.78	41.00 ± 2.12 *
relative (%)	0.03 ± 0.002	0.02 ± 0.002	0.01 ± 0.002	0.01 ± 0.001
Periovarial Fat				
absolute (g)	1.40 ± 0.27	2.46 ± 0.23 * *	–-	–-
relative (%)	0.66 ± 0.11	1.13 ± 0.09 * *	–-	–-
Epididymal Fat				
absolute (g)	–-	–-	3.67 ± 0.11	2.36 ± 0.21 * * *
relative (%)	–-	–-	0.98 ± 0.04	0.73 ± 0.05 * *

Data in Table 2 are expressed as means ± SEM, significant differences between groups are designated as: * * * for $p \leq 0.001$; * * for $p \leq 0.01$; * for $p \leq 0.05$ (MEL-group vs INT-group). Relative weight (%) = absolute weight (g or mg)/ body weight ¥ 100. MEL-melatonin, INT-intact, n-number of animals.

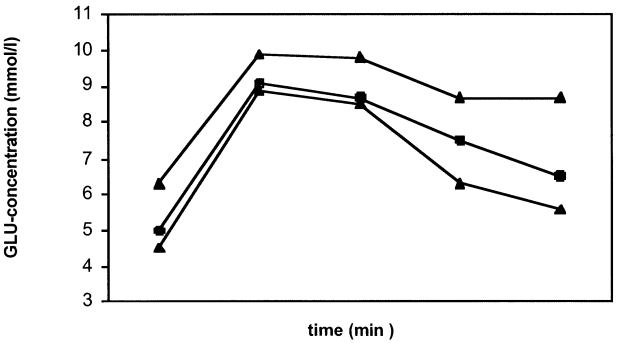

Fig. 1. The effect of 2.5-month long MEL administration on body weight of male and female Sprague-Dawley rats. Data in Figure 1 are expressed as means ± SEM. Significant differences between groups are designated as: * * for $p \leq 0.01$; * for $p \leq 0.05$.

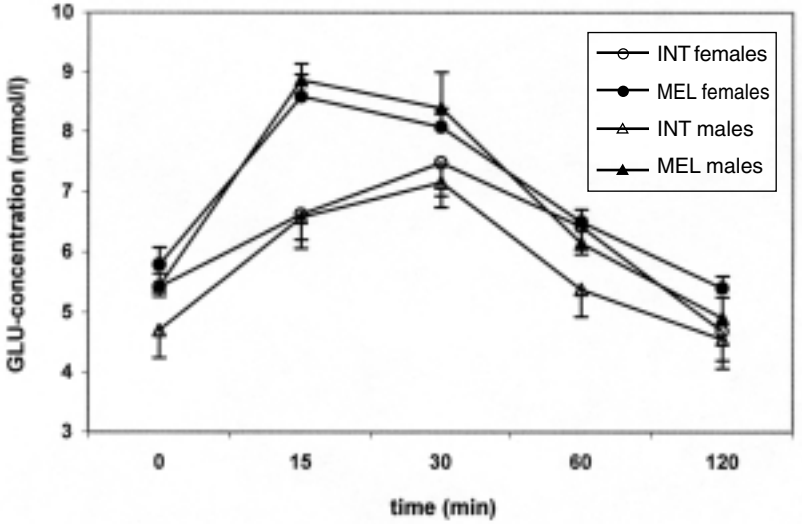

Fig. 2. The physiological course of average OGTTs in male and female SD rats.
Data in Fig. 2 are expressed as means ± SEM. Abbreviations: GLU-glucose, INT-intact, MEL-melatonin.

activity of glycogen phosphorylase recorded by Mustonen et al. (2002) in MEL males of Wistar rats. The serum glucose and insulin concentrations were not influenced. As to lipid metabolic parameters, the serum triacylglycerol concentration in males was reduced, the liver phospholipid content in females was increased, the serum and liver cholesterol levels in both males and females were not influenced as well as tissue malondialdehyde concentration. The effect of MEL on glucose tolerance test was not prominent. Prior to MEL administration the course of OGTTs in rats of both sexes was within the physiological range,

Table 3
The effect of MEL administration on water/ MEL solution and food intake

Experimental group	Daily water/ MEL solution intake per rat (ml)		Daily food intake per rat (g)	
	in week 6	in week 10	in week 6	in week 10
INT-female rats	26.47 ± 1.09	31.20 ± 1.69	18.73 ± 0.64	20.93 ± 1.08
MEL-female rats	30.08 ± 1.05	29.60 ± 1.44	20.90 ± 0.53 *	19.97 ± 1.06
INT-male rats	43.40 ± 0.76	39.67 ± 0.92	27.00 ± 1.74	26.20 ± 0.92
MEL-male rats	38.70 ± 1.41	42.10 ± 1.62	24.10 ± 0.79	26.20 ± 0.74

Data in Table 3 are expressed as means ± SEM, significant differences between groups are designated as: * for $p \leq 0.05$ (MEL-group vs INT-group). Water/MEL solution and food intake were measured during 24-hour period.

after 9 weeks of MEL administration the deviations from normal values were recorded only in 1 female and 2 males. In the group of MEL males a significant decrease of absolute liver weight connected probably with liver glycogen content decrease and absolute adrenal weight decrease which we do not know to explain were recorded. The absolute heart muscle weight decrease in MEL males and on the other hand the increase of the absolute and relative heart muscle weight in MEL females could be partially caused by the lipid content changes those were not measured in this organ. Continuous MEL administration increased the liver

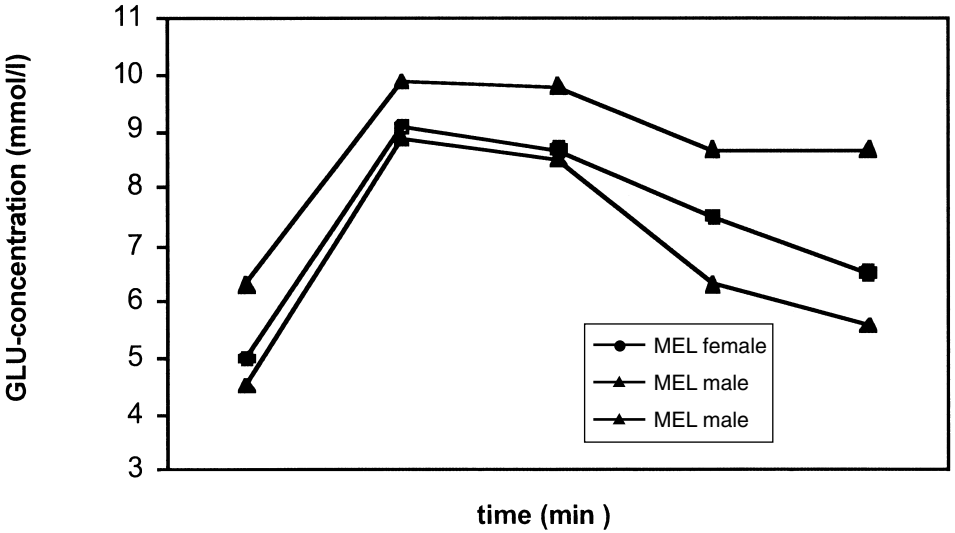

Fig. 3. The abnormal course of OGTTs in MEL-female and MEL-male SD rats.
There are individual values of glucose concentrations in 1 MEL-female and 2 MEL-male rats in Fig. 3.

phospholipid and diacylglycerol concentration in young male and female Wistar rats kept under LD regimen (Mustonen et al. 2002); in our work we recorded the liver phospholipid content to increase in MEL females. During our experiment, a temporary increase in food and water intake in 6th week of MEL administration was recorded in females but not in males, in 10th week of experiment no difference was recorded. MEL significantly decreased the body weight and absolute as well as relative epididymal fat mass in males without the change in food and water intake. In females MEL increased the periovarial fat mass but did not alter the body weight in comparison with controls. Mustonen et al. (2002) reported a decreased activity of hepatic lipase in young Wistar females due to effect of MEL released from subcutaneous implants. This decreased liver triacylglycerol utilization and increased triacylglycerol transport from the liver to peripheral tissues. We assume that this metabolic change could increase the periovarial fat mass in MEL females in our experiment. A similar mechanism of body fat accumulation was reported in photoperiodically active mammals before the winter onset (Nieminen et al. 2001). Mustonen et al. (2002) did not find a change in plasma insulin concentration in MEL females. We found no reports on plasma leptin levels in young females with prolonged MEL administration. Prolonged MEL administration (0.2 m g/ml in tap water) to 3-month-old male SD rats did not alter the plasma insulin and leptin concentration, body weight and relative intraabdominal fat mass in comparison with controls (Rasmussen et al. 2001). Fabis et al. (2002) found a significant serum glucose level increase in male Wistar rats after single MEL administration at a dosage 0.5 and 1.0 mg/kg b.w. The higher MEL dose administration increased the insulin level, too. They found a significant increase in free, total, esterified and HDL cholesterol and a decrease in serum free fatty acids.

We recorded a distinct sexual dimorphism in metabolic response of young SD rats of both sexes like Mustonen et al. (2002) did in young Wistar rats. Different effects of exogenous MEL in young males and females arise from differences in energy metabolism, hormonal status and physiological functions of both sexes.

In young rats (as well as in other mammalian species) the production of endogenous MEL is sufficient and prolonged administration of exogenous MEL is useless. MEL administered

in tap water is absorbed in gastrointestinal tract which itself is a source of synthesis and release of MEL in enterochrmaffin cells (Raikhlin and Kvetnoy 1976; Bubenik 1980). Gastrointestinal MEL regulates many physiological functions of digestion and nutrient absorption; its receptors in gastrointestinal tract have similar characteristics as MEL receptors in central nervous system (Motilva et al. 2001). The fact remaining unclear is whether the MEL synthesis in gastrointestinal tract decreases with aging similarly to the MEL synthesis in epiphysis.

Both the pineal MEL synthesis and secretion decrease with aging (the levels are significantly decreased in middle age) while the adiposity (particularly visceral adiposity) and plasma insulin and leptin levels increase with aging in humans, primates as well as in rats. These changes are often associated with glucose intolerance, and insulin resistance, respectively; with diabetes, dyslipidemia and other pathologies. From this point of view the MEL administration could delay or prevent some of these negative metabolic changes associated with aging (Wolden-Hanson et al. 2000). These authors administered MEL to 10-month-old (middle-aged) SD rats at a dose of 0.4 m g/ml in tap water continuously for 12 weeks. Three months of MEL administration increased the locomotor activity, body core temperature and morning plasma corticosterone level, and on the contrary decreased the body weight, relative intraabdominal fat mass and plasma insulin and leptin levels to the values of young rats. The intraabdominal fat mass in their experiment was expressed as the sum of omental, mesenteric, retroperitoneal, perirenal and epididymal fat mass. The mass of all types of fat tissues in MEL groups was lower compared to controls, this difference was significant for epididymal fat mass. Interestingly, the decrease in intraabdominal fat mass was accompanied neither by changes in total adiposity nor by changes in water and food intake. The pineal MEL synthesis decrease with aging can alter the physical activity and metabolism with subsequent body weight and visceral adiposity increase connected with negative metabolic changes (Wolden-Hanson et al. 2000). The decrease in body weight, relative intraabdominal fat and plasma insulin and leptin concentrations were recorded after 12 months of MEL administration at a dose of 4 and 0.4 m g/ml in tap water (Rasmussen et al. 1999) or at a dose of 0.2 m g/ml in tap water (Rasmussen et al. 2001) administered to 10-month-old SD male rats.

Different results of experimental studies dealing with the influence of prolonged MEL administration could arise from the age of experimental animals (old vs. young), the dose of administered MEL (physiological 10^{-9}M – 10^{-11}M vs. pharmacological 10^{-5}M – 10^{-7}M, (Hill and Blask 1988)), the time of MEL administration (continuously vs. discontinuously - in our experiment from 15:00 h to 08:00 h – in the period of increased sensitivity of organism to MEL), the length of MEL administration (short-term vs. prolonged or long-term) or from the diet used. The selected animal species (Sprague-Dawley, Wistar etc.), sex and light regimen have to be considered, too. The pineal MEL levels in middle-aged female rats (12-month-old) range between levels of young adult and old rats (Reiter et al. 1981), whereas in the male rats of the same age a loss of night MEL plasma peak was found (Rasmussen et al. 2001).

In our work, the prolonged MEL administration to young rats decreased particularly the body weight, the absolute organ weights (liver, heart muscle and adrenals) and epididymal fat mass in males. In females, MEL increased the periovarial fat mass and heart muscle weight and did not influence the body weight. We found sex dimorphism in the effects of prolonged MEL administration to young SD rats.

The basic research and evaluation of side effects of MEL require further experiments to validate the administration of MEL in the treatment of diseases (e.g. of insomnia). The MEL effect on carbohydrate metabolism in diabetic patients which could possibly impair their metabolic condition, remains questionable (Guardiola-Lemaitre 1997).

Vplyv prolongovane aplikovaného melatonínu na metabolické parametre a hmotnosti orgánov mladých potkanov oboch pohlaví kmeňa Sprague-Dawley

Otázka zavedenia melatonínu (MEL) do liečby ostáva stále otvorená. Zvlášť analyzované by malo byť dlhodobé podávanie MEL. Cieľom práce bola analýza účinku nízkych dávok MEL, 4 m g/ml pitnej vody, podávaného 70 dní, denne od 15.00 do 8.00 h (od 8.00 do 15.00 h pili vodu) na vybrané metabolické parametre u potkanov oboch pohlaví kmeňa Sprague-Dawley. Okrem koncentrácie/obsahu triacylglycerolov, fosfolipidov, celkového cholesterolu, malonyldialdehydu, glukózy a glykogénu v sére a v tkanivách sme stanovili hladinu kortikosterónu a inzulínu v sére, hmotnosti vybraných orgánov, periovariálneho a epididymálneho tuku a celkovú hmotnosť zvierat. Kontrolným skupinám nebol MEL podávaný. Pred aplikáciou MEL a po 9 týždňoch podávania MEL boli vykonané orálne glukózové tolerančné testy (OGTTs).

Potkany oboch pohlaví vo veku 5 týždňov adaptované na štandardné prostredie zvieratníka a umelý svetelný režim 12 h svetlo – 12 h tma (L:D-12:12 h) boli kŕmené MP diétou s obsahom tuku minimálne 2,5% a napájané pitnou vodou resp. roztokom MEL ad libitum. Potkany boli 2-krát týždenne vážené, bol sledovaný príjem vody a potravy. Po 10-tich týždňoch boli usmrtené rýchlou dekapitáciou medzi 08:00-11:00 h, orgány a tkanivá boli zvážené, v sére, v pečeni, myokarde a kostnej dreni (femur) boli stanovené hore uvedené metabolické parametre.

Podávanie MEL signifikantne znížilo koncentráciu triacylglycerolov v sére a obsah glykogénu v pečeni u samcov; u samíc zvýšilo obsah fosfolipidov v pečeni. MEL podstatne neovplyvnil hodnoty ostatných metabolických ukazovateľov. 2,5-mesačné podávanie MEL signifikantne zvýšilo u samíc hmotnosť myokardu a periovariálneho tuku; u samcov signifikantne zredukovalo hmotnosť nadobličiek, pečene, myokardu a epididymálneho tuku spolu s ich telesnou hmotnosťou od 19. dňa do konca pokusu. Telesná hmotnosť samíc pijúcich MEL bola približne rovnaká ako u kontrol. U samíc pijúcich MEL bol zvýšený príjem potravy, na hranici signifikancie bol i príjem vody v 6. týždni pokusu. U samcov pijúcich MEL sa príjem potravy a vody v tomto období nelíšil od kontrolnej skupiny.

Prolongovaná aplikácia MEL nemala výrazný vplyv na reguláciu glykémie. Pred podávaním MEL mali krivky OGTTs normálny priebeh, po 9 týždňoch podávania MEL sme v skupine samíc zaznamenali 1 abnormálnu krivku (z 9 jedincov) a u samcov 2 abnormálne krivky (z 9 jedincov).

Zaznamenali sme sexuálne rozdielny účinok prolongovane podávaného MEL hlavne na telesnú hmotnosť, hmotnosť bieleho tukového tkaniva, periovariálneho a epididymálneho, a srdcového svalu.

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References

ACUNA-CASTROVIEJO, D, REITER, RJ, MENENDEZ-PELAEZ, A, PABLOS, MI, BURGOS, A 1994: Characterization of high-affinity melatonin binding sites in purified cell nuclei of rat liver. J Pineal Res. **16**: 100-112

AOYAMA, H, MORI, N, MORI, W 1987: Anti-glucocorticoid effects of melatonin in adult rats. Acta Pathol Jpn **37**: 1143

AOYAMA, H, MORI, N, MORI, W 1988: Effects of melatonin on genetic hypercholesterolemia in rats. Atherosclerosis **69**: 269

BAILEY, CJ, ATKINS, TW, MATTY, AJ 1974: Melatonin inhibition of insulin secretion in the rat and mouse. Hormone Res **5**: 21-28

BARTLETT, GR 1959: Phosphorus assay in column chromatography. J Biol Chem **234**: 466-468

BECKER ANDRÉ, M, WIESENBERG, I, SCHAEREN-WIEMERS, N, ANDRÉ, E, MISSBACH, M, SAURAT, JH, CARLBERG, C 1994: Pineal gland hormone melatonin binds and activates an orphan of the nuclear receptor superfamily. J Biol Chem **269**: 28531-28534

BIZOT-ESPIARD, JG, DOUBLÉ, A, COUSIN, B, LESIEUR, D, GUARDIOLA-LEMAITRE, B, DELAGRANGE, P, KTORZA, A, PÉNICAUD, L 1998: Lack of melatonin effects on insulin action in normal rats. Horm Metab Res **30**: 711-716

BUBENIK, GA 1980: Localization of melatonin in the digestive tract of the rat. Effect of maturation, diurnal variation, melatonin treatment and pinealectomy. Horm Res **12**: 313-32

DUBOCOVICH, M 1988: Pharmacology and the function of melatonin receptors. FASEB J **2**: 2765-2773

DIAZ, B, BLAZQUEZ, E 1986: Effect of pinealectomy on plasma glucose, insulin and glucagon levels in the rat. Horm Metab Res **18**: 225-229

EBADI, M., GOVITRAPONG, P., PHANSUWAN-PUJITO, P., NELSON, F., REITER, R.J. 1998: Pineal opioid receptors and analgesic action of melatonin. J Pineal Res **24**: 193-200

FABIŚ, M, PRUSZYŃSKA, E, MAĆKOWIAK, P 2002: In vivo and in situ action of melatonin on insulin secretion and some metabolic implications in the rat. Pancreas **25**: 166-169

FLEUR, LA, SE, KALSBEEK, A, WORTEL, J, VLIET VAN DER, J, BUIJS, RM 2001: Role for the pineal and melatonin in glucose homeostasis: pinealectomy increases night-time glucose concentrations. J Neuroendocrinol **13**: 1025-1032, 2001.

FRANKEL, BJ, STRANDBERG, MJ 1991: Insulin release from isolated mouse islets in vitro: no effect of physiological levels of melatonin or arginine vasotocin. J Pineal Res **11**: 145-148

GUARDIOLA-LEMAITRE, B. 1997: Toxicology of melatonin. J Biol Rhythms **12**: 697-706

GUILLEMIN, R, CLAYTON, GW, SMITH, JD, LIPSCOMB, HS 1958: Measurement of free corticosteroids in rat plasma. Physiological validation of the method. Endocrinology **63**: 349-355

HILL, SM, BLASK, DE 1988: Effects of the pineal hormone melatonin on the proliferation and morphological characteristics of human breast cancer cells (MCF-7) in culture. Cancer Res **48**: 6121-6126

IIZUKA, Y 1996: Effect of melatonin on serum glucose and insulin levels in rats. Medicine and Biology **133**: 65-67

KAWASHIMA, K., MIWA, Y FUJIMOTO, K, OOHATA, H, NISHINO, H, KOIKE, H 1987: Antihypertensive action of melatonin in the spontaneously hypertensive rats. Clin Exp Hypertens **A9**: 1121-1131

LAGNEUX, C, JOYEUX, M, DEMENGE, P et al. 2000: Protective effects of melatonin against ischemia-reperfusion injury in the isolated rat heart. Life Sci **66**: 503-509

LIMA, FB, MACHADO, UF, BARTOL, I, SERAPHIM, PM, SUMIDA, DH, MORAES, SMF, HELL, NS, OKAMOTO, MM, SAAD, MJA, CARVALHO, CRO, CIPOLLA-NETO, J 1998: Pinealectomy causes glucose intolerance and decreases adipose cell responsiveness to insulin in rats. Am J Physiol **275** (Endocrinol Metab **38**): E934-E941

MAESTRONI, GJM 1993: The immunoendocrine role of melatonin. J Pineal Res **14**: 1-10

MILCU, SM, NANU-IONESCU, I, MILCU, I.1971: The effect of pinealectomy on the plasma insulin in rats. In: Woltensholme, GEW, Knight, J: The Pineal Gland. Churchill Livingstone, Edinburgh, pp. 345-377

MORI, N, AOYAMA, H, MURASE, T, MORI, W 1989: Anti-hypercholesterolemic effect of melatonin in rats. Acta Pathol Jpn **39**: 613-618

MOTILVA, V, CABEZA, J, LASTRA ALARCÓN DE LA, C 2001: New issues about melatonin and its effects on the digestive system. Current Pharm Design **7**: 909-931

MUSTONEN, AM, NIEMINEN, P, HYVÄRINEN, H 2002: Effect of continuous light and melatonin treatment on energy metabolism of the rat. J Endocrinol Invest **25**: 716-723

NIEMINEN, P, KÄKELÄ, R, MUSTONEN, AM, HYVÄRINEN, H, ASIKAINEN, J 2001: Exogenous melatonin affects lipids and enzyme activities in mink (*Mustela vison*) liver. Comp Biochem Physiol Part C **128**: 203-211

PESCHKE, E, FAUTECK, JD, MUSSHOFF, U, SCHMIDT, F, BECKMANN, A, PESCHKE, D, 2000: Evidence for a melatonin receptor within pancreatic islets of neonate rats: functional, autoradiographic, and molecular investigations. J Pineal Res **28**: 156-164

RAIKHLIN, NT, KVETNOY, IM 1976: Melatonin and enterochromaffin cells. Acta Histochem **55**: 19-24

RASMUSSEN, DD, BOLDT, BM, WILKINSON, CW, YELLON, SM, MATSUMOTO, AN 1999: Daily melatonin administration at middle age suppresses male rat visceral fat, plasma leptin, and plasma insulin to youthful levels. Endocrinology **2**: 1009-1012

RASMUSSEN, DD, MITTON, DR, LARSEN, SA, YELLON, SM 2001: Aging-dependent changes in the effect of daily melatonin supplementation on rat metabolic and behavioral responses. J Pineal Res **31**: 89-94

REITER, RJ, CRAFT, CM, JOHNSON, JEJ, KING, TS, RICHARDSON, BA, VAUGHAN, MK 1981: Age-associated reduction in nocturnal pineal melatonin levels in female rats. Endocrinology **109**: 1295-1297

REITER, RJ 1992: The aging pineal gland and its physiological consequences. BioEssays **14**: 169-175

RICHARDSON, BA, STUDIER, EH, STALLONE, JN, KENNEDY, CM 1992: Effects of melatonin on water metabolism and renal function in male Syrian hamsters (*Mesocricetus auratus*). J Pineal Res **13**: 49-59

ROE, JH, DAILEY, R 1966: The determination of glycogen with anthrone reagent. Analyt Biochem **15**: 245-250
SAARELA, S, REITER, RJ 1994: Function of melatonin in thermoregulatory processes. Life Sci **54**: 295-311
SATCH, K 1978: Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta **90**: 37-43
TAN, DX, CHEN, LD, POEGELLER, B, MANCHESTER, LC, REITER, RJ 1993a: Melatonin: a potent, endogenous hydroxyl radical scavenger. Endocrin J **1**: 57-60
TAN, DX, POEGELLER, B, REITER, RJ, CHEN, LD, CHEN, S, MANCHESTER, LC, BARLOW-WALDEN, LR 1993b: The pineal hormone melatonin inhibits DNA-adduct formation induced by the chemical carcinogen safrole in vivo. Cancer Lett **70**: 65-71
VAUGHAN, MK, RICHARDSON, BA, CRAFT, CM, POWANDA, MC, REITER, RJ 1982: Interaction of ageing, photoperiod and melatonin on plasma thyroid hormones and cholesterol levels in female Syrian hamsters. Gerontology **28**: 345
VIJAYALAXMI, B, THOMAS, CR, REITER, RJ, HERMAN, TS 2002: Melatonin: from basic research to cancer treatment clinics. J Clin Oncol **20**: 2575-2601
ZLATKIS, A, ZAK, B, BOYLE, AJ 1953: A new method for the direct determination of cholesterol. J Lab Clin Med **41**: 486-490
WADE, GN, BARTNESS, TJ 1984: Seasonal obesity in Syrian hamsters: effects of age, diet, photoperiod, and melatonin. Am J Physiol **247**: R328-R334
WOLDEN-HANSON, T, MITTON, DR, McCANTS, RL, YELLON, SM, WILKINSON, CW, MATSUMOTO, AM, RASMUSSEN, DD 2000: Daily melatonin administration to middle-aged male rats suppresses body weight, intraabdominal adiposity, and plasma leptin and insulin independent of food intake and total body fat. Endocrinology **2**: 487-497
YU, HS, REITER, RJ 1993: Melatonin: Biosynthesis, physiological effects and clinical applications. CRC Press, Boca Raton, FL