

# Effect of melatonin administration on activities of some lysosomal enzymes in the mouse

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## Abstract

**OBJECTIVES:** Changes in the activity of  $\beta$ -glucuronidase, N-acetyl- $\beta$ -glucosaminidase, cathepsin D and L, alanine aminopeptidase and lysosomal acid lipase in lysosomal fractions of the liver and kidneys of mice, which were administered 20 mg/kg b.w. of exogenous melatonin (N-acetyl-5-methoxytryptamine) for 7 and 14 days were investigated.

**METHODS:** The slices of the liver and kidney were homogenized in 0.1M phosphate buffer, pH 7.0. Homogenates were subjected to differentiated centrifuging and determination of studied enzymes.

**RESULTS:** Melatonin caused lowering of the activity of all the investigated lysosomal enzymes in the liver and kidney.

**CONCLUSION:** Administration of melatonin was caused the lowering of the activity of the investigated lysosomal enzymes in comparison with values in control groups.

## Introduction

It is known that melatonin (N-acetyl-5-methoxytryptamine) is a hormone synthesized in pineal gland, from which it is released to blood and cerebrospinal fluid. The physiological role of melatonin consists, among others, in neutralizing the influence of cancerogenic substances [1], intensifying of the immunological system [2] and the protection of organism from the effect of free radicals [1, 3–5]. At present melatonin has also a therapeutic use [6–8]. It is applied in the treatment of sleep disorders [8, 9], jet-lag [10], malignant neoplasm [11–13], Alzheimer's [14] and Parkinson's diseases [8, 9, 15].

Lysosomal space is a system which actively participates in the adaptive reaction of organism, burdened by environmental factors, generated in natural or artificial way [16–19]. The present diagnostic methods observe the changes in the activity of the enzymes of lysosomal space for estimation of the range and rate of biochemical transformations in cells [20–22]. Lysosomal enzymes are also the object of studies of stress reactivity and different diseases [23–25].

In connection with these data we examined the activity of some enzymes of lysosomal fraction of the liver and kidneys of the mice subjected to the administration of exogenous melatonin.

## Material and methods

The study was carried out on 40 male and 40 female randomized 8 week old mice, 20–22 g b.w. The animals were bred in the Institute of Genetics and Animals Breeding of the Polish Academy of Sciences in Jastrzebiec. Mice were kept in standard conditions of the farm in a ventilated room at 21°C with 12 h daylight and 12 h darkness. They were fed standard "Murigran" feed – 16% of protein, (Animal Food Company, Lomna near Warsaw, Poland) and had constant access to water. Minerals and vitamin supplements covered the nutritional needs of the animals. All animals received good veterinary care.

Mice were divided into eight groups (I – IV males and V – VIII females; n = 10 in each group) and received according to the following scheme:

- I control 100 ml 0.01% ethanol 7 days
- II melatonin 20 mg/kg b.w. 7 days
- III control 100 ml 0.01% ethanol 14 days
- IV melatonin 20 mg/kg b.w. 14 days
- V control 100 ml 0.01% ethanol 7 days
- VI melatonin 20 mg/kg b.w. 7 days
- VII control 100 ml 0.01% ethanol 14 days
- VIII melatonin 20 mg/kg b.w. 14 days

The male (I, III) and female (V, VII) of the control groups received everyday 100 ml of 0.01% ethanol solution per os by micropipette, between at 10:00–11:00 a.m.

At the same time, the experimental males (II, IV) and females (VI, VIII) received 20 mg/kg b.w. of exogenous melatonin (N-acetyl-5-methoxytryptamine, Sigma-Aldrich-Chemie GmbH, Steinheim, Germany) dissolved in 100 ml of 0.01% ethanol.

After a suitable time, the mice were killed by breaking the spinal cord and slices of the liver and kidney were immediately taken. The liver slices were perfused with 0.9% NaCl solution cooled to +5°C and similarly with the slices of kidney were suspended in 0.1 M phosphate buffer, pH 7.0 at the temperature +5°C at ratio 500 mg tissue/5 ml buffer. The whole was homogenized at +5°C in the Potter homogenizer with a teflon piston at 200 rotations/min.

The liver and kidney homogenates were subjected to differentiated centrifuging according to [26] method.

In the lysosomal fractions of the liver and kidney the activities of glycosidases:

$\beta$ -glucuronidase (BGRD, EC 3.2.1.31), N-acetyl- $\beta$ -glucosaminidase (NAG, EC 3.2.1.10) according to [27] method, lysosomal acid lipase (LL, EC 3.1.1.3) according to [28] method, proteolytic enzyme: alanine aminopeptidase (AAP, EC 3.4.1.12) according to [29] and cathepsin D and L (Cath.D, EC 3.4.23.5 and Cath.L, EC 3.4.22.15) were estimated. The activity of Cath. D and L was determined by the modified method of [30] using as substrate 2% azocasein in 6 M urea.

Enzyme activity was expressed in nmols/mg of protein/hour. All substrates were from Serva Feinbiochemica GmbH & Co., Heidelberg, Germany. Protein was also determined in the lysosomal fractions [31]. The results obtained were analyzed statistically according to Student's t test.

The experiment has been confirmed by the Academy Ethics Commission for Animals Research of the Swietokrzyska Academy in Kielce.

## Results

As can be seen from Tables 1–4 everyday administration of exogenous melatonin for 7 and 14 days caused characteristic lowering in the activity of all the investigated lysosomal enzymes in the liver and kidney of male and female mice, in comparison with control mice. The highest changes of the activity were observed after 7 days of experiment.

## Discussion

Melatonin regulates and stabilizes plenty of the functions of organism. Recent investigations have also shown that melatonin administration in small

**Table 1.** Activity ( $\bar{x} \pm SD$ ) of lysosomal enzymes (nmol/mg of protein/h) in the liver and kidney of the males after 7 days melatonin administration; control = 100%; n in each group = 10;

Enzyme	Melatonin Liver			Melatonin Kidney		
	Control		%	Control		%
<b>BGRD</b>	1.40 ± 0.280	0.760 ± 0.230***	<b>54</b>	1.81 ± 0.417	1.60 ± 0.502	<b>88</b>
<b>NAG</b>	0.251 ± 0.040	0.160 ± 0.051**	<b>64</b>	2.29 ± 0.274	1.31 ± 0.129***	<b>57</b>
<b>Cath.Dand L</b>	0.033 ± 0.001	0.018 ± 0.002***	<b>54</b>	0.044 ± 0.015	0.012 ± 0.005***	<b>27</b>
<b>AAP</b>	0.055 ± 0.012	0.033 ± 0.007**	<b>60</b>	0.061 ± 0.019	0.023 ± 0.007***	<b>38</b>
<b>LL</b>	0.203 ± 0.060	0.091 ± 0.031***	<b>45</b>	0.365 ± 0.023	0.292 ± 0.096*	<b>80</b>

\* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001 – statistically confirmed differences;

**Table 2.** Activity ( $\bar{x} \pm SD$ ) of lysosomal enzymes (nmol/mg of protein/h) in the liver and kidney of the males after 14 days melatonin administration; control = 100%; n in each group = 10;

Enzyme	Melatonin Liver			Melatonin Kidney		
	Control		%	Control		%
<b>BGRD</b>	1.57 ± 0.300	1.22 ± 0.400*	<b>78</b>	1.70 ± 0.502	1.44 ± 0.565*	<b>85</b>
<b>NAG</b>	0.300 ± 0.050	0.237 ± 0.037*	<b>79</b>	2.60 ± 0.859	2.31 ± 0.903	<b>89</b>
<b>Cath.Dand L</b>	0.045 ± 0.003	0.033 ± 0.002**	<b>73</b>	0.050 ± 0.017	0.038 ± 0.017*	<b>76</b>
<b>AAP</b>	0.053 ± 0.019	0.035 ± 0.011**	<b>66</b>	0.066 ± 0.019	0.042 ± 0.012**	<b>64</b>
<b>LL</b>	0.230 ± 0.030	0.188 ± 0.017*	<b>82</b>	0.400 ± 0.136	0.364 ± 0.108	<b>91</b>

\* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001 – statistically confirmed differences;

**Table 3.** Activity ( $\bar{x} \pm SD$ ) of lysosomal enzymes (nmol/mg of protein/h) in the liver and kidney of the females after 7 days melatonin administration; control = 100%; n in each group = 10;

Enzyme	Melatonin Liver			Melatonin Kidney		
	Control		%	Control		%
<b>BGRD</b>	1.53 ± 0.407	0.860 ± 0.137***	<b>56</b>	2.07 ± 0.189	1.31 ± 0.506**	<b>63</b>
<b>NAG</b>	0.464 ± 0.138	0.293 ± 0.045**	<b>63</b>	2.06 ± 0.137	1.44 ± 0.106**	<b>70</b>
<b>Cath.Dand L</b>	0.015 ± 0.003	0.006 ± 0.002***	<b>40</b>	0.086 ± 0.025	0.025 ± 0.012***	<b>29</b>
<b>AAP</b>	0.019 ± 0.008	0.010 ± 0.004***	<b>53</b>	0.077 ± 0.027	0.024 ± 0.011***	<b>31</b>
<b>LL</b>	0.400 ± 0.141	0.276 ± 0.126**	<b>69</b>	0.218 ± 0.139	0.124 ± 0.012***	<b>57</b>

\* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001 – statistically confirmed differences;

**Table 4.** Activity ( $\bar{x} \pm SD$ ) of lysosomal enzymes (nmol/mg of protein/h) in the liver and kidney of the females after 14 days melatonin administration; control = 100%; n in each group = 10;

Enzyme	Melatonin Liver			Melatonin Kidney		
	Control		%	Control		%
<b>BGRD</b>	1.60 ± 0.403	1.34 ± 0.614*	<b>84</b>	2.01 ± 0.304	1.86 ± 0.648	<b>93</b>
<b>NAG</b>	0.510 ± 0.145	0.459 ± 0.132	<b>90</b>	1.99 ± 0.708	1.67 ± 0.508*	<b>84</b>
<b>Cath.Dand L</b>	0.014 ± 0.003	0.010 ± 0.002**	<b>71</b>	0.100 ± 0.034	0.073 ± 0.031**	<b>73</b>
<b>AAP</b>	0.023 ± 0.006	0.015 ± 0.004**	<b>65</b>	0.099 ± 0.032	0.068 ± 0.034**	<b>69</b>
<b>LL</b>	0.375 ± 0.103	0.326 ± 0.123	<b>87</b>	0.210 ± 0.098	0.168 ± 0.056*	<b>80</b>

\* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001 – statistically confirmed differences;

doses has not only a stimulating influence on immune reactions of organism, but also – thanks to its antioxidant properties, it neutralizes the harmful influence of stress [32–34]. It restores the hormonal homeostasis of the organism [35, 36]. This influence depends on the doses used, but first of all it depends on the time of administration of exogenous melatonin [37, 38]. This demonstration of the immunological role of melatonin has become the basis for the application of this hormone in the therapy of many diseases, first of all in neoplasm immunotherapy [2, 39–41].

Lysosomal space plays a decisive role in the defensive processes of the organism, controls of secretion of endocrine glands, transportation and liquidation of foreign substances and used cellular organelles [42, 43]. It participates actively in adaptive reactions of the organism, and maintains the stability of the organism in different environmental conditions.

Our results indicate that melatonin had a significant influence on the activity the investigated glycosidases (BGRD, NAG), proteolytic enzymes (AAP, Cath.D and L) and lysosomal acid lipase of the lysosomal compartment. Exogenous melatonin, administered for 7 and 14 days in dose of 20 mg/kg b.w. decreased the activities of all the investigated enzymes of lysosomal space in the liver and kidney male and female mice.

We think that the revealed changes in the activity of the investigated enzymes in the liver and kidney of mice are one of the elements of the adaptation reaction to biological stress caused by the excess of exogenous melatonin. Observing the dynamics of changes of these enzymes it is possible to differentiate the physiological changes from the pathological changes creating damage to the cells [44, 45].

The results obtained suggest that some lysosomal enzymes can be used to study the direction and intensity of i.e. stressful reaction. The lysosomal enzymes appear to be among these interesting indicators. Due to the fact that mechanisms which direct and regulate the activity of the lysosomal enzymes it is advisable to carry out further investigations of the lysosomal enzyme compartment as the equalizing system in disturbed situations. This is an interesting result from the physiological and pharmacological points of view.

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