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**I. I. Zamorskii**Bukovyna State Medical University,  
Chernivtsi, Ukraine**EFFECT OF MELATONIN ON THE  
ACTIVITY OF MARKER ENZYMES IN THE  
NEURONAL PLASMATIC MEMBRANES  
UNDER CONDITIONS OF ACUTE HYPOXIA  
AND VARYING PHOTOPERIODIC  
DURATION**

**Keywords:**  $\text{Na}^+$ ,  $\text{K}^+$ ATPase,  
5'nucleotidase, rats forebrain,  
melatonin, acute hypoxia,  
photoperiod.

**Abstract.** The effect of a single-shot intraperitoneally administration of melatonin in a dose of 1 mg/kg on the activity of  $\text{Na}^+$ ,  $\text{K}^+$ ATPase and 5'nucleotidase (5'N) in the forebrain of juvenile male white rats has been investigated under conditions of acute hypoxia. Such studies have been carried out against a background of a varying duration of the photoperiod during one week. It has been established that constant darkness prevents an inhibition of the activity of  $\text{Na}^+$ ,  $\text{K}^+$ ATPase caused by acute hypoxia inducing and promoting an activation of 5'N. The administration of melatonin likewise constant darkness against a background of acute hypoxia prevents a decrease of the activity of  $\text{Na}^+$ ,  $\text{K}^+$ ATPase and increases the activity of 5'N.

**Introduction**

$\text{Mg}^{2+}$  dependent adenosine5'triphosphatase, which is activated by sodium and potassium ions, ( $\text{Na}^+$ ,  $\text{K}^+$ ATPase, the sodium pump) [EC 3.6.1.37] and 5'nucleotidase [EC 3.1.3.5] are the proteins of the plasmatic membrane, causing a high dependence of the functioning of these enzymes on the membranous lipid setting. This makes it possible to characterize the structural functional state of the plasmatic membranes according to the activity of the specified enzymes. Therefore,  $\text{Na}^+$ ,  $\text{K}^+$ ATPase and 5'nucleotidase are referred to marker enzymes of the plasmatic membranes [4]. Furthermore,  $\text{Na}^+$ ,  $\text{K}^+$ ATPase is a key enzyme of neurons which defines the level of their functional activity [7]. At the same time, adenosine is formed under the action of 5'nucleotidase. It manifests neuroprotective and antioxidant properties, favouring the entry of oxygen and energy substances to the tissues [10]. The effect of the pineal hormone melatonin on the ATPase activity (including  $\text{Na}^+$ ,  $\text{K}^+$ ATPase) was demonstrated for the first time over twenty years ago [1, 2]. On the basis of such findings it was suggested that there existed antioxidant activity in melatonin which was later confirmed [2, 8, 15]. Our own trials [13] corroborated the protective action of melatonin to the activity of the marker enzymes of the plasmatic membranes under conditions of acute hypoxia that is conducive to the onset of oxidative stress and intensification of free radical oxidation of macromolecules.

**Purpose**

Since a photoperiod is a modulator for the formation of endogenous melatonin [5, 14], the object

of our research became a study of the influence of melatonin on the activity of  $\text{Na}^+$ ,  $\text{K}^+$ ATPase and 5'nucleotidase in the rat forebrain under conditions of acute hypobaric hypoxia against a background of an altered photoperiodic duration.

**Material and methods**

The experiments were carried out on 107 juvenile male white rats aged 5,5–6,0 weeks and weighing 65–75 g. Only animals with average resistance to hypoxia were used in the experiment. Acute hypoxic hypobaric hypoxia, equivalent to the altitude of 12000 m was modeled in an altitude chamber. The rats were kept on the "high-altitude plateau" up to the second agonal inhalation followed by a "descent" to the previous zero altitude. Thirty minutes prior to the simulation of acute hypoxia part of the animals was administered melatonin ("Sigma", USA) intraperitoneally in a 0.1% ethanol solution in a dose of 1 mg/kg of the body weight. Three different photoperiodic modes were used during one week prior to the action of acute hypoxia for the purpose of simulating photoperiodic changes in the animals' organisms. The first group of rats (36 animals) was kept under conditions of an ordinary change of the light and dark phases of a 24 hour period during the spring-summer period of the year. The Light-Dark ratio made up 16 hours : 8 hours. The second group (36 animals) was exposed to the action of constant artificial lighting of 500 lux during a 24 hour period. The third group (35 animals) was caged in constant diurnal darkness. Access to the animals of the last group was accomplished only under a faint red light

(2 lux). The animals were decapitated in 30 minute after discontinuing the effect of acute hypoxia. The removed brain was rinsed in a cold physiological solution and preserved in liquid nitrogen. The activity of the enzyme was evaluated in the supernatant which was available after centrifugation of a homogenate weight of the forebrain at 900 g during 15 min. The forebrain weight was homogenized in 0.25 M tris-HCl buffer (pH 7.4). The activities of Na<sup>+</sup>, K<sup>+</sup>ATPase and 5'nucleotidase were determined on the basis of an elevated amount of inorganic phosphate (P<sub>i</sub>) in the process of reaction and were expressed in nmol P<sub>i</sub> that was formed during one min per one mg of protein [9, 6]. The incubation medium of two ml in volume intended for the assessment of the activity of Na<sup>+</sup>, K<sup>+</sup>ATPase contained 50 mM tris-HCl buffer (pH 7.4), 3 mM ATP, 150 mM NaCl, 15 mM KCl; whereas for the evaluation of 5'nucleotidase — 50 mM tris-HCl buffer (pH 7.4), 2 mM AMP, 1 mM MgSO<sub>4</sub>. A quantitative determination of P<sub>i</sub> was carried out by means of the colorimetric method [3]. The content of protein was defined according the method of O. H. Lowry et al. Statistical processing of the obtained findings was fulfilled with the aid of the program "STATISTICA 6.0".

### Results and discussion

In accordance with the obtained findings (Table) the activity of Na<sup>+</sup>, K<sup>+</sup>ATPase decreased due to the effect of acute hypoxia, most essentially under conditions of constant lighting, remaining unchanged under conditions of constant darkness. The activity of

5'nucleotidase exposed to acute hypoxia didn't change considerably under ordinary lighting conditions and under constant darkness, whereas under conditions of continuous lighting it increased. The administration of melatonin without the effect of acute hypoxia did not reliably change the activity of Na<sup>+</sup>, K<sup>+</sup>ATPase and simultaneously elevated the activity of 5'nucleotidase, especially under conditions of constant darkness. The introduction of melatonin against a background of acute hypoxia modulation eliminated the negative influence of hypoxia on the activity of Na<sup>+</sup>, K<sup>+</sup>ATPase and promoted an augmented activity of 5'nucleotidase. Thus, the effect of acute hypoxia on the activity of the marker enzymes of the forebrain cells depends on the duration of the photoperiod. Constant lighting decreases resistance of the rats' forebrain neurons to acute hypoxia at that, whereas constant darkness is conducive to improved adaptation of the rats to acute hypoxia. Simultaneously, melatonin counteracts the inhibition of the Na<sup>+</sup>, K<sup>+</sup>ATPase activity of the forebrain neurons in case of acute hypoxia and promoted an increase of the activity of 5'nucleotidase.

The inhibited activity of Na<sup>+</sup>, K<sup>+</sup>ATPase in the brain following the effect of acute hypoxia agrees with bibliographical findings and arises due to ATP deficiency as well as a probable destruction of neuronal plasmatic membranes, as a result of the action of free radicals [7]. At the same time, dependence of the effect of acute hypoxia on the duration of the photoperiod may be indicative of intensified resistance of neurons to acute oxygen deprivation during darkness at the expense of the

Table

The activity of Na<sup>+</sup>, K<sup>+</sup>-ATPase and 5'-nucleotidase in the rat forebrain upon the administration of melatonin (1 mg/kg) under conditions of acute hypobaric hypoxia and varying lighting (mean ± SEM, n = 7)

Lighting conditions	Character of influence	Activity Na <sup>+</sup> , K <sup>+</sup> -ATPase (mkmol P <sub>i</sub> per min per 1 mg of protein)	Activity 5'-nucleotidase (mkmol P <sub>i</sub> per min per 1 mg of protein)
Habitual lighting	Control	0.48±0.026	0.74±0.032
	Melatonin	0.44±0.024	0.79±0.030
	Hypoxia	0.37±0.018 <sup>1</sup>	0.77±0.036
	Melatonin and hypoxia	0.69±0.039 <sup>1, 3</sup>	0.87±0.038 <sup>1</sup>
Constant lighting	Control	0.46±0.024	0.70±0.029
	Melatonin	0.43±0.027	0.76±0.036
	Hypoxia	0.22±0.014 <sup>1, 4</sup>	0.79±0.033 <sup>1</sup>
	Melatonin and hypoxia	0.47±0.030 <sup>3, 5</sup>	0.69±0.028
Constant darkness	Control	0.52±0.026	0.79±0.031
	Melatonin	0.46±0.034	0.97±0.044 <sup>1</sup>
	Hypoxia	0.52±0.032 <sup>6</sup>	0.82±0.027
	Melatonin and hypoxia	0.47±0.031 <sup>5</sup>	0.76±0.038 <sup>2</sup>

**Footnotes.** <sup>1</sup> P < 0.05 vs the parameters in the control animals under the same conditions of lighting; <sup>2</sup> P < 0.05 vs the parameters under conditions of constant darkness after the administration of melatonin without hypoxia or after hypoxia without the administration of melatonin; <sup>3</sup> P < 0.05 vs the parameters after hypoxia without the administration of melatonin under the same conditions of lighting; <sup>4</sup> P < 0.05 vs the parameters after hypoxia without the administration of melatonin under habitual lighting conditions; <sup>5</sup> P < 0.05 vs the parameters after hypoxia with the administration of melatonin under habitual lighting conditions; <sup>6</sup> P < 0.05 vs the parameters after hypoxia without the administration of melatonin under constant lighting

probable effect of melatonin. Such an assumption is corroborated by our data associated with the administration of melatonin which is synthesized in darkness [14]. An analysis of the obtained findings connected with the introduction of melatonin suggests that this hormone, probably, stimulates the formation of adenosine in the brain by way of augmenting the activity of 5'nucleotidase [13]. Acute hypoxia contributes to manifestations of such an action of melatonin on the activity of 5'nucleotidase and adenosine synthesis in the neurons of the brain. Adenosine possesses marked protective and neuromodulating properties, the ability to regulate cell energy homeostasis [10], just like melatonin does [14, 15]. Therefore intensifying adenosine synthesis under the action of melatonin may characterize one of the many neuroprotective and adaptogenic mechanisms of the effect the pineal hormone — melatonin. Simultaneously, melatonin eliminates the negative influence of oxygen starvation on the activity of  $\text{Na}^+$ ,  $\text{K}^+$ ATPase improving the functioning of neurons. Melatonin may realize such an antihypoxic effect at the expense of its marked antioxidant properties [8, 12, 15], that counteract the breakdown of plasma membrane enzymes by free radicals which are formed in acute hypoxia. The obtained findings melatonin research are indicative of its neuroprotective activity.

### The conclusions

1. The constant darkness prevents an inhibition of the activity of  $\text{Na}^+$ ,  $\text{K}^+$ ATPase caused by acute hypoxia inducing and promotes an activation of 5'nucleotidase.

2. The administration of melatonin likewise constant darkness against a background of acute hypoxia prevents a decrease of the activity of  $\text{Na}^+$ ,  $\text{K}^+$ ATPase and increases the activity of 5'nucleotidase.

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### ВПЛИВ МЕЛАТОНІНУ НА АКТИВНІСТЬ МАРКЕРНИХ ФЕРМЕНТІВ НЕЙРОНАЛЬНИХ ПЛАЗМАТИЧНИХ МЕМБРАН ЗА УМОВ ГОСТРОЇ ГІПОКСІЇ І РІЗНОЇ ТРИВАЛОСТІ ФОТОПЕРІОДУ

I. I. Заморський

**Резюме.** Досліджено вплив однократного внутрішньочеревного введення мелатоніну в дозі 1 мг на кг маси тіла на активність  $\text{Na}^+$ ,  $\text{K}^+$ АТФази і 5'нуклеотидази у клітинах переднього мозку статевонезрілих самців білих щурів за умов гострої гіпоксії. Такі дослідження були проведені на фоні зміненого впродовж тижня фотоперіоду. Встановлено, що постійна темрява попереджає пригнічення активності  $\text{Na}^+$ ,  $\text{K}^+$ АТФази, яке викликається гострою гіпоксією, та сприяє активації 5'нуклеотидази. Введення мелатоніну так само, як й постійна темрява, на фоні гострої гіпоксії запобігає зниженню активності  $\text{Na}^+$ ,  $\text{K}^+$ АТФази та підвищує активність 5'нуклеотидази.

**Ключові слова:**  $\text{Na}^+$ ,  $\text{K}^+$ АТФаза, 5'нуклеотидаза, передній мозок щурів, мелатонін, гостра гіпоксія, фотоперіод.

### ВЛИЯНИЕ МЕЛАТОНИНА НА АКТИВНОСТЬ МАРКЕРНЫХ ФЕРМЕНТОВ НЕЙРОНАЛЬНЫХ ПЛАЗМАТИЧЕСКИХ МЕМБРАН В УСЛОВИЯХ ОСТРОЙ ГИПОКСИИ И РАЗЛИЧНОЙ ДЛИТЕЛЬНОСТИ ФОТОПЕРИОДА

И. И. Заморский

**Резюме.** Исследовано влияние однократного внутривентриального введения мелатонина в дозе 1 мг на кг массы тела на активность  $\text{Na}^+$ ,  $\text{K}^+$ АТФази и 5'нуклеотидазы в клетках переднего мозга неполовозрелых самцов белых крыс при острой гипоксии. Такие исследования были проведены на фоне измененного в течение недели фотопериода. Установлено, что постоянная темнота предупреждает угнетение активности  $\text{Na}^+$ ,  $\text{K}^+$ АТФази, вызванное острой гипоксией, и способствует активации 5'нуклеотидазы. Введение мелатонина так же, как и постоянная темнота, на фоне острой гипоксии предотвращает снижение активности  $\text{Na}^+$ ,  $\text{K}^+$ АТФази и повышает активность 5'нуклеотидазы.

**Ключевые слова:**  $\text{Na}^+$ ,  $\text{K}^+$ АТФаза, 5'нуклеотидаза, передний мозг крыс, мелатонин, острая гипоксия, фотопериод.

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