Effects of Geroprotectors on Age-Related Changes in Proteolytic Digestive Enzyme Activities at Different Lighting Conditions

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We studied the effect of melatonin and epithalon on age-related changes in proteolytic digestive enzyme activity in the pancreas and gastric mucosa of rats kept under different lighting conditions. In rats kept under standard illumination, pepsin activity and the total proteolytic activity in the stomach and pancreas increased by the age of 12 months, but then decreased. Constant and natural lighting disturbed the age dynamics of proteolytic digestive enzyme activity. Administration of melatonin and epithalon to animals exposed to constant lighting restored age dynamics of pepsin activity and little affected total proteolytic activity.

Key Words: *proteolytic digestive enzymes; melatonin; epithalon; illumination regimes; aging*

Light is an environmental factor that regulates periods of activity, reproduction, migration, molting, and other biological processes in animals. Reaction of the organism to changes in lighting conditions is mediated by melatonin, a pineal gland hormone. Synthesis of melatonin shows circadian fluctuations and occurs during dark time. Light induces the effect of "physiological pinealectomy", *i.e.* suppression of the synthetic function of the pineal gland [7,12]. Melatonin, the main hormone of the pineal gland, regulates various biological processes. GIT secretes even greater amounts of melatonin than the pineal gland, but this secretion shows no circadian rhythms. Disorganization of circadian rhythms of melatonin secretion can negatively affect GIT function and even provoke ulcers [10]. Moreover, melatonin produces a geroprotective effect [1]. Synthetic tetrapeptide from the pineal gland, Ala-Glu-Asp-Gly (epithalon), stimulates the synthetic function of the pineal gland and increases the release of melatonin into circulation [4]. It has been demonstrated that epithalon regulates activity of GIT enzymes during aging [5].

Here we studied the effect of different lighting regimes and geroprotectors melatonin and epithalon on age-related dynamics of proteolytic digestive enzyme activity in rats.

MATERIALS AND METHODS

The study was performed using instrumental and analytical facilities of the Center of Shared Use of Scientific Equipment, Institute of Biology of and in strict adherence to the principles of Declaration of Helsinki, World Medical Association [6].

Experiments were performed on male and female outbred rats obtained from the N. N. Petrov Oncology Institute [9] and maintained on standard vivarium diet with free access to water. At the age of 1 month, the animals were randomly divided into 3 groups and kept under different illumination conditions: standard (12 h light:12 h dark, SI), constant illumination (CI), and natural illumination (NI) of Karelia Republic. Starting from the age of 4 months, all groups were divided into subgroups. Subgroup 1 rats received melatonin (Sig-

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ma) dissolved in drinking water to a concentration of 10 mg/liter during nighttime; group 2 rats were treated with epithalon (0.1 μg subcutaneously; St. Petersburg Institute of Bioregulation and Gerontology) 5 days a week every month [2]. Group 3 rats served as the control: Some animals received injection in the same volume of saline at the time of epithalon injection, the others received drinking water without melatonin during nighttime (placebo).

At the age of 6, 12, and 18 months, the rats were decapitated and samples of the pancreas and gastric mucosa were taken. Pepsin activity and total proteolytic activity (TPA) were measured by accumulation of tyrosine released during hemoglobin hydrolysis. Enzyme activity was expressed in μmol hydrolysis products formed over 1 min per 1 g wet tissue (μmol/min/g).

The groups were compared using nonparametric Mann–Whitney test.

RESULTS

In postnatal ontogeny, pepsin activity in the gastric mucosa of rats maintained at SI reached a maximum in mature animals (12 months) and then decreased. It should be noted that the age of 12 months in rats falls on the spring, and 6 and 18 months on the fall. Despite seasonal changes in illumination were excluded throughout animal life in our experiments, age-related changes in pepsin activity in the stomach were characterized by circannual periodicity.

Changes in pepsin activity depended on illumination conditions. CI inverted the age-related dynamics (Table 1): activity of this enzyme decreased by the age of 12 months and increased again in aged (18-month-old) rats. In young (6-month-old) and mature rats maintained at NI, pepsin activity remained practically unchanged, but differed from that in rats maintained at SI. These changes could be related to a unique photoperiodicity in northwest Russia (Karelia) characterized by long day length in the spring and summer and short day length during the fall-winter period.

Geroprotectors produced opposite effects on age-related changes in pepsin activity depending on lighting regimen. Under conditions of SI, melatonin and epithalon reduced activity of the enzyme in mature animals, without changing age-related dynamics of this parameter in general. Under conditions of CI, geroprotectors led to a decrease in this parameter, thereby restoring the age dynamics of changes in pepsin activity, which can prevent the development of pathological processes during aging. Administration of melatonin and epithalon to rats kept under conditions of NI had no significant effect on enzyme activity in comparison with placebo.

Similar to pepsin in the stomach, TPA in the pancreas in rats kept at SI increased by the age of 12 months and decreased in 18-month-old animals (Table. 2). CI and NI disturbed this rhythmicity. Changes in seasonal and circadian biorhythms accompanied by suppression of melatonin production accelerate aging and reduce lifespan [8]. At CI, TPA considerably decreased in mature animals and increased in aging rats in comparison with SI. In young rats kept at NI, TPA significantly surpassed that in SI; but in mature and aging animals, TPA decreased and remained at the same level (Table. 2).

In animals kept at SI and treated with melatonin, TPA decreased in mature rats and increased in aging rats in comparison with placebo. In contrast to melato-

TABLE 1. Pepsin Activity (μmol/min/g) in the Stomach of Rats of Different Ages Maintained at Different Illumination Regimens and Treated with Melatonin and Epithalon (*M*±*m*)

Illumination mode	Age, months	Placebo	Melatonin	Epithalon
SI	6	46.60±7.25	50.78±2.71	45.23±5.01
	12	67.42±2.65^{6}	60.60±6.17	53.75±3.77*
	18	31.08±4.15^{12}	51.50±2.73*	41.21±7.19
NI	6	57.60±1.78	62.45±4.05	55.05±1.73
	12	56.01±2.21^{+}	50.61±6.90	49.98±5.71^{+}
	18	43.11±4.846^{6,12}	50.55±1.67	52.12±1.97
CI	6	53.63±3.01	53.90±2.54	50.39±4.28
	12	42.76±6.23^{+}	51.76±1.74	52.17±2.34
	18	53.18±4.78^{+}	40.45±5.74	33.96±4.15*

Note. Here and in Table 2: $p<0.05$ in comparison with *placebo under the same conditions, $^{+}$placebo at SI; superscript 6 and 12: in comparison with the specified age at the specified illumination regime in rats receiving placebo.

TABLE 2. TPA (μmol/min/g) in the Pancreas of Rats of Different Ages Maintained at Different Illumination Regimens and Treated with Melatonin and Epithalon (*M*±*m*)

Illumination mode	Age, months	Placebo	Melatonin	Epithalon
SI	6	70.43±3.06	78.59±3.93	66.76±4.72
	12	89.20±4.41[6]	70.53±4.20*	74.02±5.27
	18	62.99±2.38[12]	71.06±5.10	63.97±2.36
NI	6	85.99±4.18[+]	77.92±1.92	77.27±3.95
	12	77.31±4.85	67.19±1.66	76.31±3.36
	18	76.84±5.22[+]	61.75±6.37	62.10±1.04*
CI	6	70.19±6.54	67.31±3.91	65.27±1.14
	12	61.65±4.09[+]	66.82±6.10	66.53±3.05
	18	83.40±8.98	88.14±3.65	77.12±4.62

nin, epithalon did not change the age dynamics of TPA in rats maintained at SI. In animals maintained under conditions of CI, geroprotectors produced no significant effect on TPA. It should be noted that administration of melatonin and epithalon to rats maintained at NI reduced TPA in mature and aging animals, thereby restoring the age-related rhythmicity of this parameter.

Our results show a decrease in pepsin activity in the stomach and TPA in the pancreas in rats during aging. The age-related dynamics of enzyme activity was characterized by seasonal rhythmicity. Pepsin activity and TPA decreased during fall (6 and 18 months) and increased during spring (12 months). Different lighting regimens modulated age-related changes in activity of digestive proteolytic enzymes. CI inverted the age-related dynamics, while in rats maintained at NI, gradual decrease in pepsin activity in the stomach and TPA in the pancreas was observed. The changes observed under these lighting conditions can be interpreted as disturbances in the age dynamics of enzyme activity that can lead to health deterioration and initialization of various pathological processes. We believe that the observed regularities are primarily related to changes in melatonin synthesis by the pineal gland in response to changes in lighting conditions. It should be emphasized that more extreme lighting conditions caused effects that were more negative. It is known that the gastrointestinal tract along with independent melatonin synthesis can accumulate the hormone of epiphyseal origin from the circulation [10]. Changes in melatonin concentration under the action of CI or peculiar NI in Karelia led to disruption of the age dynamics of proteolytic enzyme activities in the stomach and pancreas. Administration of geroprotectors to rats maintained at SI led to an increase in proteolytic digestive enzyme activity. The observed changes can be related to the capacity of melatonin to enhance the secretion of digestive enzymes [11]. The use of melatonin and epithalon against the background of suppression of the synthetic function of the pineal gland under conditions of CI produced beneficial effect only on pepsin activity.

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