Role of Methionine in Nocturnal Melatonin Peak in the Pineal Gland

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 5, pp. 493-494, May, 2004
Original article submitted August 15, 2003

Methionine dose-dependently stimulated O-methylation of hydroxyindoles in the pineal gland and contributed to the nocturnal melatonin peak in adult male Wistar rats. Methionine is involved in the maintenance of diurnal rhythms by regulating biochemical transformations of indoles in pinealocytes. Our findings provide theoretical basis for new applications of methionine.

Key Words: *methionine; melatonin; pineal gland*

Methionine is an essential sulfur-containing amino acid supplied with food products. Intensive consumption of methionine is followed by a linear increase in the plasma methionine concentration [6]. Metabolism and biological activity of methionine are studied in details. S-Adenosyl-methionine (SAM) is an active form of methionine produced during the interaction of 1-methionine with ATP. SAM synthesis is the major pathway of methionine conversion. It should be emphasized that metabolism of methionine in this reaction 4-fold surpasses its incorporation into proteins [4]. SAM is the major source of methyl (-CH_3)-groups in the organism. This compound plays a role in methylation, transmethylation, and remethylation [2].

High content of SAM in the pineal gland of humans and animals provides O-methylation of pineal hydroxyindoles with the formation of methoxyindoles [5]. Previous experiments revealed diurnal variations in the content of SAM in rat pineal gland. SAM concentration is maximum during daytime and minimum at night. A correlation was found between the content of SAM and intensity of melatonin biosynthesis [3,10]. Inhibition of epiphyseal melatonin synthesis in animals caused by continuous light exposure or β-adrenoblocker propranolol decreased SAM concentration in the pineal gland [10].

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Since O-methylation of N-acetylserotonin in the pineal gland is a key reaction of melatonin biosynthesis, and SAM is a source of methyl groups in this reaction, this amino acid can play a role in the nocturnal melatonin peak.

Here we studied whether methionine can increase the nocturnal melatonin peak.

MATERIALS AND METHODS

Experiments were performed on 90 adult male Wistar rats. The animals were kept in a vivarium under standard light/dark regimen (14-h light and 10-h dark). Methionine (30 and 140 mg/kg, Oktyabr') was dissolved in 2% starch and given *per os* by the end of the daytime for 5 days. Control animals received an equivalent volume of the solvent.

The rats were decapitated according to general ethical principles of experiments on animals [1]. Since the concentration of indoleamines in the pineal gland undergoes diurnal variations, the animals were killed at night (0.00-3.00) at a dim red light, *i.e.* when functional activity of the pineal gland is maximum. The pineal glands were immediately isolated and treated by routine biochemical methods [7]. The intensity of methylation was estimated fluorometrically by the content of hydroxyindoles (serotonin and N-acetylserotonin) and their methylated derivatives (5-methoxytryptamine and melatonin) in the pineal gland. We isolated the

0007-4888/04/1375-0431

TABLE 1. Effect of 5-Day Treatment with Methionine on Nocturnal Melatonin Peak in the Pineal Gland of Adult Rats ($\bar{X}\pm S_x$)

Group	Indole concentration in the pineal gland, ng/mg tissue				
	serotonin	N-acetyl-serotonin	melatonin	5-methoxy-tryptamine	5-HIAA and 5-MIAA
Control (n=10)	14.79±1.50	5.45±0.47	2.52±0.35	11.71±1.46	4.28±0.34
Methionine 30 mg/kg (n=9)	14.65±0.47	4.98±0.52	4.74±0.37*	12.20±1.08	4.46±0.27
140 mg/kg (n=9)	31.72±3.89*+	4.44±0.64	6.49±0.50*+	15.26±1.82°	8.91±0.71*+

Note. *p<0.001 and °0.05<p<0.01 compared to the control; +p<0.001 compared to 30 mg/kg methionine.

total fraction containing 5-hydroxy- and 5-methoxyindole acetic acid (5-HIAA and 5-MIAA, respectively). Pure indoles (Sigma) served as the standard. The measurements were performed with pineal glands from 3 animals. Fluorescence was recorded on a BIAN-130 fluorometer at 365 and 470 nm. Indole concentration was expressed in ng/mg wet tissue.

The results were analyzed by Student's t test.

RESULTS

Administration of methionine modulated biochemical transformations of indoles in the pineal gland and intensified melatonin biosynthesis (Table 1).

It should be emphasized that administration of methionine even in low (physiological) dose significantly increased melatonin concentration in the pineal gland (p<0.001), but the content of other indole fractions (serotonin, N-acetylserotonin, 5-methoxytryptamine, 5-HIAA, and 5-MIAA) remained within the limits of individual variations typical of control animals. Methionine in high (pharmacological) dose increased hormonal activity of the pineal gland and significantly elevated concentrations of serotonin and its methylated derivatives melatonin and 5-methoxytryptamine (and, probably, 5-MIAA, because the content of the total fraction of indolylacetic acids in the pineal gland also increased). The intensity of melatonin secretion in the pineal gland directly depended on the dose of methionine. Melatonin concentration in control rats was taken as 100%. The content of melatonin in group 2 and 3 animals was 188 and 257%, respectively.

These data show that methionine, which serves as a source of methyl groups in the organism, intensifies O-methylation of hydroxyindoles in the pineal gland and determines the nocturnal melatonin peak.

The lipotropic effect of methionine is probably associated with its contribution to the nocturnal melatonin peak, since this hormone possesses hypocholesterolemic, hypobetalipoproteinemic, and antisclerotic properties.

Our results indicate that methionine is an important physiological factor involved in the maintenance of biological rhythms and determining the melatonin peak. Our findings provide theoretical basis for new applications of methionine for increasing nocturnal melatonin peak.

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