

## AGE-RELATED DIFFERENCES IN THE EFFECT OF EPITHELAMIN ON SEROTONIN METABOLISM IN THE RAT PINEAL GLAND

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The pineal gland occupies a central place in the regulation of the neuroendocrine and immune systems [8, 11, 13]. The functional activity of the pineal diminishes with age [1]. Pinealectomy shortens the life span of animals [12], whereas administration of the pineal hormone melatonin to old mice lengthens it [11]. Experiments on mice of different strains and on rats have shown that the use of the pineal polypeptide preparation epithalamin, which is obtained from the bovine pineal gland and is free from melatonin [7] lengthens the life span of the animals and inhibits spontaneous and induced carcinogenesis in them [1, 2, 10]. Epithalamin also possesses immunostimulant [10], anti-gonadotrophin [3], and certain other properties and has been successfully used clinically in the treatment of several diseases [6]. However, the mechanism of action of epithalamin has not yet been adequately studied. Our previous investigations showed that injection of epithalamin into young male rats causes significant changes in biosynthesis and metabolism of indoles in the pineal gland itself [4, 9], indicating participation of these biologically active substances in the mechanism of action of epithalamin.

This paper describes the results of a comparative study of indole biosynthesis and metabolism in the pineal gland of rats of different ages and the effect of epithalamin on these processes.

### EXPERIMENTAL METHOD

Experiments were carried out in the dark period of the year (November-January) on male Wistar rats of different ages: 4-5 months (body weight  $221 \pm 3$  g) and 18-20 months ( $391 \pm 5$  g). The animals were kept in the animal house at room temperature on an ordinary diet and with natural alternation of day and night. Epithalamin, dissolved in 0.9% sodium chloride solution, was injected subcutaneously in a dose of 0.25 mg/100 g body weight daily at 10 a.m. for 5 days. Intact rats served as the control. The animals were killed by decapitation after midnight, i.e., during the period of maximal pineal activity [13]. Serotonin and its metabolic products – N-acetylserotonin (N-Ac), melatonin, 5-methoxytryptamine (5-MT), and also a combined fraction consisting of 5-hydroxy- and 5-methoxyindoleacetic acid (5-HIAA and 5-MOIAA) – were determined fluorometrically, as described previously [4]. The intensity of fluorescence was measured on a BIAN-130 fluorometer, using filters with wavelengths of 365 and 470 nm. Indole concentrations in the pineal were expressed per milligram wet weight of tissue. The experimental results were subjected to statistical analysis by Student's *t* test.

### EXPERIMENTAL RESULTS

The experimental results, given in Table 1, show that serotonin metabolism in the pineal gland of old rats differs significantly from that in young, sexually mature animals. Whereas active conversion of serotonin through N-Ac into

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TABLE 1. Effect of Epithalamin on Parameters of Serotonin Metabolism in Pineal Gland of Young and Old Rats

Age of animals, months	Conditions	Concentration of indoles in pineal gland, ng/mg tissue				
		serotonin	N-Ac	melatonin	5-MT	5-HIAA + 5-MOIAA
4—5	Control (n = 8)	3.65±0.18	1.70±0.26	2.54±0.19	7.13±0.39	16.96±1.07
	Epithalamin (n = 13)	5.10±0.55 <sup>a</sup>	2.77±0.37 <sup>b</sup>	3.61±0.31 <sup>a</sup>	7.83±0.58	18.53±1.22
18—20	Control (n = 6)	5.20±0.98	3.39±1.41	1.66±0.23	3.43±0.30	14.84±1.40
	Epithalamin (n = 7)	5.93±1.14	2.28±0.19	2.32±0.25 <sup>c</sup>	3.08±0.74	14.02±1.57

Legend. Difference from control for corresponding age significant: <sup>a</sup>p < 0.01, <sup>b</sup>p < 0.001, <sup>c</sup>0.05 < p < 0.1.

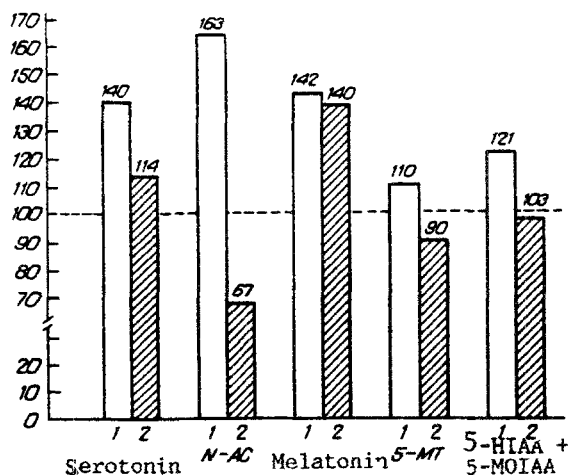


Fig. 1. Percentage content of serotonin and its metabolic products in pineal gland of young sexually mature (1) and old (2) male rats under the influence of epithalamin injections. Unshaded columns — young sexually mature, shaded columns — old rats. Horizontal broken line denotes levels of test substances in intact animals of corresponding age, conventionally taken as 100%.

melatonin takes place in the pineal of young mature rats, old animals are characterized by accumulation of serotonin in the gland because of reduction of its utilization along the N-acetylation pathway and subsequent O-methylation.

The comparative study of the effect of epithalamin on indoleamine metabolism in the pineal gland of rats at different ages revealed significant differences in its response to injection of the peptide preparation. Unlike in young sexually mature animals, in which daily injections of epithalamin for 5 days caused an increase in the nocturnal concentrations of serotonin, N-Ac, and melatonin in the pineal gland, in old rats under similar experimental conditions a tendency was observed for only the melatonin level to increase, whereas concentrations of serotonin and the other metabolic products studied did not differ statistically significantly from those observed in intact animals.

If the concentration of the indoles studied in intact animals is conventionally taken to be 100%, it will be clear (Fig. 1) that injection of epithalamin caused an increase in the melatonin concentration in the pineal of animals of both age groups, but the mechanism of this increase has certain age differences. For instance, in young mature rats receiving epithalamin, the levels both of serotonin and of its metabolic products along the N-acetylation pathway followed by O-methylation is observed, evidence in support of activation of serotonin formation from tryptophan and its subsequent conversion through N-Ac into melatonin. The increase in the melatonin concentration in the pineal of old animals under the influence of epithalamin evidently takes place through the more intensive conversion of N-Ac into melatonin, i.e., the point of application of epithalamin in the serotonin → N-Ac → melatonin reaction in old animals is predominantly the last stage. Meanwhile, in neither young nor old animals had epithalamin any marked effect on other pathways of serotonin metabolism in the pineal, namely the reaction of direct O-methylation of serotonin with the formation of 5-MT, and oxidation deamination followed by O-methylation with the formation of 5-HIAA and 5-MOIAA. Incidentally, our observa-

tions agree with those made previously which showed an increase in the melatonin concentration in the blood serum of old rats under the influence of epithalamin [5].

It can thus be concluded from the results of this investigation that the action of epithalamin at the pineal level, despite its age-related differences, consists of potentiation of the biosynthesis of melatonin and its secretion by pinealocytes. This mechanism with an ultrashort link between pineal peptides and indoles may be realized through the many different biological effects of epithalamin.

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