EFFECT OF PINEAL PEPTIDE PREPARATION EPITHALAMIN ON SEROTONIN METABOLISM IN THE RAT PINEAL GLAND

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The role of the pineal gland (epiphysis) as a central regulator of endocrine functions is currently being widely studied. The function of the pineal gland as a gland of internal secretion is generally accepted, but discussions are still proceeding on the number of hormones synthesized and secreted by it. Most investigators regard melatonin as the principal mediator of the various influences of the pineal gland on the neuroendocrine system, but it has been shown that other serotonin metabolites may also be secreted by the gland [11]. Finally, there is evidence that some effects of the epiphysis are the result of its secretion of peptide hormones [1, 3, 7, 8]. It must be emphasized that data on interaction between these two classes of compounds, produced in the pineal gland, are virtually nonexistent. On the basis of analysis of relations between indoleamines and oligopeptides contained in the same granules in the pinealocytes, it has been suggested that indoles modify permeability of the pinealocyte cell membranes for peptides and thereby facilitate entry of the latter into the bloodstream [10]. Meanwhile, nothing is known about the possible effect of pineal peptides on indole production and metabolism in the pineal gland.

The aim of this investigation was to study the effects on these processes of the Soviet peptide preparation epithalamin (sanctioned by the Pharmacological Committee, Ministry of Health of the USSR, Protocol No. 16, dated September 23, 1988, for medical administration to patients). This preparation was obtained from bovine pineal glands and consists of a combination of peptides, possessing distinct biological effects, notably antigonadotropic, immunostimulating, psychotropic, and antitumor effects [1-6].

EXPERIMENTAL METHOD

Experiments were carried out on 60 mature male Wistar rats weighing 220-250 g in the dark time of the year (November). The animals were kept in the animal house at room temperature on a normal diet, under conditions of natural daylight and darkness. Daylight lasted 9 h and darkness 15 h. Epithalamin, dissolved in 0.9% sodium chloride solution immediately before injection, was injected subcutaneously in a dose of 0.25 mg/100 g body weight once a day at 10 a.m. for 5 days. Intact animals served as the control.

Assuming that the concentration of indoles in the pineal gland undergo significant fluctuations during the 24-h period, the rats were killed by decapitation at the time of maximal activity of the gland (after midnight, between that time and 3 a.m.), under red light [11, 12]. Serotonin and its metabolic products — 5-methoxytryptamine (5-MT), melatonin, N-acetylserotonin (N-AS), and 5-hydroxy and 5-methoxyindoleacetic acid (5-HIAA) and 5-MOIAA) were determined in the pineal gland fluorometrically [9]. Fluorescence was measured on a BIAN-130 fluorometer, using filters with wavelengths of 365 and 470 nm. Concentrations of indoles were expressed per milligram wet weight of tissue. The experimental results were subjected to statistical analysis by Student's *t* test.

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TABLE 1. Effect of Epithalamin on Parameters of Serotonin Metabolism in Rat Pineal Gland

Group of animals	Character of procedure	Statistical parameter	Concentration of indoles in pineal gland, ng/mg tissue					
			5-MT	melatonin	serotonin	N-AS	5-HIAA, 5-MOIAA	total of fractions
I	Intact control (n = 8)	$\bar{x}\pm S_{\bar{x}}$	7,13±0,39	2,54±0,19	3,65±0,18	1,70±0,26	16,96±1,07	32,00±1,57
II	Single injection of epithalamin (n = 8)	$\bar{x}\pm S_{\bar{x}}$	6,62±0,58	3,15±0,21	4,24±0,27	2,03±0,19	18,65±1,71	35,00±1,59
		P_{I-II}	—	0,05	0,1	—	—	—
II	5 daily injections of epithalamin (n = 13)	$\bar{x}\pm S_{\bar{x}}$	7,83±0,58	3,61±0,31	5,10±0,55	2,77±0,37	18,53±1,22	38,76±3,65
		P_{I-III}	—	0,01	0,02	0,05	—	—
		P_{I-III}	—	—	—	0,05	0,1 —	—

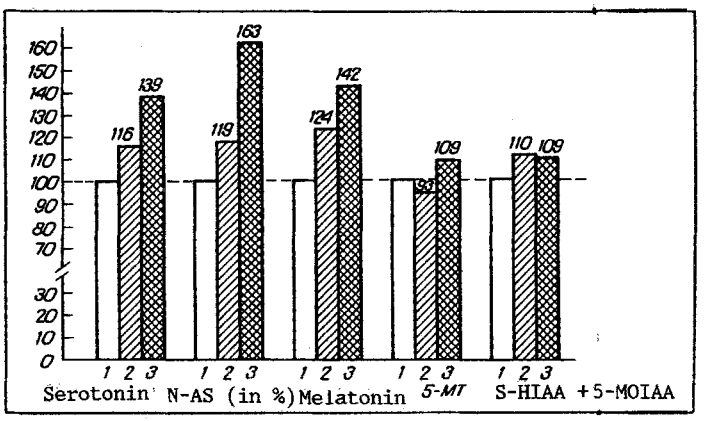

Fig. 1. Concentrations (in per cent) of serotonin and its metabolites in rat pineal gland after single (2) and 5 daily injections (3) of epithalamin 1) intact control.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1. They show that a single injection of epithalamin caused an increase in the melatonin concentration in the pineal gland of the experimental rats, the serotonin level showed a tendency to rise, but concentrations of the remaining serotonin metabolites studied did not differ statistically significantly from values in the control animals. Injection of the preparation for 5 days led to an increase in pineal levels of serotonin, N-AS, and melatonin, but there was no effect on the 5-MT concentration or the total indoleacetate fraction.

The relative percentages of individual indole fractions in the pineal gland are shown graphically in Fig. 1, from which it can be seen that epithalamin increased the relative percentages both of serotonin itself and of its metabolic products along the N-acetylation and subsequent O-methylation pathway, with the formation of N-AS and melatonin, but had no significant effect on the other metabolic pathways (direct O-methylation of serotonin with 5-MT formation, and also oxidative deamination followed by O-methylation with the formation of 5-HIAA and 5-MOIAA).

The results of this investigation show that epithalamin, a polypeptide extract of the pineal gland, has a marked stimulating action on melatonin biosynthesis in the pineal gland; the intensity of this effect, moreover, is proportional to the duration of injection of the preparation. This means that an ultrashort connection exists between pineal peptides and indoles, and the point of application of pineal peptides in the pinealocytes is primarily the reaction of conversion of tryptophan into serotonin and its subsequent metabolism into N-AS and melatonin. In our view, stimulation of pineal melatonin production lies at the basis of the mechanisms of the therapeutic action of epithalamin.

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MODIFICATION OF ION-TRANSPORTING SYSTEMS OF HUMAN ERYTHROCYTES DURING KEEPING

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The last decade has seen a sharp increase in the number of diagnostic kits, associated with determining the functional state of cell membranes. In one case, these diagnostic kits have been designed for analysis of the efficacy of therapeutic preparations (for example, agonists and antagonists of different classes of receptors, organic calcium antagonists cardiac glycosides, and so on), in another case to detect abnormalities in the functioning of certain membrane-bound systems. In clinical practice, virtually the only object accessible for studies of this kind is the blood cells. The reason why they can be used is that the level of activity of membrane-bound systems in the blood cells and in cells functionally significant for the pathogenesis of the disease of the organs under investigation correlate. In particular, this approach is currently being used for the differential diagnosis of essential hypertension. In an experimental model of essential hypertension, namely spontaneous genetic hypertension of rats, changes detectable in the blood cells in most cases can also be detected in smooth muscle cells, nerve endings, epithelial cells of the renal tubules, i.e., in organs directly related to the functioning of the systems for long-term regulation of the blood pressure [2, 5, 11].

The introduction of these methods into practice on a large scale depends on a number of factors and, in particular, on the stability of the method and the stability of the parameter determined in the case of storage of blood cells.

In this paper we give data showing that if heparinized blood is kept for three days on ice, there is no change in the rate of functioning of Na^+/Li^+ antitransport, Na^+/K^+-cotransport, and Na^+,H^+-exchange in the erythrocytes. Data were obtained previously on the heterogeneity of the kinetic parameters of these carriers in rats with spontaneous hypertension and in humans with essential hypertension [2-4, 7, 8, 10]. The results of the present investigation indicate that the tests may be used to determine the functional state of cell membranes in the investigation of large population groups, for the stability of the parameters determined is such that material can be accumulated, and this greatly simplifies problems related to its collection and transport.

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