EFFECT OF MELATONIN ON MITOTIC DIVISION OF GEL'SHTEIN 22A ASCITES HEPATOMA CELLS

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The action of melatonin on mitosis of cells of the ascites form of Gel'shtein 22A mouse hepatoma was studied. Male C3HA mice with ascites for 7 days were used. A stathmokinetic reaction of the ascites hepatoma cells were found after intraperitoneal injection of melatonin. In concentrations of 200 and 100 μg/g body weight, melatonin was shown to cause an increase in the mitotic index and accumulation of mitoses at the metaphase stage 3 h after injection.

KEY WORDS: Melatonin; stathmokinetic effect; ascites hepatoma.

Hormones play an important role in the regulation of the mitotic regime of the tissues of multicellular organisms [1, 4, 6]. There is conflicting evidence on the character of action of the pineal gland hormone melatonin (N-acetyl-5-methoxytryptamine) on mitotic division. Some workers have shown that melatonin changes the mitotic index (MI) of animal tissues [5, 13], but others found no such effect [7, 8, 10, 12]. There is also evidence of the induction of a stathmokinetic reaction by melatonin. For instance, complete delay of mitosis under the influence of melatonin at the metaphase stage has been found in onion root cells [7]. Also there are reports in the literature that melatonin can exert an antitumor action when administered to animals [2, 9, 11]. The presence of a stathmokinetic reaction and of an antitumor effect of melatonin suggests that it may act in a similar way to colchicine. However, investigation of the action of this substance on animal cells *in vitro* and *in vivo* has not revealed a stathmokinetic effect [5, 8, 10, 12, 13]. Information on the effect of melatonin on the mitotic regime of tumors is not available. It was therefore decided to study the character of the action of melatonin on mitosis of ascites hepatoma 22A cells.

METHODS

Experiments were carried out on the ascites form of Gel'shtein hepatoma 22A [3]. C3HA male mice weighing 20 g were used. A tumor was transplanted into intact animals on the 10th day after inoculation, by injecting 0.3 ml of ascites fluid, containing about $40 \cdot 10^6$ cells, intraperitoneally into each animal. Mice with a 7-day tumor were used in the experiments. The synthetic melatonin used was from Calbiochem (USA). It was dissolved in hot physiological saline (0,9% NaCl), which was brought to a boil, then allowed to cool to room temperature, and injected intraperitoneally in a dose of 0.5 ml. The control physiological saline was treated in the same way. Injections were always given at the same time of day, in the morning. Two concentrations of melatonin were used: 200 and 100 μg/g body weight.

Preliminary experiments showed that 3 h is long enough for the stathmokinetic reaction of colchicine to be exhibited. After intraperitoneal injection of colchicine in a dose range from 0.1 to 10 μg/g body weight complete delay of mitosis took place at the metaphase stage. The time of exposure for melatonin was therefore chosen to be 3 h also. The animals were killed 3 h after injection of the hormone, ascites fluid was removed from their peritoneal cavity, and films were made on slides, fixed with methanol, and hydrolyzed in 1 N HCl for 1 min at 60°C, after which they were stained with toluidine blue. MI was determined by counting the number of mitoses in 5000 nuclei. The results were expressed in promille and as percentages of phases of mitosis in 100 mitoses examined. Statistical analysis of the data was carried out by Student's method.

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TABLE 1. MI and Ratio of Phases of Mitosis after Exposure for 3 h to Melatonin

Conditions	Number of animals	MI, ‰	Distribution of phases of mitosis		
			prophase	metaphase	ana-telophase
Control	6	11,37±1,48	18,17±0,91	52,33±4,39	29,50±3,79
Melatonin 200 μg/g	6	21,37±1,88	4,67±0,88	83,00±3,88	12,33±3,82
P		0,002	<0,001	<0,001	0,01
100 μg/g	6	18,50±2,36	11,50±2,01	68,50±2,49	20,00±2,17
P		<0,05	0,01	0,01	0,05

RESULTS

The concentrations of melatonin used caused an increase in MI, an increase in the relative number of metaphases, and a decrease in the number of prophases and ana-telophases, evidence of delay of mitosis at the metaphase stage. The data showing changes in the parameters of the mitotic regime are given in Table 1. They show that the most marked changes were observed when the higher concentration of melatonin was used.

The results of this investigation thus show that melatonin induces a definite stathmokinetic reaction in cells of Gel'shtein 22A ascites hepatoma. The absence of a similar reaction in other types of cells, as other workers have described, can probably be attributed to particular features of the action of melatonin, as a hormone which differs in its action on different cells.

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