CYTOSTATIC ACTION OF 5-HYDROXYTRYPTAMINE (SEROTONIN) ON TISSUE CULTURES

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Serotonin (5-hydroxytryptamine hydrochloride) causes a transient and reversible decrease in the number of prophases in a culture of HeLa cells. With an increase in the serotonin concentration, mitotic activity of cells of HeLa, CaVe, AS, and FK cultures may be sharply and irreversibly suppressed, causing death of the culture. It is postulated that serotonin reversibly blocks transition of the cells from a period of the mitotic cycle.

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It has previously been shown that serotonin (5-hydroxytryptamine hydrochloride), when added to a nutrient medium in a concentration of 1000 μg/ml, completely arrests growth of a HeLa culture [7].

To continue to the study of the mechanism of action of serotonin on the tumor and normal cell, in the present investigation we studied its effect on cell multiplication in culture.

EXPERIMENTAL METHOD

Monolayer cultures on cover slips in Carrel flasks were used in the experiments. The nutrient liquid was synthetic medium No. 199 and human blood serum (20%). Together with fresh nutrient liquid, serotonin obtained by M. F. Petrova in the Laboratory of Chemistry of Natural Substances of this Institute, was added to 2- or 3-day cultures. At the same time, fresh nutrient medium without serotonin was added to the control cultures. After 1-6 h the cultures were fixed in Bouin's fluid. Mitoses were counted in 5000 cells. Most of the experiments were performed on the HeLa strain. In addition, strains CaVe (from carcinoma of the stomach) [2], AS (from angiosarcoma) [3], and FK (from rat fibroblasts taken from the capsule formed around a lung abscess) [1] were used.

EXPERIMENTAL RESULTS

TABLE 1. Action of Serotonin in Different Concentrations on Mitotic Activity of HeLa Cells

Duration of action (in h) and concen. of serotonin (in μg/ml)	No. of HeLa cultures	Mean mitotic index (in %)	P	Mean prophase index	P
3; 25	6	30,3	0,08	5,7	<0,001
3; 50	5	29,7	0,01	4,7	0,002
3; 100	7	32,6	0,3	3,4	<0,001
Control	5	37,5	—	9,1	—
6; 25	5	34,4	0,1	7,1	>0,1
6; 50	6	35,8	0,1	6,6	0,1
6; 100	6	41,1	0,1	7,8	>0,1
Control	6	36,4	—	9,7	—

No significant differences in mitotic index were found between HeLa cultures treated with serotonin for 1 and 2 h and the controls, even when a high concentration was present in the medium. Serotonin caused a considerable decrease in the number of cells in prophase 3 h after the beginning of its action (Table 1). The mean prophase index of the cultures (number of prophases per 1000 nuclei) fell more severely the higher the concentration of serotonin. Deviations from the intact control were statistically significant for all concentrations used – 25, 50, and 100 μg/ml, expressed as serotonin hydrochloride, or 21, 42, and 83 μg/ml of the pure base (serotonin hydrochloride contains 83% of the pure amine). Although changes were observed in the total number of mitoses (mean mitotic index;

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TABLE 2. Action of Serotonin in a Concentration of 1000 μg/ml on Mitotic Activity of Cultures

Expt. No.	Strain	No. of cultures	Time of action (in h)	Mean mitotic index (in %)		P
				expt.	control	
1	HeLa	6	1	31,8	34,3	0,23
2	HeLa	6	2	32,3	34,0	0,78
3	HeLa	5	3	12,0	33,5	<0,001
4	HeLa	5	6	4,4	36,4	<0,001
5	CaVe	5	6	5,4	31,4	<0,001
6	AS	5	6	11,0	38,8	0,001
7	FK	5	6	5,6	26,6	<0,001

see Table 1), they were less distinct. Six hours after addition of serotonin no changes were present in the number of prophases. The mean mitotic index in experiments with 25 and 50 μg/ml also was equal to this index in the control, while in the experiments with 100 μg/ml it was higher than the control value.

Hence, the moderate cytostatic effect of serotonin observed 3 h after its administration in high concentrations evidently does not produce irreversible changes in the cells but is temporary in character.

In experiments in vivo the cytostatic action of serotonin revealed the same reversibility and short duration when its effect was studied on regeneration and mitotic activity of the epidermis in tadpoles [4].

A further increase in serotonin concentration in the nutrient fluid may, however, cause irreversible changes in the cells. A serotonin concentration of 1000 μg/ml, for instance, may cause a considerable decrease in mitotic index (Table 2), and death of the culture after approximately 20 h. In this case also, changes in the number of mitoses also appeared 2 and 3 h after the beginning of treatment.

There is reason to suppose that blocking of the mitotic cycle under the influence of serotonin, leading to a reduction in the index between the 2nd and 3rd hours of its action on the cell, occurs at the shortest phase of the cycle G_2, lasting about 3 h in HeLa cells [8]. The premitotic period G_2, according to published findings, is very sensitive to radiation [9]. If, therefore, temporary and reversible blocking of cells in phase G_2 occurs under the influence of serotonin, which is usually given as a radioprotective agent before irradiation, especially in experiments in vitro [6], this blocking may play the role of the principal reaction in the mechanism of the protective action of serotonin [5]. However, this hypothesis must be verified in special experiments to study the effects of serotonin on the mitotic cycle.

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