COMPARATIVE ACTION OF INHIBITOR AND STIMULATOR IN EHRLICH'S ASCITES TUMOR CELLS ON CELL DIVISION IN THE TUMOR

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The study of the chalone system of tissues, which is concerned directly with regulation of proliferative processes and tissue homeostasis, is nowadays considered important. However, some aspects of this problem have not been adequately studied. One of them is the location of tissue-specific inhibitors of cell division in the cell and the principle governing their interaction with stress hormones.

According to some workers chalones are located on the cell surface [2, 4, 6]. Other workers have demonstrated the inhibitory action of components of the cell nuclei on cell division [5].

The view is widely held in the literature that adrenalin plays an essential role in the mechanism of the biological effect of chalones [7, 8]. Meanwhile, investigations have demonstrated that interaction between chalones and stress hormones is more complex than was hitherto considered [1, 3].

The object of this investigation was to study the action of cytoplasmic and nuclear fractions of Ehrlich's ascites tumor (EAT) cells of mice on cell division in this tumor and on its dependence on adrenalin.

EXPERIMENTAL METHOD

Noninbred male albino mice of the same age (1.5-2 months) and weighing 18-20 g were used. The animals were kept in 12 h of daylight (6 a.m. to 6 p.m.) and 12 h of darkness and were fed ad lib. for two weeks before the experiment began. The test object was a diploid strain of EAT adapted to conditions of culture *in vivo* and *in vitro*. The tumor was transplanted by intraperitoneal injection of 0.2 ml of ascites fluid containing about 10^7 tumor cells, every 7 days of development of the tumor. To study tissue specificity of the chalone-containing preparation (CCP) a strain of Novikoff's ascites hepatoma, transplanted similarly into C3HA mice, was used.

To obtain the nuclear and cytoplasmic fractions ascites fluid extracted from the peritoneal cavity on the 13th day of tumor growth was centrifuged at 1000 rpm for 5 min on a TsAK-1 centrifuge. The supernatant was poured off and the residue resuspended in physiological saline at 0-4°C and centrifuged at 1000 rpm for 5 min. The supernatant was again poured off and the residue resuspended in a solution of 0.32 M sucrose and 0.003 M $MgCl_2$ in sterile distilled water (pH 7.2-7.4) in the ratio of 1:4 (one part of residue to four parts of solution). The suspension was incubated at 37°C for 15 min to swell the cells and facilitate destruction of the dense cell membrane of EAT cells. After incubation for 15 min at 37°C the suspension was homogenized vigorously for 20 min in a glass-Teflon homogenizer at 0°C. The homogenate was centrifuged for 4 min at 1000 rpm to remove cell fragments. The supernatant was centrifuged at 3000 rpm for 10 min. The residue of nuclei was diluted with the solution of sucrose and $MgCl_2$ (pH 7.2-7.4) in the ratio of 1:20 and kept at 0°C before injection.

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TABLE 1. MI in EAT 4 h after Intraperitoneal Injection of Nuclear and Cytoplasmic Fractions in EAT Cells

Fraction	MI ($M \pm m$) ‰	Percent of control	P
Control	15,3±1,01	—	—
Nuclear fraction	29,8±1,93	195,0	<0,001
Cytoplasmic fraction	4,4±0,91	18,8	<0,001

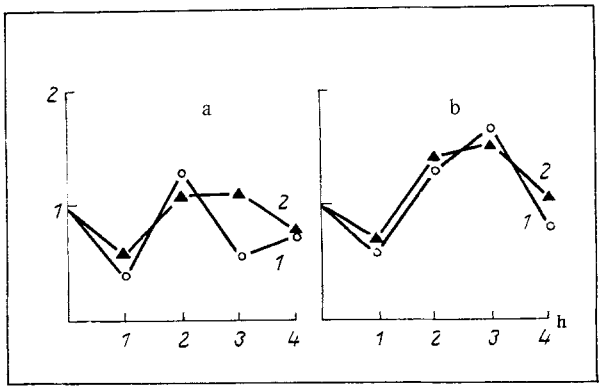

Fig. 1. Action of cytoplasmic fraction of EAT cells on mitotic activity in culture of EAT cells without adrenalin (a) and in the presence of adrenalin (b). 1) 0.5 ml of cytoplasmic fraction, 2) 0.1 ml of cytoplasmic fraction. Here and in Figs. 2 and 3: abscissa, time after addition of fraction to culture (in h); ordinate, ratio of MI in experiment to MI in control.

The cytoplasmic supernatant was diluted with the same solution in the ratio of 1:5 (final dilution 1:20) and kept at 0°C before injection. Corresponding fractions were obtained in the same way from Novikoff's ascites hepatoma in order to study the tissue specificity of CCP from EAT. To standardize the experimental conditions the fractions were isolated from a strictly definite number of cells.

Cells obtained from the tumor on the 5th-6th day of growth were cultured by the method described previously [3].

To analyze mitotic activity in EAT films were prepared from the tumor, fixed with methanol, and stained with methylene blue. In each preparation 5000 cells were analyzed and the mitotic index (MI) calculated in promille. In each experiment *in vitro* an average of three parallel tests were carried out. Allowing for repetition of the experiments, each point corresponded to 3 to 12 tests. The significance of differences in the parameters was calculated by the Student–Fisher method. Differences were considered significant at the $P \leq 0.05$ level.

EXPERIMENTAL RESULTS

Determination of MI 4 h after intraperitoneal injection of 1 ml of nuclear and cytoplasmic fractions and of the control solution (sucrose + $MgCl_2$) showed that the nuclear fraction almost doubled the level of mitotic activity, whereas the cytoplasmic fraction led to a sharp decrease in the number of mitoses in the EAT cells (Table 1).

The cytoplasmic fraction of EAT cells thus has an inhibitory action on mitosis.

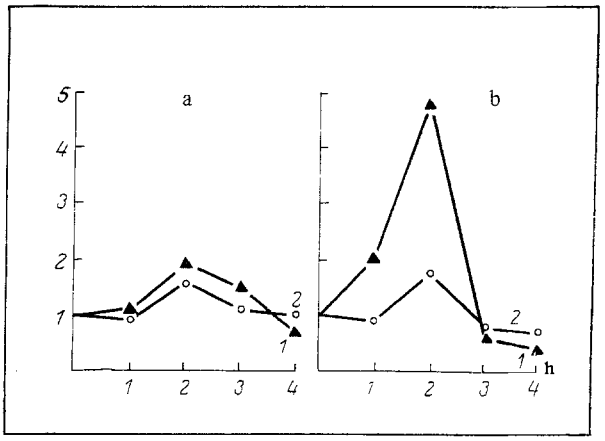

Fig. 2. Action of nuclear fraction of EAT cells on mitotic activity in culture of EAT cells in the absence (a) and presence of adrenalin (b). 1) 0.5 ml of nuclear fraction, 2) 0.1 ml of nuclear fraction.

The next experiments were undertaken in a culture of EAT cells. The action of the cytoplasmic and nuclear fractions on cell division in doses of 0.5 and 0.1 ml was studied in the presence of adrenalin (2.5 μg/ml culture medium) and in its absence. A solution of sucrose and $MgCl_2$ (0.5 and 0.1 ml respectively) was added to the control samples. MI was analyzed 1, 2, 3, and 4 h after addition of the fraction to the culture.

It will be clear from Fig. 1 that 1 h after addition of the cytoplasmic fraction a significant fall in MI was observed. Mitotic activity 1 h after addition of cytoplasmic fraction in a dose of 0.5 ml was reduced by 62%, and in a dose of 0.1 ml by 39%. After 2 h, MI in the experiment was now higher than MI in the control (by 32 and 8% respectively), evidence that the action of the cytoplasmic inhibitor is reversible. The latent period of action of the inhibitor on cells in culture, it will be noted, was much shorter than *in vivo*. The presence of adrenalin was not essential for manifestation of the inhibitory effect. Addition of adrenalin in the tests with cytoplasmic fraction did not potentiate its inhibitory effect but led to a more marked subsequent rise of mitotic activity, which lasted until the 3rd hour of the experiment. Consequently, adrenalin definitely modifies the inhibitory action of cytoplasmic fraction on mitosis.

It will be clear from Fig. 2 that the nuclear fraction, when added to the culture, caused a sharp rise in MI after 2 h: by 92% in a dose of 0.5 ml and by 59% in a dose of 0.1 ml. By 4 h after addition of the fraction MI returned to its control level, evidence that the stimulating action of the nuclear fraction is of short duration. The combined action of nuclear fraction and adrenalin potentiated the stimulating effect and shortened the latent period of action of the stimulator: With a dose of nuclear fraction of 0.5 ml, an increase in mitotic activity by 98% was observed 1 h after the experiment began, and by 2 h MI was increased by 4.8 times. At the same time the period of increased mitotic activity was shortened: MI returned to its control level 3 h after the experiment began. It follows from these data that adrenalin modifies the character of action not only of the inhibitor, but also of the stimulator of division of EAT cells isolated from them.

In the next experiment the tissue specificity of action of the cytoplasmic and nuclear fractions on cell division in EAT was studied. Corresponding fractions were isolated from cells of Novikoff's hepatoma 22A and the culture of EAT cells was treated with them. It will be clear from Fig. 3 that the cytoplasmic fraction of hepatoma 22A cells, unlike that of EAT cells, had no effect on cell division in the EAT culture. The cytoplasmic fraction of EAT cells thus contains an inhibitor of cell division, with reversible action on the late G_2 phase of EAT cells, which is tissue-specific. Addition of the nuclear fraction of hepatoma 22A cells to a culture of EAT cells, like addition of the nuclear fraction of EAT cells, gave a similar effect, namely an increase in mitotic activity in this culture. Consequently, the action of the nuclear fraction of EAT cells on cell division is tissue-nonspecific.

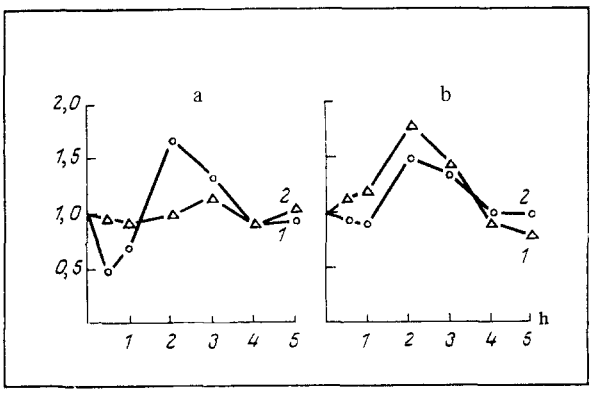

Fig. 3. Action of cytoplasmic (a) and nuclear (b) fractions of EAT cells (1) and of Novikoff ascites hepatoma 22A cells (2) on mitotic activity of EAT cells in culture.

These experiments showed that cytoplasmic fraction of EAT cells contains an inhibitor of cell division which is tissue-specific, a characteristic feature of the biological effect of chalones. Meanwhile the nuclear fraction of these cells contains a stimulator of cell division which is not tissue-specific. However, the action of both inhibitor and stimulator is modified by adrenalin.

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