EFFECT OF INTENSITY OF PROTEIN SYNTHESIS ON INCREASED RESISTANCE OF TISSUE CULTURE CELLS

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Addition of meradine, a compound with anabolic action, in a dose of 5 μg/ml to the culture medium of HeLa cells caused an increase in resistance of the cells to the harmful action of 200-400 μg/ml chlortetracycline. Meradrine does not exhibit its protective action after its combined administration with puromycin, which indicates that the resistance of the cells to the harmful agent is directly dependent on the intensity of protein synthesis.

A previous communication [3] described the stimulant effect of 8-mercaptoadenine (meradine) on cell metabolism and, in particular, on protein synthesis in cells. Later experiments showed that meradine also causes a nonspecific increase in cellular resistance to various harmful agents (the action of certain viruses and antibiotics in very high doses, etc.). It was postulated that the increase in resistance of the cells is associated with stimulation of their protein synthesis by the action of meradine.

The object of the present investigation was to test this hypothesis experimentally.

EXPERIMENTAL METHOD

HeLa cells were used because of the rapidity of their proliferation and the high intensity of their metabolism. In the experiments of series I the resistance of cells, stimulated by meradine, to high doses of chlortetracycline, an antibiotic with a many-sided mechanism of harmful action [4], was determined. Meradine was added in a dose of 5 μg/ml for 4 h to a 3-day culture of HeLa cells, after which the culture medium was replaced by a fresh medium containing 200-400 μg/ml chlortetracycline. In the experiments of series II, after treatment with meradine the culture medium was replaced by one containing puromycin, an inhibitor of protein synthesis [2], for 30 min, after which it was again changed for a medium containing chlortetracycline in the doses specified above.

The results of the tests were read 24 h after addition of the chlortetracycline. The change in the number of living cells was determined by staining with a 1% aqueous solution on erythrosin, and the mitotic activity was investigated by counting the number of different phases of mitosis (in one experiment the number of dividing cells per 3000 interphase cells for each group was counted). The results given below are the mean values of not less than five repeated experiments. The results of all the counts were subjected to statistical analysis by the Student–Fisher method.

The dose of puromycin was titrated in preliminary experiments using methionine-S^{35}. The results were obtained by measuring the change in intensity of incorporation on a gas-flow counter.

EXPERIMENTAL RESULTS

The experimental results given in Table 1 show that the number of cells still remaining viable after exposure to the harmful action of chlortetracycline in groups 5, 8, and 11 (the metabolism of the cells in

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TABLE 1. Changes in the Number of Living Cells, Mitotic Activity, and Individual Stages of Mitosis in HeLa Cell Lines under the Influence of Meradine, Puromycin, Chlortetracycline, and Their Combinations

Group No.	Dose of chlortetracycline (in μg/ml)	Dose of meradine (in μg/ml)	Dose of puromycin (in μg/ml)	Number of living cells (in thousands/ml)	Mitotic activity (sum of all phases of mitosis per 1000 interphase nuclei)	Number of prophases per 100 dividing cells	Number of metaphases per 100 dividing cells	Number of anaphases and telophases per 100 dividing cells
1	—	—	—	377,3±13,2	19,2±1,5	17,4±3,6	58,3±4,4	24,3±0,6
2	—	5	—	358,6±16,5	22,1±1,5	16,1±3,7	65,3±4,3	18,6±0,7
3	—	—	5	309,1±11,0	21,0±2,3	13,2±4,1	80,8±3,7	6,0±0,2
4	200	—	—	324,5±19,8	11,0±1,3	19,1±4,6	62,3±5,9	18,6±0,4
5	200	5	—	392,7±33,0	20,7±1,5	13,0±3,8	61,7±4,5	25,3±1,2
6	200	5	5	244,2±11,0	17,8±2,8	13,9±5,1	82,1±5,2	4,0±0,3
7	300	—	—	270,6±14,3	15,1±2,8	15,7±4,7	64,8±4,5	19,5±0,3
8	300	5	—	336,6±12,1	21,1±2,0	14,8±5,3	60,3±6,4	24,9±1,0
9	300	5	5	222,2±7,7	13,5±2,1	11,3±2,3	83;0±3,4	5,7±0,4
10	400	—	—	191,4±14,3	8,7±1,4	23,9±8,5	53,1±7,9	23,0±5,7
11	400	5	—	299,2±9,9	16,3±2,4	15,8±3,1	57,9±4,0	26,3±1,8
12	400	5	5	171,6±9,9	8,2±1,2	12,5±5,0	80,0±6,3	7,5±1,6

Note. For counting the number of living cells, n = 20; for counting the total number of mitoses and the number of individual phases, n = 14.

these groups was preliminarily stimulated by the addition of meradine) was significantly higher ($a > 0.999$) than in groups 4, 7, and 10, respectively, which did not receive this preliminary treatment. In groups 5, 8, and 11 the morphology of the cell layer was much better preserved, there were fewer degenerating cells, and the mitotic activity was higher than in groups 4, 7, and 10, respectively. Preliminary cultivation of the cells for 4 h in a medium containing meradine in a dose of 5 μg/ml thus protects them adequately against the harmful action of large doses of chlortetracycline.

The experiments using the labeled precursor methionine-S^{35} showed that the addition of 2.5 μg/ml puromycin for 30 min to the culture of HeLa cells did not change the intensity of their protein synthesis. The addition of puromycin in a dose of 5 μg/ml reduced protein synthesis by 48%, and in a dose of 10 μg/ml by 68%.

The action of puromycin in a dose of 5 μg/ml on protein synthesis in cells stimulated by meradine was determined. The incorporation of methionine-S^{35} was increased by 53% over the control 5 h after addition of meradine. When puromycin was added to the cells for 30 min in a dose of 5 μg/ml after the preliminary treatment with meradine for 5 h, their protein synthesis was at the rate of 64% of the control. Stimulation of protein synthesis caused by the addition of meradine was thus completely blocked by puromycin in a dose of 5 μg/ml.

It will be clear from Table 1 that when chlortetracycline was added to HeLa cells in which the stimulation of protein synthesis by meradine had been abolished by addition of 5 μg/ml of puromycin (groups 6, 9, and 12), the number of viable cells remained the same as after chlortetracycline had acted on cells unprotected by meradine (groups 4, 7, and 10, respectively). The difference between the numbers of living cells in groups 6, 9, and 12, on the one hand, and groups 5, 8, and 11, on the other, is absolutely significant ($a > 0.999$).

After the successive action of 5 μg/ml puromycin and chlortetracycline on cells stimulated by meradine, the mitotic activity of groups 9 and 12 was depressed by about the same amount as by chlortetracycline alone in groups 7 and 10. The difference between the level of mitotic activity of cells with protein synthesis stimulated (groups 8 and 11) and inhibited (groups 9 and 12) is absolutely significant ($a > 0.98$). In these experiments treatment with 5 μg/ml puromycin caused mitosis to be arrested at the metaphase stage, in agreement with data in the literature [5]. It was therefore decided to determine the change in the ratio between the different stages of the mitotic cycle under the influence of meradine, chlortetracycline, and combinations of these substances with puromycin. These results, given in Table 1, show that when chlortetracycline was added after meradine (groups 5, 8, and 11), the number of anaphases and telophases per 100 dividing cells was between 25 and 27 respectively, i.e., virtually the same as in the control. If, on the

other hand, chlortetracycline was added after meradine and puromycin, the percentage of anaphases + telophases was 4-7 (groups 6, 9, and 12), the same as after treatment with puromycin alone (6% in group 3). The difference between groups 5, 8, and 11, on the one hand, and groups 6, 9, and 12, on the other, is absolutely significant ($a > 0.999$).

These experiments thus showed that under the influence of meradine, the resistance of the cells to the harmful action of chlortetracycline was increased. The preliminary addition of meradine to the culture prevented the decrease in the number of living cells, the depression of mitotic activity, and damage to the cell layer by the action of chlortetracycline. This protective action of meradine is not exhibited after its combined administration with puromycin. Treatment with puromycin thus abolishes the increased resistance of the cells to chlortetracycline produced by meradine. According to Andreeva [1], puromycin abolishes the increase in the thermostability of the sartorius muscle induced by 2-benzylbenzimidazole hydrochloride or by 4-methyluracil.

The fact that these results are similar in principle confirms the nonspecific character of the cellular resistance associated with an increase in the intensity of intracellular protein synthesis. Where synthesis was inhibited by the combined administration of meradine and puromycin, then as the results of counting the living cells (in groups 6, 9, and 12) showed, their resistance to the harmful action of large doses of chlortetracycline was actually slightly lower than the resistance of the cells in groups 4, 7, and 10, not receiving preliminary treatment. This observation correlates with the results of determination of the intensity of methionine incorporation, for in these experiments the intensity of protein synthesis after the combined administration of meradine and puromycin also was slightly lowered by comparison with the control. The high level of correlation observed between the results, taking into account the specificity of action of puromycin, indicates that the cellular resistance to the harmful agent is directly dependent on the intensity of protein synthesis.

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