EFFECT OF MEDIATORS ON PROCESSES OF CELL DIVISION

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A number of papers have appeared during the past few years, on regulation by the nervous system of cell division processes in the organism. I. A. Alov [1] and M. K. Zakharov have shown that changes in the number of mitoses of epithelial cells of the cornea and tongue occur as a result of direct stimulation of the nerves supplying these tissues. A number of authors [2-7] have demonstrated reflex depression of the number of cells entering into mitosis following the application of external stimuli. This reflex reaction is effected through the adrenals, which secrete increased amounts of adrenalin into the blood stream; denervation of the adrenals prevents reflex inhibition of cell division. Administration of adrenalin lowers the mitotic activity of the cornea, skin, tongue, and intestine [2].

In the present paper we describe the results of a study of the effects of mediators on processes of nervous regulation of mitotic activity.

EXPERIMENTAL METHODS

Mitotic counts were performed on the cornea of white mice. A drop of physiological saline was placed on the left eye, or (for experiments on the action of adrenalin) a drop of the preservative solution, of the appropriate concentration of chloretone and hydrochloric acid. A solution of mediator was placed on the right eye.

The mice were killed 1-1 $\frac{1}{2}$ hours later, at the same time (17 animals). Mitotic counts were performed for full thickness sections of cornea, for an area of 1.54 mm^2, and the mitotic phase coefficient was evaluated (ratio of the first two phases of mitosis to the last two). Standardization of counts was achieved by shifting the fields of vision along the meridians of the cornea. Comparison of the mitotic counts for the left and right eyes permitted of an appreciation of the effects of adrenergic and cholinergic preparations on cell division. Preliminary studies showed that the differences between the mitotic counts for normal animals (10 mice) for the left and right eyes did not exceed $\pm$ 4.5%.

The results found in experiments in which adrenalin solutions (1:1000 to 1:100,000) were applied (27 animals) showed that adrenalin sharply depresses the mitotic count. Its effect depends on its concentration and on the duration of its action.

The number of cells in mitosis falls by 35-45%, as compared with the control, an hour after application of adrenalin, and there is a preponderance of cells in the later stages of mitosis (ana- and telophase). Maximum suppression of mitotic activity, up to and including complete arrest, is observed 1 $\frac{1}{2}$ hours after application of adrenalin, 1:1000. With diminishing concentrations of adrenalin (1:10,000 to 1:100,000) the inhibitory effect somewhat diminishes, chiefly as a result of the appearance towards the end of the 1 $\frac{1}{2}$ hour period of new mitoses (pro- and metaphase). It can be concluded from the sequential disappearance and appearance of the various mitotic phases, and from an analysis of the absolute counts, that adrenalin retards the entrance of cells into the mitotic process. Those processes which have already begun proceed to completion in the ordinary way, notwithstanding the presence of adrenalin.

Some of the results of this series of experiments are presented in Table 1.

TABLE 1

Changes in the Mitotic Count of Mouse Corneas 1 1/2 Hours After Application of Adrenalin

No. of experiment	Adrenalin concentration	Number of mitoses and phase coefficient K		Change (as % of control)
		left side (control)	right side (experiment)	
4	1:1000	13; K=0.3	4; K=0.3	— 69.2
5	1:1000	276; K=0.9	9; K=1.2	— 96.2
10	1:1000	177; K=1.3	0	—100
11	1:1000	159; K=0.9	0	—100
12	1:1000	199; K=1.4	0	—100
13	1:1000	34; K=1.0	0	—100
66	1:1000	107; K=1.4	0	—100
67	1:1000	50; K=5.2	0	—100
68	1:1000	20; K=4.0	0	—100
69	1:1000	104; K=2.1	4	— 96.1
6	1:100 000	249; K=1.1	148; K=1.4	— 40.5
7	1:100 000	176; K=1.4	176; K=1.3	— 0
51	1:100 000	123; K=1.3	85; K=1.1	— 30.9
52	1:100 000	96; K=2.1	15	— 84.4
53	1:100 000	92; K=1.7	63; K=2.0	— 31.5

In the second series of experiments (35 experiments in all) we studied the effect of acetylcholine and carbocholine on cell division. At low concentrations (1:10,000 to 1:50,000) carbocholine did not give clear-cut effects 1 to 1 1/2 hours after application to the cornea. The number of mitoses was raised (up to 161% of control) in 8 of 19 experiments, lowered in 8 experiments, by 42%, and unchanged in 3 experiments. These variable results were evidently due to differences in the initial levels of mitotic activity. It is noteworthy that increase in the number of motises was observed with relatively low mitotic activity (Experiments Nos. 31, 32, 34, and 36), whereas in most of the experiments in which carbocholine depressed mitotic activity, the latter was at a relatively high initial level (Nos. 30, 35, and 41). Some of the results obtained in this series are presented in Table 2.

The impression is given that carbocholine has a normalizing effect on mitotic activity, raising it when the initial level is low, and depressing it when it is high. In only 3 of 19 experiments did carbocholine appear to have no effect.

TABLE 2

Change in Mitotic Count of the Cornea 1 1/2 Hours After Application of Carbocholine

No. of experiment	Concentration of carbocholine	Number of mitoses and phase coefficient K		Change (as % of control)
		left side (control)	right side (experiment)	
30	1:10 000	166; K=1.6	136; K=1.0	— 18.1
31	1:10 000	71; K=1.4	103; K=1.1	+ 45.1
32	1:10 000	82; K=1.6	197; K=1.7	+140.2
33	1:10 000	130; K=2.9	126; K=2.0	— 3.0
34	1:10 000	100; K=2.2	131; K=4.7	+ 31.0
35	1:10 000	198; K=1.4	146; K=2.2	— 26.2
36	1:10 000	109; K=1.3	118; K=2.0	+ 8.2
37	1:10 000	74; K=2.5	49; K=2.3	— 33.8
41	1:25 000	210; K=1.4	121; K=1.9	— 42.4
42	1:25 000	98; K=1.9	97; K=1.4	— 1.0
43	1:25 000	78; K=1.3	156; K=2.7	+100
44	1:25 000	70; K=1.9	183; K=1.5	+161.4

TABLE 3

Change in Mitotic Count of the Cornea $1\frac{1}{2}$ Hours After a Single Application of Acetylcholine

No. of experiment	Concentration of acetylcholine	Number of mitoses and phase coefficient K		Change (as % of control)
		left side (control)	right side (experiment)	
54	1:1000	49; K=5.1	79; K=2.4	+ 61.2
55	1:1000	100; K=3.5	92; K=1.7	— 8
56	1:1000	75; K=1.6	42; K=1.6	— 44
57	1:10 000	58; K=3.1	76; K=4.8	+ 31
58	1:10 000	41; K=5.8	36; K=5.0	— 12.2
59	1:10 000	68; K=1.3	64; K=1.5	— 5.9
60	1:10 000	31; K=1.2	55; K=2.1	+ 77.4
61	1:50 000	90; K=1.7	102; K=1.8	+ 13.3
62	1:50 000	73; K=1.4	38; K=0.9	— 47.9
64	1:50 000	44; K=2.1	54; K=2.2	+ 22.7
65	1:50 000	22; K=0.8	110; K=1.3	+400

Very similar results were obtained with acetylcholine (1:1000 to 1:50,000). In 5 of 11 experiments the mitotic count was raised $1\frac{1}{2}$ hours after application of acetylcholine, and in 6 it was lowered. As for carbocholine, the final effect appeared to depend on the initial mitotic activity (Table 3).

Our experiments thus show that mediators have a definite effect on processes of cell division in the organism. The mediators, which are the terminal effectors of the nervous mechanisms, are evidently able to affect the intensity of cell division processes in tissues. Adrenergic substances (adrenalin) inhibit entrance of cells into mitosis, but where the process has already begun it proceeds to completion in the ordinary way. The effect of cholinergic substances is not so clear-cut, and the results do not permit of the drawing of any definite conclusions as to their effect on mitotic processes.

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