MONOAMINE AND DIAMINE OXIDASE ACTIVITY IN A CULTURE OF CHICK FIBROBLASTS INFECTED WITH Rous Sarcoma VIRUS

E. A. Soloimskaya and O. K. Kuznetsov

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Infection of a culture of chick fibroblasts with Rous sarcoma virus has a substantial influence on monoamine oxidase activity in the cells. During the first 2-4 days after infection activity of this enzyme increases, and on the 8th-21st day, when most cells of the monolayer have been transormed into malignant, a marked inhibition of activity of the enzyme is observed. The increase in diamine oxidase (histaminase) activity during the first days after infection, which is replaced by inhibition of activity in the later periods of cultivation, was not significant.

Mitochondrial enzymes monamine oxidase (MAO) and diamine oxidase (DAO), which catalyze the breakdown of biogenic amines, participate in many physiological and pathological processes. In particular, their activity in the tissues of animals has been found to vary under the influence of carcinogenic agents [8].

It was decided to investigate whether changes in the activity of these enzymes take place in cultures of chick embryonic cells during malignant transformation under the influence of Rous virus.

EXPERIMENTAL MATERIAL AND METHOD

Rous sarcoma virus (RSV), strain D-5, was used as culture fluid from an infected culture of chick embryonic cells (CEC). The virus was assayed by titration in a CEC culture on the basis of the formation of foci of transformation.

The cell culture was prepared from trypsinized pieces of myodermal tissue of 10-day chick embryos. The cells were grown in Eagle's nutrient medium containing 10 % calf serum, 100 units penicillin, and 100 units streptomycin, pH 7.3-7.8.

Infection of the Culture and Determination of Its Enzymes. A monolayer of a flask culture (114 cm^2) of 1-day CEC was infected with $10^{4.5}$ plaque-forming units (PFU_{50}) of RSV and incubated for different times at 37°C. The nutrient medium for infection of the cells was a mixture of Eagle's medium containing 10% tryptose phosphate broth (Difco) and 3% inactivated (30 min at 56°C) calf serum. The enzymes were determined on the 2nd, 4th, and 8th days after infection of the culture (incubation without subculture) with RSV.

To rule out the effect of degenerative changes due to natural aging of the monolayer cultures some of them were regularly subcultured into other flasks, and on the 15th and 20th days after infection (on the 4th day after the preceding subculture) activity of the enzymes in the cells was again determined. Uninfected cultures, incubated under the same conditions as the infected, served as the control.

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TABLE 1. MAO and DAO Activity in a CEC Culture Infected with RSV (Mean Results of 4-7 Experiments)

Time after infection of culture (in days)	Conditions of cultivation		MAO activity (in μg serotonin decomposed in 2 h at 37°C, (2.5× 10^6 cells)	P	DAO activity (in μg histamine decomposed during 2 h at 27°C (2.5× 10^6 cells)	P
2	Without subculture	Experiment	10,4±0,5	0,01	1,4±0,2	0,1
		Control	7,6±0,5		1,2±0,1	
4		Experiment	11,5±0,5	0,05	2,0±0,3	0,1
		Control	9,6±0,3		1,4±0,3	
8		Experiment	3,0±0,5	0,05	0,7±0,1	0,1
		Control	6,0±0,9		1,0±0,1	
15	With subculture	Experiment	11,0±1,2	0,01	1,3±0,4	0,5
		Control	17,0±0		0,9±0	
21		Experiment	7,4±0,4	0,001	4,0±0,4	0,1
		Control	10,6±0,2		3,0±0,2	

Note: Similar results were obtained by the use of tyramine and noradrenalin as substrates and these results are not therefore given in the table.

To determine the enzymes in the cells of the monolayer, after removal of the nutrient medium all traces of it were removed by rinsing with physiological saline. The cells were stripped from the cover slip by means of a rubber rod and immersed in 0.1 M phosphate buffer, pH 7.4, in an estimated number of about 2.5×10^6 cells. The material was homogenized in tubes by agitation with glass beads for 10 min. The supernatant obtained after centrifugation of the homogenate for 15 min at 2000 g was used for assay of the enzymes. Enzyme activity was determined by methods described previously [7]. The substrates used were serotonin, noradrenalin, and typamine for MAO and histamine for DAE. Activity of the enzyme was judged from the loss of substrate in the reaction mixture during the period of incubation. Activity was expressed in micrograms of the corresponding substrate broken down by 1 ml of the test material during incubation for 2 h at 37°C.

EXPERIMENTAL RESULTS AND DISCUSSION

Microscopic foci of morphological transformation, increasing in size during incubation of the culture, appeared after the second or third day in the CEC culture infected with RSV, and by the end of the second week most of the cells of the monolayer were replaced by transformed cells [3, 4].

MAO and DAO were detected in normal and virus-infected cells throughout the 21 days that the culture remained under observation (Table 1). Differences between the DAO activity of the experimental and control cultures were not significant. Activity of MAO in the infected cells differed significantly from that in the control. On the 2nd-4th day after infection MAO activity was significantly higher than in the uninfected cells. After 8 days, when multiple foci of transformation, visible to the unaided eye, had appeared in the culture activity of the enzyme was reduced by 1.4-2 times compared with the unifected culture. By the 15th-20th day the virus-infected culture consisted mainly of activity proliferating, transformed cells. The MAO activity of the experimental cultures of this period, as before, remained below that of the control cultures.

The results thus indicate that activity of the enzyme DAO, which inactivates histamine in the cells, does not change significatnly during malignant transformation of cells in vitro under the influence of RSV. At the same time, activity of MAO, which inactivates biogenic amines by oxidative deamination, is significantly increased during the first days of cultivation of the infected cells, but later, with an increase in the number of transformed cells in the culture, it falls below the level in normal cells. This confirms that the development of virus infection of the cell leads to reorganization of its metabolism. Chick fibroblasts, infected with RSV, are known to possess increased aerobic glycolytic activity [5, 6], an increased RNA content [4, 10, 13], and intensified protein synthesis [10].

There is much evidence that changes in MAO and DAO activity in mammals are frequently accompanied by pathological symptoms [1, 5, 6, 9] connected with metabolism of endogenous amines and with a disturbance

of nervous control. The discovery of a high level of MAO activity in the normal tissue culture and low activity in cells transformed by RSV thus deserves attention. It is difficult, however, at present to explain the disturbance of MAO biosynthesis in connection with virus carcinogenesis.

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