Evidence of inhibition and induction of microsomal enzymes in *Tetrahymena*

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Summary. Microsomal cytochrome $b560_{ms}$ of the unicellular organism *Tetrahymena* was studied after triamcinolone treatment. One day of treatment decreased the enzyme concentration (nmol/mg protein); however, exposure for 2 or 3 days enhanced it. Three days of exposure increased the total protein content without further increase of microsomal enzyme concentration. The experiments demonstrate the possibility of microsomal enzyme inhibition and induction in *Tetrahymena*.
Key words. Tetrahymena; enzyme inhibition; enzyme induction; microsomal enzymes.

The microsomal enzymes collaborate in a great variety of catalytic and biosynthetic processes. Their operation in higher organisms has long been known, and recently one microsomal enzyme has also been demonstrated in the unicellular organism *Tetrahymena*[1,2]. In cells of higher organisms microsomal enzyme activity is inducible with certain substrates, for example with steroid hormones. Steroid treatment thus results in an increased microsomal enzyme activation. In earlier studies with *Tetrahymena*[3] we were able to induce cytosolic receptors, which were otherwise not detectable, by steroid treatment (imprinting). Therefore, we attempted to induce microsomal enzyme activity in this organism by short-term and long-term exposure to a steroid hormone.

Induction of cytochrome $b560_{ms}$ in *Tetrahymena* by triamcinolone treatment

Period of treatment (nmol/mg) (days)	Time of assay after treatment (days)	Protein content (mg/ml)	Cytochrome $b560_{ms}$ content. (nmol/mg protein)	(nmol/mg protein/ml)
0	1	10.10	0.478	4.7
0	2	11.61	0.382	3.2
0	3	10.85	0.425	3.9
0	4	10.46	0.470	4.4
1	1	11.72	0.363	3.1
1	2	10.91	0.351	3.2
1	3	7.51	0.130	1.7
1	4	8.35	0.154	1.8
2	1	9.97	0.598	6.0
3	1	15.22	0.527	3.5

Tetrahymena pyriformis GL cells were maintained in a medium containing 0.1% yeast extract and 1% Bacto-tryptone (Difco, Michigan, USA), and at the logarithmic phase of growth (2-day-old cultures) were inoculated into media containing 10^{-6} M triamcinolone acetonide (Richter, Budapest), for 1, 2, or 3 days at 28 °C. After treatment the cells were returned to plain medium for different time periods (changing the medium at the 2nd day), and were assayed for enzyme activity. Control cells were handled similarly in each experiment, without triamcinolone treatment. Experiments were done with a time-lag, which provided the possibility of measuring at the same time. The experiments were repeated twice; the results in the table show the mean values.

Tetrahymena cells were disintegrated and homogenized for 40 sec in 30% glycerol containing Losina solution by an ultrasonic disintegrator, and the samples were diluted to 25 mg/ml protein content. Protein was determined by the method of Lowry et al.[4] using bovine serum albumin as the standard.

Tetrahymena cytochrome $b560_{ms}$ content was determined by the $Na_2S_2O_4$ – reduced minus oxidized difference spectrum in a Zeiss UV-VIS Specord spectrophotometer, with an extinction coefficient of 216 $mM^{-1}cm^{-1}$ between 425 and 410 nm, using the method of Fukushima et al.[1]. The results were expressed as specific content, nmol cytochrome per mg protein.

One-day treatment with triamcinolone was followed within 24 h by a concentration decrease of cytochrome $b560_{ms}$, and a still greater decrease was observed 3 and 4 days later (table). However, prolongation of triamcinolone exposure to 2 or 3 days gave rise to a considerable increase in enzyme concentration (nmol/mg protein).

Considering the microsomal enzyme concentration per mg protein, there is similar increase after 2- or 3-day treatment, relative to the untreated control. However, there is a difference between the results of two and of three days' treatment when the changes in total protein content are considered. The 2 days' hormone exposure resulted in a cytochrome $b560_{ms}$ increase, with a total protein content equal to the control. After a 3-day treatment the cellular protein content showed an enormous increase, which was not paralleled by the enzyme activity change. This either suggests that 3-day triamcinolone treatment promoted the increase of proteins other than microsomal, too; or, if the total protein content is accepted as an index of the number of cells, the microsomal enzyme concentration per cell decreased.

In conformity with earlier observations in receptor induction studies[3], the steroid required 3 days to develop the inducer effect. The lasting and increasing enzyme inhibitor effect of 1-day steroid treatment cannot be explained on the basis of the present findings. The fact nevertheless remains that the cytochrome $b560_{ms}$ enzyme of *Tetrahymena* was inhibited or induced by steroid treatment. Both actions were long lasting, and took effect in the progeny generations; this is analogous with the persistence of neonatal enzyme induction in the target cells of adult higher organisms[5-7].

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