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A Functional Study of the Dexamethason e-Induced Steroid Receptor in *Tetrahymena*

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Synopsis. Tetrahymena doesn't contain steroid receptors, however these develop in it after steroid treatment. This was demonstrated earlier by receptor-kinetic analysis. Presently a functional index, PAS-positivity was studied. The PAS positivity of the cells decreases under the effect of the first encounter with the ethanol solved form of dexamethasone, however increased significantly in the case of the second treatment, indicating the presence (development) of receptor.

Although the hormone receptors are characteristic of higher organisms, similar structures also occur in invertebrates and unicellulars (Csaba 1980, 1985, 1986). Specific insulin receptors were demonstrated in *Neurospora crassa* (Fawell et al. 1988, Fawell and Lepard 1988, McKenzie et al., 1988) and specific appearing steroid receptors in *Saccharomyces cerevisiae, Candida albicans* and *Trichomonas vaginalis* (Ford et al. 1987, Feldman et al. 1982, Loose et al. 1981). We reported earlier that the unicellular *Tetrahymena*, which is able to respond to several polypeptide and amino acid hormones, had not a detectable steroid receptor at primary interaction with a steroid hormone, but did form a specific binding site for the steroid on lasting exposure (Csaba et al. 1985). In the present study we investigated the functional expression of the induced steroid binding site by comparison to the response of not previously treated (imprinted) *Tetrahymena* cells.

Material and Methods

Tetrahymena pyriformis GL cells cultured in yeast extract containing Bactotryptone medium at 28°C were treated in the logarithmic phase of growth with 10^{-6}M dexamethasone (Serva, Heidelberg) for 72h, returned to plain medium for 48h, and re-exposed to dexamethasone for 24h. Since dexamethasone was dissolved in presence of 0.07 per cent ethanol, an ethanol control series, too, was set up in addition to the absolute (untreated) control cultures. Cells fixed 24h after the first and the second dexamethasone treatment were treated with PAS reagent for a quantitative cytophotometric assay. A Zeiss cytophotometer, which was connected with a HP41 CX calculator for data processing, was used. Twenty cells were assayed in each group and each assay was performed in three replicates, thus each column of the attached diagram (Fig. 1.) represents a mean value for 60 cells.

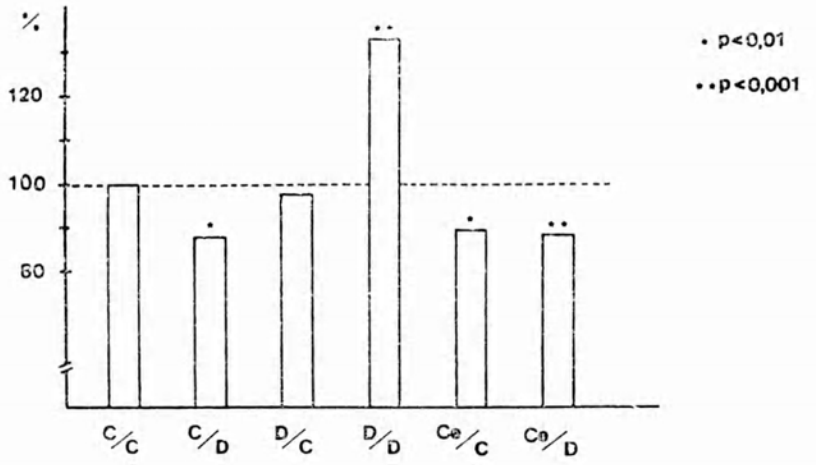

Fig. 1. PAS positivity of *Tetrahymena* cells evaluated by quantitative cytophotometry related to the double untreated control (C/C) as 100. C — untreated, D — dexamethasone treated, Ce — ethanol control

Results and Discussion

As shown in earlier studies (Csaba and Kovács 1979), the hormones influence the glucose metabolism not only in higher organisms, but also in unicellular ones. Thus the PAS reaction, which detects the intracellular accumulation of glycogen, can serve as indicator of glycocorticoid action. In the present experiment the PAS-positivity of *Tetrahymena* was significantly decreased after primary interaction with dexamethasone but since the cells of the ethanol control series showed a similar decrease, this phenomenon was clearly unrelated to hormone action. However, the second exposure to dexamethasone resulted in a biologically

and statistically equally significant increase in PAS-positivity over the control. It follows that the induced steroid receptor, whose formation had been substantiated earlier by receptor kinetic evidence (Csaba and Inczefi-Gonda 1989, Csaba et al. 1985) was functioning in *Tetrahymena* as a genuine receptor, also in respect of mediation of the signal (hormone) molecule. This did, of course, presuppose the collaboration of the signal mediation structures involved in the transfer of information from the receptor to the intracellular functional unit. This is not surprising, if it is taken into consideration that the human oestrogen receptor is able to precipitate the adequate function also after transplantation into yeast (Metzger et al. 1988) cells.

As the cells had been studied certain time after treatment, many new generation developed. Thus the probability of the cell presence of the originally imprinted generation at the time of the assay was as low as 0.097 per cent. It follows that practically all cells involved in reexposure to dexamethasone belonged to a progeny generation and those assayed after reexposure also represented progeny cells. The fact that the latter still showed indications of an increased synthesis of storage of glycogen 24 h after dexamethasone supports the implication of a durable receptor level action of the hormone.

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