Acta Protozool. (2001) 40: 221 - 224

ACTA
PROTOZOOLOGICA

Short Communication

Effect of Epidermal Growth Factor (EGF) on *Tetrahymena pyriformis*

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Summary. *Tetrahymena* has receptors for hormones characteristic in higher animals and signal transduction systems for provoking response. In the present experiments EGF, a potent mitogen of epithelial cells were studied. In a 24 h experiment 1 h treatment with different concentrations of EGF (0.1-10 μg) significantly decreased the growth of *Tetrahymena*. Five microgram EGF treatment 24 h after 5 μg EGF pretreatment (imprinting) also significantly reduced the amount of cells, and this was moderately, however significantly less, than without the repeated treatment. After 48 h there was not difference between the amount of single or double treated cells. EGF was chemorepellent in chemotaxis experiments and 4 h EGF pretreatment abolished this effect, possibly by down regulation. The experiments call attention to the effect of EGF at a very low phylogenetic level, however the direction of the effect was the opposite of the effect in higher animals.

Key words: cell growth, growth factors, chemotaxis, evolution, *Tetrahymena.*

INTRODUCTION

The unicellular ciliated *Tetrahymena* produce, store and secrete hormone-like materials known at higher level of phylogeny. These hormones are insulin (LeRoith *et al.* 1983), adrenocorticotrophic hormone (LeRoith *et al.* 1983), endorphin (Csaba and Kovács 1999), relaxin (LeRoith *et al.* 1983), endothelin (Kőhidai and Csaba 1995), interleukin-6 (Kőhidai *et al.* 2000), serotonin (Brizzi and Blum 1970, Csaba and Kovács 1994), melatonin (Kőhidai *et al.* 2001) histamine (Hegyesi *et al.*1999) etc. and it also has receptors for binding these hormones (Csaba 1985, 2000; Christopher and Sundermann 1995). The information borne by the hormones is transmitted by second messenger systems (Kuno *et al.* 1979, Kovács and Csaba 1990), consequently the cell can respond to the hormonal stimuli. The first encounter between the cell and the hormone develops the hormonal imprinting (Csaba 1985, 2000) which is transmitted to hundreds of progeny generations, causing easier recognition of the given hormone and stronger response of the cell.

Epidermal growth factor (EGF) is a peptide molecule present in the cell membrane and after detachment it becomes a hormone, influencing the velocity of cell

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division, first of all in the case of epithelial cells (Cohen 1983, Soler and Carpenter 1994). It seems to be ubiquitous in the animal world. Considering that *Tetrahymena* can react to many hormones (Csaba 1985, 2000) and that EGF-receptor-like structures were demonstrated (Hide *et al.* 1989) in an infectious protozoan, *Trypanosoma brucei* it was reasonable to study the effect of EGF on *Tetrahymena*, a noninfectious unicellular animal, which - being a ciliate - is at one of the highest steps of unicellular evolution.

MATERIALS AND METHODS

Cells and culturing. *Tetrahymena pyriformis* GL cells were maintained in axenic cultures containing 1% tryptone and 0.1% yeast extract (Difco, Michigan, USA). Cells of logarithmic growth phase (48 h) cultures were assayed. The starting cell density of cultures was 10^2 cell/ml in the assay of growth, in chemotaxis assay it was 10^4 cell/ml.

Hormones and buffers. For treatments and pretreatments of cultures [Cys(Acm)$^{20, 31}$]-epidermal growth factor fragment 20-31 (Sigma, USA; hereafter EGF) was used in 5 μg/ml concentration.

Pretreatments with EGF. For the treatment with EGF we applied two setups: (1) Cultures were pretreated with (0.1, 1.0, 2.5, 5.0 and 10 μg/ml) EGF for 1 h. The control group was treated with the solvent, fresh culture medium. Then the samples were washed trice with phosphate buffered saline (PBS) and the pretreated cultures were maintained in 96 wells of plastic plates for 24 h. (2) In an other setup cultures pretreated for 4 h with EGF and the controls were maintained for 24 or 48 h. After these periods the cultures were reexposed to EGF or culture medium in the next combinations: C/C; C/EGF; EGF/C; EGF/EGF.

For studying the multiplication of cells the colorimetric MTT method was used. In this procedure the water soluble tetrazolium salt, 3-[4,5-dimethylthiazol-2yl]-2.5-diphenyltetrazolium bromide (MTT; Sigma Chemicals, St. Louis, USA) is converted to an insoluble purple formazan by mt-dehydrogenase enzymes of the cell (Denizot and Lang 1986). The incubation time with 0.3 mg/ml MTT was 180 min then the plates were centrifuged for 5 min (2500 x g). The supernatant was discarded and the precipitate was dissolved in sodium duodecyl sulphate. The samples were evaluated in Elisa reader (at 540 and 620 nm).

Chemotaxis assay. Cells pretreated with 5 mg/ml for 4 h and the controls were washed and cultured for 24 h. After this incubation the chemotactic responiveness of *Tetrahymena* cells was evaluated in a two-chamber capillary assay developed by us (Kőhidai *et al.* 1995). In this assay, tips of a multi-8-channel micropipette served as inner chambers filled with different concentrations of the attractant (0.1, 1.0, 5.0 μg/ml EGF or control substance), while wells of a plastic plate were the outer chambers filled with cells. The incubation time was 20 min. Samples were fixed in 4% formaldehyde containing PBS. The number of positively responding cells was counted in samples by Neubauer hemocytometer.

Statistical evaluation. Experiments evaluating effects of EGF and pretreatment on multiplication were done in 16 parallels. Chemotaxis assays were repeated in five times in two parallels. ANOVA method of Origin 4.0 was used to evaluate statistical sigificance. In the Figures the next marks refer to significant differences: x - $p<0.05$; y - $p<0.01$; z - $p<0.001$.

RESULTS AND DISCUSSION

EGF is a growth factor, a potent mitogen for cells of epithelial origin. It is bound by specific membrane receptors, the intracellular part of which is a tyrosin kinase (Soler and Carpenter 1994). By the help of this, its message is transmitted by a signal cascade (Ruff-Jamison *et al.* 1995) into te nucleus. However, sometimes it can act through the mediation of adenylate cyclase-cAMP system (Budnik and Mukhopadhyay 1991). As it was known that *Tetrahymena* has receptors for hormones of higher level animals and signal transduction systems, it seemed to be worth to study the effect of EGF on this cell.

Different concentrations of 1 h EGF treatment significantly decreased the multiplication of *Tetrahymena* (Fig. 1). The hormone was effective at very low concentration (0.1 μg/ml) and the effect was increased at 2.5 μg, however further increase of the dose did not influence the effect. One day after 1 h treatment with 5 μg EGF (Fig. 2) there was present a reduced number of cells (EGF/C), similar to the case, when the cells first time met the hormone (C/EGF). When EGF was given again to previously EGF treated cells (after imprinting) a further moderate, however significant decrease could be observed (EGF/EGF). Two days after imprinting (Fig. 2) the cell number was also less than in the controls (EGF/C) but repeated EGF treatment (EGF/EGF) did not influence this strongly, however significantly. This means that EGF is not a stimulator, but an inhibitor of cell division in *Tetrahymena* and it has only a very limited imprinter effect.

EGF was discovered and is recorded as a mitogen factor for epithelial cells. However, also in mammals in some cases it can inhibit cell division (Hirai *et al.* 1988, Ponec *et al*. 1988). This latter effect was manifested in *Tetrahymena*. The real importance of this phenomenon is not known, however, it was described earlier that *Tetrahymena* produces growth inhibitory factors at stationary phase (Saitoh and Asai 1980, Schousbue *et al*. 1998) and these factors were not chemically defined.

It was observed (Andersen *et al.* 1984) that platelet-derived growth factor (PDGF) is a very active chemoattractant in *Tetrahymena*. This was the reason

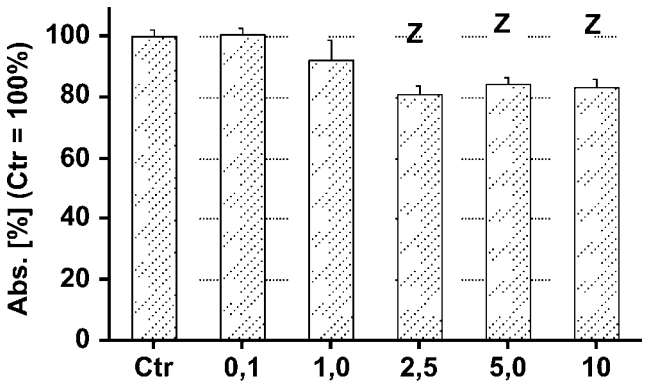

Fig. 1. Effect of 1 h pretreatment with different concentrations of EGF on the multiplication of *Tetrahymena* (z = p<0.001)

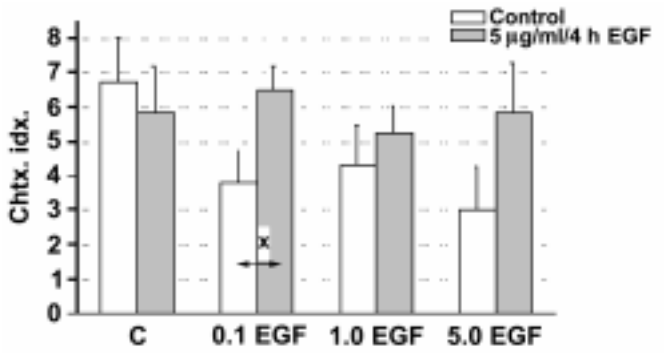

Fig. 3. Chemotactic responsiveness of control and 5µg/ml EGF pretreated (for 4 h) *Tetrahymena* cells 24 h after the pretreatment, to different concentrations of EGF (abscissa, in µg/ml; x = p<0.05)

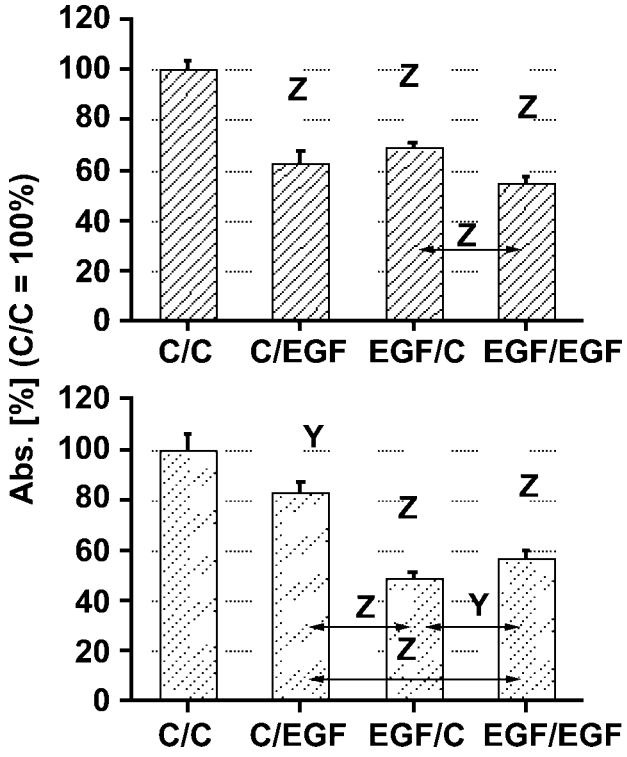

Fig. 2. Effect of 4 h treatment with 5 µg/ml EGF on the multiplication of *Tetrahymena* cells pretreated with 5 µg/ml EGF 24 (above) or 48 h before the second treatment (y = p<0.01; z= p<0.001)

why we studied the chemosensory response to EGF. In our experiments EGF was unequivocally chemorepellent, independent on the concentration administered (Fig. 3). However, 24 h after imprinting with 5 µg EGF, the repellent effect was not significant, which makes likely that the first treatment (imprinting) abolished the sensitivity of cells to EGF. It is possible that the duration of treatment (4 h imprinting) was too much causing intensive down regulation of receptors, which did not allow the recognition of EGF as ligand.

Considering both (growth and chemosensory) results, they show the repressive effect of EGF to *Tetrahymena*.

The experiments demonstrate the ability of *Tetrahymena* to react to a hormone having role in multicellular animals and emphasize the possibility of disparate response of cells to a hormone at different levels of phylogeny.

Acknowledgement. This work was supported by the National Research Fund (OTKA T-024064 and T-017773), Hungary.

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Received and accepted on 17th April, 2001