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Interrelationship of Hormone Concentration, Hormonal Imprinting and Receptor Down-regulation in *Tetrahymena*

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Synopsis. Higher concentrations (10^{-8}-10^{-5}M) of insulin down regulated the insulin binding sites of *Tetrahymena* one day after treatment, however concentrations of 10^{-6}-10^{-18}M caused imprinting with higher binding capacity. After one week imprinting had been observed as a consequence of treatment by each dose. The experiments demonstrate, that (1) very low (100 femtomole) insulin concentration is enough for provoking imprinting and (2) down regulation is a response to hormone excess already at the level of *Tetrahymena*.

In higher organisms the primary interaction between the still immature, but genetically encoded receptor and the adequate hormone takes place in the perinatal period and gives rise to hormonal imprinting, which accounts for receptor amplification, i.e., for establishment of the hormone binding capacity characteristic of adulthood (Csaba 1980, 1986 a). Since unicellular organisms are, by nature, lacking genetically encoded hormone receptors, they present membrane-associated protein configurations of varied quality as hormone binding structures, and transmit these to many progeny generations (Csaba 1985, 1986 b, Csaba et al. 1982). The establishment of such receptor "memory" is being influenced by many factors, of which the concentration of the hormone was the subject of the present study.

Materials and Methods

Tetrahymena pyriformis GL cells maintained in 1% peptone plus 0.1 yeast extract containing Bacto-tryptone medium (Difco, Michigan, U.S.A.) at 28°C were treated (or not treated, as control) with different insulin (Semilente MC, Novo,

Denmark) concentrations (range: 10^{-3}-10^{-13}M) for 1 h (sample size: 10 ml, volume 4×10^5 cell/ml), returned to plain medium for 24 h, then incubated in presence of fluoresceine-isothiocyanate-labeled insulin (FITC, BDH, England) for 1 h, and assayed for intensity of fluorescence in a Zeiss Fluoval cytofluorimeter, which was connected with a HP41CX calculator, programmed for the statistical evaluation of mean values, standard deviation, and significance of inter-group variation. At three levels of treatment (10^{-13}, 10^{-8} and 10^{-3}M) the fluorescence assay was done one week later, too. Twenty cells were assayed at each level of treatment in five replica experiments, thus the values shown in Fig. 1 represent means for 100 cells.

Results and Discussion

The experimental results have demonstrated that already a very low (100 fmol) concentration of insulin was sufficient to induce a lasting imprinting, which did not diminish after transmission to as many as 70 generations within a week's time. It was also demonstrated that insulin imprinting did not become stronger with the concentration increase, and exposure to higher insulin concentrations ($10^{-3}$$10^{-5}$M) even gave rise to down-regulation within 24 h (Fig. 1). This seems logical,

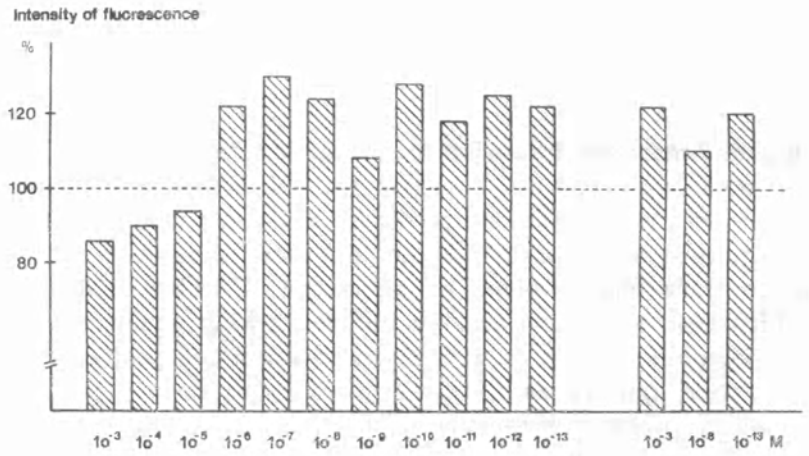

Fig. 1. Binding of insulin to *Tetrahymena* imprinted by different concentrations of insulin one day (left side) and one week (right side) after treatment, related to the control as 100%

if the appearance of coated pits and internalization — in every respect similar to those occurring in higher organisms — in *Tetrahymena* is taken into consideration (Csaba et al. 1984). The critical threshold between the imprinting and down-regulatory effects of insulin on the unicellular is to be sought within the concentration range 10^{-5}-10^{-6}M

but, after one week, the down-regulation elicited by high insulin level also transformed to an imprinting effect. Since, in view of this, imprinting was apparently independent of the applied hormone concentration, the presence, or rather the quality of the hormone seemed to play the decisive role. Since the "recognition" of, or "memory" of, useful and noxious environmental molecules is of vital importance for the survival and reproduction of a protozoon, the dependence of the imprinting mechanism on qualitative rather than quantitative factors seems to be wholly justified from the biological point of view.

Analysis of the present results from the angle of down-regulation — a mechanism which has been well established in higher organisms (G a m m e l t o f t 1984, G o r d e n et al. 1980, M a r s h a l l and O l e f s k y 1980) — permits the conclusion that such a mechanism also operates at the unicellular level, at which it would also render the target cell refractory to excessive hormonal influence. Since down-regulation begins to operate already at the primary interaction of the target cell with the hormone if the latter is present in excess, there is reason to postulate that down-regulation is not so much an integral part of hormonal regulation as response shown to hormone excess also by "naive" — not encoded — cells after presentation of a hormone binding (receptor) structure (C s a b a and K ő h i d a i 1986). The membrane of *Tetrahymena* being extraordinarily dynamic, exposure to insulin (or another hormone) for 1h seems to be sufficient to initiate either down-regulation or hormonal imprinting, depending on the applied concentration of the hormone.

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