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# Impact of the Length of Exposure to Peptides of Different Molecular Mass on the Establishment of Imprinting in *Tetrahymena*

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*Synopsis.* Treatment of *Tetrahymena pyriformis* cells with oxytocin, insulin, thyrotropic hormone (TSH) or bovine serum albumin (BSA) for different times resulted in negative imprinting by oxytocin, whereas in positive imprinting by the other three molecules. The optimal time period for positive imprinting was 60 min, and prolongation of exposure beyond 60 min accounted for down-regulation in the case of oxytocin and insulin, whereas the imprinting potential of the non-hormone peptide BSA was still present at 240 min.

The collection and processing of environmental information is vitally important for the survival of unicellular organisms and species. Communication with the environment is furnished mainly by certain, in all probability randomly formed, patterns in the membrane of the unicellular, which are able to receive information by interaction with environmental signal molecules (Koch et al. 1979). The signal molecules participate in the amplification of the receiver structures by transforming the originally non-specific binding site to a specific receptor (Lenhoff 1968, 1974, Csaba 1980, 1984). The primary interaction of a binding structure with the signal molecule gives rise to imprinting, which accounts for amplification of the receptor and for a consequent alteration of the binding capacity and response of the cell to the signal molecule on subsequent exposure (Csaba 1985, 1986). Thus the cell acquires a receptor-level "memory" of primary interaction with the

signal molecule and transmits it to several hundreds of offspring generations (C s a b a et al. 1982).

Many signal molecules possess an imprinting potential, but the interrelatedness of imprinting with the size and quality of the molecule, and/or with the duration of contract between cell and signal molecule is still obscure. The present experiments were performed to throw more light on this problem.

## Material and Methods

*Tetrahymena pyriformis* GL cells, maintained in 0.1 per cent yeast extract containing 1 per cent Bacto tryptone medium (Difco, Michigan, U.S.A.) were used in the logarithmic phase of growth.

The cells were treated with $10^{-6}$M oxytocin (Gedeon Richter Ltd. Budapest), insulin (Semilente, MC, Novo, Copenhagen, Denmark), thyrotropin (TSH, Ambinon-Organon Oss, Holland) or bovine serum albumin (BSA, HUMAN, Budapest) for 10, 30, 60 or 240 min. after which they were returned to plain medium for 24 h. Subsequently the daughter cells of the pretreated cells were fixed in 0.4 per cent formaline containing phosphate buffer solution (PBS, pH 7.2), washed in two changes of PBS, and incubated for 1 h in presence of fluorescein-isothiocyanate-labeled (FITC, BDH, London, England) peptide, the same as used for pretreatment. After incubation, the cell suspensions were washed in several changes of PBS, spread on slides, and dried.

The binding of the FITC-labeled peptide was assessed by cytofluorimetry, using a Zeiss Fluoval cytofluorimeter, which was connected with a HP-42CX calculator for statistical analysis of inter-group variation by Student's t-test and analysis of variance. Twenty cells were assayed for fluorescence in each group and each assay was performed in flour replicates. Thus each column diagram in Fig. 1 represents the mean value for 80 cells.

## Results

Of the four peptides tested in the present study three, insulin, TSH and BSA, induced a positive imprinting (Fig. 1) after exposure for 60 min. No positive imprinting took place on shorter exposures for 10 and 30 min, and only imprinting by BSA was still positive after 240 min. Oxytocin induced consistently a significant negative imprinting. Insulin accounted after 10 min for a negative imprinting, after 30 min for binding comparable to the control, after 60 min for a positive imprinting and after 240 min the value was comparable to the control.

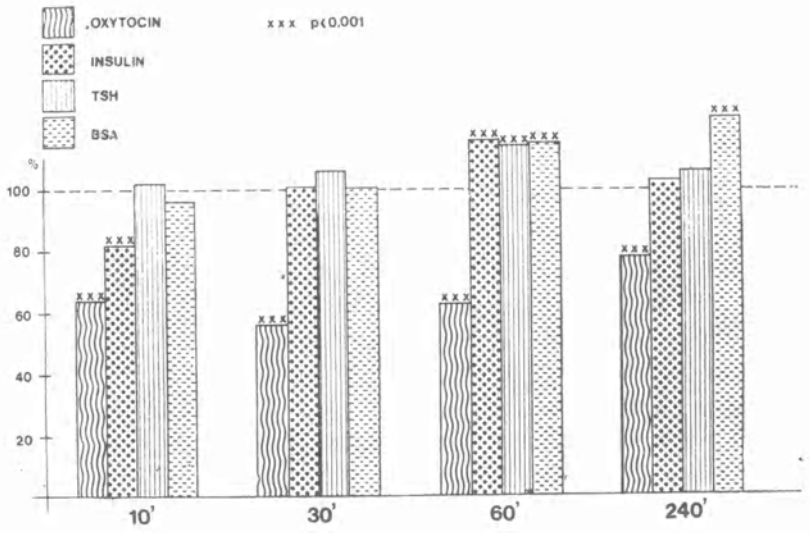


Fig. 1. Impact of treatment (imprinting) with $10^{-6}$M concentration of different peptides for different times on the FITC-peptide binding of *Tetrahymena pyriformis* 24 h after exposure, related to the control as 100

## Discussion

The peptide concentration used ($10^{-6}$ M) was high enough for induction of imprinting, and — as demonstrated earlier (Csaba and Kőhidai, 1986) — also sufficient for initiation of down-regulation. The trend of imprinting may as well be positive as negative, and account for a greater or lesser binding (or cellular response), depending on the nature of the imprinter, or on the species of *Tetrahymena* used (Csaba and Kovács 1987, Kovács and Csaba 1987). Imprinting was usually positive with *Tetrahymena pyriformis*.

Taking into consideration the molecular mass of the imprinters (insulin 6000, TSH 28 000; BSA 67 000 dalton), it appears that the imprinting potential of peptides (and of the protein BSA) did take effect in that molecular mass range, without appreciable differences between the levels. It is also obvious that such peptides or BSA fail to induce positive imprinting within 30 min or less time.

It also deserves mention that oxytocin, whose molecular mass is about 1000 dalton, induced a negative imprinting in a relatively short time in practically all conditions of the experiment. This effect was, however, in all probability unrelated to the molecular mass, since pep-

tides of a much lesser molecular size, such as di-, tri- and pentapeptides an even amino acids, were able to induce a positive imprinting in *Tetrahymena* in earlier studies (Csaba et al. 1986, Csaba and Darvas 1987). It appears that the imprinting potential is associated with the structural properties (amino acid sequence, steric configuration) rather than with the molecular mass of a peptide. It ought to be mentioned in this context that insulin, too, can induce a negative imprinting, e.g. in *Tetrahymena thermophila* (Csaba and Kovács 1987, Kovács and Csaba 1987), whereas it always imprints positively *Tetrahymena pyriformis*, the species used in the present study.

Another important conclusion emerging from this study is that the non-hormone (non-signal) molecule BSA behaved different from the two hormone-like peptides after imprinting for 240 min, probably on the ground that, unlike oxytocin and insulin, it did not give rise to down-regulation after 60 min. It follows that there is a difference in the imprinting potential of signal and non-signal molecules of higher vertebrates in *Tetrahymena*. This does not, of course, exclude that non-hormone polypeptides, too, may possess an imprinting potential (Csaba et al. 1985).

The experimental results uncequivocally indicate that the time factor plays an important role in the issue of imprinting by a given concentration of peptide. The molecular mass of the hormone (peptide molecule) seems to play a lesser role than its chemical nature. The imprinting potential differs between peptide molecules of signal and non-signal nature, and certain signal molecules may even account for down-regulation on lasting exposure.

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