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THE EFFECT OF VARYING ILLUMINATION ON IMPRINTING OF *TETRAHYMENA* BY INSULIN

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Tetrahymena pyriformis cultures were imprinted with insulin. Hormone binding was reduced in the dark, but alternation of dark and light periods were in this respect more effective than the dark itself. The deviations observed may be attributed, besides the reduced insulin binding by the imprinted cells in the dark, to an enhanced binding by the non-imprinted control cells kept in the dark. It is suggested that the dark-induced structural transformation of the membrane, manifesting among others in a changed hormone binding, may be caused by alterations in haem synthesis due to varying illumination.

Environmental effects on cells are of decisive importance for the development of cell function in both unicellular and multicellular organisms. The extraneous parameters, including ambient temperature, pH and ion saturation, may take up values varying between wide limits; their variation from the optimum may cause in cells changes manifesting themselves biochemically and functionally.

The above changes apply to unicellular organisms with a special accentuation, for these organisms must develop an ability to adapt, often very rapidly, to the outworld varying frequently. In the present experiments we used as model cell the unicellular organism *Tetrahymena pyriformis*, which had been the object of our previous investigations into hormonal effects [1].

Hormonal imprinting has been demonstrated in both unicellular and multicellular organisms. It indicates an enhanced responsiveness — memory — which is acquired by cells in an early phase of their development, when they meet the "imprinting" substance, usually a hormone.

The imprinted cells and their progeny will remember the cell-hormone meeting through generations and will respond with an enhanced reaction to repeated hormonal effects [2, 3].

To be imprintable, the cell needs an intact membrane, [4], and changes at other levels, viz. the cytoplasm and the nucleus, are also essential for imprint-

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ing. The evolution of imprinting may be disturbed by environmental changes perturbing the membrane structure [5, 6].

Changing illumination is one of the extrinsic factors compelling the cell to respond and adapt to its environment. In *Tetrahymena*, haem is synthesized by a photosensitive reaction. An intermediary metabolite of haem synthesis is protoporphyrin IX, a substance that, integrating in the cell membrane, may change membrane structure and fluidity [7].

In the present work we examined the effects of changing illumination, effects supposedly mediated by changes in haem synthesis, on the hormonal imprinting attainable with insulin, a hormone of polypeptide nature.

Materials and methods

The GL strain of *Tetrahymena pyriformis* was cultured at 28 °C in a medium containing 1% Bacto Tryptone (Difco) and 0.1% yeast extract; 24 h cultures were used.

Three main stages of experiment were distinguished: (i) adaptation period, i.e. the period preceding hormonal treatment and aimed at adapting to the prescribed environment (24 h); (ii) period of imprinting, i.e. treatment with insulin (Semilente Novo, Copenhagen, Denmark) for 60 min; (iii) transfer into fresh medium (24 h).

The effect of changes in illumination on imprinting was examined by changing the illumination of cultures in consecutive periods of time. A group with unchanged illumination (without dark period) served as control and another group of cultures growing in the dark throughout displayed the effect of uninterrupted lack of illumination.

The scheme of experiment is set out in Table I.

The cells left to rest for 24 h were fixed in 4% formalin diluted in PBS (PBS = 0.05 M phosphate buffer of pH 7.2 containing 0.9% NaCl) and washed with PBS. The cells were then incubated for 1 h in the presence of insulin labelled with FITC (fluorescein isocyanate BDH, London, England).

The degree of insulin binding was estimated by using a cytofluorimeter built together with Hewlett–Packard 41C minicomputer. The fluorescence of 20 cells was measured in each group, and each experiment was performed five times. Thus, each column in Figs 1 and 2 indicates the mean value for the fluorescence of 100 cells.

Results

In Experiments 1, 2 and 3, cells had different times for adaptation to the dark (Table I). In Experiment 1, the cells kept in the dark constantly during adaptation, insulin treatment and the subsequent 24 h period displayed a reduced hormone binding after the three periods, in contrast to the control groups which were kept in natural illumination throughout. In the latter group the hormone binding was enhanced (Fig. 1), owing to a successful imprinting.

In Experiment 2, in which illumination was withdrawn only during the period of imprinting, the reduction in hormone binding was well-defined, although the cells were kept in the dark for a considerably shorter time than

Table I

Scheme of experiment

Experiment No.	Periods of experiment		
	adaptation	imprinting	culturing (24 h)
1	dark	dark	light
2	light	dark	light
3	dark	light	light

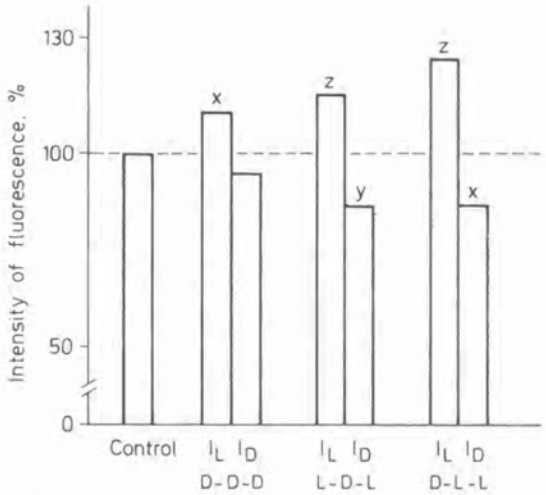

Fig. 1. Binding of FITC-labelled insulin to *Tetrahymena* cells kept in the dark for various periods of time. Binding to the control cells kept illuminated throughout is taken as 100%. C = Control; I = insulin-treated; L = light; D = dark; x, $P < 0.05$; y, $P < 0.01$; z, $P < 0.001$

the cells in Experiment 1. The insulin binding was significantly less than in the control groups, the illumination of which was not interrupted.

In Experiment 3 we wished to obtain new knowledge on the time dependence and the dynamics of the effect under study. We examined whether it is enough to keep the cells in the dark during the period of adaptation to reduce imprinting and the consequent increased hormone binding. The results showed that in this experiment, too, the withdrawal of illumination did exert its imprinting-reducing effect. The difference from the control values was significant.

Discussion

Environmental factors are of decisive importance for living organisms. Even complex biological processes, like the evolution, are, to some extent, directed by environmental factors, which owe their leading role to the fact

that they act at the cellular level by ensuring the dynamic stability of the cell.

The decisive character of environmental factors applies particularly to unicellular organisms, for the survival of these organisms depends on the environmental parameters. Rapid adaptation to environmental changes is a prerequisite for the survival of both the individual and the species.

During hormonal imprinting the cell acquires a special "memory". When it meets the signal molecule again, it will remember the first meeting and usually respond with an altered, usually enhanced, reaction. The enhanced reaction is first of all an increased binding of the given hormone [8], and further parameters may also show alterations [9, 10]. The mechanism of imprinting is only partially known; effects of the given hormone at the membrane level may play a role [4], but modifications in the intracellular space and at the nuclear level may contribute to the phenomenon [6].

The hormonal action, the undisturbed imprinting among them, is a membrane-bound process, therefore, it needs a "physiological" membrane structure, which is indispensable for hormone binding [11]. Every effect that changes the composition and structure of the membrane (effects of temperature, chemicals, ect.) causes a well-demonstrable disturbance in the process of imprinting [4, 5].

Considering that according to literary data *Tetrahymena* is sensitive to illumination [12, 13], we searched in the present work for an answer to the question whether a change in its illumination is capable of causing a structural change of the membrane observable as a change of hormonal imprinting.

The imprinting observed in illuminated *Tetrahymena* supports our earlier experimental results [2, 3]. The insulin binding by the imprinted organism during the second meeting was considerably higher than the binding by the nonimprinted control. On the other hand, imprinting in the dark was followed by a well-defined decrease in insulin-binding capacity, which was independent of the length of the period of imprinting. (The results were approximately the same either 24 h or 1 h was the duration of keeping in the dark.) Therefore, it must be the light-adapted membrane of *Tetrahymena* that is capable of accepting the positive imprinting effect of insulin.

In the experiment in which imprinting was performed in the dark and the cells were kept in the dark for 24 h both before and after the imprinting period, the negative change in insulin binding was less than in the experiments in which cells were kept in the dark only in the 1 h period of imprinting or during the 24 h period of adaptation (Fig. 1). This variation of response suggests that, though, adaptation to the dark is the primary factor causing an alteration in imprinting. *Tetrahymena* adapts to the uninterrupted dark and compensates its effects. Alternating of illumination and dark, on the other hand, seems to induce a considerably more intense effect than keeping in the dark itself.

The deviation between dark-adapted and light-adapted non-imprinted controls proved that a change had happened in the membranes of the cells kept in the dark (Fig. 2). The change in hormone binding was similar in tendency of extent and opposite in direction, compared to the change demonstrated in the case of imprinted cultures. The insulin binding by *Tetrahymena* cells kept in the dark continuously was approximately the same as that by the cells illuminated without interruption. The hormone bound by non-imprinted cells illuminated for only 1 h and kept in the dark before and after imprinting, on the other hand, considerably exceeded the amount of hormone bound by the non-imprinted cells kept illuminated throughout. Furthermore, the hormone binding of the *Tetrahymena* cells kept in the dark for 24 h was still more.

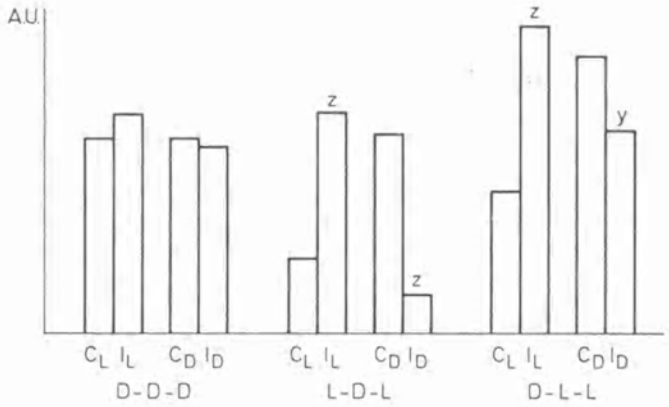

Fig. 2. Comparison of the absolute values of FITC-labelled insulin binding. For abbreviations and explanations, see Fig. 1

Our previous experiments [4, 14] and experiments reported by others [15, 16] have shown that *Tetrahymena* adapts to environmental changes well. This means that a disturbance in its membrane is followed by a steady state, which tends to compensate the changes and, just like the earlier steady state, ensures the life conditions for the unicellular, unless the membrane is impaired again. The changes to and fro, on the other hand, are poorly tolerated by the *Tetrahymena* membrane.

We observed the effect of the dark (in Experiments 1 and 3) and that of imprinting (Experiments 1, 2 and 3) after an interval of 24 h or more. During that time 5 or 6 new generations of *Tetrahymena* came about. Thus, the indi-

viduals that were examined for hormone binding were not the same as those that had undergone the environmental change. This means that, in accordance with our earlier conclusions [17], the changes caused in *Tetrahymena* remained detectable in the progeny through a number of generations. It seems that the periodicity of illumination, i.e. a stimulus regarded as not too strong, was strong enough to induce a change in the responsiveness of the unicellular, a change, which may persist over generations.

The changes discussed above are only selections from changes induced by environmental stimuli. It is not easy to explain the mechanism changing the responsiveness of cells for generations. The synchronizing effect of light and dark [18] must not be disregarded in this respect. Synchronization might favour the selection of a population which is characterized by a special membrane structure. Possible affections at the nuclear level should also be taken into consideration. Nevertheless, a change in the activity of haem synthesis, a photosensitive process, seems to be the most probable influencing factor. Changes in haem synthesis may disturb the membrane structure by integrating into the membrane protoporphyrin IX, an intermediary metabolite of haem synthesis. Protoporphyrin integration is an illumination-dependent process [12], which modifies the membrane structure, viz. impairs its structural stability, on which the undisturbed hormonal imprinting relies.

In conclusion, effects on hormonal imprinting induced by changes in the illumination of *Tetrahymena* cells were examined in the present work. Imprinting by insulin failed to develop in cells kept in the dark and illuminated alternatively while developed but weakly in those kept in the dark constantly. It seems that the dark-induced change in membrane structure is the more pronounced the more striking is the stress acting on the membrane. Furthermore, membrane restauration leads to a steady state, which follows the stimulus rapidly. Besides the reduced insulin binding by imprinted cells, a simultaneous increase in the insulin binding by non-imprinted cells is a characteristic phenomenon following changes in the illumination of *Tetrahymena* cells.

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