"Memory" of First Interaction with Physiological or Biologically Active Foreign Molecules (Benzpyrene, Gibberelline) in a Unicellular (*Tetrahymena*) Model System

G. Csaba *, G. Németh *** and P. Vargha **

Department of Biology *, and Biometry and Clinical Epidemiology Unit **, Semmelweis University of Medicine, Nagyvárad tér 4, H-1445 Budapest; Department of Medical Biology ***, University of Medicine, Szeged

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Gibberelline, prednisolon and benzpyrene stimulated the growth of *Tetrahymena* at the first interaction, while epinephrine did not. On a second exposure following upon the first 24 h treatment after a rest period of 6 h, epinephrine, too, developed a stimulatory action, and the other three molecules gave rise to a still greater increase in the growth rate. Obviously, imprinting effected by the first interaction accounts for a greater responsiveness to physiological materials (hormones) also if these are indifferent in respect of the given function, and non-physiological, but biologically active materials can also give rise to imprinting in exposed target cells.

The first interaction with a hormone alters the target cell's response to it on subsequent exposure(s), depending on the enhancing or depressive effect of the first stimulus. This phenomenon has been referred to as "hormonal imprinting" [1]. Interaction with the adequate concentration of the adequate – physiological – hormone for an appropriate time period usually results in amplification of the receptor [2], whereas interaction with a molecule structurally different from the hormone, but capable of binding to its "receptor" site, usually depresses cellular response to subsequent exposure(s). Since not only the cells are directly involved in hormonal imprinting, but also their progeny generations show the characteristic behavioural change induced by imprinting, the existence of a receptor "memory" has been postulated [4]. In mammalian and avian species, hormonal imprinting takes place during the perinatal period, while in unicellular organisms at the first interaction with the hormone. Thus unicellular animals, whose preceding interaction with a given ligand can be ruled out with certainity, can be utilized as an experimental model for the follow-up of certain cellular events taking place at he higher levels of phylogenesis.

Earlier experimental observations have shown that *Tetrahymena* is capable of (a frequently specific) interaction with certain hormones of higher animals, with which it had not previously been in contact [5, 6]. This can be explained by postulating presence in the unicellular organism of non-specific membrane structures capable of binding the given hormone, and amplification of these "receptor" structures by the first interaction. An alternative explanation may be derived from Koch's dynamic receptor pattern generation theory [7, 8], according to which transient structures arisen by conformational changes of membrane proteins serve as receptors for an infinite variety of ligands; interaction of a hormone with such a structure may result in the latter's stabilization. Consideration should also be given to the experimental observation of Roth and his group [9] who, prompted by our finding that *Tetrahymena* responded adequately to insulin [1], examined the unicellular for presence of that hormone, and did in fact find in it insulin or an insulin-like molecule by immunological approach. In view of this Roth and colleagues have suggested that since. *Tetrahymena* secretes insulin, it necessarily should possess receptors interacting with it. While we accept the experimental facts, we can not agree with this suggestion, on the ground that such a mechanism would be perfectly aimless and unnecessary, not only from theoretical point of view, but considering that *Tetrahymena* moves very fastly.

To obtain more information on the problem, we investigated the (receptor-mediated) response of *Tetrahymena* to molecules not present either in its body or its environment, by exposure of the unicellular to certain biologically active materials, which do not occur in the organism of animals.

Subculture-derived mass cultures of *Tetrahymena* pyriformis GL strain, maintained for 3 days in 1% Bacto trypton (Difco, Michigan) medium at 27 °C, were used. The mass cultures were incubated for 24 h in a medium containing 10^{-9} M gibberelline A_3 (Phylaxia, Budapest) 10^{-9} M, 3–4 benzpyrene (Ega-Chemie, Steinheim, Albuch) 10^{-9} M epinephrine (Richter, Budapest), or 5 µg/ml prednisolon (di-Adreson-F-Aquosum, Organon-Oss), were washed in three changes of plain medium, and were subcultured 6 h later for a second 24 h treatment with the materials indicated in Table 1, at the above con-

Reprint requests to Prof. Dr. G. Csaba.

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Table I. Influence of treatment with physiological and biological active non-physiological materials on the clonal growth rate of the *Tetrahymena*. (The control value repesents the mean rate for 16 cultures.)

First treatment (in mass culture)	–	–	–	–	–	epi.	gibb.	predn.	benzp.
Second treatment (in subculture)	–	epi.	gibb.	predn.	benzp.	epi.	gibb.	predn.	benzp.
Growth rates	12.5	13.4 / 13.5	14.4 / 14.2	15.4 / 16.4	17.8 / 18.5	16.3	17.8	19.9	20.0

Abbreviations: epi., epinephrine; predn., prednisolon; benzp., benzpyrene; gibb., gibberelline. The growth rates shown in the upper row apply to treated for 24 h in mass culture and, after return to plain medium for 6 h, for another 24 h in subculture. The values shown in the lower row apply to cells treated only in subculture. There is no significant difference between the two values in any relation. Growth rate = the number of cells originated from one cell after 24 h.

centrations. Cultures not treated for a second time after cloning, and others not treated in mass culture, only after cloning, were also set up to furnish an untreated control for each experimental group. The clonal cell counts were determined in each series after 24 h. Twenty clones were considered in each group, and the significance of inter-group differences was evaluated by variance analysis.

Since inter-group variation was less than 1 ($F_{15;\,304} = 0.75$) between the untreated control groups (mean total: 12.5), it was possible to compare the treated groups directly, without correction.

Variance between the treated and control groups proved to be $F_{31;\,608} = 35.5$ ($P < 0.001$); for total treatments, the variance factor was 4.14. The latter value was used in comparison between pairs.

Clonal growth rates were similar in subcultures set up in plain medium after treatment with the examined active substances in mass culture, and in those exposed to the active substances without previous treatment in mass culture. With the exception of the epinephrine-treated groups, in which a single treatment did not appreciably enhance cell growth, the growth rate increased significantly ($P < 0.001$) over the control in all first-treated groups, especially in those exposed to benzpyrene.

Two exposures to the same active substance always resulted in a significantly ($P < 0.001$) greater stimulation of cell multiplication than a single treatment; epinephrine, too, accounted for an appreciable growth stimulation on second exposure.

Gibberelline and benzpyrene are non-physiological, but biologically active at the unicellular (*Tetrahymena*) level, while epinephrine and prednisolon, tested for comparison, can be regarded as physiological, since their presence in *Tetrahymena* has been substantiated (epinephrine) or postulated (glycocorticoids). Epinephrine, not previously known for cell growth stimulatory action [4] was tested as a molecule acting through the membrane receptor, while prednisolon, long known as a cell growth stimulant, as one acting through cytosolic receptors. The two treatments applied in the experiment were separated by an interval of 6 h, during which at least one generation change (division) of *Tetrahymena* cells took place in the normal medium, to which they had been returned between the treatments.

Of the materials tested, gibberelline, prednisolon and benzpyrene, in rising sequence, stimulated the growth of *Tetrahymena* (Table I) on the first exposure, and epinephrine, too, was found to stimulate it on the second exposure. The second treatment with the former three molecules also resulted in a greater growth stimulation, essentially in the above sequence.

These observations permit the following conclusions:

1. A physiological molecule may give rise to imprinting and, consequently, to increased responsiveness on a next exposure, also when seemingly indifferent in respect of the given function on first exposure.

2. Non-physiological, but biologically important (active) molecules can also stimulate the multiplication of *Tetrahymena*. However, it should be taken into consideration that both non-physiological molecules tested in this study act as cell growth stimulants in their own systems (gibberelline – plants; benzpyrene – cancer).

3. A non-physiological, but biologically active molecule may give rise to imprinting at the first interaction with the target cell exactly as a physiological one, and may elicit as a result an increased cellular response on the next exposure. Since in all

probability neither gibberelline nor benzpyrene occur in the body or immediate environment of *Tetrahymena*, it seems highly probable that *Tetrahymena* is able to respond to non-self (foreign) molecules, either by naturally presenting in its membrane or cytosol a non-specific "receptor" pattern to which the ligand is able to bind, or by presenting such a structure by reassembly of a detail of membrane or cytosol in response to interaction with the ligand. The structure having once acted as, or assembled to, a receptor than persists in the cell and at least in its first progeny generations as well, to judge from the circumstance that *Tetrahymena* undergoes four (and even more under the influence of stimulating treatment) generation changes within 24 h [10]. The nature and precise localization of the "receptor", through which *Tetrahymena* interacts with foreign materials are naturally obscure. Probably the non-physiological materials tested acted on *Tetrahymena* at other than receptor level, although binding of benzpyrene to cellular receptors has been suggested [11], and the sterane-like structure of gibberreline [12] also seems to be suitable for such binding. The fact nevertheless remains that *Tetrahymena* behaved as if it possessed a receptor "memory".

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