Transmission of Hormonal Imprinting in *Tetrahymena* Cultures by Intercellular Communication

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Tetrahymena, Hormonal Imprinting, Cell-to-Cell Communication

The primary interaction of cultured *Tetrahymena* cells with a hormone (insulin in the present case) gives rise to hormonal imprinting, which accounts for a considerable increase in the later hormone binding capacity of the cells. Mixed culturing of imprinted and not imprinted (virgin) cells results in transmission to the latter of the information mediated by imprinting and thereby in a considerable increase in hormone binding capacity over that of pure hormone-preexposed cell cultures. The material substrate of intercellular information transmission is a cellular secretion which appears in the nutrient medium, and is increasingly released in presence of not imprinted (virgin) cells in the mixed culture.

Introduction

The primary interaction of a cell with a certain signal molecule (hormone) gives rise to hormonal imprinting, which alters the later response, and/or hormone binding capacity of the cell [1–3]. In unicellulars, the direct issue of hormonal imprinting is either the formation of specific hormone receptors from formerly non-specific membrane patterns [4, 5] or the amplification of performed nutrient receptors for hormone reception, whereas in higher organisms, imprinting takes place in the perinatal period, in which the genetically encoded receptor pattern is still plastic, and requires hormonal presence for stabilization [6].

Imprinting develops extraordinarily rapidly in the unicellular *Tetrahymena*. Exposure to a hormone for 1 h is sufficient to induce a durable imprinting which lasts over as many as 500 generations [7]. Imprinting endows the cell with a "memory" of the hormone, which accounts for an increased response to the latter on reexposure(s).

It follows from the foregoing considerations that hormonal imprinting, once established, is transmitted from parent-to-daughter cells. The mechanism of transmission is, however, still obscure because, in principle it may take place at membrane level, by reassembly of the transmitted membrane (receptor) pattern in the daughter cells, or – given that the membrane-received information is transmitted to the nucleus – at gene level as well.

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Fletcher and Greenan [8] demonstrated that the post-receptorial mechanism (protein kinase activity) which follows upon binding of the hormone to the cellular receptor, also appeared in (cultured) cells which did not themselves bind the hormone. This supported the hypothesis that the cell-cell transmission of information is also involved in hormonal effects [9]. Evidence was presented that in Chang liver and Chinese hamster ovary cell cultures imprinting was transmitted to cells which did not themselves interact with the hormone [10]. The gap junctions present in the monolayers of these cell lines could account for the transmission of information, in which contacts between cell processes and coated pits may probably also play a role.

Since no gap junction-like contacts have been observed between ciliated unicellular organisms, such as the *Tetrahymena,* there is reason to postulate that in populations of these, cell-cell contact is based on conjugation and/or on extracellular secretions, as was supposed earlier [11–14].

The main purpose of the present study was to clarify whether, further to the (vertical) parent-to-daughter cell transmission of hormonal imprinting, some other mechanism, *e.g.* intracellular communication, could also accomplish the (horizontal) cell-cell transmission of the information carried by hormonal imprinting among *Tetrahymena* cells, and if they could, which were the events involved.

Materials and Methods

Tetrahymena pyriformis GL cells, cultured in 1% yeast extract containing Bacto tryptone medium at 28 °C, were used in the logarithmic phase of growth.

Hormonal imprinting was induced by exposure to 0.14 IE (10^{-6} M) insulin (Insulin Semilente MC, Novo, Copenhagen) for 1 h. Subsequently the cells were washed in Losina solution and were returned to plain medium. Of both control and insulin-treated cultures 0.5 ml cell suspension of 2×10^5/ml density was inoculated into 10 ml growth medium. In a third series 0.25 ml control plus 0.25 ml insulin-treated cell suspension was set up in 10 ml growth medium. After culturing for 1 day, the cells were fixed in 4% neutral formaline (pH 7.2, in 0.05 M PBS) for 5 min, washed in two changes of PBS, and incubated in presence of FITC-labeled insulin for 1 h at room temperature (FITC/protein ratio: 0.12; protein concentration: 0.4 mg/ml). After incubation, the cells were washed in three changes of PBS, spread on slides, dried, and assayed for intensity of fluorescence. Three replica assays, each covering 60 cells per group, were performed to construct the histograms, and two further assays covered 20 cells per group. The values shown in the enclosed figures represent the mean of five replica experiments.

In the second experimental series the cells were treated exactly as in the first series, but after 1-day culturing in the plain medium the control, insulin-treated and mixed cultures alike were passed through a Millipore filter of 1.2 μm pore diameter, and the cell-free filtrate (10 ml in each case) was seeded with *Tetrahymena* cells, added in 0.5 ml suspension of 10^5/ml cell density. One day later the cells were fixed and incubated in presence of FITC-insulin as described above.

The intensity of fluorescence was determined in a Zeiss Fluoval cytofluorimeter, connected with a Zeiss MFV 4001 signal amplifier and a digital processor for signal transformation. The transformed signals were recorded, and processed, in a Hewlett Packard HP 41C calculator for evaluation of mean values, standard deviation and significance of intergroup variations.

Results and Discussion

One-hour treatment of the *Tetrahymena* cells with insulin increased FITC-labeled insulin binding significantly over the control, conform to earlier observations in pertinent studies [4]. The FITC-insulin binding capacity of the mixed cultures, set up from equal counts of cells pretreated and not pretreated with insulin, increased significantly over that of the pure insulin-treated cell cultures, indicating that (1) the insulin-mediated information was transmitted to the control (not pretreated) cells and (2) the presence of untreated (control, virgin) cells enhanced the effect of insulin in the entire population (Fig. 1).

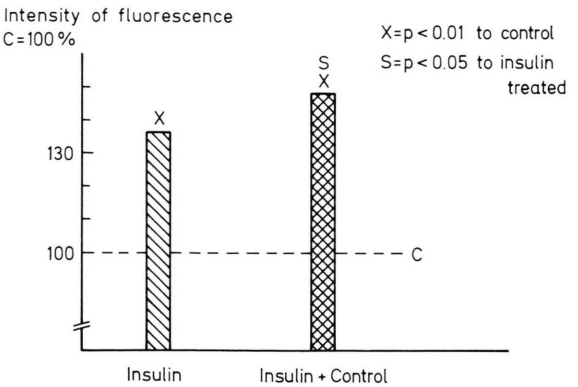

Fig. 1. FITC-insulin binding of insulin-treated and mixed (insulin-treated + control) *Tetrahymena* populations one day after treatment.

Analysis of the histograms (Fig. 2) revealed that while the insulin binding capacity of the control cells was fairly homogeneous, that of the pure insulin-treated and mixed (insulin-exposed + control) cultures showed considerable variations. At the same time, the histograms of the pure insulin-treated and mixed cultures showed a shift to the right (towards higher binding values). The fact that, despite the

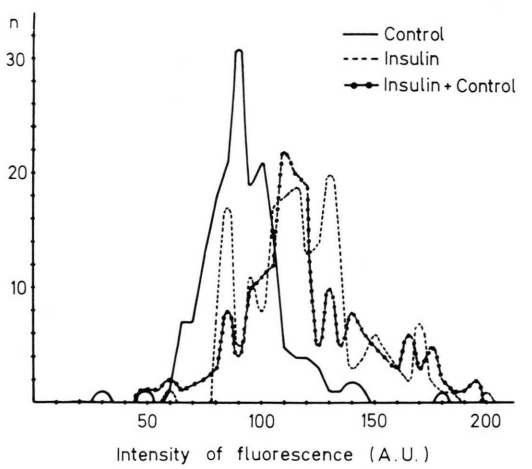

Fig. 2. Proportion of FITC-insulin binding of control, insulin-treated and mixed (insulin-treated + control) *Tetrahymena* populations. A.U. = arbitrary unit.

considerable standard deviation, both experimental cultures showed a significant binding increase over the control can be ascribed to the association of the greater variations with the increase in binding capacity. Absence of two peaks in the histograms of the treated cultures excluded a possible combination of low fluorescence in control progeny cells with a higher fluorescence in the progeny of insulin-preexposed cells after one day of mixed culturing. This accords well with the significant binding increase in the mixed cultures, and with the close resemblance of the histograms for the pure insulin-pretreated and mixed cultures, which differed only in a shift of the latter to the right (towards higher binding values) rather than to the left (towards the control).

It is known that the *Tetrahymena pyriformis* GL cells multiply asexually, without conjugating with one another [15]. If follows that, unless a short-term direct contact occurs, the cell-cell transfer of information presupposes an extracellular communication. Evidence of this has emerged from culturing "virgin" *Tetrahymena* cells in the cell-free filtrates of untreated, pure insulin-treated and mixed cultures. The FITC-insulin binding of the virgin cells grown in the filtrate of the insulin-preexposed culture increased significantly (Fig. 3) over the control, but the absolute value of the increase was relatively low (8%) and considerably less than that measured in the cultures directly exposed to insulin (36%). A much greater increase (about 53%) was observed in the population set up in the filtrate of the mixed culture, but it did not differ significantly from the value (48%) assessed in the original mixed culture. It follows that while the after-effect of insulin exposure is minimal in the growth medium, the factor(s) appearing in the medium of the mixed cultures account for practically the same effect as mixed culturing itself. This implies that (horizontal) cell-cell communication was mediated by a factor secreted into the maintenance medium and that the secretion of that factor was obviously enhanced by the presence of virgin cells in the mixed culture.

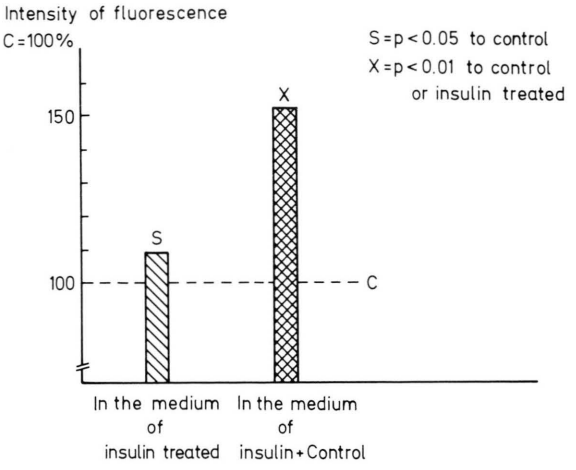

Fig. 3. FITC-insulin binding of *Tetrahymena* populations cultivated in the medium of insulin-treated or mixed (insulin-treated + control) *Tetrahymena* cultures after one day cultivation.

Thus the present experimental observations support the hypothetical conclusion that transmission of imprinting from imprinted to virgin cells can take place by intercellular communication not only in mammalian cell lines [10], but also in cultured populations of the unicellular *Tetrahymena*. In view of this, current conceptions on the transfer of imprinting by membrane-level (self-assembly) or gene-level mechanisms should be completed by a third alternative of transmission *via* cellular secretion(s), whose release seems to be considerably enhanced by presence of virgin cells not possessing the hormone-mediated information.

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