

# EFFECTS OF INSULIN AND HISTAMINE IN THEMSELVES AND IN COMBINATION ON THE GLUCOSE METABOLISM OF *TETRAHYMENA*

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**Abstract.** The glucose metabolism and the related intracellular processes of *Tetrahymena pyriformis* GL cells are measurably influenced by several hormones of higher organisms, among others by the endocytosis stimulating [hormone histamine, and the glucose metabolism regulating hormone insulin. Histamine does not interfere with the glucose metabolizing action of insulin, but markedly enhances the utilization of glucose, to judge from a significant decrease in the PAS-positive (hexose) component of histamine-exposed *Tetrahymena pyriformis* GL cells.

**Keywords:** Tetrahymena — glucose metabolism — histamine — insulin

## Introduction

The hormones and hormone-like compounds of higher organisms are able to elicit an adequate response also in unicellulars. For example, insulin influences the glucose metabolism [4, 9] and histamine the endocytosis [2, 14] of the unicellular *Tetrahymena pyriformis*.

Combined treatment of unicellulars with these two hormones could throw light on the problem whether or not histamine modifies insulin influence on the glucose metabolism, either by latering the latter's receptor-bound endocytosis [12, 19], or by some other mechanism.

The action of both test hormones, however, is determined by the intracellular conditions and by certain factors acting on the function(s) of the extracellular space (nutrient medium). Thus, extracellular presence (and concentration) of glucose may alter the intensity of the intracellular processes [9] of similar degree to the number and size of those particles, which initiate endocytosis [17, 18]. The duration of hormone action also plays a decisive role, since cellular response greatly depends on the length of hormone exposure.

In the present study *Tetrahymena pyriformis* was used as model cell to examine the influence of insulin and histamine in themselves, and in combination with one another, on the glucose metabolism of the unicellular,

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with special regard to the duration of hormone action. The hexose component of the intracellular oligo- and polysaccharides was measured by the periodic acid-Schiff (PAS) reaction, and the results read by cytophotometry.

### Materials and methods

*Tetrahymena pyriformis* GL cells, maintained in 0.1% yeast extract containing 1% Bacto-Trypton medium (Difco, Michigan, USA) for 24 h at 28 °C, were used in the logarithmic phase of growth. The cultures were washed in Losina-Losinsky solution [15], and incubated in the presence of  $10^{-6}$  M insulin (Novo, Semilente; Copenhagen, Denmark),  $10^{-6}$  M histamine (Reanal, Budapest), or 1 mg/ml glucose (Reanal, Budapest). The cells were assigned to 12 experimental groups (Nos 2–13) and one control group (No. 1) for the following treatments:

A. The cells of groups 2–7 were incubated for 10 min in the presence of glucose (group 2), histamine (group 3), insulin (group 4), glucose + histamine (group 5), glucose + insulin (group 6) or histamine + insulin (group 7).

B. Other cultures (Nos 8–13) were, after the first 10-min exposure, washed and reexposed immediately according to the following schemes of treatment: glucose—glucose (group 8), histamine—histamine (group 9), insulin—insulin (group 10), glucose + histamine—insulin (group 11), histamine—insulin + glucose (group 12), histamine—insulin (group 13). After treatment the cells were washed, spread on slides, dried, fixed in ethanol, and tested for hexose content with the periodic acid-Schiff (PAS) reaction as described by MacManus. The amount of PAS-positive material was determined by scanning cytophotometry at 546 nm, using a Zeiss (Jena) cytophotometer. Twenty cells were assayed by cytophotometry in each group, and each experiment was performed in three replicates, thus the enclosed figure shows the mean values for 60 cells in each group. The inter-group differences were analyzed for significance by Student's *t*-test.

### Results and discussion

The purpose of the study was to examine the vertebrate hormones insulin and histamine, and extracellular glucose, for influence on the glucose metabolism of the *Tetrahymena*, by detecting intracellular hexose with the PAS reaction in differently treated cells. Untreated cells maintained in glucose-free medium were used as control. Results related to the control, and the significances of inter-group differences are shown in Fig. 1 (the group designations are the same as described in "Materials and methods").

In accordance with earlier experimental observations [2, 14], histamine stimulated the endocytosis of the *Tetrahymena* in exactly the same manner as in higher organisms. It is known that histamine develops action via the cAMP mechanism [5], which also mediates the action of insulin at the unicellular level [6]. Accordingly, the cAMP-mediated processes may stimulate not only the incorporation of glucose, but also the phagocytic activity, the induction of which is probably the main effect of histamine [5, 6].

The polypeptide hormone insulin acts on the cellular glucose metabolism by a membrane-receptor-mediated effect, which is the issue of different mechanisms. First, by binding to the receptors, insulin modifies the glucose-carrier processes, increasing thereby the intracellular-glucose transport [11].

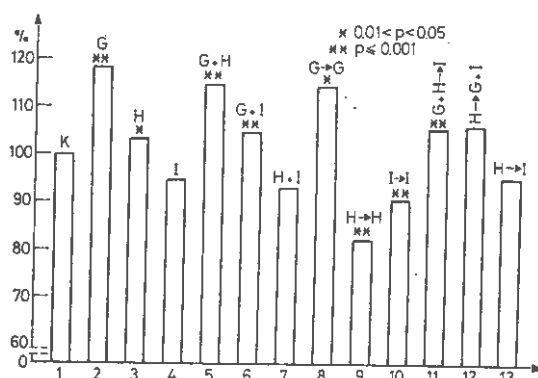


Fig. 1. Effect of treatment with histamine, insulin and glucose, alone and in various combinations, on the PAS-positivity of *Tetrahymena* relative to the control as 100

Secondly, it acts on the membrane proteases, intracellular kinases and phosphatases, and influences thereby the intracellular utilization and storage of the incorporated glucose [10]. Thirdly, internalization of membrane-receptor-bound insulin by endocytosis modifies the intracellular proteolytic activity in a manner characteristic of insulin action [12, 19]. The membrane of the *Tetrahymena* either possesses or forms — under hormonal influence — specific binding sites (receptors) for both histamine [3, 7, 13] and insulin [8].

The incorporation of extracellular glucose can also take place by two different mechanisms, i.e. via carrier transport (20) or via endocytosis, inside pinocytotic vesicles [1, 16].

With these facts in mind, the present experiments were performed to clarify whether (1) insulin and histamine modified one another's influence on the glucose metabolism of the *Tetrahymena* by coincident effects; (2) the action of either hormone was modified by the presence of extracellular glucose, and (3) the time factor (length of hormone exposure) played a notable role in the supposed insulin-histamine interactions.

The cells treated with *glucose* alone for 10 min (group 2) showed a significant increase in PAS-positivity, which was not considerably changed by reexposure to glucose (group 8), indicating that saturation with glucose had taken place in the first 10 min (Fig. 1).

Exposure to *histamine* for 10 min (group 3) increased PAS-positivity slightly over the control, owing either to the endocytosis stimulant (pinocytotic glucose influx enhancing) effect of the hormone, or to the activation of glucose incorporation by histamine-induced cAMP-elevation [5, 6]. However, reexposure to histamine (group 9) accounted for predominance of the hormone's metabolic activator action, to judge from the drastic concentration decrease of intracellular PAS-positive material, to the lowest level relative to all experimental groups.

*Insulin* measurably enhanced glucose utilization on a single short-term exposure (group 4). Since this effect involved depression of the PAS-positive component, the hormone obviously enhanced the utilization rather than the storage of glucose [9]. Reexposure to insulin (group 10) accounted for a somewhat greater decrease in PAS-positivity, indicating that the length of exposure to the hormone did play a role in the intensity of its effect.

We relied on the above results as reference values to evaluate the effects of combined treatment, which were either synergistic or antagonistic, depending on the applied combination. The effects of the combined treatments were realistically shown by the changes in the quantitative relations of the PAS-positive component.

The two test hormones, insulin and histamine, acted differently on the cells in the presence of extracellular glucose. The cells exposed to histamine + glucose (group 5) showed a greater increase in the amount of PAS-positive component than those exposed to insulin + glucose (group 6). Since the PAS-positivity of the cells exposed to *histamine + glucose* did not differ practically from that of the cells exposed to glucose alone, histamine had obviously no perceivable influence on glucose metabolism.

*Insulin + glucose* depressed PAS-positivity to a lesser degree than insulin alone, for, naturally, the presence of extracellular glucose enhanced the storage rather than the utilization of the intracellular reserves.

Exposure to *insulin + histamine* in the absence of glucose (group 7) depressed PAS-positivity to a similar degree as insulin in itself. It follows that histamine did not markedly interfere with the glucose utilization activating effect of insulin, nor did it notably enhance the endocytotic uptake of the latter.

The cells preexposed to *histamine + glucose* and reexposed to *insulin* (group 11) showed a similar PAS reaction to those treated with insulin + glucose (group 6), and a decrease in PAS-positivity relative to those treated with histamine + glucose (group 5). Thus, in the combination of histamine + glucose + insulin, the effects of insulin and glucose were practically not modified by histamine.

*Histamine + insulin* (group 7) acted in a similar manner as insulin alone, i.e. this combination depressed the PAS-positivity relative to the control, too. The cells preexposed to histamine behaved similarly on reexposure to insulin (group 13) and insulin + glucose (group 12). Consequently histamine had no perceivable influence in either combination, nor did histamine and insulin exert any additive effect on the intracellular glucose metabolism.

As a summary, it could be stated that the glucose metabolism regulating vertebrate hormone insulin, and the biogenic amine histamine, also acting at membrane level, do not markedly influence each other's effects on the glucose metabolism of the unicellular *Tetrahymena*. Insulin and extracellular glucose modify the glucose metabolism of the unicellular considerably, both alone

and in combination with one another, and histamine greatly increases the glucose utilization (decreases PAS-positivity) of the unicellular on second exposure by a not yet clearly understood mechanism.

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