Cellular and Molecular Biology **32**(6), 641–645, 1986
Printed in Great Britain.

0145–5680/86$3.00+0.00

STUDIES ON THE INTERRELATIONSHIP BETWEEN HORMONAL IMPRINTING AND MEMBRANE POTENTIAL IN MODEL EXPERIMENTS ON *TETRAHYMENA*

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(*Accepted* 31 *March* 1986)

Abstract—Model experiments on *Tetrahymena* have shown that hormone binding was always associated with membrane depolarization. The degree of depolarization varied with the actual binding capacity for the hormone, and the two parameters appeared to be interrelated. An appreciable depolarization occurred always at low levels of hormone binding, and vice versa, it was less distinct when the level of binding was high. The degree of depolarization also depended on the type of the interacting hormone molecule, and other factors, such as membrane down-regulation processes, or the length of hormone exposure. A non-hormone molecule did not show the membrane polarity modifying effect, which seems to be characteristic of hormones.

Key words: *Tetrahymena*, membrane potential, GTH, TSH, insulin hormonal imprinting

ÉTUDE DE LA LIAISON ENTRE L'EMPREINTE HORMONALE ET LE POTENTIEL DE LA MEMBRANE SUR LES CELLULES DE *TETRAHYMENA*

Résumé—Des expériences modèles sur *Tetrahymena* ont montré que la liaison de l'hormone est toujours associée avec le dépolarisation de la membrane. Les degrés de dépolarisation varient avec la capacité de liaison de l'hormone, et ces deux paramètres semblant être en interrelation. Une dépolarisation significative se manifeste toujours aux bas niveaux de la liaison de l'hormone, et vice versa. C'est moins distinct quand le niveau est haut. Le degré de dépolarisation dépend aussi du type de molécule de l'hormone réagissante et d'autres facteurs, comme le processus de la régulation vers le bas de la membrane, ou la durée d'exposition, donc du temps de traitement par l'hormone. Dans ces expériences, les molécules 'non-hormones' ne montreraient pas les modifications de la polarité de la membrane, qui semblerait être caractéristique des hormones.

Mots-clefs: Empreinte hormonale, *Tetrahymena*, polarité de la membrane, down-régulation

INTRODUCTION

Uni- and multicellular organisms equally respond to external stimuli by characteristic membrane changes, which can be measured or cytochemically analyzed (Nozawa *et al.*, 1980; Köhidai *et al.*, 1986) irrespective of the chemical, mechanical, optical or other nature of the stimulus. Proteins and hormones acting as signal molecules also elicit detectable membrane changes.

Hormonal imprinting takes effect by changing—usually increasing—cellular response to the hormone on re-exposure. The change is expressed by a quantitative alteration of hormone binding or some other cellular function (Csaba, 1980, 1981). Thus, establishment of a 'memory' (Csaba, 1985) at membrane or another cellular level may be the result of different events, such as lasting modification of the membrane structure, and of certain intracellular and/or nuclear processes.

Binding to the membrane of chemical compounds, including hormones acting as signal molecules, may modify the electric charge distribution in, or the polarity of, the membrane by interfering with the membrane transport processes. The depolarization of hyperpolarization of the membrane depends greatly on the fine structure, dimensions and inner polarity of the interacting signal molecule. Accordingly, hormone molecules of different natures may dissimilarly modify the membrane potential and elicit thereby a change characteristic of the hormone involved.

Since hormonal imprinting durably transforms membrane-mediated cellular response, the question

may be justly put as to whether the electric potential of the membrane, which represents a characteristic parameter of its state, would also show a measurable change in consequence of imprinting, and of re-exposure to the hormone (which is known to amplify imprinting) as well, and whether these changes were measurable after a time sufficient for the completion of the hormone-induced membrane transformation processes.

We used *Tetrahymena pyriformis GL* cells, the well-known model cells for hormone receptor studies, to monitor imprinting by quantitative determination of fluoresceine-labeled hormone binding, and changes in the membrane potential by measuring the membrane penetrance of rhodamine.

MATERIALS AND METHODS

Tetrahymena pyriformis GL (Zeuthen) cells, grown in a 0·2% yeast extract containing 2% Bacto Tryptone medium (Difco, Michigan, U.S.A.), at 28°C were used in the logarithmic phase of growth. The cells were assigned to three main groups; the first group was not treated, so as to serve as a control, and the second and third groups were exposed to the hormone once and twice, respectively. The following hormones were used: insulin (Semilente, Novo; Copenhagen, Denmark), TSH (thyrotropin, Ambinon, Organon Oss, Holland), GTH (Gonadotropin, FSH+LH, 50+50%, Pergonal, Serono, Rome; Human, Budapest). Bovine serum albumin (BSA, Reanal, Budapest) was tested for comparison as a non-hormone protein. The applied concentration was uniformly 10^{-6}M and exposure always lasted 1 hr. After treatment, part of the cells was returned to plain medium for 24 hr, fixed in 4% formaline, washed in three changes of phosphate buffer (0·05 M phosphate buffer, pH 7·2, in PBS + 0·9% NaCl), exposed for 1 hr to fluoresceine-isothiocyanate-labeled (FITC, BHD, London, England) hormone or BSA, washed again in several changes of PBS, spread on slides, dried, and examined for hormone binding by cytofluorimetry. An Olympus MMPS cytofluorimeter, connected with Canon Canola SX 320 apparatus, was used. The other part of the cells was washed in 1 mM, pH 7·2, Tris/HCL buffer, resuspended at 10^4 cell/ml density, treated with 0·25 mM rhodamine 6GO (Chroma, F.R.G) containing (pH 7·2) 1 mM Tris/BCl buffer for 30 min, and assayed for the fluorescence associated with membrane-potential-dependent dye penetrance in a FS 501 S fluorescence spectrophotometer (Union Giken, Japan) at 550 nm with a constant temperature of 25°C (Ainchi *et al.*, 1980). A Sord M223 micro-computer was connected with the spectrophotometer for data recording and evaluation.

Each experiment was performed in three replicates, and 30 cells were assayed for intensity of fluorescence in each series, thus the values of FITC-labeled hormone binding and membrane-potential-dependent rhodamine penetrance equally represented the mean of 90 readings.

RESULTS

A. *Hormone binding* (Fig. 1)

A single exposure to insulin altered considerably the binding relations of labeled insulin, but two exposures to it on 2 successive days did not alter the binding capacity relative to the control.

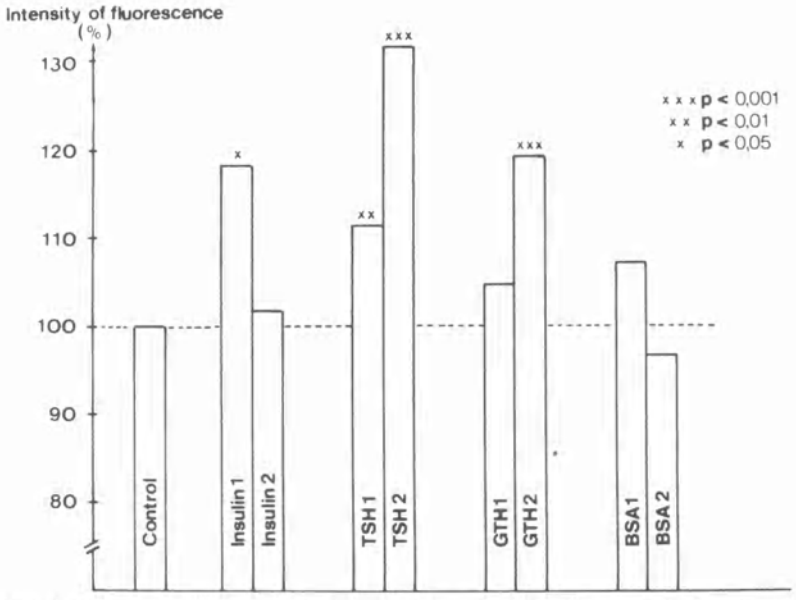

Fig. 1. FITC-labeled hormone binding of single or double treated *Tetrahymena* cells. Ins, insulin: TSH, thyrotropin: GTH, gonadotropin; BSA, bovine serum-albumin; 1, single treated, 2, double treated cells.

TSH, in contrast, increased the binding capacity significantly on a single exposure, and to a still greater degree on re-exposure.

GTH acted in a similar manner to TSH, but a binding increase, of a measurable degree after primary exposure, became significant only after the second exposure.

The non-hormone, non-signal molecular BSA accounted for a slight, but not significant increase on first exposure, and for a decrease rather than increase in binding capacity on re-exposure.

B. *Membrane potential changes* (Fig. 2)

The hormones tested unequivocally developed a membrane depolarizing action, but to different degrees, depending on their nature.

The depolarizing effect of insulin was considerable at the first treatment, and still greater at the second treatment.

TSH showed peak depolarizing effect, relative to all hormones tested, at primary exposure, and a lesser, but relative to the control still appreciable, effect at second exposure.

GTH behaved similarly to TSH also in this respect, having acted more strongly at the first than at the second treatment.

BSA again acted differently from the signal molecules in that it gave rise to slight hyperpolarization rather than depolarization of the membrane at both the first and second exposure.

DISCUSSION

Hormonal imprinting occurs at the primary interaction of the target cell with the given signal molecule, and this event is stored by a special 'memory', which causes the cell to respond to re-exposure(s) by an increase in hormone binding or other function (Csaba, 1980, 1981). The phenomenon of hormonal imprinting is presumbly due to the segregated or joint effect of several mechanisms, such as the membrane structure modifying effect of the hormone, changes in the concentration and/or localization of membrane-bound ions, gene level fixing of the information, and/or alteration of certain intracellular parameters (Berridge and Irvine, 1984; Csaba, 1985). The effect of primary exposure (hormonal imprinting) is durable, and is demonstrable after a much longer time (Csaba *et al.*, 1982) than considered in the present experiments (24 hr).

The actual electric potential of the membrane also depends on several factors, such as the nature, immersion and distribution of proteins in the phospholipid bilayer, and the associated circumstances of transmembraneous Na^+, K^+, Ca^{2+} and/or

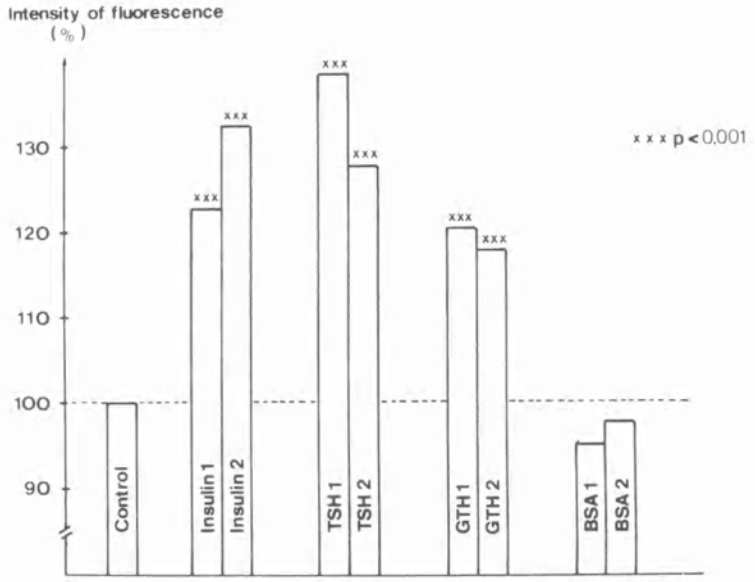

Fig. 2. Fluorescent activity of rhodamine 6GO in the incubation solution, containing hormone treated *Tetrahymena* cells. Ins, insulin: TSH, thyrotropin: GTH, gonadotropin: BSA, bovine serum-albumin; 1, single treated, 2, double treated cells.

Cl^- flow. The ion transport may be mediated by the membrane proteins in different forms in that: (i) the proteins may form a channel for penetration of the ions; (ii) they may orient ion migration by their position, or (iii) certain protein-linked enzymes may furnish appropriate conditions for transmembranous ion transport (Campbell, 1983a or b). Anion binding sites, too, play an important role in membrane polarity (Berridge and Irvine, 1984). All these mechanisms cooperate in the establishment of a characteristic membrane charge distribution and ion permeability.

The membrane-level effects of hormones alter the potential and the dynamics of the membrane by modifying the structure (Helmreich, 1976; Koch *et al.*, 1979) and the ion distribution (Campbell, 1983; Schlatz and Marinetti, 1972) thereof.

It has been suggested that the membrane-potential-dependent penetrance of rhodamine is a reliable indicator of membrane polarity. Pertinent observations in the present study have substantiated a close interrelationship between membrane potential changes and the properties of the modifying molecule.

Although all three hormones tested accounted for a distinct depolarization, the degree of depolarity changed with the type of the molecule, and was closely related with the latter's imprinting powers.

Interaction of the hormone with the membrane resulted in the latter's depolarization at both the first and second treatment, but the depolarizing effect was greater when the level of hormone binding was lower, i.e. after primary exposure to TSH and GTH, and after second exposure to insulin. The control-level binding capacity of the cells twice exposed to insulin remains to be explained, but accords well with the down-regulation mechanism demonstrated earlier in the insulin reception of the *Tetrahymena* (Csaba and Köhidai, 1986). (Down-regulation which accounted for disappearance from the surface, and migration into intracellular pools via membrane flow of the superficial insulin binding structures took effect exactly 1 day after insulin treatment.) The quantitative decrease in insulin binding capacity increased rather than decreased the extent of depolarization. Reduced depolarization was, however, associated with the groups binding the greatest amounts of hormone, owing presumably, to a local abundance of receptors.

Thus the three hormones tested influenced the membrane potential in two different ways. While insulin developed the greater depolarizing action at the second exposure, TSH and GTH developed it at the first exposure, which accounted for down-regulation at the second treatment. The dissimilar effects can be attributed to the unlike dimensions, amino acid sequences and steric structures of the hormone molecules, and probably also to the dissimilar localizations of their surface electric charges. Insulin, being a relatively low molecular weight polypeptid, obviously acted on the membrane differently from the large-molecular glycoprotein hormones TSH and GTH. The similar behaviour of TSH and GTH is not surprising, for they have a common alpha chain and analogous sequences in the beta chain (Frieden, 1976). The structural resemblance of the two hypophyseal hormones can also account for their cross-imprinting effects reported earlier (Csaba *et al.*, 1979; Kovács and Csaba, 1985).

Naturally, not only the hormones themselves, but also the receptors involved in their binding (and formed and amplified by imprinting) differ in respect to structure and composition. This offers another explanation for the variation of membrane potential changes with the type of the signal molecule.

The non-hormone, non-signal molecule, BSA, which displayed only a weak, temporary imprinting effect in earlier (Csaba *et al.*, 1985) and present studies, failed to depolarize the membrane. Taking into consideration the extraordinary selectivity of the membrane in respect to interacting environmental molecules, it appears that BSA was in all probability not able to modify the membrane structure as radically as did the hormone-like compounds. Theoretically this implies that the membrane depolarizing property may have played a role in the evolution of signal molecules, but at the same time the association of imprinting with depolarization differences seems unlikely, for the hormones did account for depolarization at both primary and secondary interaction.

Summarizing, the present experiments have again substantiated the suitability of the *Tetrahymena* for model experiments (Csaba, 1985) on receptor–hormone interaction. The membrane of the *Tetrahymena* responds selectively to the imprinting effects of the different molecules by changing not only its binding relations, but also its membrane potential. However, the polarity changes seem to depend greatly on the nature of the imprinting molecule and on the length of exposure as well. It appears that membrane depolarization is a general effect of hormone-like molecules, but varies in extent with the type of the interacting molecule.

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