INFLUENCE OF PHENOTHIAZINES AND LOCAL ANESTHETICS ON LECTIN BINDING TO TETRAHYMENA SURFACE MEMBRANE

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Abstract—Treatment of Tetrahymena pyriformis cells with phenothiazines (trifluoperazine, proporciacine, chlorpromazine) or local anesthetics (dibucaine, procaine, lidocaine, tetracaine) gave rise to alterations in the membrane dynamic structure well-portrayed by changes in lectin binding capacity. Comparison of the binding relations of Phaeosolus and Helix lectins to the drug-treated cells with those of TSH to TSH-treated cells revealed a parallelism (chlorpromazine, dibucaine-increased Phaeosolus lectin binding; dibucaine-increased Helix lectin binding; tetracaine, lidocaine-decreased Phaeosolus and Helix lectin binding), which has suggested the responsibility of the same chemical structures, N-acetyl-D-galactosamine containing G_{M2} gangliosides for lectin and hormone binding.

Key words: Tetrahymena, membrane perturbation, lectin binding, G_{M2} gangliosides

INFLUENCE DES PHÉNOTHIAZINES ET ANESTHÉSIQUES LOCAUX SUR LA CAPACITÉ DE LA LIAISON LECTINE DE LA SURFACE DE LA MEMBRANE DE TETRAHYMENA

Résumé—Après traitement par des phénothiazines (trifluoperazine, proporciacine, chlorpromazine) et des anesthésiques locaux (dibucaine, procaine, lidocaine, tétracaine) de Tetrahymena pyriformis qui provoquent une inhibition de l'activité de la membrane, on peut, avec les lectines, observer des changements caractéristiques de la membrane cellulaire. Comparant nos résultats avec la liaison hormonale des cellules prétraitées de la même façon et imprégnées hormonalement (TSH), on peut observer un parallélisme entre les lectines Phaeosolus ou Helix, et la liaison TSH (chlorpromazine, dibucaine-liaison élevée de la lectine Phaeosolus; dibucaine-liaison élevée de lectine Helix; tétracaine, lidocaine-liaison abaissée de la lectine de Phaeosolus et de Helix). Ce travail nous permet de tirer la conclusion que dans les deux cas les mêmes structures chimiques, les gangliosides G_{M2} contenant la N-acétyl-D-galactosamine, peuvent être responsables de la formation de la liaison.

Mots-clés: Tetrahymena, perturbations de membrane, liaison de lectine, G_{M2} gangliosides

INTRODUCTION

The existence of cells is determined by the relationship with its milieu. The changes of this dynamic relation definitively affect the physiological processes and adaptability of the cell. The information produced by the environmental alterations is transferred into the cell through the plasma membrane, leading to modified membrane composition with adequate membrane fluidity.

Certain phenothiazines and local anesthetics undergo significant alterations in Tetrahymena surface membranes (Muto et al., 1983). In a previous paper we have shown that the membrane perturbation alters the hormonal imprinting in Tetrahymena plasma membrane (Nozawa et al., 1985). In the present work, membrane changes elicited by these drugs were studied and characterized by measuring the binding of several lectins to Tetrahymena surface membrane.

MATERIALS AND METHODS

Tetrahymena pyriformis GL cells, cultured in 0.1% yeast extract containing 1% Bacto Trypton medium (Difco; Michigan, U.S.A.) at 28°C, were used. One-day cultures were treated for 1 hr either with phenothiazine derivatives, such as trifluoperazine, proporciacine and chlorpromazine (all of them were purchased from Yoshitomi Pharmaceutical Co., Tokyo) at 200, 50 and 25 μM concentrations, respectively, or with the following local anesthetics,
uniformly at 200 μM concentration: dibucaine hydrochloride (Teikoku Chemical Inc., Tokyo); procaine hydrochloride (Iwaki Pharmaceutical Co., Tokyo); lidocaine hydrochloride (Fujisawa Pharmaceuticals, Tokyo); tetracaine hydrochloride (Kyorin Pharmaceutical Co., Tokyo). A control culture was treated with ethanol alone (Reimal, Budapest) at 0.4% final concentration. After treatment, 500 μl samples of each culture were transferred to normal medium for 24 hr, fixed in 4% formalin, and washed in two changes of PBS (0.05 M phosphate buffer containing 0.9% NaCl, pH 7.2).

The lectin binding studies were performed with the following fluorescein-isothiocyanate-(FITC)-labeled lectins: Concanavalin A, Datura stramonium, Lens culinaris, Pisum sativum, Phaseolus vulgaris, Lycopersicon esculentum, Helix pomatia. All lectins were applied at 0.4 mg/ml concentration for 1 hr. After incubation the lectin-treated culture samples were washed in several changes of PBS, spread on slides, dried, and examined for lectin binding by cytofluorimetry, using a Zeiss Fluovar Fluorometer connected with a HP-41C calculator for evaluation of the results. Twenty cells were examined for fluorescence measurement in each group, and each experiment was performed with three replicates.

RESULTS AND DISCUSSION

The informative process, running from the outer cell membrane to the nucleus, mediated by membrane flow, is very important in the vital phenomena of eukaryotic cells. Membrane-bound enzymes like guanylate cyclase are considered to be influenced by this process (Schultz and Klumpp, 1984). Other intracellular materials influencing enzyme activity can also join this chain, such as calmodulin-Ca²⁺ complex (Klee et al., 1980; Klee and Vanaman, 1982; Kovács et al., 1984). As a consequence of damaging this system, there is a disproportionate synthesis of membrane proteins, leading to the altered composition of the membrane. These changes influence the saccharide components of the membrane, and consequently their number and distribution refer to the different quantitative and local alterations of membrane proteins, indirectly. These differences can be characterized by measuring the binding of lectins of defined ligand specificity. Nevertheless, this binding is affected by the polar head groups of neighboring phospholipids in connection with the saccharides in the membrane (Sundler, 1982).

In the present experiment, phenothiazines, such as trifluoperazine, proprerazaine and chlorpromazine were used. These substances cause membrane perturbation.

The local anesthetics cause, in protozoa, deciliation (Thompson et al., 1974), mucocyst release (Satir et al., 1976), and alter the direction of ciliary movement (Browning and Nelson, 1976), and since they inhibit guanylate cyclase activity through the calmodulin–Ca²⁺ system (Muto et al., 1983), they develop these effects in all probability at the same level.

We examined three phenothiazine derivatives and four local anesthetics for influence on the membrane of *Tetrahymena* by the binding studies. We selected the lectins which reflected most precisely the modifications of the membrane saccharide components.

The cells pretreated with phenothiazines showed greatest binding affinity for *Phaseolus* and *Helix* lectins (Fig. 1), which bind specifically to N-acetyl-galactosamine. Binding affinity was lowest for *Datura* lectin in cultures pretreated with trifluoperazine and proprerazaine, and for ConA in those pretreated with chlorpromazine, although in the latter *Datura* lectin binding was also low.

The local anesthetics caused an increase in *Datura, Phaseolus* and *Helix* lectin binding (Fig. 2), but did not appreciably alter the binding of *Lens*...
lectin. Tetracaine generally depressed the lectin binding capacity of the cells, to the greatest degree for Helix lectin, and least for Lens lectin, whose binding value approximated the control level. Lidocaine proved to be more depressive, for only Lens lectin binding increased over the control in the cultures treated with it; the binding minima were represented by Datura and Helix lectin in this series. Procaine, remarkably, accounted for a 'dissociation' of the binding relations of Phaseolus and Helix lectin, which are of identical ligand specificity, for the cells treated with it showed maximal binding affinity for Phaseolus lectin, and minimal for Helix lectin.

These observations support the earlier implication that compounds acting presumably on the same target cells, but differing from one another in chemical structure, have a dissimilar influence on the sugar components of the cell membrane. Similarities were, nevertheless, also observed in the behaviour of the differently treated cell groups. Binding affinity was generally similar for Phaseolus and Helix lectins, owing presumably to their identical ligand specificity, and was greatest for Phaseolus lectin in five of the seven groups. Next to Phaseolus lectin, Lens lectin was bound to a greater degree than the other lectins in four groups (chlorpromazine, procaine-, tetracaine-, lidocaine-pretreated cultures). It follows that not only N-acetyl-β-galactosamine, which binds Phaseolus and Lens lectins, but also β-glucose, α-mannose and N-acetyl-α-glucosamine tended to accumulate on the membrane of the Tetrahymena under the influence of the applied treatments.

The primary interaction of Tetrahymena with a hormone (imprinting) gives rise to formation—or amplification—of an adequate membrane receptor which persists over many progeny generations (Csaba, 1980, 1981, 1984; Csaba et al., 1980). Hormonal imprinting presupposes the intact, physiological state of the cell membrane, and is biased by adverse influences such as heating, cooling (Kovács et al., 1984; Nozawa, 1980), exposure to ergosterol (Kovács et al., 1984; Nozawa, 1980), phenothiazines and local anesthetics (Nozawa et al., 1985). With these experimental facts in mind, the present findings have disclosed a certain parallelism in the binding behaviour of TSH and lectins. The binding relations of TSH to TSH-pretreated cells appear to be the same as those of the Phaseolus and Lens lectins to the cells pretreated with local anesthetics (chlorpromazine and dibucaine enhanced also that of Helix lectin, whereas tetracaine and lidocaine depressed the binding of both lectins).

Since imprinting by TSH can also take place in the presence of guanylate cyclase inhibitors (Nozawa et al., 1985), although in a somewhat modified form, there is reason to postulate that similar changes in cellular hormone and lectin binding capacity are associated with changes (increase or decrease) in the same membrane components. According to present knowledge, the TSH binding site is a glycoprotein formed by four structural units of dissimilar (high or low) affinities for TSH (Pekonen and Weintraub, 1979). The gangliosides (Meldolesi et al., 1976; Mullin et al., 1976), which are highly complex sphingolipids having sialic acid molecules on their oligosaccharide part (Lehninger, 1979), and containing also β-galactose and N-acetyl-β-galactosamine (Lehninger, 1979; Schachter and Roseman, 1981), help to form the TSH binding site.

Authors generally agree that sialic acid influences the high affinity TSH-binding structures, but opinions have been divergent on the trend and extent of its influence. While certain authors have described sialic acid as one prerequisite of the functioning receptor (Consiglio et al., 1979; Tate et al., 1975), others have regarded it as a neutral component of the latter (Amir et al., 1976), and again others as an inhibitor of hormone binding (Moore and Feldman, 1976).

The role of the Ga3 gangliosides has also been differently interpreted. Certain investigators believe that these are possible components of the low affinity binding site (Pekonen, 1980). The terminal group of all ganglioside varieties is sialic acid or α-galactose, with the exception of the Ga32 ganglioside, in which it is N-acetylgalactosamine. It appears that, like increased binding to TSH in the cited experiments, that of the Phaseolus and Helix lectins was also associated with the presence of that ligand on the terminal chain of Ga32. Thus Ga32 is presumably one of the molecules which forms the TSH receptor.

The local anesthetics influence not only the guanylate cyclase activity of the membrane, but also its fluidity (Sekiya and Nozawa, 1983). To obviate the risk of misinterpretations arising from that dual action, comparison with materials fluidizing the membrane to a similar degree, without inhibiting the activity of guanylate cyclase, is helpful. Ethanol is such an agent (Nozawa et al., 1979; Goto et al., 1983); in the present experiment it enhanced the binding of ConA, but depressed that of all other
lectins except *Pistia* below the control level (Fig. 3).

Since the lectin binding pattern of the ethanol-treated cells differed considerably from that shown by cells treated with guanylate cyclase inhibitors, the membrane fluidizing action had, obviously, only a negligible impact on the events.

Summarizing, we may state that the applied phenothiazines and local anesthetics caused, by inhibition of intracellular guanylate cyclase activity, membrane changes reliably portrayed by alterations in lectin binding capacity. Comparison of the binding relations of *Phaseolus* and *Hetz* lectins to the drug-pretreated cells to those of TSH to TSH-pretreated cells has indicated the responsibility of the same chemical structures—N-acetyl-galactosamine containing *GM* gangliosides—for lectin and hormone binding to *Tetrahymena*.

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REFERENCES


