

DRUG INDUCED MEMBRANE PERTURBATION IN
TETRAHYMENA PYRIFORMIS. STUDIES ON
LECTIN BINDING

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Cells are covered by membranes, their receptors and marker structures have an important role in the complexity of the cell and the environmental factors. Dynamic changes of these behavioural data make the membrane possible to rapid structure-correction. On the other hand such important structural characteristics are also present conservation of which is the basic condition of living cell. This "conservative" plastic membrane is the essential element of integrity of both the unicellular and multicellular organisms. This integrity may be influenced by: 1/the internal regulation of cellular life-processes and 2/ the external milieu.

The unicellular organisms are very sensitive to changes of the behavioural conditions. This is the case in our model object, the Tetrahymena pyriformis, too. /2/.

Different drugs can modify the membrane by reacting with the membrane or the associated enzymes. These perturbations are well characterized by lectin binding because of the structural changes of the membrane alter the position or density of several saccharides, particularly the organization or composition change of these molecules in the unit-membrane. Temperature alterations also transform the membrane composition. Long-term heat exposure decreases the fluidity, while the cold-shock renders it more fluid /5/.

In the following we shall describe the effects of these two different environmental factors - drugs and temperature changes -, how they can modify the membrane structure, especially the orientation or number of saccharides in it.

Phenothiazines like trifluoperazine, propericiazine and chlorpromazine or some local anesthetics - dibucaine, tetracaine, lidocaine and procaine - are able to perturb the membrane composition by way of inhibition of membrane associated guanylate cyclase /4/. We could illustrate the result of these effects by the binding profile of applied lectins.

After phenothiazine treatment we can register a special binding for certain lectins /Fig.1/. Maximal lectin binding was observed for Phaseolus and Helix lectins with the treatment of all kinds of phenothiazines. The ligand of these two lectins is the same saccharide, N-acetyl-galactosamine.

Minimal binding differences depend on the type of phenothiazine molecule. In the case of trifluoperazine and propericiazine the Datura, in the case of chlorpromazine the Concanavalin A and the Datura presented the lowest level.

Local anesthetics produced agreeing and discordant data, too. /Fig.2/. By the influence of dibucaine three lectins: Datura, Phaseolus and Helix represented the maximal lectin binding, while the other pole was presented in the Lens lectin. Action of tetracaine /Fig.3/ results a sinking of all the bindings, Helix presents the minimal point and Lens the maximal point of this profile.

In the lidocaine treated cells /Fig.4/ the decrease of the lectin binding was the most explicit. Only the Lens binding values elevate over the control level - this is the maximum point-, the lowest binding has been shown by Datura and Helix. The group of procaine treated cells /Fig.5/ was the only where the binding of Phaseolus and Helix opposed, Phaseolus has the maximal Helix the minimal values.

If we compare these results to the thyrotropic hormon /TSH/ binding values of TSH treated - imprinted /1/ - and certain phenothiazine or local anesthetics treated cells /4/, the increase and decrease seems to be parallel with lectin binding profile of Phaseolus and Helix lectins. Both ligands are glycoproteins, N-acetyl-galactosamine molecules. Since the TSH-receptor contains a ganglioside-group, it seems to be possible that the ganglioside is just the size G_{M2} , containing also a N-acetyl-galactosamine in terminal position.

The drug-effects investigated above are specific, enzyme-linked membrane perturbations. We can demonstrate this by ethanol treatment, which also perturbs the membrane, but by another mechanism /Fig.6/.

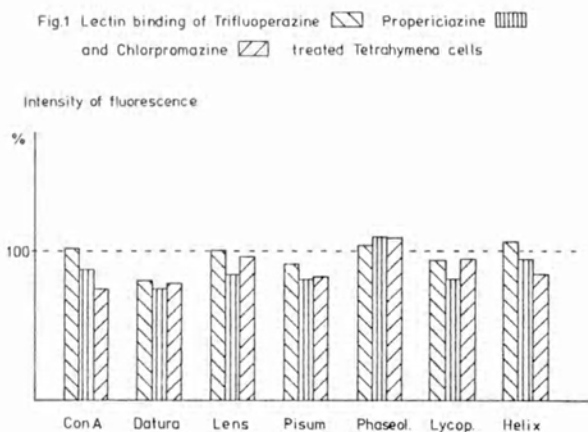


Fig 2 Lectin binding of Dibucaine treated Tetrahymena cells

Fig 3 Lectin binding of Tetracaine treated Tetrahymena cells

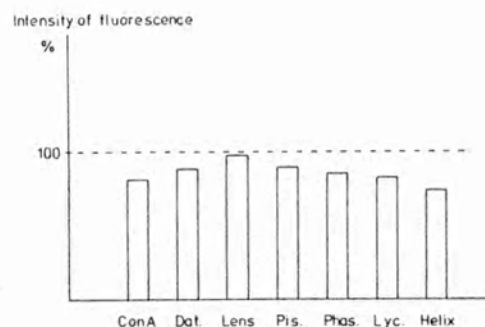
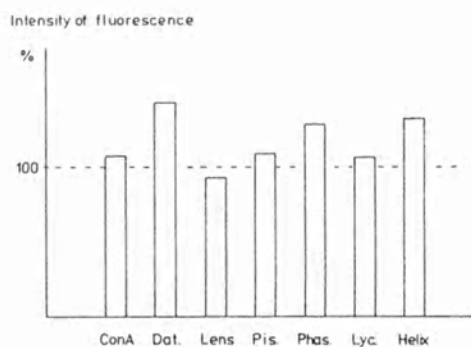


Fig. 4 Lectin binding of Lidocaine treated Tetrahymena cells

Fig 5 Lectin binding of Procaine treated Tetrahymena cells

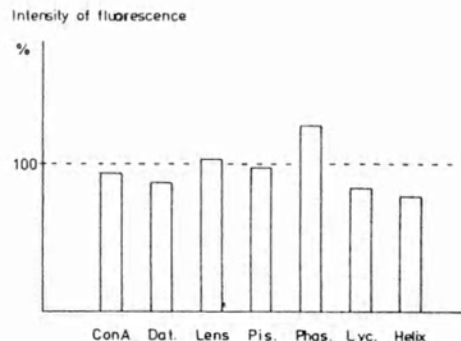
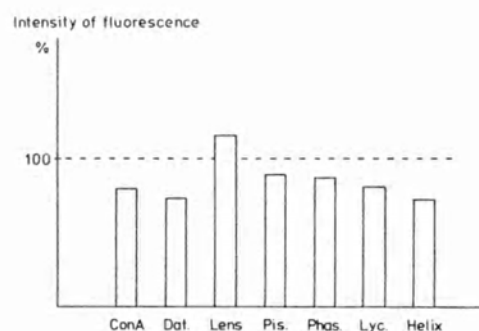


Fig.6 Lectin binding of Ethanol treated Tetrahymena cells

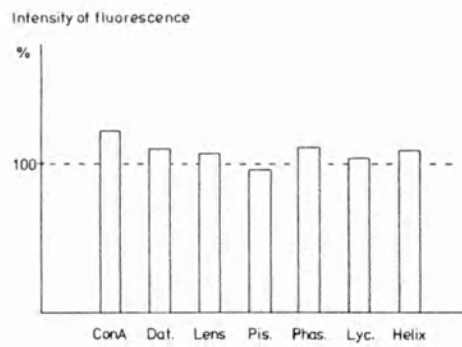


Fig.8 Effect of short-term cold-shock (28°C→15°C) on lectin binding of Tetrahymena cells

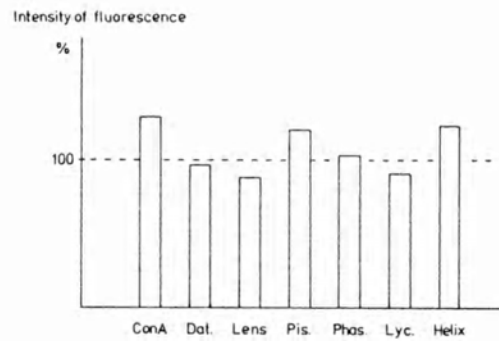


Fig.10 Effects of long-term cold-shock (15°C) and Ergosterol treatment on lectin binding of Tetrahymena cells

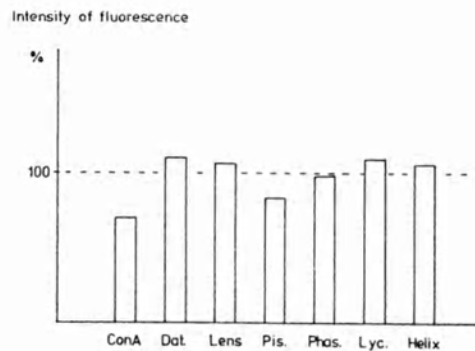


Fig.7 Effect of long-term cold-shock (15°C) on lectin binding of Tetrahymena cells

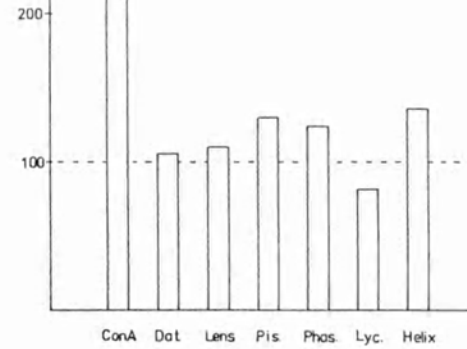


Fig.9 Effect of short-term heat-shock (15°C→28°C) on lectin binding of Tetrahymena cells

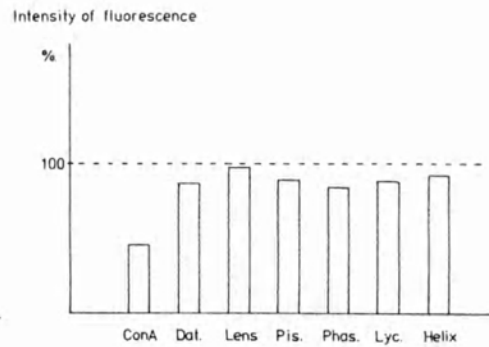
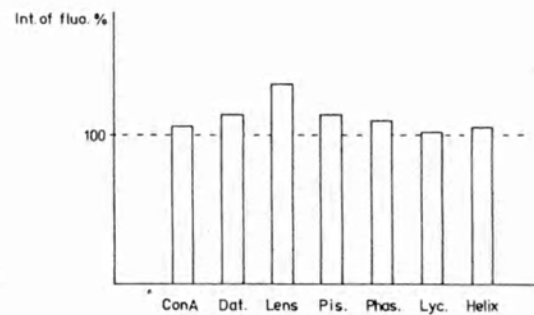


Fig.11 Effects of short-term heat-shock (15°C→28°C) and Ergosterol treatment on lectin binding of Tetrahymena cells



In this case the Concanavalin A presents the highest binding level indicating that ethanol treated membrane is abundant in D-glucose and D-mannose molecules, which are able to bind this lectin.

In further experiment we observed the altered lipid-phospholipid composition of membrane influenced by ergosterol treatment or temperature changes. These membrane alterations were studied also with the help of lectins.

Ergosterol treatment induces a new, rigid membrane in the following ways: 1/ indirectly - helps the new neutral lipids to build in the membrane; and 2/ directly - when the ergosterol molecules themselves change places with tetrahymenol, the special lipid of Tetrahymena /3/. Temperature adaptations are also able to transform the organization of membrane structure: warm environment increases its fluidity, in cold conditions it becomes more rigid. But these changes are transient, after certain time the cell adapts to the shift temperature by modifying the structure of membranes. Finally, the result of these modifications is that warm conditions increase the rigidity, and the cold culturing the fluidity of the membrane to its original state. The reason of this is the flow of microsomal lipids towards the cell surface. Between these two effects /the ergosterol and temperature impression/ there was a relation in respect of membrane fluidity and structure, considering their saccharide components.

The permanent or the transient effect of cold /Fig.7-8/ resulting a more fluid membrane, increases the binding of Concanavalin A, Pisum and the Phaseolus and Helix lectins, presumably by changing the organization of glucose, mannose and their derivatives as well as N-acetyl-galactosamine in the membrane.

The thermo-shock /Fig.9/ - heating - causes the opposed alterations of saccharide composition according to lectin binding.

The effect of ergosterol /Fig.10-11/ stiffening of membrane depends also on the other parameters, like temperature. The main result is, nevertheless, the high level of Datura, Lens and Lycopersicon lectin binding and consequently the increasing of N-acetyl-glucosamine molecules in this membrane.

All these results fortify our opinion that the environmental factors have a special importance in the determination of membrane-processes.

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