Short communication

Effects of Insulin and Glucagon on Elasticity of Lipid Bilayers Modified by Rat Liver Plasma Membrane Fragments

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In our preceding works we studied effects of insulin on viscoelastic characteristics of lipid bilayers (Hianik et al. 1987) and insulin-induced changes in mechanical characteristics of lipid bilayers modified by rat liver plasma membrane fragments (Hianik et al. 1988). The Young modulus of elasticity in direction perpendicular to membrane plane, $E_\perp$, showed significant changes as compared to the initial state of bilayer lipid membranes (BLM). The changes were up to threefold when rat liver plasma membrane fragments were incorporated into BLM.

To demonstrate the specificity of the hormone-BLM interaction, changes induced by another hormone, glucagon, in $E_\perp$ of both nonmodified membranes and those modified by rat liver plasma membrane fragments were studied. The hormone has an opposite effect as compared with that of insulin. When bound to membrane receptor, glucagon induces glycogenolysis, resulting in elevated blood glucose levels (Hanč and Pádr 1982). Consequently, a comparison of the effects of the two hormones, insulin and glucagon, on BLM elasticity may provide significant information concerning the major step involved in the reception process, namely the hormone-receptor interaction. No similar comparison of the two hormones at the membrane level has been done as yet.

Membranes were formed at room temperature ($T = 20\,°C$) according to Mueller et al. (1962) on a circular hole ($d = 0.5$ mm) in the wall of a teflon cup, dividing it into two identical compartments (5 ml each).

BLM were formed of a mixture of egg lecithin (Plant of Chemical Preparations, Kharkov, USSR) with cholesterol (weight ratio 4:1) in *n*-heptane (Fluka; 20 mg/ml). Krebs-Ringer phosphate solution prepared with redistilled water was used as buffer. Salt concentrations in the buffer were (mmol/l): NaCl,

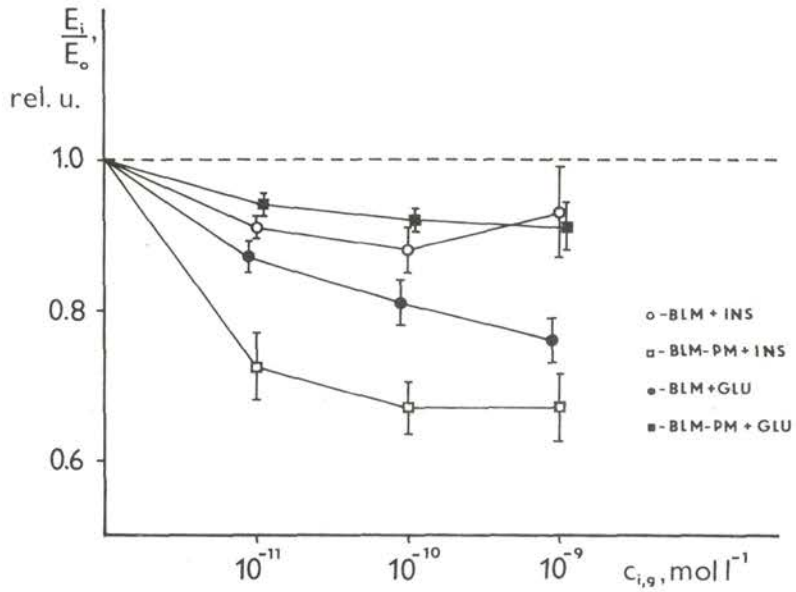

Fig. 1. The dependence of relative changes in Young modulus of elasticity, E_i/E_o, on insulin (empty symbols) and glucagon (filled symbols) concentration. Circles represent the depencence for non-modified lipid bilayers and squares that for lipid bilayers modified by rat liver plasma membrane fragments (concentration of membrane proteins 60 μg per 1 ml lipid solution).

134; KCl, 1.68; $MgSO_4$, 0.4; Na_2HPO_4, 0.6; and KH_2PO_4, 0.149; pH of the solution was adjusted to 7.4. The buffer was supplemented with bovine albumin (final concentration 0.05 %) to reduce hormone adsorption to glass walls of the experimental cell and to raise hormone stability. Insulin (Calbiochem) and glucagon (Elanco) both diluted in Krebs-Ringer solution supplemented with albumin, were added to one cup compartment to give sequential final concentrations of 10^{-11}; 10^{-10}; and 10^{-9} mol/l. The same volume of buffer was simultaneously added to the other compartment to balance hydrostatic pressure. Hormones were added to the electrolyte approx. 30 min after membrane formation. Any subsequent hormone additions were also made in 30 min intervals. Within the same intervals values of membrane $E_\perp$ were read from continuous records.

The dissociation constant of insulin (pK = 5.4) suggests that, under the conditions used in our experiment (pH = 7.4), the insulin molecule contains two elementary negative charges (Wu and Yang 1981). This suggestion concerning insulin adsorption has also been supported by our experiment (Hianik et al. 1987). That is why $CaCl_2$ was added to compensate for possible negative surface

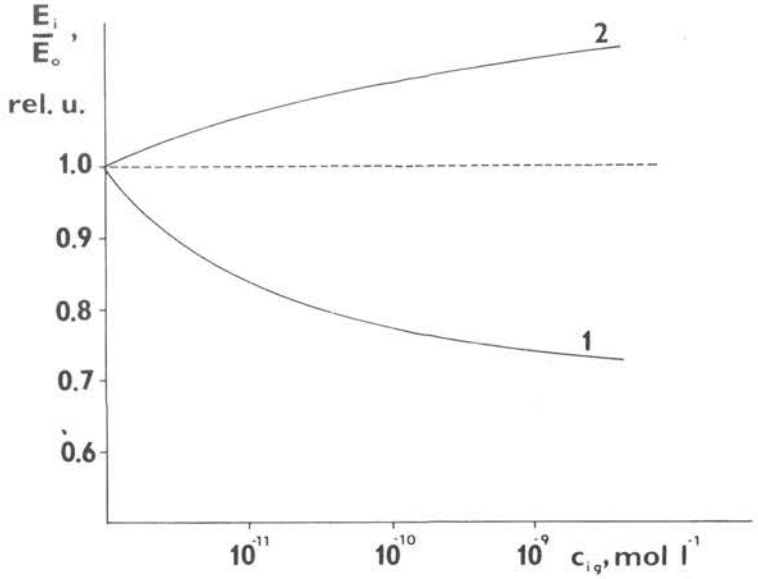

Fig. 2. The dependence of relative changes in Young modulus of elasticity, E_i/E_o, on the concentration of insulin (curve 1) and glucagon (curve 2) due solety to hormone-receptor interaction (obtained by subtracting the dependence of insulin and glucagon shown in Fig. 1).

charge on BLM; it was added to the Krebs-Ringer solution to give a final concentration of 10 mmol/l.

Rat liver plasma membrane fragments isolated according to Emmelot et al. (1974) were also added to lipid solution (60 μg membrane protein per 1 ml lipid solution).

BLM $E_\perp$ was measured with the special electrostriction method according to Passechnik and Hianik (1977), which is based on measuring the third harmonic which is also a component of the current flowing through the membrane. When studying the changes of the viscoelastic properties of the membranes induced by various agonists, one should bear in mind that the third harmonic may become generated even if the agonist causes a non-linear conductivity of the membrane (Passechnik et al. 1985). We used a special method, described in the above work, to test the effect of the hormone with BLM induced changes in the viscoelastic properties of the membrane.

Each experimental series used measurements on 8—10 membrane specimens. Mean values and mean quadratic deviations were calculated.

Fig. 1 shows dependence of the relative change in Young modulus of elasticity E_i/E_o on insulin and/or glucagon concentration for both non-modified membranes and those modified by rat liver plasma membrane fragments. BLM

formation, hormone concentrations as well as any other experimental conditions were identical for both hormones. It is obvious from Fig. 1 that glucagon interaction with non-modified BLM (non-specific binding) induces a larger relative change in modulus of elasticity than does non-specific insulin interaction. In contrast, the presence of membrane fragments raises E_i/E_o for glucagon interaction (the same symbol E_i is used for glucagon interaction; it stands for modulus of elasticity $E_\perp$ after 30 min of glucagon action, 10^{-10} mol/l).

By its binding to lipid bilayer, glucagon induces changes in the ordering of the hydrophobic region resulting macroscopically in a decrease of the Young modulus of elasticity similarly as it was the case with insulin-BLM interaction. This decrease is maximal for glucagon concentration of 10^{-9} mol/l; this contrasts with insulin with minimal values of $E_\perp$ being measured at 10^{-10} mol/l insulin.

The reason for the much more intense action of glucagon on lipid bilayer ordering as compared to that of insulin may be sought in structural differences between the hormones. Glucagon consists of a linear chain of amino acids (29 in number) allowing its easier incorporation into BLM as compared with insulin, the latter containing two polypeptide chains (chain A contains 21, chain B 30 amino acids) linked by disulphide bridges (Hanč and Pádr 1982).

Upon the interaction with membrane fragments-modified BLM, the two systems behave in an entirely different manner. With insulin, threefold changes in parameter E_i/E_o are observed as compared with those during the interaction with non-modified BLM. On the other hand, glucagon induces an opposite effect in the presence of membrane fragments: parameter E_i/E_o changes significantly less. Considering that the measured value of E_i/E_o results from contributions of changes in modulus of elasticity by both specific and non-specific interaction of the hormone with BLM, we may try to estimate changes in E_i/E_o due solely to hormone-receptor interaction. This estimate is obtained by subtracting the dependence for insulin (Fig. 1, empty symbols □ — ○) and those for glucagon (filled symbols, ■ — ●). The resulting relationships are shown in Fig. 2. It is obvious that insulin binding to its receptor reduces the value of the Young modulus of elasticity of BLM, while glucagon binding has an opposite effect.

It can be concluded that the antagonist action of insulin and glucagon is also reflected in their interaction with membranes.

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