ON THE ROLE OF GLYCOPROTEINS IN HORMONE ACTION: STABILIZATION OF ACTH STIMULATION OF ADENYLATE CYCLASE IN ISOLATED ADRENOCORTICAL PLASMA MEMBRANES BY CONCANAVALIN A

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1. Introduction

Many of the functional properties of plasma membrane glycoproteins have been elucidated by the use of specifically interacting proteins (or glycoproteins), namely, the lectins [1]. An important plasma membrane function is signal transferring during or following hormonal stimulation. Cuatrecasas [2,3] has shown that Concanavalin A and wheat germ agglutinin influence insulin binding to and action on isolated fat cells, as well as the adenylate cyclase activity of their membranes. Lyle and Parker reported that human lymphocytes showed an increase in intracellular cAMP after exposion to Concanavalin A [4].

Our study demonstrates a protective effect of Concanavalin A on the ACTH response of adenylate cyclase in purified bovine adrenocortical plasma membrane. Although the nature of this protective effect is unclear, we think, that the fact may be of interest for the following reasons: first, it may be that other hormone sensitive adenylate cyclase preparations, most of them being also quite labile, share the possibility of being protected by either Concanavalin A or other lectins, second, lectin treatment may stabilise other labile membrane functions, and third, this kind of effect has not been described before.

Abbreviations: $ACTH_{1-24}$ = Adrenocorticotrophin-(1–24)-tetracosipeptide, cAMP = 3′5′-cyclic-adenosine monophosphate. Other abbreviations according to the suggestions of the IUPAC-IUB Commission on Biochemical Nomenclature, Eur. J. Biochem. 1(1967)375.

2. Material and methods

Plasma membranes of bovine adrenal cortex were purified by a zonal centrifugation procedure as previously described [5].

Adenylate cyclase activity was determined with a modification of the method of Salomon and Rodbell [6]. The incubation mixture contained 15 mM Tris–HCl, pH 7.5, 15 mM creatine phosphate, 20 U/ml creatine phosphokinase, 2.5 mM $MgCl_2$, 0.5 mM cAMP, 0.5 mM (α-^{32}P)ATP (30–50 cpm/pmol), $ACTH_{1-24}$, NaCl and Concanavalin A (Boehringer) at various concentrations, and 10–30 μg of membrane protein in a final volume of 100 μl. The reaction was initiated by the addition of the membranes and stopped by heating the samples to 95°C for one minute in the presence of 150 μl of stopping solution (10 mM Tris-HCl, pH 7.5, and 3 mM [^{3}H]cAMP (40 cpm/nmol)). cAMP was isolated as follows: The samples were centrifuged to remove denatured protein, poured on Dowex 50 WX4 (200–400 mesh) columns (1 ml) and the columns then washed with 2.5 ml of water. With a further 2.5 ml of water the cAMP was eluted directly onto columns of neutral aluminia (equilibrated with 0.4 M imidazole–HCl, pH 7.2) from which cAMP was eluted with 2.5 ml of 0.4 M imidazole–HCl, pH 7.2 into scintillation vials. Recovery of cAMP was between 40 and 60%.

Protein was determined by the Lowry method [7]. Radioactivity measurements were done in a Nuclear Chicago Isocap scintillation counter with either Aquasol (NEN) or Rotiszint (Roth Chem.) as scintillation fluid.

3. Results

Preincubation of purified bovine adrenocortical plasma membranes at 30°C leads to a partial inactivation of their adenylate cyclase and, more important, the loss of its stimulation by ACTH. This loss can be considerably diminished by the action of Concanavalin A. Figure 1 shows two experiments which clearly demonstrate the protective effect of the lectin on the ACTH stimulated adenylate cyclase activity.

The basal activity, which is also diminished by preincubation at 30°C can be kept constant if the preincubation medium contains 100 mM NaCl as shown in fig.2. NaCl alone, however, does not protect

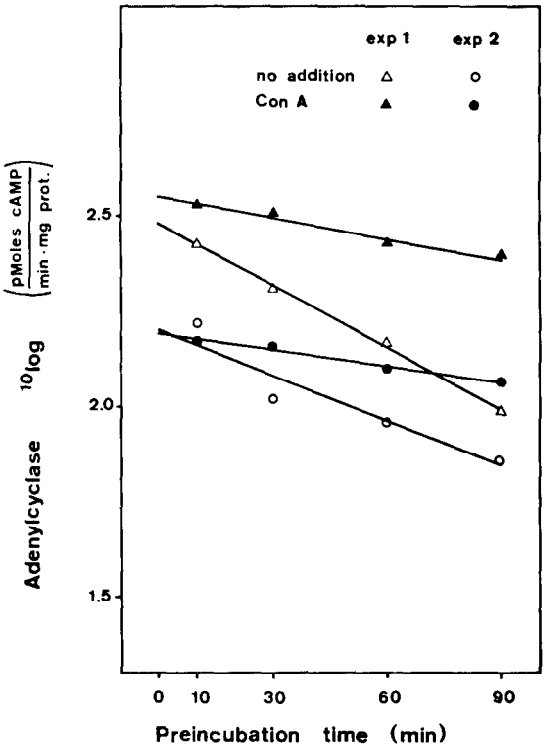

Fig.1. Stabilization of ACTH stimulated adenylate cyclase activity of bovine adrenocortical plasma membranes by Concanavalin A during preincubation at 30°C. Conditions of preincubation: bicarbonate buffer pH 7.5, 5 mM, with or without the addition of 625 μg/ml Concanavalin A and 100 mM NaCl. Membrane protein: 592 (exp. 1) and 496 (exp. 2) μg/ml during the preincubation (148 and 124 μg/ml during adenylate cyclase assay). $ACTH_{1-24}$: 5×10^{-6} M (during the assay only).

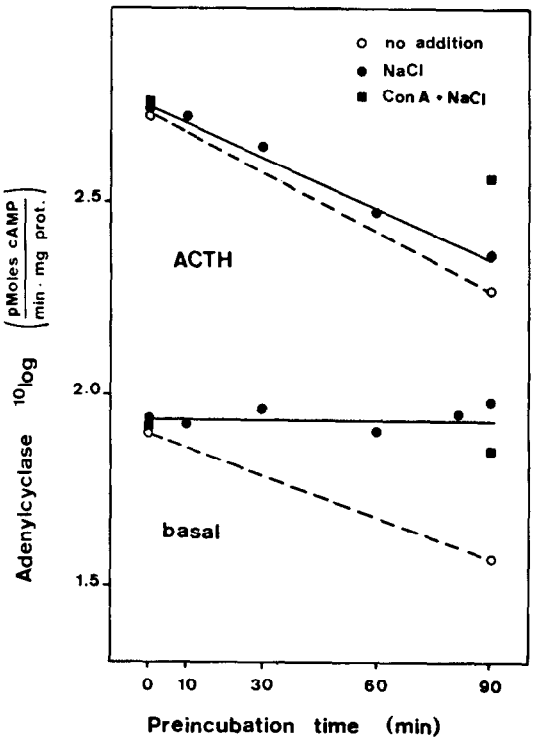

Fig.2. NaCl addition during preincubation at 30°C: effects on basal and $ACTH_{1-24}$ activated adenylate cyclase activity: Conditions of preincubation: bicarbonate buffer pH 7.5, 5 mM, with or without 100 mM NaCl (and 625 μg/ml Concanavalin A). Membrane protein: 616 μg/ml (154 μg/ml in the assay), $ACTH_{1-24}$ in the assay: 5×10^{-6} M.

the ACTH stimulability of adenylate cyclase activity, whereas Concanavalin A in the same experiment does (fig.2).

The protective effect of Concanavalin A is dose dependent in a way shown in fig.3, i.e., an optimum is reached at about 60 μg/ml.

The protective properties of Concanavalin A for ACTH activated adenylate cyclase can also be shown when the membranes are stored for 24 h at 4°C. Membranes stored with 625 μg of Concanavalin A per ml (and 100 mM NaCl) retain 1.5 to 4 times more ACTH activated adenylate cyclase activity compared to membranes stored in pure bicarbonate buffer.

The protection can be inhibited by the addition of methyl-D-mannoside (0.5 M) in the preincubation buffer. There is no significant change in the apparent affinity for ACTH in both membranes stored with or

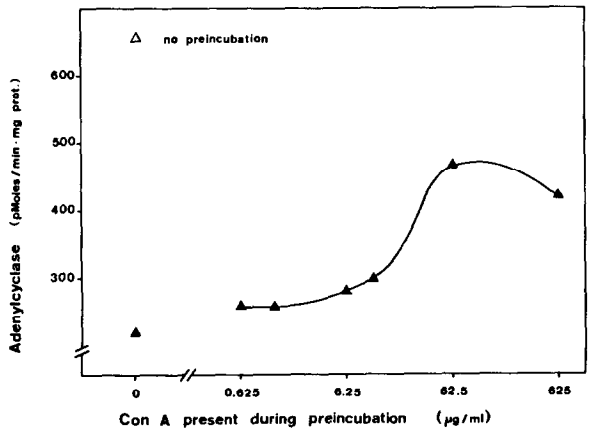

Fig.3. Concanavalin A dose dependency of the stabilization of $ACTH_{1-24}$ stimulated adenylate cyclase activity during preincubation at 30°C. Preincubation was carried out in 5 mM bicarbonate buffer pH 7.5 and different Concanavalin A concentrations (NaCl was 100 mM or 10 mM at 625 or 62.5 μg/ml Concanavalin A respectively). Membrane protein was 1.1 mg/ml (270 μg/ml in the assay). $ACTH_{1-24}$ in the assay: 5×10^{-6} M.

without the addition of Concanavalin A compared to freshly isolated membranes; the ACTH concentration of 5×10^{-6} M gives a maximal stimulation in all conditions.

4. Discussion

As already pointed out in the introduction, an interpretation of the effects of Concanavalin A is very difficult since the cause(s) for the loss of stimulation of adenylate cyclase by ACTH are unclear. There are several possible reasons: (a) loss of cofactors (Guanylnucleotides, Mg^{2+}, Ca^{2+}, etc.), (b) loss of receptor or enzyme molecules and (c) changes influencing the 'coupling' of the hormonal stimulus to the adenylate cyclase. This coupling is still a very unknown process. It seems that the lipid environment is important [8] and it may be that the coupling process requires a loose structural contact of either the receptor molecules among themselves ('receptor clustering' [9]) or of the receptor molecule with the enzyme. An argument in this direction is the fact that, normally, adenylate cyclase loses its hormone responsiveness upon solubilization or dispersion by detergents. It is very probably that a structural organisation in the plasma membrane would be lost upon storage and this might be the reason for a reduced effectiveness of coupling and thus a reduced hormone response.

We have some indication from electron microscopy that Concanavalin A might stabilise or form glycoprotein patches. These patches might be the regions where either the structural linkage or the lipid environment of receptor and enzyme molecules are better preserved. In these regions the coupling of the hormonal stimulus to the adenylate cyclase would be more efficient and/or the binding of cofactors [10] would still be more intact.

The role of glycoproteins for signal transferring thus remains unclear and much further experimental effort will be necessary to bring new insights.

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