INTERACTION OF PREGNANCY PROTEINS WITH PHYTOHEMAGGLUTININ P AND CONCANAVALIN A

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KEY WORDS: pregnancy proteins; trophoblast-specific β-glycoprotein; α_2-glycoprotein; α-fetoprotein; lectins: phytohemagglutinin and concanavalin A.

A trophoblast-specific β-glycoprotein (TBG) and an α_2-globulin associated with pregnancy (α_2-GP), the concentration of which rises sharply in pregnancy, have been found in the blood serum of pregnant women [4, 5, 11]. They are glycoproteins, but the composition of their carbohydrate components has received little study [8, 11]. TBG inhibits degranulation of mast cells when incubated together [3], whereas α_2-GP inhibits proliferation of lymphocytes stimulated by phytohemagglutinin P (PHA) or concanavalin (con A), both in the presynthetic period and during DNA synthesis [1, 7, 11]. They possess marked immunosuppressive properties in cultures of lymphocytes stimulated by mitogens and by allogeneic cells [6, 9, 14, 15].

The aim of this investigation was to analyze interaction of pregnancy proteins with lectins PHA and con A.

EXPERIMENTAL METHOD

TBG and α_2-GP were studied, and α-fetoprotein (AFP) was used as the control; the latter, on affinity chromatography on con A-Sepharose can be separated into two isoforms: one reacting and one not reacting with con A, probably due to the presence of a terminal mannopyranoside residue in the con A-reacting isoform [13].

The first two proteins were isolated by replica chromatography on Sephadex G-200 (Pharmacia, Sweden) and negative immunoaffinity chromatography on Sepharose 4B, with high-avidity rabbit antibodies against human serum proteins bound to it. The protein concentration was determined in the eluates and adjusted by ultrafiltration (XM-50 membranes, Diaflo, USA) to 50 μg/ml. To study AFP, a diagnostic serum for primary carcinoma of the liver and teratoblastoma (N. F. Gamaleya Research Institute of Epidemiology) was used, when the AFP concentration was about 50 μg/ml. Antisera were obtained by immunizing rabbits with purified proteins together with Freund's complete adjuvant, and antibodies against TBG, α_2-GP, and AFP were isolated on an immunosorbent [10]. The PHA P was obtained from Difco, USA and Reakhim, USSR, the con A was obtained from Sigma, USA. To study interaction of prgenancy proteins with the lectins, low-voltage versions of linear [12] and comparative cross immunoelectrophoresis [2] were used. In the latter case, blocks of gel containing PHA or con A were placed in front of the plate after immunoelectrophoresis in the first direction. The 1% agarose was type I, from Sigma, USA. To study interaction of lectins with serum proteins from pregnant women, sera were obtained from women in the third trimester of pregnancy and antibodies obtained against them [10]. Immunoelectrophoresis was carried out in barbiturate and Tris-barbiturate buffers [2].

EXPERIMENTAL RESULTS

Pregnancy proteins and AFP have affinity for con A (Fig. 1). The highest concentrations of con A causing "invagination" of the precipitation line formed by antigen and the corresponding antibodies (about 50 μg/ml of each protein) were 80, 180, and 500 ng of the lectin for TBG, α_2-GP, and AFP respectively. Consequently, they possess more than one receptor for interaction with a lectin, corresponding in its chemical composition to mannose [7]. Only α_2-GP and TBG interact with PHA, and the concentrations of lectin causing changes in the precipitation lines were 20 and 5 ng for them respectively. They may indicate that these proteins have more marked affinity for PHA than for con A. The carbohydrate content in TBG, α_2-GP, and AFP is 29.3, 9.9,

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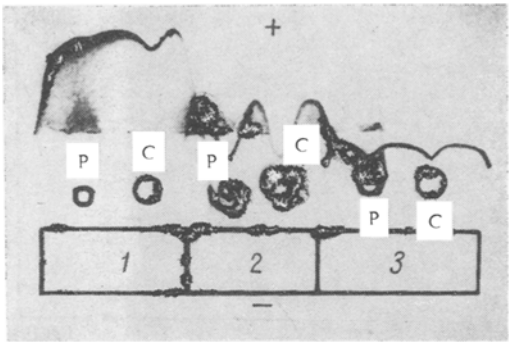

Fig. 1. Comparative characteristics of affinity of pregnancy proteins with PHA and con A. 1) AFP; 2) α_2-GP; 3) TBG. P) PHA (1 μl of lectin with concentration of 10 mg/ml added to each well). C) Con A (10 μl of lectin with a concentration of 10 mg/ml added to each well).

and 4.3%, respectively [11]. Incidentally, for all proteins a linear relationship exists between the protein and lectin concentrations (measured as the area of change of the precipitation line). The reaction of these proteins with con A may perhaps reflect the quantity of mannose analog present in the molecules of these glycoproteins. PHA has been shown to have affinity for N-acetyl-D-galactosamine [7].

Reactions of the proteins with lectins are determined by the number of receptors for lectin in the protein molecule: If one receptor is present a soluble lectin–ligand complex is formed, which is bound only by lectin immobilized on an insoluble carrier, whereas if two receptors or more are present, the lectin acts like an antibody, causing the formation of an affinity precipitate [7]. The best way to determine the number of receptors for lectins is to use immobilized lectins.

An excess of con A causes disappearance of the TBG precipitate and well-marked cathodal migration of precipitates of α_2-GP and AFP (Fig. 2A). If PHA is present in excess in the intermediate gel the precipitation of TBG and α_2-GP disappear, but the AFP precipitates is virtually unchanged in area (Fig. 2B). In this case a massive affinity precipitate is formed on the lower border of the intermediate gel, containing PHA; its arrangement by electrophoretic mobility corresponds to that of α-GP and TBG. This phenomenon indicates that receptors for con A in the α_2-GP molecule are nonhomogeneous. Part of the molecule of this protein probably contains not more than one receptor for con A or contains no such receptor. TBG and α_2-GP either contain more than two receptors for PHA, or these receptors are dominant in their molecule. The properties of AFP correspond to reaction of this protein with PHA and con A. However, it can be postulated that the number of receptors for PHA in its molecule is small, or that a soluble lectin–ligand complex is formed on interaction betwen antigen and PHA.

TBG has a heterogeneous structure: It consists of immunochemically identical components with electrophoretic mobility of α- and β-globulins. If barbiturate buffer is used, the α-component of TBG does not appear, but by contrast it is clearly visible if Tris-barbiturate buffer is used. It was postulated previously that the α-component of TBG can be revealed only after addition of 6-kilodalton polyethylene glycol to the gel and Tris-barbiturate buffer [11]. Both forms of TBG have marked affinity for con A and PHA.

It has been shown that TBG and α_2-GP have marked immunosuppressive properties [6, 9, 14, 15]. However, most observations are based on the results of inhibition of the blast trasformation reaction, stimulated by PHA or con A, in the presence of these proteins. It has been shown [9] that inhibition of the blast-transformation reaction of lymphocytes by PHA does not take place in the presence of TBG, if protein and lectins are added simultaneously to the cells. If the lectin is added first, marked inhibition of the blast-transformation reaction is observed after addition of TBG [9]. Considering the high affinity between PHA and TBG, the significance of the immunosuppressive properties of this protein may be questioned. Similar doubts may also be expressed as regards α_2-GP, for we know that it causes immunosuppression of the immune response even without the use of these mitogens [11]. All that can be noted is that before proteins are used as inhibitors of the blast-transformation reaction of lymphocytes, stimulated by PHA or con A, it is necessary to make sure that they have no affinity for these lectins.

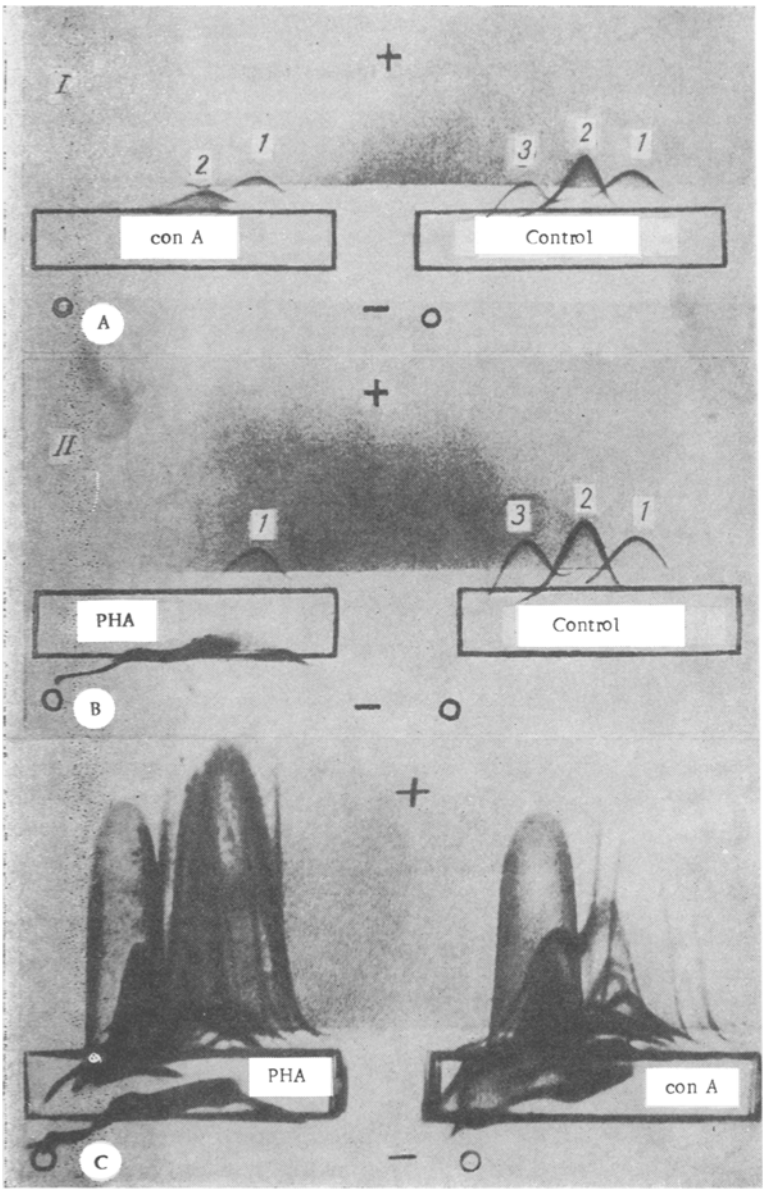

Fig. 2. Interaction of pregnancy proteins and human serum proteins with lectins. I) Reaction of pregnancy proteins with con A; II) reaction of pregnancy proteins with PHA; III) reaction of serum proteins with lectins. Control – intermediate gel without lectin. Concentration of lectin in intermediate gel 30 mg/ml. 1) AFP, 2) α_2-GP, 3) TBG. A, B) Mixture of monospecific antibodies and mixture of pregnancy proteins used; C) antibodies against serum proteins of pregnant women and mixture of serum from pregnant women used.

The possibility cannot be ruled out that stimulation of the blast-transformation reaction is more complex in character than the results of direct interaction between mitogen and lymphocyte membrane, for it has been shown [9] that if lymphocytes are cultured in a 3% solution of human serum albumin, the blast-formation reaction is much weaker than when fetal calf serum is used. At this point we can suggest either that albumin is unsuitable for use as nutrient medium, or that the lectin must be bound with a certain receptor before it can interact with the lymphocyte membrane.

It is very important to analyze also the character of interaction of lectins with human serum proteins, for this problem still awaits study. Figure 2C shows that the majority of human serum proteins (not excluding pregnancy proteins also) interact with con A. On this basis it can be postulated that they all have more than

one receptor for this lectin. Far fewer human serum proteins interact with PHA. Consequently, TBG and α_2-GP are among the few serum proteins which possess at least two receptors to react with PHA, and this phenomenon can be used to isolate them in the pure form.

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EFFECT OF CALCIUM CHANNEL BLOCKERS ON THE POSITIVE INOTROPIC EFFECT OF IONOPHORE A23187 IN THE MYOCARDIUM

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KEY WORDS: ionophore A23187; calcium antagonists; force of contraction; myocardium

The carboxyl ionophore A23187 increases the force of contraction and Ca-current in the myocardium [2, 5]. The action of ionophore A23187 is due to its ability to form complexes with Ca^{++} ions [4, 9]. It has been shown on artificial media that ionophore A23187 forms complexes not only with Ca^{++} ions, but also with other bivalent cations; ions which block the Ca-current in the myocardium, moreover, form more stable complexes with the ionophore than with Ca^{++} [9]. Ionophore A23187 also binds with a number of organic compounds, including the well-known calcium antagonists verapamil and its derivative D-600 [8].

The aim of this investigation was to study the ability of Zn^{++} and Mn^{++} ions and organic compounds fenigidine and D-600 to inhibit the positive inotropic effect of ionophore A23187 and also to compare the ability of calcium antagonists to inhibit myocardium contraction and the effect of the ionophore.

EXPERIMENTAL METHOD

Experiments were carried out on atrial strips of Rana ridibunda. The technique of recording mechanical activity and the conditions of stimulation were described previously [1]. The original physiological saline

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