Thus, SKF 10.047, a sigma receptor agonist, can activate the ODC of loach embryos. The ODC-activating action of SKF 10.047 is stereospecific and it depends on the stage of embryonic development. A further study of the character of aggregation of embryonic cells under the influence of polyamines (and/or their derivatives) and discovery of the mechanism of their action on embryonic cells would seem to be important.

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DETERMINATION OF AMYLOID P-COMPONENT IN BLOOD PLASMA BY ELISA

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KEY WORDS: amyloid P component; enzyme immunoassay; serum amyloid P component

The amyloid P component (AP), found in amyloid deposits, is structurally and antigenically similar to serum amyloid P-component (SAP), which is a normal blood plasma glycoprotein [15]. It is claimed that SAP is the precursor of AP in systemic amyloidosis [9]. There is evidence that AP is not only formed in the tissues during the development of systemic amyloidosis, but it is also a normal component of the basement membranes of the renal tubules, skin, lungs, and intestine [2].

The disappearance of AP from the basement membrane of the renal glomeruli is associated with the development of an inherited nephropathy (Alport's disease) [10]. SAP is a glycoprotein with mol. wt. of 250 kD, consisting of 10 identical subunits which, by noncovalent binding, form two pentameric structures. C-reactive protein and certain other mammalian protein molecules, linked together on this basis to form a class of proteins known as pentraxins [11], have a similar structure. A characteristic feature of AP is its conservatism in evolution, for identical proteins are found in all vertebrates, amphibians, and fishes investigated, and this has attracted attention to the study of its biological function [13]. It has been shown that SAP is produced by hepatocytes, it takes part in immunoregulation, by inhibiting the proliferative response of lymphoid cells to mitogenic stimulation [8], and it can undergo calcium-dependent binding with various ligands [1]. Normally the SAP concentration remains quite stable at 50-70 μg/ml [3]. Serum levels of SAP are extremely low in neonates and a little higher in children [12]. The SAP level is lower in women than in men [12]. Several methods have been developed to determine the SAP concentration, including rocket immunoelectrophoresis, nephelometry, radioimmunoassay, and enzyme immunoassay [3, 4, 7, 14]. However, there is little information on the SAP concentration in diseases in man. Some preliminary results indicate an increase in the SAP concentration in Waldenstrom's macroglobulinemia, rheumatoid arthritis, and malignant neoplasma [6, 7]. This confirms the need for further improvement in methods used to determine SAP in order to estimate its concentration in various human diseases.

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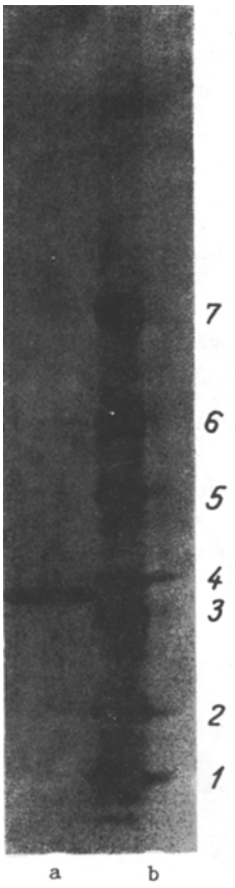

Fig. 1. Polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate, purified AP (a), and reference proteins (b). 1) 14 kD; 2) 20 kD; 3) 24 kD; 4) 29 kD; 5) 36 kD; 6) 45 kD; 7) 66 kD.

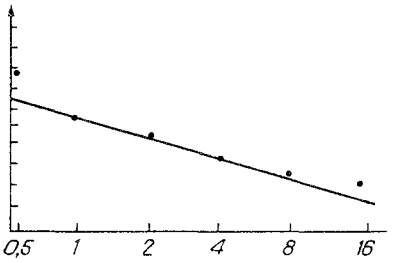

Fig. 2. Calibration curve of AP. Abscissa, AP concentration (in μg/ml); ordinate, relative optical density (E_{405}), in %.

EXPERIMENTAL METHOD

AP was isolated from the spleen of a patient dying from secondary amyloidosis by affinity chromatography on sepharose 4B [8]. The purity and molecular weight of the preparation were determined by polyacrylamide gel electrophoresis with sodium dodecylsulfate [5], using standard markers ("Sigma," USA). The protein was identified by means of commercial antiserum to AP by double immunodiffusion in 1% agarose, containing 0.01 M EDTA. The following reagents were used to determine SAP by ELISA: commercial antiserum to AP ("Dakopatts," 0.01 M phosphate-buffered saline (PBS), pH 7.4; 0.01 M citrate buffer, pH 4.8; 2% bovine serum albumin (BSA) in PBS, monospecific goat antibodies to rabbit immunoglobulin, labeled with peroxidase ("Sigma," USA), 0.005% hydrogen peroxide and 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonate (ABTS). A polystyrene microplateau (Flow Laboratories, Great Britain) was used. The modified method of enzyme immunoassay was carried out as follows. Into each of the 96 wells

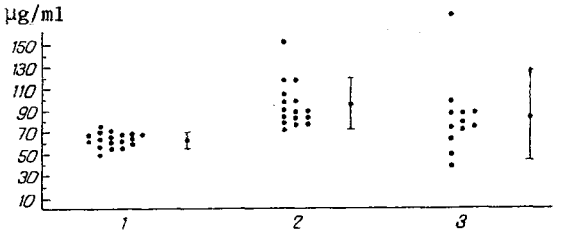

Fig. 3. SAP levels in blood donors (1) and patients with amyloidosis (2) and rheumatoid arthritis (3). Ordinate, SAP concentration in μg/ml in sera from healthy blood donors and patients (individual values and M ± σ).

of the microplateau 50 μl of protein AP in PBS in a concentration of 2 μg/ml was added and the samples were incubated overnight at 4°C in a humid chamber. After adsorption of the AP the wells were washed twice with 250 μl of PBS each time and blocked with 2% BSA for 30 min. The purified AP protein and the sera for analysis were diluted immediately before application to the plateau. The test sera and antisera were diluted with the aid of 2% BSA. To plot the calibration curve serial dilutions of AP were prepared from 1 to 16 μg/ml, and an equal volume of rabbit monospecific antiserum to AP in a dilution of 1:1000 was added. To determine the SAP concentration in the samples the sera were diluted 1:4, 1:8, 1:16, 1:32, and 1:64 and an equal volume of antiserum to AP in a dilution of 1:500 was added. The samples thus prepared were introduced into the wells in a volume of 50 μl in each case, in duplicates. The microplateau was incubated for 18 h at 4°C, washed off twice with PBS, again blocked with 2% BSA for 30 min, after which 50 μl of peroxidase-labeled rabbit antibodies in a dilution of 1:1000 was added to each well. The microplateau was incubated for 3 h at room temperature, and washed off twice with PBS and once with citrate buffer, after which the substrate mixture consisting of ABTS and H_2O_2 was added. After 30 min the optical density was measured at a wavelength of 405 nm, using the Behring ELISA Processor II (West Germany).

EXPERIMENTAL RESULTS

The purified amyloid P component was detected on electrophoresis as a single band corresponding in molecular weight to one subunit of pure 25 kD protein (Fig. 1). The presence of a single line of precipitate also was found by double immunodiffusion in agarose using monospecific antiserum to P component, further evidence of the purity and specificity of the preparation.

As can be seen from Fig. 2, in the AP concentration range from 1 to 16 μg/ml a linear dependence of the optical density on the logarithmic AP concentration was observed. Dilutions of the sera to be analyzed were selected so that the values of the optical density would fall within the linear region of the calibration curve. It was established that at a serum dilution of 1:16, SAP can be detected in concentrations from 16 to 260 μg/ml. When commercial antiserum to the P-component was used in a 1:1000 dilution, the value of the optical density at maximum binding ranged from 0.6 to 0.8 unit. It was established that increasing the concentration of AP immobilized on the solid phase from 2 to 10 μg/ml does not lead to any substantial increase in the sensitivity and specificity of the method.

To characterize the method SAP was determined in pooled sera from normal blood donors, and sera from patients with amyloidosis and rheumatoid arthritis. It will be clear from Fig. 3 that the SAP concentration in the donors' sera ranged from 45 to 73 μg/ml (average 61.1 ± 1.8 μg/ml). Similar results for the SAP concentration in the donors were obtained by Levo and coworkers [7]. The SAP concentration in patients with amyloidosis varied from 70 to 149 μg/ml (average 91.1 ± 5.6 μg/ml), significantly higher than in the donors. In rheumatoid arthritis, more substantial fluctuations of the SAP concentration were observed: from 33 to 171 μg/ml, but the mean SAP concentrations (76.8 ± 12.2 μg/ml) was significantly higher than in the donors.

The results thus indicate that the SAP concentration in the sera of patients and healthy blood donors can be determined by the use of the method of enzyme immunoassay developed by the writers. The ability of AP to undergo adsorption on polystyrene suggested that its concentration could be determined without any additional binding with other ligands, thus significantly simplifying the performance of the reaction.

The preliminary results are evidence of an increase in SAP concentration in amyloidosis and during chronic inflammatory diseases of the joints, and they thus indicate that determination of SAP in chemical practice is worthwhile.

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