INVESTIGATION OF MEMBRANE-ACTIVE COMPONENTS FROM BLOOD SERUM OF PATIENTS WITH CHRONIC RENAL FAILURE

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UDC 616.61-008.64-036-12-07:616.15-008.6-074

KEY WORDS: chronic renal failure; medium-sized molecules; gel filtration; conductance; bilayer phospholipid membranes.

The terminal stage of chronic renal failure (CRF) is characterized by changes in the electrolyte composition of the extracellular fluid, disturbance of the acid-base balance, and also anemia as a result of disturbance of hematopoiesis or of increased erythrocyte destruction under the influence of membrane-active substances which accumulate in the body [1, 5]. Long-term observations on patients undergoing periodic hemodialysis have shown that despite lowering of their urea and creatinine levels to subnormal values and normalization of the acid-base and electrolyte balance, such patients develop complications such as encephalopathies and polyneuropathies. Many investigators link these complications with the accumulation of toxic substances in the blood because they diffuse inadequately through the semipermeable membrane during hemodialysis. It has been suggested that correlation exists between the accumulation of these substances in the body and progression of changes in the nervous system of patients with CRF [6, 8].

To shed light on the nature and mechanism of action of these toxic substances, an investigation was carried out to study the possible effect of whole serum from patients with CRF and healthy subjects, and also components of the serum on conductance of bimolecular phospholipid membranes (BPM).

EXPERIMENTAL METHOD

Blood serum from patients with the terminal stage of CRF and clinically healthy subjects (blood donors) was investigated. The blood was centrifuged at 3000 rpm for 20 min and the serum kept at −45°C until required for use.

Chromatography was carried out on Sephadex G-15 (from Pharmacia, Sweden), and the pH of the buffer solutions was measured by means of a PHM-3 pH meter (from Radiometer, Denmark). Fractions were detected on a "Uvicord-II" instrument (from LKB, Sweden) at a wavelength of 280 nm. An instrument of the OP-205 type (Hungary) was used as high-ohmic electrometer. BPM were obtained from phospholipids isolated from the white matter of bovine brain by the method in [7], with an initial concentration of 20 mg/ml. The membrane was formed on holes in Teflon foam, placed in a glass cuvette with the electrolyte; the area of cross section of the holes was 1.7 mm^2. Conductance and transmembrane potential of BPM were measured by the usual method [5]. The serum was fractionated by gel-chromatography on a column measuring 1.0 × 100 cm, and 0.01 M sodium acetate buffer, pH 6.7 was used for elution (elution rate 14 ml/h). The volume of serum applied to the column was 1 ml.

EXPERIMENTAL RESULTS

During fractionation of serum, not counting high-molecular-weight proteins and immunoglobulins eight or nine peaks, conventionally numbered according to the order of elution, were observed (Fig. 1). Comparing fractionation curves of blood serum of patients with CRF and healthy subjects showed that peaks 3 and 4 were

Laboratory of Protein and Peptide Chemistry, Institute of Bioorganic Chemistry, Academy of Sciences of the Uzbek SSR. Department of Biophysics, Institute of Biochemistry, Academy of Sciences of the Uzbek SSR. Department of Surgery, Faculty of Medicine, Tashkent Medical Institute. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 10, pp. 82-83, October, 1982. Original article submitted March 5, 1982.

 0007-4888/82/9410-1408$07.50

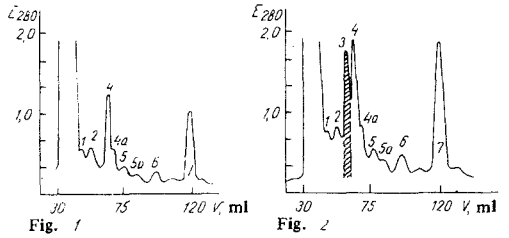

Fig. 1. Gel chromatography of healthy human blood serum.

Fig. 2. Gel chromatography of blood serum from patients in terminal stage of CRF.

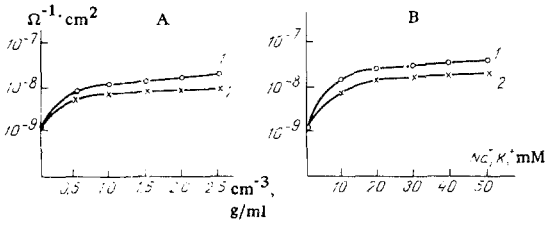

Fig. 3. Conductance of BPM as a function of concentration of fraction CM-3 and concentration of Na^+ and K^+ ions in medium. A) Conductance of BPM (in $\Omega^{-1} \cdot cm^2$) as a function of concentration of fraction CM-3 (in g/ml): 1) in presence of 10 mM K^+; 2) curve in presence of 10 mM Na^+; B) conductance of BPM as a function of concentration (in mM) of K^+ (1) and Na^+ (2) ions, in the presence of 2 ml of fraction CM-3.

considerably enlarged in the blood of patients with CRF (Fig. 2). To assess the molecular weights of these peaks, the ratio V_e/V_0 (ratio of elution volume to the free volume) was calculated and compared with the corresponding ratio for known substances: peak 3 corresponded in its V_e/V_0 ratio to vitamin B_{12} and angiotensin II (mol. wt. 1357.4 and 1368.0 daltons respectively), peak 4 to bradykinin and oxytocin (mol. wt. 1000.6 and 1000.7 daltons respectively). Peaks from 5 to 7 corresponded to substances with mol. wt. below 300 daltons. The components of fractions 1-4 were conventionally called average molecular-weight, those of fractions 5-7 low molecular-weight.

The membrane activity of the fractions and whole serum was studied by its action on conductance of BPM. Conductance of BPM, bathed with a solution of 25 mM Tris-HCl, pH 7.3, was $2.37 \times 10^9\ \Omega^{-1} \cdot cm^2$. Addition of fractions and whole serum from healthy subjects to the cuvette up to 1 ml caused no marked changes in conductance of BPM.

The addition of blood serum from patients with CRF in medium without K^+ and Na^+ ions to the cuvette did not change membrane conductance, but contact of 0.4 ml of serum from a patient with CRF with the membrane for 8 min led to its rupture. On the addition of serum from patients with CRF in a dose of 0.4 ml to medium containing 10 mM K^+ or Na^+, an increase in conductance of BPM was observed by one order of magnitude on average. On addition of low molecular weight fractions from blood serum of patients with CRF to the solution bathing the BPM, no change was observed. In a study of average molecular-weight fractions an effect was found only in the case of addition of fraction 3, designated average molecular weight (CM-3), which was not found in healthy human blood.

Membrane conductance, when modified by fraction CM-3, was increased after 6 min on average by one order of magnitude (Fig. 3A) in the presence of K^+ and Na^+ ions. Changes in conductance of BPM were studied in the presence of a linear increase in concentrations of fraction CM-3 and of the concentrations of ions in the medium (Fig. 3).

In the presence of Ca^{++}, membrane conductance was virtually unchanged. The membrane potential established on the BPM in a tenfold gradient was 47-50 mV relative to K^+ and 39-40 mV relative to Na^+.

The membrane activity of serum from patients with CRF, revealed by these experiments, evidently lies at the basis of its lytic action on erythrocytes. In their effect these toxins are similar to the widely distributed protein cytotoxic factors from microorganisms [2] and also from snake venoms [3].

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LIPID METABOLISM IN CELLS OF THE ATHEROSCLEROTIC HUMAN AORTA. EXPERIMENTS IN PRIMARY CULTURE

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UDC 616.132-004.6-07:616.132-008.939.15

KEY WORDS: atherosclerotic lesion; lipid metabolism; blood plasma.

The accumulation of lipids, mainly cholesterol esters, in the aortic wall is a characteristic feature of atherosclerosis [4, 6]. It is nowadays considered that the chief source of the accumulating lipids is the low-density lipoproteins circulating in the blood plasma [3, 7]. Meanwhile other possible mechanisms of the increased lipid content in the atherosclerotic aorta cannot be ruled out, including a disturbance of intracellular lipid metabolism in the affected regions.

This paper describes an attempt to compare lipid metabolism in cells of both unaffected and atherosclerotic regions of the human aorta. The investigations were carried out in primary culture, in which the cells preserve their similarity with cells in vivo in many properties. The fact that all cells in culture are under identical conditions allows the intensity of the lipid metabolism of cells from unaffected regions of the aorta and from lipid patches and atherosclerotic plaques to be compared.

EXPERIMENTAL METHOD

Cells of the intima and media of the aorta were isolated separately by dispersion with collagenase and elastase as described in [1]. The isolated cells were transferred to plastic Petri dishes (Falcon, USA), 60 mm in diameter, in a density of 0.5×10^6 to 1×10^6 cells per dish. The cells were cultured in 5 ml of medium 199 containing embryonic calf serum and 2.5 μg/ml fungizone, 100 μg/ml kanamycin, and 2 mM glutamine (all reagents from Gibco, USA) at 37°C in an atmosphere of 5% CO_2 and 95% air, saturated with water. On the 7th day the medium was changed and 40 μCi/ml of the sodium salt of [^{3}H]-acetic acid (from the Radiochemical Centre, Amersham, England) was added; the specific radioactivity of the preparation was 300 mCi/mmole. Incubation with labeled acetate continued to 6 h. At the end of incubation the medium was poured off and the dishes were washed three times with isotonic Dulbecco phosphate buffer, pH 7.4 (from Gibco, USA). The cells were suspended with 0.25% trypsin (from the same firm) and the suspension was centrifuged (200g, 10 min) and resuspended in isotonic phosphate buffer. Lipids were extracted from the cells by the method in [2]. The principal

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(Presented by Academician of the Academy of Medical Sciences of the USSR E. I. Chazov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 94, No. 10, pp. 83-85, October, 1982. Original article submitted March 16, 1982.

0007-4888/82/9410-1410$07.50