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ELECTROPHORETIC ANALYSIS OF LEUKOCYTE PROTEIN COMPOSITION IN DOWN'S SYNDROME

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UDC 616.899.65-07:616.155.3-008.939.6-074:543.544

KEY WORDS: Down's syndrome; aneuploidy; leukocytes; proteins.

Comparative electrophoretic analysis of polypeptide gene products in cells from patients and normal individuals in a promising approach to the study of changes in the function of the genetic apparatus of the cells in various diseases and, in particular, in those due to aneuploidy, one of the consequences of which is realized in Down's syndrome. When using this approach, as a rule investigators have turned to methods of two-dimensional electrophoresis in order to resolve the maximal possible quantity of cell proteins [5, 9, 10]. Unfortunately, previous investigations on cultures of different types of cells from human adults and embryos either revealed no changes in the protein composition of cells receiving an extra 21st chromosome [9, 10] or revealed an increased presence of only one or two proteins [5], i.e, far fewer than might be expected from the dose effect of genes expressed on the 21st chromosome [9, 10].

Using uncultured human peripheral blood cells, the writers succeeded for the first time, by one-dimensional electrophoresis, in finding a protein in leukocytes of patients with Down's syndrome that cannot be detected in normal human leukocytes [2]. In the investigation described below this feature of the leukocytes of these patients is analyzed and the causes of disagreement between the theoretically expected differences in the protein composition of normal and trisomic cells and those actually found by two-dimeniosnal electrophoresis [5, 9, 10], are discussed.

EXPERIMENTAL METHOD

Venous blood (5-15 ml) from healthy donors aged 20-40 years and from patients with Down's syndrome (8-18 years) was mixed with heparin (Richter, Hungary, 1000 IU/ml) in the ratio of 33 IU heparin to 1 ml blood, and transferred into tubes and incubated at 37°C for 2 h to sediment the erythrocytes. The leukocytes were washed off with 0.15 M NaCl and the residual erythrocytes hemolyzed in 0.035 M NaCl to produce a homogenate of leukocytes which, after freezing in water with 1 mM PMSF (phenylmethylsulfonyl fluoride), was either used immediately for electrophoresis [6], or acid extracts were prepared from it beforehand in 0.4 N HCl (°C, 30 min), and these were precipitated in 25% TCA (0°C, 12 h). These residues and residues of proteins of leukocyte homogenates not extracted with acid, were dissolved in Laemmli's protein buffer [6].

Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. P. Bochkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 1, pp. 50-52, January, 1985. Original article submitted May 14, 1984.

0007-4888/85/9901-0061$09.50

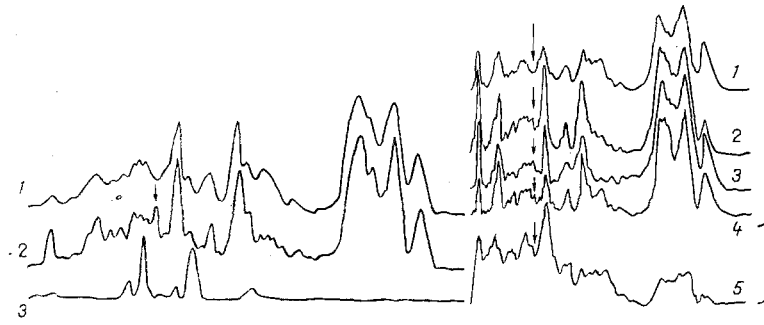

Fig. 1

Fig. 2

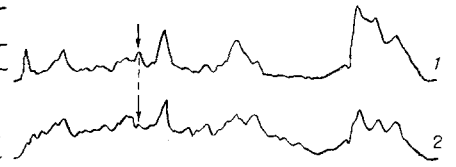

Fig. 3

Fig. 1. Densitograms of gels after electrophoresis of proteins of acid extracts of leukocytes from patients with Down's syndrome and healthy subjects. 1) Protein of healthy bood donor, 2) patient's proteins, 3) marker proteins (kilodaltons): bovine serum albumin 67, catalase 60, ovalbumin 43, aldolase 40, chymotrypsinogen A 25.

Fig. 2. Densitograms of gels after electrophoresis of proteins of acid extracts and acid-unextractable proteins from leukocytes, 2-4) proteins of extracts of patient's leukocytes, 5) acid-insoluble proteins of patient's leukocytes.

Fig. 3. Densitograms of gels after electrophoresis of proteins of leukocyte homogenates from patients with Down's syndrome (1) and with chronic myeloblastic leukemia (2).

The gels after electrophoresis were subjected to densitometry on a Gilford (England) spectrophotometer at 570 nm after staining with Coomassie blue R-250.

EXPERIMENTAL RESULTS

Densitograms of the gels obtained by electrophoresis of proteins of acid extracts of healthy human leukocytes, leukocytes from a patient with Down's syndrome, and of marker proteins are illustrated in Fig. 1 (from top to bottom). On the basis of these data the molecular weight of a polypeptide (marked by an arrow on the trace) obtained from the patient's leukocytes but not detectable in those of the normal subject, was determined (53 ± 1 kilodaltons). Densitograms of gels of proteins in acid extracts of leukocytes from a single healthy subject and from three patients with Down's syndrome and a densitogram of the gel after electrophoresis of proteins remaining in the residue after acid extraction of the patient's leukocytes are given in Fig. 2. The polypeptide of interest to us is acid-soluble and is evidently completely extracted from patients' leukocytes with 0.4 N HCl, for it is not found in the residue. This indicates that it is evidently a neutral or basic protein.

The occurrence of this polypeptide differed in leukocytes from different patients (Fig. 2). In normal human leukocytes (12 subjects) it either could not be detected at all or it was present only in traces. We were able to identify a polypeptide with a molecular weight of 53 kilodaltons in the leukocytes of all 20 patients with Down's syndrome studied. This protein can thus serve as a biochemical marker of Down's syndrome.

However, the use of data on the presence of this protein in leukocytes is possible only as an additional test for the diagnosis of Down's syndrome or as a biochemical feature of the pathogenesis of this disease, for a protein with a molecular weight of 53 kilodaltons is detectable in an appreciable amount on electrophoresis of homogenates of leukocytes from persons who, although not clinically healthy, do not suffer from Down's syndrome. One such example is given in Fig. 3, in which this particular protein was present on the densitogram of proteins from leukocytes of a patient with chronic myeloblastic leukemia.

These findings necessitated a detailed study of the prevalence of the protein with a molecular weight of 53 kilodaltons in the leukocytes of persons classed as normal during the diagnosis of Down's syndrome. Such an investigation should shed light on the mechanisms responsible for the presence of this protein in leukocytes of patients with Down's syndrome. Discovery of the protein in leukocytes of patients with leukemia (two cases) presupposes a definite similarity between the biochemical changes in this disease and in trisomy for the 21st chromosome. In this connection reports of the increased prevalence of leukemias and of neoplastic diseases in general [1, 7] or of transient leukemoid reactions [8] in patients with Down's syndrome must be mentioned.

Having set out to discover all possible changes in the character of protein synthesis in aneuploid cells, Weil and Epstein [9, 10] used the method of two-dimensional electrophoresis to analyze the proteins of cultured fibroblasts obtained from five healthy subjects and five patients with trisomy for chromosome 21. Labeling of the proteins with [^{35}S]methionine and visual comparison of autoradiographs obtained after different exposures of the gels after electrophoresis of cell lysate proteins, were used. These workers concluded that among the 850 proteins discovered, they could not find one which, in all the cell lines studied, differed appreciably in its presence in diploid and aneuploid fibroblasts. To explain the fact that two-dimensional electrophoresis did not reveal proteins that are represented 1.5 times more strongly in trisomic cells than would correspond to the dose of genes located on the 21st chromosome, these workers postulated that their method is capable of testing only twofold or more differences in protein content [10]. By subjecting the gels to densitometry, we were able to find a protein in leukocytes of patients with Down's syndrome by one-dimensional electrophoresis that could not be detected in normal subjects [2]. However, this protein could not be synthesized in trisomic fibroblasts.

Klose et al. [5], to analyze protein differences between human cells in culture (diploid and with trisomy for chromosome 21), later used two-dimensional electrophoresis with staining of the proteins with Coomassie blue; in this way they were able to detect differences of 1.5 times in protein content. However, having detected about 800 polypeptides by electrophoresis, they were able to find only one protein that was represented more strongly in all seven lines of trisomic fibroblasts, and two proteins represented more strongly in all four lines of trisomic amniotic fluid cells. Having estimated that 1.7% of the active genes of the genome are present on chromosome 21 [10], it was suggested that changes would be found in the representation of 14 proteins in trisomy under conditions of resolution of about 800 proteins [5, 10]. Disagreement between the experimental results and those expected theoretically could arise from the fact that the quantity of protein products of most genes expressed on chromosome 21 is so low that they were not revealed by the methods used. This possibility has not previously been analyzed. A second possible cause of disagreement could be a change in expression of the genes during cell culture. In this connection it is interesting to note that interlinear variability of protein composition was found to be higher during culture of aneuploid cells than of diploid cells [5], which the authors cited associate with greater weakening of the stability of regulation of protein metabolism in aneuploidy under *in vitro* conditions. Analysis of proteins of fibroblasts from dizygotic twins (a normal individual and a patient with trisomy for chromosome 21) revealed the greatest differences: Four proteins in cells with trisomy were not detected in normal fibroblasts [10]. Consequently, to analyze the role of aneuploidy in changes in the protein composition of the cells it is more promising, in our view, to use uncultured cells.

The human peripheral blood leukocytes which we used likewise could facilitate the discovery of protein differences between normal and trisomic cells even with only a small number of resolvable proteins by one-dimensional electrophoresis. Unfortunately the protein which we found in the leukocytes of patients with Down's syndrome cannot be identified with proteins more strongly represented in cutaneous fibroblasts and amniotic fluid cells trisomic for chromosome 21 [5] and also in fibroblasts of fetal lung of a twin with trisomy [9, 10], because the authors cited do not give the molecular weight of these proteins. All that can be stated is that the protein which we found in the leukocytes of patients with Down's syndrome and of two patients with leukemia is identical in molecular weight to a protein discovered by electrophoresis and with the aid of monoclonal antibodies is spontaneous teratocarcinomas and in tumor cell lines belonging to different species and induced by chemical agents, by radiation, and by viruses [3, 4, 11].

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ACTIVATION OF THE CAFFEINE SITE OF THE SARCOPLASMIC RETICULUM AT LOW MAGNESIUM ION CONCENTRATION

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UDC 612.744.2.015.31:[546.41+546.46].014.46:615.214.31:547:857

KEY WORDS: sarcoplasmic reticulum; electromechanical coupling; caffeine; magnesium ions.

Studies of skeletal muscle fibers deprived of their membranes have shown that contraction of these fibers can be induced by a reduced Mg^{++} concentration in the medium. This is connected with the release of Ca^{++} ions from the sarcoplasmic reticulum (SR), which takes place under these circumstances [7]. Contraction of such fibers and Ca^{++} release can also be induced by caffeine [4]. Caffeine releases Ca^{++} ions from the isolated fraction of terminal cisternae of SR [8]. It has been shown in experiments on terminal cisternae that caffeine has an uncoupling action on active Ca^{++} transport. Caffeine does not act on active Ca^{++} transport in the longitudinal tubules of SR [3]. Hence the importance of a study of the effect of Mg^{++} ions on active transport of Ca^{++} by different fractions of SR.

EXPERIMENTAL METHOD

The caffeine-sensitive fraction of SR membranes was isolated from white muscles of rabbit hind limbs. The issue was homogenized as described previously [2]. To increase the yield of membranes 10 mM caffeine was added to the homogenization medium. The total membrane sedimented by centrifugation from 10,000g to 36,000g was extracted in the cold in medium containing 0.6 M KCl, 0.1 mM EDTA, 0.2 mM $CaCl_2$, human serum albumin (0.6 mg/ml), and 5 mM histidine (pH 7.2). The membrane suspension was centrifuged at 11,000g (20 min) to sediment the caffeine-sensitive fraction, and then again to 40,000g (60 min) to sediment caffeine-resistant membranes (light fraction). The residues thus obtained were suspended in medium containing 25% of glycerin (vols. 5), 0.1 mM EDTA, 0.2 mM $CaCl_2$, and 10 mM histidine (pH 7.2 at 4°C). For further purification the fractions were layered in a centrifuge tube above 4 ml of the same medium and centrifuged at 36,000g (60 min). The caffeine-sensitive fraction was obtained as the residue, and the light fraction as a thick suspension in the lower part of the tube. ATPase activity and the mean efficiency of Ca^{++} transport by SR membranes were determined by pH-metry [1]. The incubation medium contained 100 mM NaCl, 4 mM $MgCl_2$, 20 mM sodium oxalate, 25 μM $CaCl_2$, 2 mM ATP, 20-30 μg/ml of SR protein, and 2.5 mM imidazole (pH 7.05 at 37°C). The protein concentration was measured by the biuret reaction.

EXPERIMENTAL RESULTS

Previously, using an apparatus whereby ATP hydrolysis and Ca^{++} transport by SR fragments could be recorded simultaneously by means of Ca-selective and pH-electrodes, introduced into the same cell, the writers showed that caffeine inhibits uptake of Ca^{++} ions without affecting ATP hydrolysis, i.e., it has an uncoupling action on active Ca^{++} transport [3]. In that way the effect of caffeine can be estimated quantitatively by its effect on the mean efficiency of active Ca^{++} transport (Ca/ATP), which can easily be determined by pH-metry. A pH-metric record of ATP hydrolysis during active Ca^{++} transport by the fraction of terminal cisternae and longitudinal tubules of SR is shown in Fig. 1. Clearly, if the oxalate concentration in the incubation in medium was 20 mM, caffeine reduced the efficiency of active Ca^{++} transport by the fraction of terminal cisternae by about two-thirds but had relatively little

Laboratory of Physical Chemistry of Biomembranes, Faculty of Biology, M. V. Lomonosov Moscow University. (Presented by Academician of the Academy of Medical Siences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 1, pp. 53-55, January, 1985. Original article submitted April 21, 1984.

 0007-4888/85/9901-0064$09.50