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Erythrocyte alkaline phosphatase in patients with myotonic muscle disorders.

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Key words: Erythrocyte alkaline phosphatase, myotonic muscle disorders, kinetics, interaction membrane-enzyme.

Abstract. The allosteric behaviour of the p-nitrophenyl-phosphatase (E.C.3.1.3.1.) from membrane erythrocytes was investigated in the following multisystemic diseases: myotonic dystrophy, limb-girdle muscular dystrophy, Charcot-Marie-Tooth and juvenile spinal muscular atrophy; in myotonia congenita, which is not a multisystemic disease, and in healthy controls. The Hill coefficient in F^- inhibition in controls was different from that in multisystemic diseases patients but not from that in myotonia congenita patients. Changes in the cooperative type kinetics would suggest that the interaction membrane-enzyme in controls and in patients with neuromuscular disorders is only different for multisystemic diseases.

Fosfatasa alcalina en eritrocitos de pacientes con enfermedades musculares miotónicas

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Palabras clave: Fosfatasa alcalina de eritrocitos, desórdenes musculares miotónicos, cinética enzimática, interacciones membrana-enzima.

Resumen. Se investigó el comportamiento alostérico de la p-nitrofenil fosfatasa (E.C.3.1.3.1.) en las membranas de los eritrocitos provenientes de pacientes con las siguientes enfermedades neuromusculares multisistémicas: distrofia miotónica, distrofia muscular de cintura, enfermedad de Charcot-Marie-Tooth y atrofia muscular espinal juvenil, no-multisistémicas (miotonía congénita) y en controles sanos. El coeficiente de Hill en la inhibi-

ción por F- de los controles fue diferente del de los pacientes con enfermedades multisistémicas, pero no difirió del encontrado en pacientes con miotonía congénita. Las modificaciones en el coeficiente de Hill están relacionadas con cambios en la cinética enzimática; los resultados obtenidos sugieren una diferente interacción membrana-enzima solo en el caso de enfermedades multisistémicas.

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INTRODUCTION

Neuromuscular diseases include muscular dystrophies; Duchenne, Becker, myotonic and limb-girdle diseases are among them. They belong to a group of inherited diseases characterized by progressive weakness and degeneration of the skeletal muscle. Juvenile spinal muscular atrophy belongs to the group of motor neuron diseases and Charcot-Marie-Tooth disease belongs to the group of peripheral nerve diseases. In these two diseases, the same symptoms that show in the diseases of the previous group, arise from defects in the nerves that control the muscles.

These are multisystemic diseases because the symptoms, besides muscle weakness, involve other organs or organ systems such as testicular atrophy in myotonic dystrophy or heart disease in Duchenne muscular dystrophy (DMD).

The genetic defects associated with DMD may be detected not only in muscle cells but also in a variety of non-muscle cells. For instance, abnormalities in membrane erythrocytes have been reported, including modifications in the allosteric behaviour of membrane-bound enzymes (7).

Modifications in the value of the Hill coefficient for erythrocyte alkaline phosphatase have been related to changes in the membrane structure (5).

The purpose of this work is to determine the value of the Hill coefficient for the erythrocyte membranes from patients suffering the following neuromuscular diseases: myotonic dystrophy (MyD), limb-girdle muscular dystrophy (LGMD), juvenile spinal muscular atrophy (JSMA), Charcot-Marie-Tooth disease (CMT) and myotonia congenita (MC). For this purpose the action of the inhibitor fluoride on the erythrocyte alkaline phosphatase is studied.

The value of the Hill coefficient in patients with multisystemic neuromuscular diseases were found to be different from the value corresponding to controls.

MATERIALS AND METHODS

Patients with the following diagnoses were studied: Normal controls, myotonic dystrophy, limb-girdle dystrophy, juvenil spinal muscular atrophy, Charcot-Marie-Tooth

disease and myotonia congenita. In each case the diagnosis was made by rigorous clinical and laboratory methods.

Blood samples (5-10 ml) were collected by venepuncture into tubes containing heparin. The plasma was separated by low speed centrifugation (500 x g, 15 min, 4°C). Red cell ghosts were prepared by the method of Dodge *et al* (2). They were washed twice with a mixture of 0.002 mol/l Imidazole buffer, pH 7.6; 0.001 mol/l cysteine and 0.001 mol/l ethylenediamine tetraacetic acid and then suspended in 0.05 mol/l Tris-(hydroxi-methyl)-amino-methane, pH 7.6. The determination of 4-nitrophenyl-phosphatase activity was performed on suspensions of red cell ghosts which had been either freshly prepared or kept at -25°C for up to four weeks. The standard reaction mixture was the following: Tris-(hydroximethyl)-aminomethane, pH 7.6, 50 mmol/l; $MgCl_2$, 1.67 mmol/l; KCl, 20 mmol/l; p-nitrophenylphosphate, 3 mmol/l and 0.1 ml of ghost suspension in a final volume of 0.75 ml. The mixture was incubated at 37°C for 1 hr and the reaction was stopped by the addition of 0.1 ml of 50% trichloroacetic acid. The mixture was centrifuged for 30 min. and the supernatant was separated. The p-nitrophenol formed in the reaction was spectrophotometrically determined by measuring the optical density at 420 nm of 0.5 ml of the supernatant mixed with 1 ml of 0.5 N NaOH. Inhibition by fluoride was investigated by measuring the activity in the presence of fluoride concentrations from 0.33 to 2.00 mmol/l. Total ghost protein was determined by the method of Lowry *et al.*, (10). The value of the Hill coefficient was calculated as described by Farias *et al.*, (3). The arithmetical mean ± SD was used for statistical evaluation. For comparison between controls and patients, a **t** test was used.

RESULTS

Hill coefficient in muscular dystrophies

The effect of F^- on the alkaline phosphatase from erythrocytes in MyD, LGMD, and in normal controls is shown in Fig. 1a, where the enzymatic activity, expressed as relative rate, is plotted against F^- concentration. Hyperbolic curves were obtained for both muscular dystrophies and a sigmoidal curve was obtained for controls.

Fig. 1b shows the same data plotted as log $(v/V_o\text{-}v)$ against log $[F^-]$. Slopes of -1.55; -1.43 and -2.00 were obtained for myotonic dystrophy, limb-girdle muscular dystrophy and controls, respectively.

The effect of F^- on the alkaline phosphatase for Duchenne muscular dystrophy (DMD) had been investigated in previous works (6, 7). Table I shows the values of the Hill coefficient (n) reported in that work as well as those obtained in the present work. The Hill coefficient for muscular dystrophy patients are significantly different from the Hill coefficient for normal controls.

TABLE I
VALUES OF N OF ALKALINE PHOSPHATASE INHIBITED BY F^- FOR MUSCULAR DYSTROPHY PATIENTS AND CONTROLS

Subjects	n
DMD patients (6)	1.43 ± 0.24^a
MyD patients (5)	1.49 ± 0.15^b
LGMD patients (3)	1.46 ± 0.27^c
Controls (5)	2.12 ± 0.07^d

Values are given as mean ± SD. The number of samples is given in parentheses. Values are statistically significant at $p < 0.001^{(a,d)}$; $p < 0,01^{(b,d)}$; $p < 0.05^{(c,d)}$.

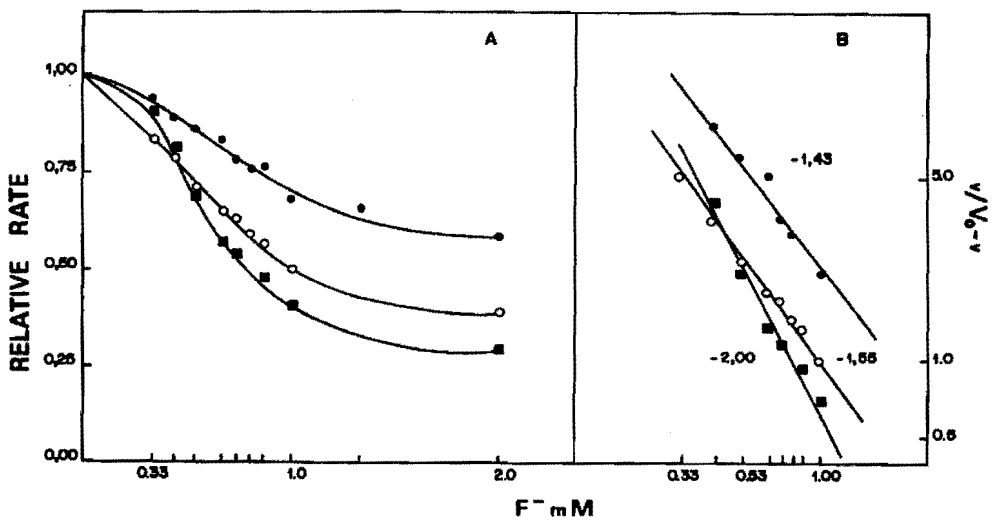

Fig. 1. Inhibition of 4-nitrophenylphosphatase by F^-. Enzyme preparation from MyD (o--o); LGMD (•--•) and control (■--■).
A. Direct plot of relative rate as function of (F^-).
B. Plot of v/Vo -v as function of F^- on logarithmic coordinats.
The slope of each line is indicated in the figure.

Hill coefficient in other neuromuscular diseases

Fig. 2 shows log (v/Vo-v) against log [F^-] for Charcot-Marie-Tooth (CMT), juvenile spinal muscular atrophy (JSMA) and myotonia congenita (MC). A slope of -1.88 was obtained for alkaline phosphatase in CMT, a disease of peripheral nerves and a slope of -1.65 was obtained for alkaline phosphatase in JSMA, a motor neuron disease. The slope in MC included among other myopathies (11), was not different from the slope in controls, that is n = - 2.00 (Fig. 1).

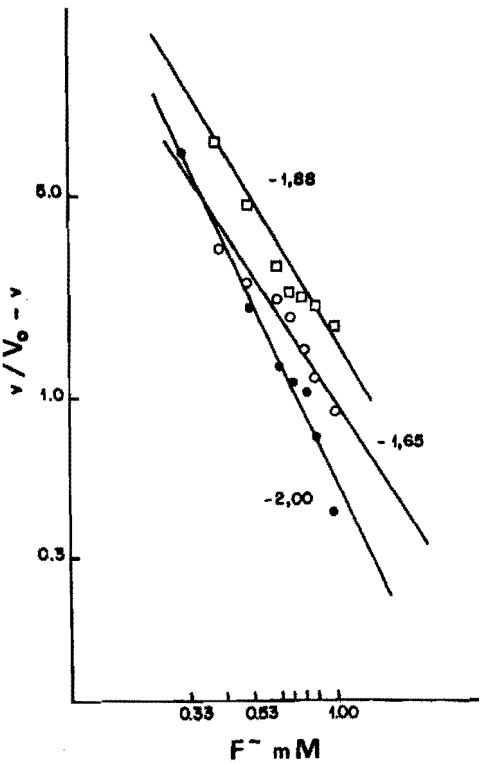

Fig. 2. Hills plot for the inhibition by F^- of the p-nitrophenylphosphatase from MC(•--•); CMT(□--□) and JSMA (o--o). The slope of each line is indicated in the figure.

DISCUSSION

Muscular dystrophies are a group of hereditary muscle disorders which vary in age of onset, initial muscles involved, inheritance patterns and rate of progression. The disease gene related to myotonic dystrophy has been genetically linked to the long arm of chromosome 19. Besides muscular symptoms, patients suffering MyD have other organs affected, including heart and brain.

Dystrophin is the product of the DMD gene, which is found in the X-chromosome. Abnormalities in its production lead to either Duchenne or Becker muscular dystrophies. Symptoms are pelvic girdle weakness and progressive affection of other skeletal muscles. A great proportion of patients with DMD are mentally retarded (13, 1).

Inheritance of LGMD appears to be autosomal recessive: several disorders may be caused by different gene defects. Major symptoms are shoulder and hip girdle weakness. The severity and rate of progression are variable. The heart muscle and the respiratory function may also be affected.

CMT, in its most common form, is caused by a defect in chromosome 17. It is inherited as a dominant disease and affects the peripheral nerves that control muscle contraction and sensations. The major symptoms are weakness and atrophy of the muscles in hands, feet and legs; gastrointestinal symptoms also appear.

JSMA causes degeneration of the lower motor neuron of the spinal cord, resulting in weakness or paralysis of the muscles in limbs and trunk. Its mildest form (type III) is autosomal recessive. The genetic defect is in chromosome 5q11-q13.

The symptoms of myotonia congenita are difficulties in motion after periods of rest and muscle stiffness.

MyD, DMSD, BMD, LGMD, CMT and JSMA are multisystemic diseases because they affect more

than one organ system. On the other hand, symptoms of myotonia congenita are limited to skeletal muscles.

Although MyD and DMD affect mainly the muscles, abnormalities of membrane erythrocytes have been also reported in patients suffering these diseases. A defect in a protein kinase corresponds to alterations in the phosphorylation of the erythrocytes in MyD patients (12). Dystrophin, the protein product of the DMD gene is thought to belong to a family of membrane cytoskeletal proteins. It is associated with a sarcolemma glycoprotein in a similar way as ankyrin is associated with red cell membranes (8).

In previous works, (6, 4), both the allosteric behaviour of the p-nitrophenyl phosphatase of erythrocyte membranes, determined by means of the Hill coefficient, and the Arrhenius plot between 16° and 40°C were found to be different in DMD patients and in controls.

Discontinuities in the Arrhenius plot and modification in the Hill coefficient have been interpreted as changes in the enzyme structure (5). Whilst many cellular processes are function of membrane protein, alteration in lipids or in other components of the membrane may disturb them, changing the kinetics responses of the embedded enzyme (9).

In the present work, the Hill coefficient in patients with other multisystemic diseases was also found to be different from the Hill coefficient in controls. It is possible that abnormalities in the erythrocyte membrane would also be responsible for the changes in the Hill coefficient of the p-nitrophenyl-phosphatase in CMT and JSMA patients.

In the present study the Hill coefficient of the p-nitrophenyl phosphatase in myotonia congenita patients was found to be similar to that in controls. This could be related to the fact that MC is not a multisystemic disease and only affects skeletal muscles.

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