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Serum creatine kinase B subunit levels in neurogenic atrophies

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Summary. This study is an attempt to determine the creatine kinase B (CK-B) subunit levels in neurogenic atrophies. A group of 69 patients was studied and the results were compared with those in a group of 32 patients with muscle disease. The results showed that the CK-B levels are considerably higher in patients with amyotrophic lateral sclerosis ($P < 0.001$) and peroneal muscular atrophy ($P < 0.001$). Further studies in the various subgroups of neurogenic atrophies showed that, regardless of the nosological entity, the CK-B activity is considerably higher: (1) in the "widespread" as opposed to "limited" forms ($P < 0.001$); (2) in the "chronic" than in the "acute" neurogenic atrophies ($P < 0.001$); and (3) in the "active" as opposed to "residual" forms ($P < 0.02$). It is suggested that the increase of CK-B in neurogenic atrophies is a strong indication of an active regeneration process in the denervated muscle.

Key words: Muscular atrophies – Serum creatine kinase – Neurogenic atrophies

Serum creatine kinase (CK) activity has long been considered as a valuable sign in the diagnosis of myopathic as opposed to neurogenic disorders. The increase in CK activity in the serum is believed to be the result of enzyme leakage into the circulation, reflecting the extension of muscle fibre destruction.

Although CK elevation has been considered as a sign of primary muscle lesion, there are frequent reports of elevated CK activity in neurogenic atrophies of various types [12, 13, 18]. In particular, increased CK activity has been found in a considerable percentage (30%–40%) of patients with amyotrophic lateral sclerosis [2, 4, 12, 18] as well as in a number of patients with spinal muscular atrophies [9, 11, 17, 19]. However, the nature of the CK increase in neurogenic disorders remains obscure in spite of recent advances in the technology of CK isoenzyme measurements.

Creatine kinase is a dimer constituted of two subunits, M and B, muscle and brain subunits respectively. These two subunits constitute three isoenzymes: the MM or "muscle type" isoenzyme; the hybrid form, MB, or the "myocardial" isoenzyme; and the BB form, the so-called brain type isoenzyme. The aim of the present study was to determine, by a sensitive method, the CK-B subunit in various groups of neurogenic atrophies, in the hope that this approach would be helpful in understanding the pathogenetic mechanisms of these conditions.

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Materials and methods

The present study involved a group of 69 patients (49 males and 20 females) suffering from various neurogenic atrophies, and a control group of 200 individuals. In addition, in order to compare the values of CK activity in neurogenic atrophies with a comparable nosological condition, a group of 32 patients with muscle disease (14 with progressive muscular dystrophy, 7 with myotonic dystrophy and 11 with myasthenia gravis) was also studied.

The group of neurogenic atrophies consisted of 13 patients with amyotrophic lateral sclerosis, 19 with various types of sensorimotor polyneuropathy, 6 with peroneal muscular atrophy, 2 with spinal muscular atrophy and 29 patients with various radiculopathies or mononeuropathies. The number of cases, as well as their sex, is shown in Table 1.

In all cases the diagnosis was confirmed by a careful clinical, biochemical and mainly electrophysiological investigation, on an in-patient basis. The neurophysiological investigation included electromyogram, measurement of motor and sensory nerve conduction velocity, H-reflex, F-wave, and somatosensory evoked potentials.

Enzyme and isoenzyme assays

Serum samples were frozen at −25°C immediately after collection. Mercaptoethanol was added to a final concentration of 0.02 m/l 30–60 min before analysis in order to restore CK activity. Total CK activity was measured at 25°C, using the kit CK-NAC from Boehringer (Mannheim, FRG). Serum CK-B activity was determined by a new solid-phase direct enzyme immunoassay (EIA) as follows. Polystyrene or polyprophylene cuvettes were coated with anti-CK-BB IgG antibodies by incubating them with 1 ml antibody dilution in phosphate-buffered saline (PBS; 7 µg IgG/ml) for 24 h at 4°C. Normally a stock of IgG precoated cuvettes was kept at 4°C, ready for use. Patients serum (1 ml) was placed in the cuvettes and allowed to incubate for 20 h at 4°C. After removal of the serum, the cuvettes were washed with PBS/Tween (0.05%) and the NAC reaction mixture was added. The reaction was stopped after 60 min incubation at 25°C and the absorption was measured at 340 nm using the Variant/Cary spectrophotometer. For blanks, cuvettes filled with PBS or normal serum which had been preabsorbed with anti-CK-BB antibodies

Table 1. Total CK and CK-B activity in the various myopathic and neurogenic conditions

	No. of cases	Sex		Total CK activity (U/l)		CK-B activity (U/l)	
		M	F	Mean	95% confidence limits	Mean	95% confidence limits
Controls	200	112	88	31.0	28.9– 33.1	0.27	0.25–0.29
Myopathic	32	18	14				
Muscular dystrophy	14	9	5	200	79.5–502	1.60	0.55–4.86
Myotonic dystrophy	7	5	2	23.5	14.5– 38.1	0.44	0.32–0.61
Myasthenia	11	4	7	15.2	8.5– 27.0	0.18	0.11–0.31
Neurogenic	69	49	20				
Amyotrophic lateral sclerosis	13	11	2	45.8	28.9– 72.6	0.53	0.38–0.76
Polyneuropathies	19	12	7	18.2	11.0– 30.2	0.30	0.21–0.42
Peroneal muscular atrophy	6	4	2	43.7	20.0– 95.5	0.60	0.41–0.86
Spinal muscular atrophy	2	2	0	8.9		0.45	–
Radiculopathies mononeuropathies	29	20	9	23.0	17.4– 30.2	0.22	0.17–0.28

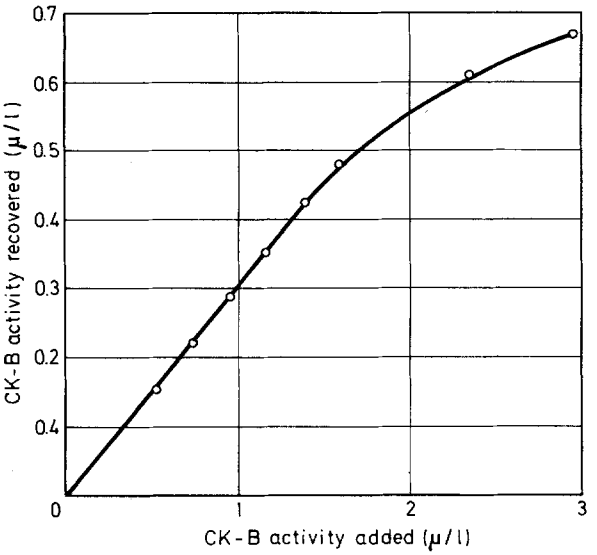

Fig. 1. Standard calibration curve for the measurement of creatine kinase B (CK-B) activity by solid–phase direct enzyme immunoassay

were used. The measured activity was then compared with a standard calibration curve. Figure 1 represents a typical standard curve obtained by adding known activities of CK-BB diluted either in 1% BSA/PBS or in preabsorbed human serum. Exactly the same curve was obtained when CK-MB activities, similarly diluted, were added and considered as CK-B = CK-MB/2. Application of patients' sera with known amounts of CK-MB activity as measured by immuno-inhibition (Merck, Darmstadt, FRG) or by immunoprecipitation [8] also resulted in the same calibration curve and was normally used as standard in routine analysis.

The CK-B subunit was thus determined irrespective of the isoenzyme type applied. This assay is, therefore, considered as a CK-B subunit assay. Its sensitivity was determined as a blank plus its threefold standard deviation to 0.15 U/l. The working range of the assay was thus 0.15–1.5 U/l. Sera with CK-B activity higher than 1.5 U/l were diluted and reassayed. Precision studies demonstrated the intraserial coefficient of variation at 0.47, 0.81, and 2.5 U/l to be 11.5%, 7.9% and 4% respectively. A preliminary report on this method and its application in the detection of CK-B activity in prostate disease has already been published elsewhere [10].

In cases where total CK activity was found to be elevated (10 U/l), the CK-MB isoenzyme was measured: (1) by the immuno-inhibition assay (Merck); (2) by the immmunotitration assay [8]. In addition, agarose electrophoresis, using the Corning system, was performed for qualitative analysis of the isoenzyme pattern.

The total CK and the CK-B activity were determined blindly, i.e. without knowledge of the diagnosis or the patient's age, sex, duration of illness or any other clinical details.

Since the values of the total CK and the CK-B activity do not seem to follow a normal distribution pattern, the statistical evaluation of our results was based on their logarithmically transformed values. Since a number of comparisons were attempted, P values of < 0.02 were considered to be significant.

Results

The results are presented in Tables 1 and 2. The total CK activity seemed to be increased in a number of patients with neurogenic atrophy, especially in cases of amyotrophic lateral sclerosis and peroneal muscular atrophy (Table 1). However, this increase was not statistically significant and the considerable variation in the values observed suggests that under these conditions the total CK activity is not uniform for all patients, but represents very high values in some patients, while the others have CK values within normal limits.

The CK-B fraction was considerably increased in cases of amyotrophic lateral sclerosis ($t = 3.62$, $P < 0.001$) as well as in cases of peroneal muscular atrophy ($t = 4.25$, $P < 0.001$). A similar increase was also observed in myotonic dystrophy ($t = 2.91$, $P < 0.005$), while the CK-B activity in the sera of

Table 2. CK-B activity in the various groups of neurogenic atrophies (statistical differences derived from logarithmic data)

	No. of cases	CK-B activity			Statistical differences
		$\bar{x} \pm$ SD (log data)	Mean values (U/l)	95% confidence limits (U/l)	
Controls	200	-0.56 ± 0.20	0.27	0.25–0.29	$t = 1.39$
Neurogenic atrophies	69	-0.50 ± 0.35	0.31	0.26–0.38	N.S.
Neurogenic atrophies					
Widespread	40	-0.39 ± 0.34	0.40	0.32–0.51	$t = 3.46$
Limited	29	-0.66 ± 0.31	0.22	0.17–0.28	$P < 0.001$
Chronic	37	-0.32 ± 0.26	0.48	0.40–0.57	$t = 3.77$
Acute	32	-0.66 ± 0.45	0.22	0.15–0.31	$P < 0.001$
Active	63	-0.47 ± 0.39	0.34	0.27–0.42	$t = 2.30$
Residual	6	-0.62 ± 0.16	0.24	0.18–0.32	$P < 0.02$

patients with muscular dystrophies was, as expected, extremely high. Electrophoresis and CK isoenzyme measurements revealed that in muscular dystrophies the elevation of the CK-B fraction was due to an increase of the CK-MB isoenzyme.

If all the neurogenic atrophies are considered as one group, their CK-B activity did not differ significantly from that of the controls.

In order to correlate the CK-B values with various clinical parameters, a statistical comparison was attempted between: (1) "widespread" as opposed to "limited" (radiculopathies and mononeuropathies) neurogenic atrophies; (2) "chronic" (duration of illness more than 6 months) versus "acute" forms; and (3) "active" as opposed to "residual" forms of the neurogenic atrophies studied.

The results of this analysis are shown in Table 2. The CK-B activity was higher: (1) in the widespread than in the limited forms ($P < 0.001$); (2) in the chronic than in the acute neurogenic atrophies ($P<0.001$); and (3) in the active ($P<0.02$) than in the residual forms, since in a group of six patients with residual neurogenic atrophy, the CK-B activity did not differ significantly from the control values, in spite of the marked and widespread atrophies observed in these cases.

Discussion

The mechanism of the frequently reported increase CK activity in neurogenic atrophies remains obscure. This is probably due to the fact that, in neurogenic atrophies, there is no enzyme leakage from extensive muscle destruction and thus the CK activity in the serum is low and difficult to detect by conventional methods.

Recently, the application of sensitive techniques has confirmed the elevation of CK activity in neurogenic disorders [2, 9, 15, 19]. In addition, in these reports and increase of CK-B activity was noted, but its interpretation is not clear.

In the present study, it was found that in some neurogenic atrophies the CK-B subunit in serum is significantly elevated. This increase indicated the presence of either the MB or the MM isoenzyme, since the sensitive screening method used detects the B subunit irrespective of the isoenzyme type derived. However, where there were high CK values, electrophoresis and CK isoenzyme measurement showed an elevation of the CK-MB isoenzyme. The increase of the CK-B found, therefore, probably originates from the elevation of the CK-MB isoenzyme.

An increase in the CK-B fraction has repeatedly been reported in muscular dystrophies, but this finding has been attributed to concomitant myocardial lesions, by a number of investigators. Since the myocardial involvement has been excluded by clinical and laboratory means in the neurogenic atrophies reported here, the observed increased in CK-B activity might be attributed to skeletal muscle.

The muscular CK isoenzyme pattern is governed by the rate of synthesis of CK-M and CK-B subunits [14]. The hybrid CK-MB, a product of a random association of the two subunits, has been related to myogenesis [5, 16]. As myogenic cells differentiate from mesenchymal cells, the CK-B synthesis increases [14]. The simultaneous increase in CK-M synthesis during myotube formation (7–8 weeks of gestation) results in the "fetal pattern" of CK-isoenzymes, characterized by an equal distribution of the three forms. From the 12th week of gestation onward, the CK-M activity increases steadily whereas the CK-B subunit remains constant throughout maturation [7].

It has been suggested by a number of authors [1, 3, 6,] that the elevation of the CK-MB isoenzyme in the dystrophic and atrophic muscle is a sign of active muscle-fibre regeneration. The present findings seem to support the hypothesis that the increase in CK-B fraction is related to regeneration of the muscle fibres. The CK-B activity was found to be elevated in the "chronic" forms of neurogenic atrophies (amyotrophic lateral sclerosis, peroneal muscular atrophy, etc), while in acute polyneuropathy of Guillain-Barré type its activity was within the normal range. This was probably due to the lack of active regeneration, which seems not to be a perpetual process, since in the six cases of residual neurogenic atrophy studied, the CK-B activity was normal in spite of the widespread and long-standing atrophies.

The finding that in "limited" neurogenic atrophies the CK-B activity was within normal limits could possibly be explained by the limited extent of the regeneration process.

The results of the present study have to be considered as evidence of a significant regeneration of the denervated muscle fibres. However, further studies are needed, in order to elucidate the nature of the regeneration process in neurogenic atrophies.

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