Intracellular Alterations of the Creatine Kinase Isoforms in Brains of Schizophrenic Patients

TATYANA P. KLUSHNIK,^{*,1} ALEXANDER YA. SPÜNDE,¹
ALEXANDER G. YAKOVLEV,¹ ZAZA A. KHUCHUA,²
VALDUR A. SAKS,² AND MARAT E. VARTANYAN¹

¹All-Union Research Center for Mental Health, Acad. Med. Sci.,
Zagorodnoe, Shosse, 2, Moscow, 113152, USSR, ²All-Union Research
Center for Cardiology, Acad. Med. Sci., Moscow, USSR

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ABSTRACT

Postmortem brain tissues of schizophrenic patients were found to contain 5–10 times less water-soluble creatine kinase (BB CK) and 1.5–3 times less mitochondrial creatine kinase as compared to control. The major part of BB CK in schizophrenic brain tissues, contrary to control, was found to be insoluble in water (particulate form of BB CK) and could be extracted from brain tissue with strong denaturing agents. The particulate form of BB CK did not have any enzymatic activity but activity was found after the solubilization of this isoenzyme. The observed BB CK translocation into the particulate inactive form and the decrease of mitochondrial CK content to schizophrenic brains may reflect changes in the synthesis and the utilization of creatine phosphate.

Index Entries: Human brain; schizophrenia; creatine kinase; enzyme translocation.

*Author to whom all correspondence and reprint requests should be addressed.

INTRODUCTION

Creatine kinase (CK, EC 2.7.3.2) is one of the most important enzymes in the energy metabolism of many cell types including those of brain (Saks et al., 1978; Wallimann et al., 1990). It maintains high local concentrations of ATP by a rapid rephosphorylation of ADP at the expense of phosphocreatine (Wallimann and Eppenberger, 1990).

Several isoforms of CK were described and characterized by tissue-specific expression. One of them, the brain isoenzyme (CK BB), is expressed in a wide variety of tissues with the highest level detected in the brain (Quest et al., 1990). The expression of CK BB is under the control of several regulatory factors including peptides, steroid hormones, and transforming factors (Kaye, 1986; Reiss, 1988).

The previous analysis of protein composition of water-soluble brain extracts by two-dimensional polyacrylamide gel (PAG) electrophoresis has revealed significant differences between the contents of CK BB in the normal and schizophrenic human brains taken by autopsy (Klushnik et al., 1991).

The purpose of this work was to investigate in detail the reason for this difference by using a method of fractional extraction of creatine kinase from the brain.

MATERIALS AND METHODS

The diagnosis of schizophrenia was based on the clinical observations in accordance with the international disease classification (ICD 9).

Ten patients were taken for the analysis in each of the two following groups:

Group 1: Patients who had no evidence of mental disorders and who died from sudden heart attack. This group served as the normal control.

Group 2: Patients with different forms of schizophrenia who died from sudden heart attack or pneumonia. Table 1 summarizes the clinical features of these patients. The diagnosis of schizophrenia was specified by psychiatrists, who observed the patients during a long period of time in the All-Union Mental Health Research Center. The psychiatrists used the generally accepted classification of JCD-9 with the rubrication of schizophrenia under 295.4—acute schizophrenia attack and 295.3—paranoid schizophrenia. Most of the patients who were observed in clinical conditions were treated with neuroleptic drugs (haloperidol, chlorpromazine, stelazine) in combination with antidepressive preparations (amithriptilline, melipromine) in medium therapeutical dosages.

Human brain tissues were obtained from autopsy and frozen at -70°C . Samples of frontal cortex (area 10 according to Brodman) from different patients were studied in each group.

Table 1
Clinical Data Pertinent to Tissue Sources

	Normal	Schizophrenia
No. of patients	10	9
Sex	7M + 3F	3M + 6F
Age (y)	56.7 $\pm$ 4.0	63.7 $\pm$ 3.2
Diagnosis (ICD)	—	295.4; 295.3
Hours, postmortem	5.9 $\pm$ 0.5	7.4 $\pm$ 0.7

Some 100–200 mg of frozen brain tissues were homogenized in 10 vol of 100 mM potassium phosphate buffer (KPB), pH 9, containing 0.2 mM EDTA, 5 mM dithiothreitol, and 0.1% Nonidet P40 in Ultraturax homogenizer. The homogenate was centrifuged at 16000g for 30 min. The supernatant containing KPB-soluble proteins was analyzed for the enzyme activities and by electrophoresis.

The pellet was washed once with KPB, resuspended in initial vol of the same buffer containing 8M urea (Urea-KPB) and centrifuged as described earlier. Supernatant obtained contained proteins solubilized in urea but not in KPB. Immediately after centrifugation 0.5–1 mL of the supernatant was applied on NAP-5 Column (Pharmacia, Sweden) to remove urea. After this procedure, a fraction of urea-soluble proteins was used in the enzyme activity and electrophoretic analysis.

For the quantitative measurement of a CK activity in KPB-soluble and urea-soluble fractions we used NAC-activated CPK test (KONE Corp., Finland).

Immunoelectroblotting was performed as described earlier (Towbin et al., 1979). Samples of KPB-soluble and urea-soluble proteins were subjected to SDS-electrophoresis (Laemmli, 1970) in 10% PAG followed by the electrophoretic transfer to nitrocellulose membrane. Immunodetection of CK enzymes on nitrocellulose was performed with polyclonal antibodies against human brain CK BB developed in rabbit (Klushnik et al., 1991) and polyclonal antibody to human heart mitochondrial CK (Saks et al., 1987) using antirabbit IgG conjugated with peroxidase (Sigma).

Electrophoresis in the agarose gel was carried out as described earlier (Khuchua et al., 1989). Visualization of CK isoenzymes was performed by using a modified commercial method of fluorescence staining technique (CIBA Corning Diagnostics Corp., Palo Alto, CA, USA).

All results were analyzed by using Student's *t*-test. Mean values and standard deviation of parameters studied are given in Table 1 and Figs. 1 and 2, which also show the statistical significance of the difference observed.

Fig. 1. KPB-extracted CK activities in normal (□) and schizophrenic (▨) brains. Total CK activity (A) was directly measured in tissue KPB extracts. The activities of BB CK (B) and mitochondrial CK (C) were calculated on the basis of the total CK activity (A) and relative content of CK isoenzymes shown in Fig. 2.

Fig. 2. The BB CK activity of particulate (urea-extractable) fraction from normal (□) and schizophrenic (▨) brains. The activity was calculated as the difference between CK BB extracted with 8M urea and KPB-soluble BB CK fractions activities.

RESULTS

The results of immunoelectroblotting presented in Fig. 3 demonstrate that the content of BB CK is significantly decreased in the KPB-extracts of schizophrenic brains. Estimation of enzyme activities in the extracts of frontal cortex of both normal and schizophrenic brains showed that the activity of CK is stable to autolysis and practically was not changed during 3–10 h of postmortem period. Total CK activity in the KPB-extracts of normal brain was equal to 356 ± 25 IU/g wet wt. In the KPB-extracts of schizophrenic brains, the activity of CK was significantly decreased and was equal to 83 ± 23 IU/g wet wt. Inasmuch as it is known that brain may contain different CK isoenzymes (Wevers et al., 1981), it was of interest to estimate the CK isoenzyme spectrum of the total KPB-extracts. The results of quantitative electrophoresis of KPB-extracts of the brain in agarose gels are shown in Fig. 4.

Two major isoforms of the CK were found in these extracts, i.e., BB and the mitochondrial CK. Tissues taken from normal individuals contained 75–85% BB and 15–25% of mitochondrial form, but in the KPB-extracts of brain of schizophrenic patients this ratio was reversed (Fig. 4). On the basis of obtained ratio and total activity of CK in protein extracts we could calculate the values of activities for both CK forms (Fig. 1). The calculated CK activity values demonstrated that the activity of BB CK is decreased 5–10 times in the schizophrenic brains as compared to control. The activity of mitochondrial isoform was also decreased but to a lesser extent. These data are in good agreement with the results of immunoblotting shown in Fig. 3.

In order to understand if observed phenomenon is a result of the real decrease of CK isoenzymes content or a result of changes in the protein solubility, the brain tissues after the primary extraction with 100 mM KPB were repeatedly extracted with 8M urea and/or mercaptoethanol. These extractions resulted in the additional release of BB CK. Such a release was not observed when KPB with different pH or containing 1–5% Triton X-100 were used. These results indicate that the total population of CK BB in the brain of schizophrenic patients is presented by both KPB-soluble and membrane-bound fractions. The latter one can be extracted only by using strong denaturing agents. The results of immunoblotting illustrating distribution of BB CK isoforms between phosphate-soluble and urea-soluble fractions are shown in Fig. 5.

The comparative analysis of KPB and urea extracts of brains revealed that the mitochondrial form of CK is completely extracted by 100 mM phosphate solution, pH 9.0, inasmuch as application of antiserum showed the presence of trace amounts of this isoenzyme in urea-containing extracts in normal and in schizophrenic brains. Along with this, it is important to note that the amount of this CK isoenzyme is decreased 1.5–

Fig. 3. The immunoblotting of BB CK from normal and schizophrenic brains extracted with potassium phosphate buffer. Numbers correspond to different patients. Proteins were extracted from normal (lines 1–5) and schizophrenic human brains (lines 6–10) with 100 mM potassium phosphate buffer and after SDS-electrophoresis were transferred to nitrocellulose membrane. All lanes contain equal quantity of total protein (20 μ g). Immunodetection was carried out with polyclonal antibodies against human brain BB CK using anti-rabbit IgG conjugated with peroxidase (see Materials and Methods).

Fig. 4. Fluorescence profiles of CK isoenzymes from normal and schizophrenic brains after electrophoresis in agarose gel. Buffer: 60 mM Tris-HCl/10 mM barbital/50 mM sodium barbital/1 mM EGTA/1 mM dithiothreitol/0.1% Triton X-100 (pH 9.0); 100 V for 30 min at 4°C.

Fig. 5. The immunoblotting of CK BB from normal and schizophrenic brains extracted with KPB and with 8M urea. Lines 1–3: Proteins extracted from the brain of a normal patient. 1–extraction with KPB, 2–subsequent extraction with 8M urea, 3–direct extraction with 8M urea (total fraction). Lines 4–6: Proteins extracted from the brain of a schizophrenic patient. 4–extraction with KPB, 5–subsequent extraction with 8M urea, 6–direct extraction with 8M urea.

3 times in schizophrenic brains as compared to control. As shown in Fig. 2, the major part of BB CK isoenzyme in normal brains was extracted by 100 mM phosphate and only trace amount is additionally extracted by solution containing 8M urea. In the case of schizophrenia the latter represents about 75% of the total amount of BB CK. The particulate (urea-extractable) fraction obtained after primary extraction of brain tissue with KPB solution did not show CK activity that reappeared only after solubilization of the enzyme with urea solution. Thus, the particulate BB CK seems to be enzymatically inactive.

Chlorpromazine and haloperidol used in regular accepted doses per kg body wt in rats for 30 d did not result in alteration of CK activity.

DISCUSSION

The present results show that BB CK in the brain of schizophrenic patients is modified or translocated from easily extractable active form into particulate inactive form.

Two isoenzymes of CK: mitochondrial CK and BB CK, compose a phosphocreatine pathway of energy transport in the brain cells that is similar to the analogous pathway in cardiac, skeletal muscle cells and other cell types (Saks et al., 1978; Wallimann and Eppenberger, 1990). The phosphocreatine pathway has been described in detail for cardiomyocytes because of easiness of quantitative determination of heart function. The idea of this "phosphagen" pathway was also formulated for nervous cells by A. Hodgkin in 1964 on the basis of experimental data showing a clear preference of Na^+ , K^+ -pump of giant axon for phospho-arginine (+ADP) in comparison with ATP as an energy source (Hodgkin, 1964). In 1964 Klingenberg and his associates (*see* Jacobs et al., 1964) described the presence in the brain of two CK isoforms: mitochondrial and BB. Mitochondrial CK is attached to the outer surface of the inner mitochondrial membrane and may acquire octameric form (Schlegel et al., 1990). These forms, however, do not differ in enzymic activity. It uses mitochondrial ATP for phosphocreatine synthesis, which is an energy carrier inside the cells (Jacobus, 1985). BB CK may be located in or connected to the surface membrane near by Na^+ , K^+ -ATPase to replenish the local pool of ATP at the expense of phosphocreatine synthesized in mitochondria (Saks et al., 1978).

Mitochondrial CK can be easily detached from the membrane with 100 mM KPB, pH 8.0–9.0 (Jacobus, 1985). We observed that about 90% of BB CK is extracted (detached from membrane) from normal brain under similar conditions. Such an easiness of BB CK extraction with low molarity saline buffers was also reported by other authors (Chandler, 1987; Miller and Wei, 1985).

In the brain of schizophrenic patients, BB CK is converted or translocated almost completely into particulate inactive form that may be solubilized and reactivated with strong denaturing agents. These changes concern specifically BB CK and are not related to mitochondrial CK. A decrease of the latter enzyme activity by a factor 1.5–3 probably shows serious disturbances in the phosphocreatine synthesis, which is the first step in the energy transport. Conversion of the major part of BB CK into particulate inactivated form may reflect defects in the energy conversion at the level of cellular membranes. Apparently, obtained results testify to serious defects in the system of intracellular energy supply in the schizophrenic brain. Earlier, it has been shown that glucose utilization is decreased in this disease.

A cause of CK isoenzyme alteration is not known as yet. We did not find any correlation between the activity of BB CK in KPB-soluble fraction and a clinical form of schizophrenia, the sex and the age of patients, or the postmortem interval. We believe that the examination of molecular and cellular mechanisms of the BB CK translocation deserve further attention and study.

SUMMARY

The content and the enzymatic activity of CK isoenzymes (BB CK and mitochondrial CK) were estimated in postmortem normal and schizophrenic brains. Water-soluble extracts of schizophrenic brain tissues contain 5–10 times less of BB CK and 1.5–3 times less of mitochondrial CK as compared to the control. The extraction of the particulate fraction of schizophrenic brain tissues with 8M urea and 2-mercaptoethanol resulted in the additional release of BB CK but not of mitochondrial CK. The particulate form of BB CK does not have any enzymatic activity, which can be restored after solubilization and subsequent elimination of urea. The observed translocation of BB CK from easily extractable active form into particulate inactive form and the decrease of mitochondrial CK content in schizophrenic brain may reflect the changes in the synthesis and the utilization of creatine phosphate.

REFERENCES

- Chandler W. L. (1987) Comparison of solutions for extracting CK, AK and LD from brain samples. *Clin. Chem.* **33**, 2304–2306.
- Hodgkin A. L. (1964) *The Conduction of Nervous Impulse*. Liverpool University Press. Liverpool, pp. 80–85.
- Jacobs H., Heldt H. W., and Klingenberg M. (1964) High activity of creatine kinase in mitochondria from muscle and brain and evidence for a separate mitochondrial isoenzyme of creatine kinase. *Biochem. Biophys. Res. Comm.* **16**, 516–521.
- Jacobus W. E. (1985) Respiratory control and the integration of heart high-energy phosphate metabolism by mitochondrial creatine kinase. *Ann. Rev. Physiol.* **47**, 707–725.
- Kaye A. M. (1986) Hormonal regulation of creatine kinase BB. *Adv. Exp. Med. Biol.* **194**, 83–101.
- Khuchua Z. A., Ventura-Clapier R., Kuznetsov A. V., Grishin M. N., and Saks V. A. (1989) Alterations in the creatine kinase system in the myocardium of cardiomyopathic hamsters. *Biochem. Biophys. Res. Comm.* **165**, 748–757.
- Klushnik T. P., Spunde A. Ya., and Yakovlev A. G. (1991) Decreasing of creatinephosphokinase BB in water-soluble fraction of schizophrenic brains revealed by two-dimensional electrophoresis. *Neurochimia*, in press.
- Laemmli U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.
- Miller J. and Wei R. (1985) Properties of creatine kinase from canine and human brain tissue. *Clin. Biochem.* **18**, 14–19.
- Quest A. F. G., Eppenberger H. M., and Wallimann T. (1990) Two different B-type creatine kinase subunits dimerize in a tissue specific manner. *FEBS Lett.* **262**, 299–304.

- Reiss N. A. (1988) Ontogeny and estrogen responsiveness of creatine kinase and glycolytic enzymes in brain and uterus of rat. *Neurosci. Lett.* **84**, 197–202.
- Saks V. A., Rosenshtaukh L. V., Smirnov V. N., and Chazov E. I. (1978) Role of creatine phosphokinase in cellular function and metabolism. *Can J. Physiol. Pharmacol.* **56**, 691–706.
- Saks V. A., Khuchua Z. A., and Kuznetsov A. V. (1987) Specific inhibition of ATP-ADP translocase in cardiac mitoplasts by antibodies against mitochondrial creatine kinase. *Biochim. Biophys. Acta* **891**, 138–144.
- Schlegel J., Wyss M., Eppenberger H., and Wallimann T. (1990) Functional studies with the octameric and dimeric form of mitochondrial creatine kinase. *J. Biol. Chem.* **265**, 9221–9227.
- Towbin H., Staehelin T., and Gordon J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Natl. Acad. USA* **76**, 4350–4354.
- Wallimann T. and Eppenberger H. M. (1990) The subcellular compartmentation of creatine kinase isoenzymes as a precondition for a proposed phosphoryl-creatine circuit, in *Isoenzymes: Structure, Function and Use in Biology and Medicine* (Wallimann, T., ed.), pp. 877–889, Wiley-Liss, New York.
- Wevers R. A., Reutelingsperger C. P. M., Dam B., and Soons J. B. J. (1981) Mitochondrial creatine kinase (EC 2.7.3.2) in the brain. *Clin. Chim. Acta* **119**, 209–223.