Muscle Creatine Kinase Isoenzyme Expression in Adult Human Brain*

(Received for publication, November 2, 1989)

Robert J. Hamburg‡, David L. Friedman‡, Eric N. Olson§+, Tony S. Ma†, M. Dolores Cortez‡, Clay Goodman∥, Peter R. Puleo‡, and M. Benjamin Perryman**

From the Departments of ‡Medicine, Molecular Cardiology Unit, and §Pathology, Baylor College of Medicine, and the ¶Department of Biochemistry and Molecular Biology, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Previous studies have suggested that MM creatine kinase is a muscle-specific protein and is not present in adult brain tissue. We have isolated a protein from human brain with an apparent molecular weight of 43,000 as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis which is identical to the muscle M creatine kinase isoenzyme subunit at all 30 sequenced amino acid residues and possesses creatine kinase enzymatic activity following non-denaturing agarose-gel electrophoresis. Immunohistochemistry localizes M creatine kinase to discrete areas of adult human brain. Northern blot analysis of both total and human placenta, heart, and brain mRNA detected M creatine kinase mRNA. However, polymerase transferase, EC 2.7.3.2) are involved in the transfer of cellular energy through the reversible transfer of a phosphate residue.

The BB creatine kinase homodimer is found in high concentrations in adult brain, placenta, smooth muscle (1), and lenticular epithelium (2). This isoenzyme is also present in embryonic tissues including cardiac and skeletal myoblast. The MB creatine kinase heterodimer is found primarily in adult human myocardium and has significant clinical utility as a diagnostic marker for acute myocardial infarction (3). The MM creatine kinase homodimer is considered to be restricted in its distribution to striated muscle (1) and is used as a definitive marker for the differentiated muscle phenotype.

The presence of both MM and BB creatine kinase in adult human and rat lenticular epithelium and that BB creatine kinase is not expressed in this tissue until the age of puberty (2). Subsequent examination of adult rat brain tissue by agarose gel electrophoresis, in situ hybridization, and immunocytochemistry detected significant amounts of MM creatine kinase within adult rat brain.1

Despite the contention that M creatine kinase is a muscle-specific protein, whether human brain tissue contains significant amounts of this isoenzyme remains controversial. The presence of BB creatine kinase has been clearly demonstrated and most authors report that BB creatine kinase is the only isoenzyme present in brain based upon chromatographic, immunological, and electrophoretic techniques (13–20). However, a few groups have reported the presence of MM creatine kinase in human brain (19–22) and Hauschka et al. (23) recently detected MM creatine kinase chloramphenicol acetyltransferase hybrid gene expression in brain tissue from transgenic mice. Our results obtained from rat brain and previous reports suggesting that adult human brain tissue...
may contain significant amounts of MM creatine kinase led us to reexamine this question.

We isolated a protein from adult human brain tissue with an apparent molecular weight of 43,000 as determined by SDS-polyacrylamide gel electrophoresis which is identical to muscle M creatine kinase at all 30 sequenced N terminal amino acid residues. This protein was shown to have creatine kinase enzymatic activity and MM creatine kinase electrophoretic mobility on nondenaturing agarose gel electrophoresis. Immunohistochemistry detected M creatine kinase in discrete areas of human brain. Preliminary in situ hybridization verifies the presence of M creatine kinase mRNA in human brain. Although M creatine kinase mRNA could not be detected by Northern blot analysis of either total or poly(A)+ RNA isolated from adult human brain tissue, polyacrylamide chain reaction amplification of cDNA synthesized from human placenta, myocardium, and brain demonstrated the presence of M creatine kinase message in brain and myocardium but not placenta which contains no detectable M creatine kinase protein. Considerable data on control of M creatine kinase gene expression suggests that most regulation occurs at the transcriptional level through the activity of a muscle-specific enhancer sequence (5-8). In initial experiments to study the control of M creatine kinase gene expression in neural cells we transfected the mouse neuroblastoma cell lines NlE115 and NS20Y with M creatine kinase-chloramphenicol acetyltransferase hybrid genes containing 4,800 bp of M creatine kinase upstream sequence and found that these sequences up-regulated expression of the reporter gene during cell differentiation. The endogenous mouse creatine kinase gene was simultaneously up-regulated during differentiation of the cultured cells.

**MATERIALS AND METHODS**

**Purification of MM Creatine Kinase from Human Brain**—Human hippocampus and temporal lobe (43 g) were homogenized in 10 volumes of 50 mM Tris-HCl (pH 8.3) containing 5 mM 2-mercaptoethanol, 2 mM EDTA, 100 µg/ml 1 chloro-3-tosylamido-7-amino-2,4,6-2-tetraheptane HCI, 50 µg/ml tosyl phenylchloroketone, and 1 mM phenylmethylsulfonyl fluoride, using a Polytron tissue homogenizer. The homogenate was clarified by velocity sedimentation (100,000 x g, 60 min) and concentrated by centrifugal filtration. The peak of creatine kinase activity from the column was eluted with Polybuffer 96 (Pharmacia) (pH 9.1). Creatine kinase was desalted and concentrated by centrifugal filtration. The desalted peak was run at 150 mV for approximately 4 h with 20-mM MOPS, 0.05 mM EDTA, 100 µg/ml 1 chloro-3-indolyl phosphate-p-toluidine in 100 mM Tris-HCl (pH 9.5), 5 mM MgCl₂, 100 mM NaCl. Purified creatine kinase was then buffer exchanged into Tris-HCl (pH 8.3) containing 50% glycerol, 25% polyethylene glycol, 0.05% sodium azide, and 0.01% sodium azide. The final protein concentration was approximately 8 mg/ml. The cDNA sequence downstream of the human M creatine kinase gene was used to prepare PCR primers using the primer." method of Feinberg and Vogelstein (32).

**Polyacrylamide Gel Electrophoresis**—The cDNA sequence downstream of the human M creatine kinase gene was used to prepare PCR primers using the primer." method of Feinberg and Vogelstein (32).
Polymerase Chain Reaction—PCR primers were selected for 100% sequence similarity with 3'-untranslated M creatine kinase gene and 3'-untranslated B creatine kinase sequences, respectively. (i) Creatine kinase M sense primer with EcoRI linker (31-mer): 5'-GAAT TCCGCT TCCTCA CCTGAA CACTCC A-3'. (ii) Creatine kinase M anti-sense primer with EcoRI linker (34-mer): 5'-GAATTC GCTGTT GCAGA CAAGA CGACA AGGG-3'. (iii) Creatine kinase B sense primer with EcoRI linker (38-mer): 5'-GAATT CCAGAC CCCAGT GGCTC GCCAG AAGAC-3'. (iv) Creatine kinase B anti-sense primer with EcoRI linker (31-mer): 5'-GAATTC GCTGAA CACTCC TCTGCT TCCTA A-3'.

PCR detection primers were selected for 100% sequence similarity with 3'-untranslated M creatine kinase and 3'-untranslated B creatine kinase sequences, respectively. Each PCR primer set is complementary to sequences internal to the amplification primers. (i) Creatine kinase M detection primer (30-mer): 5'-GGTTGGCTCGGG AGCTC TCCAT TAACT AGAGC-3'. (ii) Creatine kinase B detection primer (34-mer): 5'-CTACAG CAAGG CTGAGG GCCCT CCCAG-3'. Reverse Transcription and First Strand cDNA Synthesis—Ten micrograms of total human heart, brain, or placenta RNA was used as a template for cDNA synthesis with the creatine kinase isoenzyme-specific anti-sense primers (1 µM), 40 units of Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories)/µg of RNA, in a reaction volume of 50 µl at 42 °C for 2 h. The cDNA was extracted with equal volumes of phenol/chloroform, precipitated in ethanol, and resuspended in 25 µl of water. Five µl of the supernatant was used for each PCR reaction.

Amplification of cDNA by Polymerase Chain Reaction—cDNA synthesized from 2 µg of total RNA from each tissue was used for PCR reactions. The reaction volume was 50 µl containing 1 µM each of amplification primers, 2 units of Taq polymerase (Bethesda Research Laboratories), 50 mM KCl, 10 mM Tris (pH 9.4), 2.5 mM MgCl₂, 0.2 mM deoxynucleotide triphosphate, and 200 µCi/ml gelatin. The reaction was carried out under 1 drop of mineral oil. The amplification conditions were performed with an initial denaturing step at 93 °C for 5 min; and 4, 8, 16, 20, or 30 cycles of denaturing at 93 °C for 2 min followed by annealing and elongation at 68 °C for 6 min. At the end of each amplification cycle, the reaction products were chilled on ice.

End Labeling of Oligonucleotides—The detection primers (0.6 µM each) were end-labeled in a single reaction with 150,000 cpm of [γ-³²P]ATP to a specific activity of 2 × 10⁶ cpm/µg using a kinase reaction (33) and purified by column chromatography (Nensorb, Du Pont–New England Nuclear).

Detection of Amplified Products—Half of the amplified products were subjected to an additional cycle of 5 min of denaturation and 60 min of annealing and elugation in the presence of end-labeled detection primers (2 × 10⁶ cpm/tube). The reaction was terminated with equal volume of standard sequencing reaction stopping solution. The gel was at 83 °C for 4 min and 4 µl loaded on each lane at 8% denaturing urea-acrylamide gel (sequencing gel). Following electrophoresis, the gel was dried and exposed to Kodak X-Omat 5 film overnight at −70 °C using one intensifying screen (Du Pont). The amplification and detection primers were chosen so that M creatine kinase cDNA would be detected at a size of 145 nucleotides and B creatine kinase at 105 nucleotides.

Cell Culture and Transfection—NIH 115 and NS20Y cells derived from mouse neuroblastomas (34), were seeded at 20% confluency into 100-mm² culture dishes 24 h before transfection in 15 ml of Dulbecco's modified essential medium, 10% fetal bovine serum culture medium. Transfection was carried out with 10 µl of transfecting plasmid containing 10 µg nucleotides and 5 µl of culture medium and cells were transfected with M creatine kinase upstream sequence-chloramphenicol acetyltransferase hybrid genes as described previously (6). DNA precipitate was prepared by adding 150 µl of 0.5 M CaCl₂ to 10 µg of plasmid in 150 µl of H₂O, then slowly adding 300 µl of the following solution, 40 mM Heps, 274 mM NaCl, 10 mM KCl, 1.4 mM Na₂HPO₄, 12 mM dextrose (pH 7.5), in 6 × 50-µl aliquots and incubating at room temperature for 10 min. The DNA precipitate was added to the dishes and 24 h later medium with precipitate was removed and dishes refed with culture media. After 48 h the cells were harvested either as undifferentiated or refed with Dulbecco's modified essential medium + 1% dimethyl sulfoxide/2% horse serum to induce differentiation. Cells were allowed to differentiate for 4 days before harvesting.

Chloramphenicol Acetyltransferase Assay Constructs—Hybrid genes have been prepared as described previously (6). (i) pUC 4800-CAT: a region upstream of the M creatine kinase gene from 4800 to +1 base pairs from the transcription initiation site linked immediately upstream to chloramphenicol acetyltransferase; (ii) pSV2-CAT: simian virus 40 promoter and enhancer linked to chloramphenicol acetyltransferase; (iii) pSV0-CAT: a promoterless or enhancerless region linked to chloramphenicol acetyltransferase.

Chloramphenicol Acetyltransferase Assays—For analysis of chloramphenicol acetyltransferase enzyme activity, cells were rinsed with cold phosphate-buffered saline (140 mM NaCl, 10 mM Na₂PO₄, pH 7.2) and were suspended in 100 µl of 0.25 M Tris chloride (pH 7.8), and cells were lysed by 3 cycles of freeze-thawing. Chloramphenicol acetyltransferase activity in cell lysates was determined in a reaction mixture containing 50 µl of cell extract (after dilution to contain equivalent amounts of protein), 20 µl of 4 mM acetyl coenzyme A (Pharmacia LKB Biotechnology Inc.), and 0.2 µCi of ³²P-labeled chloramphenicol (54 µCi/mmol; Amersham Corp.) in a total volume of 170 µl. Reactions were allowed to proceed for 20 min at 37 °C, and then an additional 10 µl of 4 mM acetyl coenzyme A was added for 20 min at 37 °C. Reactions were terminated by extraction with 500 µl of ethyl acetate. The organic phase was removed, dried, and suspended in 20 µl of ethyl acetate and applied to silica gel thin-layer plates (Eastman Kodak Co.). Acetylated and unacetylated forms of chloramphenicol were separated by using chloroform/methanol (9:5:5) as the mobile phase, and were visualized by autoradiography with XAR-5 x-ray film (Kodak). The extent of conversion of substrate to products was quantitated by excising labeled spots from thin-layer plates and by liquid scintillation counting. To account for possible differences in transfection efficiencies or assay conditions among different experiments, chloramphenicol acetyltransferase activity directed by chimeric plasmids was compared with the levels of chloramphenicol acetyltransferase activity in cells transfected with pSV2-CAT in which the CAT gene is subjected to transcriptional control by the SV40 early gene promoter and enhancer. Chloramphenicol acetyltransferase activity recovered from transfected cultures was linear with respect to the concentration of plasmid DNA used in transfection.

RESULTS

DEAE-Sepharose Purification of Human Brain M Creatine Kinase—Fig. 1 shows the elution pattern of creatine kinase isoforms in human hippocampus and temporal lobe, following chromatography on DEAE-Sepharose. In Fig. 1a, total creatine kinase activity is plotted as a function of fraction number. In Fig. 1b, the creatine kinase isoenzyme content of these peaks is demonstrated by non-denaturing gel electrophoresis. At an NaCl concentration of approximately 150 mM a small peak of enzyme activity (peak A) elutes which migrates cathodic to MM creatine kinase on non-denaturing agarose gel electrophoresis. When the buffer salt concentration is increased to approximately 250 mM, the mitochondrial isofrom of creatine kinase is eluted from the column (peak B). The next peak in the elution profile (peak C), 300 mM NaCl, comigrates with skeletal muscle MM creatine kinase on non-denaturing electrophoresis. This peak represents approximately 35% of the total creatine kinase activity of this region of brain. The final peak (peak D) which elutes at 450 mM NaCl, has an electrophoretic mobility consistent with that of BB creatine kinase.

N-terminal Amino Acid Sequence of Human Brain M Creatine Kinase—To confirm the identity of the creatine kinase isoenzyme contained in DEAE-Sepharose peak (C), the protein was concentrated and subjected to SDS-polyacrylamide gel electrophoresis, lightly stained to identify protein bands and the band corresponding to a molecular weight of 43,000 N-terminal amino acid sequence data obtained from the electroeluted 43,000-dalton protein. For comparison, the published sequences for the human creatine kinase isoenzymes are listed in the lower part of the figure. The N-terminal sequence obtained from the M creatine kinase purified from human brain is identical to the known sequence for M creatine kinase.
MM Creatine Kinase in Human Brain

**Origin**

**FIG. 1.** DEAE chromatography elution profile of human brain supernatant. Brain supernatant creatine kinase isoforms were resolved by ion exchange (DEAE) chromatography (a). Individual fractions were analyzed for creatine kinase (CK) activity by spectrophotometry as described under “Materials and Methods.” Peak fractions were concentrated and desalted using centrifugal concentrations. Pooled activities were subject to agarose-gel electrophoresis to identify the isoform content of each peak (b).

**FIG. 2.** Sequence of M creatine kinase purified from human brain. The N-terminal amino acid sequence was determined for the 43,000-dalton protein (M creatine kinase) from brain using the Pictotag (Waters) method. In the top line, the N-terminal 30 amino acids are shown. In the lower part of the figure the published sequences for the three human creatine kinase isoforms are included for comparison. The extent of homology with muscle creatine kinase is shown in the right column of the figure. Note the complete identity of muscle and brain M creatine kinase isoforms.

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Sequence</th>
<th>Similarity with muscle M creatine kinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human brain M kinase</td>
<td>MFFGNTHEFELAEVEKEFIPKSKHNM</td>
<td>100%</td>
</tr>
<tr>
<td>Human Skeletal Muscle M creatine kinase (40):</td>
<td>MFFGNTHEFELAEVEKEFIPKSKHNM</td>
<td>---</td>
</tr>
<tr>
<td>Human B creatine kinase (42):</td>
<td>MFFNHHALEAQAEDEFPOLSKHNM</td>
<td>78%</td>
</tr>
<tr>
<td>Human Placenta Mitochondrial creatine kinase (43):</td>
<td>AASERRRRTPAEFPOPLHMW</td>
<td>64%</td>
</tr>
</tbody>
</table>

**Human Brain Tissue**—Following the identification of MM creatine kinase in human brain we initiated experiments to determine the localization of this protein within human brain. Fig. 3a shows a photomicrograph of a section of hippocampus which has been reacted with a rabbit polyclonal antisera specific for MM creatine kinase. The darkly staining cytoplasm within the cell body and primary processes of the pyramidal cells is the insoluble reaction product produced by the second antibody–alkaline phosphatase detection system (Fig. 3a). The rabbit polyclonal M creatine kinase antisera specificity to M creatine kinase was previously validated (35, 36). The same pattern of immunostaining was seen when sections were reacted with a mouse monoclonal anti-human M creatine kinase antibody (Fig. 3b). When preimmune rabbit serum is used no detectable reaction product is produced (Fig. 3c).

**FIG. 3.** Immunocytochemistry and in situ localization of M creatine kinase in human brain. Human brain tissue was fixed in formalin, embedded in paraffin, and sectioned at 7 μm. Three serial sections from the hippocampus are shown above. In a, polyclonal rabbit anti-human MM creatine kinase antisera was used as label with an alkaline phosphatase labeling system. In b, the tissue has been labeled with a mouse monoclonal M creatine kinase monoclonal antisera (1:100 dilution) using an alkaline phosphatase detection system. In the control experiment shown to the right (c), the tissue has been labeled with a preimmune rabbit serum and a goat anti-rabbit second antibody coupled to alkaline phosphatase (×237 magnification). Note the increased stain present in the perinuclear and proximal cell body of neuronal cells in a and b when compared to c.
Analysis of Human Brain mRNA by Northern Blot Analysis and Polymerase Chain Amplification—Total and poly(A)+ RNA from adult human hippocampus and temporal lobe, human adult skeletal muscle, and adult human myocardium, were isolated, electrophoresed on an agarose-formaldehyde gel, and transferred to a nylon membrane. The membranes were probed with 32P-labeled human 3'-untranslated M creatine kinase (135 bp). The expected 1400-bp M creatine kinase message was not present in human brain poly(A) selected or total RNA lanes. However, the expected M creatine kinase signal was present in the human skeletal muscle and human myocardial RNA. A human B creatine kinase-specific probe did detect the expected B creatine kinase message (data not shown).

In the next series of experiments we used polymerase chain reaction to amplify cDNA synthesized from human brain mRNA to detect M creatine kinase message in human brain. Ten µg of total RNA from human placenta, myocardium, and human hippocampus/temporal lobe, were used for PCR analysis. cDNA was synthesized using creatine kinase isoform-specific antisense primers as templates and amplified by PCR. PCR products were denatured, annealed, and elongated in the presence of 32P-end-labeled detection primers. Aliquots of each mixture were electrophoresed in a denaturing sequence gel and exposed to x-ray film. These results are shown in Fig. 4a. In the bottom half of Fig. 4a, B creatine kinase signal can be seen in all three tissues at 105 nucleotides (human placenta, human myocardium, and human brain hippocampus/temporal lobe). In the top of the figure the M creatine kinase signal (145 nucleotides) was present in the human myocardium and human brain but not in human placenta.

To further validate the results of the PCR experiment, Fig. 4b shows the creatine kinase isoforms present in human myocardium, brain and placenta as detected by nondenaturing electrophoresis followed by overlaying the gel with Rosalki reagent. In lane P, the placenta supernatant shows the presence of BB creatine kinase and a cathodic band corresponding to mitochondrial creatine kinase. In lane M the myocardial tissue is shown to contain MM and MB creatine kinase. In lane B, the human brain supernatant contains BB and MM creatine kinase as well as an uncharacterized cathodic isoenzyme which is immunologically related to MM creatine kinase. The separation of MM creatine kinase from the other forms is incomplete. This is, however, the brain homogenate from which MM creatine kinase was purified. Placental homogenates subjected to the identical chromatography procedures contained only the mitochondrial and BB isoform.

M Creatine Kinase Hybrid Genes Transfected into Neuronal Cell Cultures—In an initial experiment designed to examine transcriptional regulation of M creatine kinase in neuronal cells, we transfected a reporter gene linked to mouse M creatine kinase regulatory sequences into two mouse neuroblastoma cell lines, N1E115 and NS20Y, which have been used as models of neuronal cell differentiation (34). The hybrid gene, pCK 4800-CAT, contains the upstream region 4800 to +1 base pairs relative to the transcription initiation site of M creatine kinase fused immediately upstream of the reporter gene chloramphenicol acetyltransferase. The 4800-bp region contains the M creatine kinase promoter and four 5' enhancers which have been shown to specifically regulate M creatine kinase gene transcription in differentiated skeletal myoblasts. pSV0-CAT and, pSV2-CAT, were transfected in parallel as controls. Calcium phosphate precipitates of the CAT constructs were transfected (6) into undifferentiated neuronal cell lines, placed in either serum-depleted 1% dimethyl sulfoxide medium to differentiate the cells or left undifferentiated for 48 h, and harvested. Fig. 5a shows the autoradiograph of chloramphenicol acetyltransferase assays from the respective transfections in N1E115 cells. Chloramphenicol acetyltransferase analysis (Fig. 5a) showed increased chloramphenicol acetyltransferase activity in cells transfected with pCK 4800-CAT when compared to controls (pSV0-CAT). In the upper part of Fig. 5a relative amounts of the acetylated form of chloramphenicol are represented. From left to right (Fig. 5a), pSV2-CAT shows chloramphenicol acetyltransferase activity expressed in the undifferentiated and differential cell lines. pSV2-CAT expression is slightly decreased in these differentiated cell lines. pSV0-CAT shows no activity in either the undifferentiated or differentiated cells. pCK 4800-CAT had increased activity compared to both the pSV2-CAT and pSV0-CAT. The product formation was increased in the differentiated cells compared to undifferentiated cells in the pCK 4800-CAT-transfected cells. Identical results were seen in NS20Y-transfected cells (data not shown). This reporter plasmid has been inactive in all other

D. L. Friedman, R. J. Hamburg, and M. B. Perryman, unpublished data.
MM Creatine Kinase in Human Brain

were assayed as described in the text. Plasmid DNAs were transfected into proliferating undifferentiated N1E115 cells by calcium phosphate precipitation. After 48 h undifferentiated cultures (U) were either harvested or were transferred to differentiation (D)-promoting media. Transfected plasmids listed above were pSV2-CAT, pSV0-CAT, and pCK 4800-CAT. a, agarose-gel electrophoresis of supernatant from N1E115 cells (both undifferentiated and differentiated) compared to supernatant from mouse skeletal muscle. Equal amounts of protein supernatant (from N1E115 cells) were electrophoresed on 1% agarose gel, overlaid with Rosalki reagent and photographed under UV light. Lanes 1 were with lane 1. Note the increased intensity of the band in MM creatine kinase region (compare lane 2 with lane 1).

nonmuscle cell types examined (6).

The cells used as models the regulation of M creatine kinase, were previously used to study neuronal differentiation. N1E115 and NS20Y are adrenergic and cholinergic neuroblastoma cell lines, respectively. We examined the morphology of these cells throughout the experiment to ensure that these cells retained their phenotypic neuronal properties (data not shown).

We also examined the expression of the endogenous mouse M creatine kinase gene in the two differentiating neuroblastoma cell lines, N1E115 and NS20Y cells. Fig. 5d shows a nondenaturing electrophoretic separation of creatine kinase isozymes from N1E115 cell supernatants. In lane 1, the MM creatine kinase band represents the activity obtained from 3 μg of protein from undifferentiated N1E115 cells. Lane 2 shows the MM creatine kinase activity present in an equal amount of protein (expressed as international units/mg) from differentiated N1E115 cells. In lane 3, purified mouse skeletal muscle MM creatine kinase is shown. The creatine kinase activity of N1E115 cells were 672 IU/mg undifferentiated cell homogenate and 1418 IU/mg differentiated cell homogenate. The endogenous creatine kinase activity for the NS20Y cells were 291 IU/mg undifferentiated cell supernatant and 605 IU/mg for the differentiated cell supernatants. There is a relatively high level of endogenous M creatine kinase and chloramphenicol acetyltransferase assay expression in the undifferentiated neural cell lines. This may be due to high levels of expression in small number of differentiated cells in the culture or to uniform levels of expression in the entire culture. In these preliminary studies we did not differentiate between these two possibilities.

FIG. 5. Regulation of M creatine kinase enhancer in N1E115 cells. a, levels of chloramphenicol acetyltransferase assay activity in N1E115 cultures transfected with plasmid DNA constructs were assayed as described in the text. Plasmid DNAs were transfected into proliferating undifferentiated N1E115 cells by calcium phosphate precipitation. After 48 h undifferentiated cultures (U) were either harvested or were transferred to differentiation (D)-promoting media. Transfected plasmids listed above were pSV2-CAT, pSV0-CAT, and pCK 4800-CAT. b, agarose-gel electrophoresis of supernatant from N1E115 cells (both undifferentiated and differentiated) compared to supernatant from mouse skeletal muscle. Equal amounts of protein supernatant (from N1E115 cells) were electrophoresed on 1% agarose gels, overlaid with Rosalki reagent and photographed under UV light. Lanes were loaded with supernatant from undifferentiated N1E115 cells (lane 1), supernatant from differentiated N1E115 cells (lane 2), and homogenate from mouse skeletal muscle control (lane 3). Note the increased intensity of the band in MM creatine kinase region (compare lane 2 with lane 1).

We have isolated MM creatine kinase from adult human brain tissue where it is present in appreciable amounts. Initial immunolocalization studies of regions from human brain reveal that MM creatine kinase is localized to discrete regions of these tissues. Although M creatine kinase mRNA could not be detected in hippocampus by Northern blot analysis, polymerase chain reaction amplification of cDNA synthesized from human hippocampal mRNA detected M creatine kinase message. No message could be detected in placenta which contains no MM creatine kinase protein. Transient transfection of two mouse neural cell lines with an M creatine kinase upstream sequence-chloramphenicol acetyltransferase hybrid gene demonstrated that cis-acting M creatine kinase upstream sequences are sufficient to modulate transcription during differentiation of these nonmuscle cells.

The names of the cytoplasmic creatine kinase isoenzymes, MM creatine kinase for muscle and BB creatine kinase for brain, reflect the present concept of tissue-specific creatine kinase isoenzyme distribution. We have shown MM creatine kinase to be present in lens (2) and other eye tissues including cornea and retina(4) and this isoenzyme has been detected in kidney (37) and arteries (1). These data would suggest that creatine kinase isoenzymes and particularly MM creatine kinase is much less tissue-specific than previously thought.

In these studies, we found that approximately 35% of creatine kinase present in human hippocampus and temporal lobe to be MM creatine kinase. This figure may be artificially high for several reasons. The tissue was obtained approximately 24 h postmortem and a significant amount of BB creatine kinase may have lost enzymatic activity since BB creatine kinase is noted for decreased stability relative to MM creatine kinase. These purifications were performed from a specific region of human brain and this area of human brain may have markedly increased MM creatine kinase content compared to other regions of brain. Studies are underway to examine all of the major regions of human brain by in situ hybridization and immunocytochemistry for MM creatine kinase in order to address this question.

Several techniques were used to detect the presence of MM creatine kinase mRNA in brain tissue. Using in situ hybridization we were able to identify M creatine kinase mRNA message and localize it to discrete regions of human brain (data not shown). We, however, surprised that M creatine kinase message could not be detected in human brain by Northern blot analysis. There are several plausible explanations for this. First, there may be a very low copy number of mRNA encoding M creatine kinase. This interpretation is supported by the finding that brain mRNA is complex and many messages are present in low copy number (38, 39). For example, Hoffman et al. (40) reported that no signal above base line as obtained when probing brain mRNA for dystro-

Although, the protein was easily detectable using Western blot techniques. Our localization experiments also indicate that M creatine kinase message is localized to specific cell populations which represent only a small fraction of the total cell number in brain. We then chose to use polymerase chain reaction amplification of cDNA synthesized from human hippocampal, myocardial and placental mRNA. The sensitivity of this technique is not as limited by mRNA complexity as Northern blot analysis and was able to detect M creatine kinase mRNA in brain and heart but not placenta which contains no demonstrable MM creatine kinase protein.

It is currently accepted that a distal upstream M creatine kinase gene enhancer is muscle specific and is sufficient to control developmentally regulated expression of M creatine kinase in skeletal muscle (5–8). We sought to determine whether the 4800 upstream M creatine kinase sequence containing this enhancer and other regulatory elements is responsible for the transcriptional regulation of the M creatine kinase in neural tissue. Simultaneously with up-regulation of transcription of the M creatine kinase-chloramphenicol acetyltransferase hybrid gene, the two neural cells expressed phenotypic characteristics of neurons and expression of the endogenous mouse M creatine kinase gene was up-regulated. This suggests that not only is MM creatine kinase developmentally regulated in neural tissue but M creatine kinase gene upstream sequence can modulate expression of MM creatine kinase in certain neuronal cells.

The findings that M creatine kinase expression is not muscle-specific and that transfection of M creatine kinase upstream sequence can regulate transcription of a reporter gene in neuronal cells has relevance to the determination of stem cells. In this regard, the M creatine kinase enhancer has been shown to be activated by the muscle regulatory factors MyoD1 (5) and myogenin (44). These factors also have been shown to bind to sites within the enhancer core that are essential for muscle-specific activity (45). In addition to activating M creatine kinase activity expression, ectopic expression of MyoD1 and myogenin is sufficient to activate the complete muscle differentiation program. The fact that MyoD1 and myogenin are expressed exclusively in skeletal muscle implies that activation of M creatine kinase expression in neuronal cells occurs through a separate mechanism, possibly through different regulatory sequences than are used in muscle. Moreover, the complete set of skeletal muscle-specific genes is not activated in neuronal cells, which suggests that M creatine kinase can be regulated independently from other muscle genes.

Acknowledgments—We would like to thank Arnold W. Strauss (Washington University, St. Louis) for protein sequence determination, Tom Parker, Henry F. Epstein, Michael D. Schneider, and Jonah Igneu for many helpful discussions, Bruce Kenwood, Bobby Kesterson, and Molecular Analysis Incorporated (MAI) for assistance with in situ hybridization protocols, Sherry Terry for manuscript preparation, Bill Boerwinkle, Regan Ryan, and Mary Ellen Perry for laboratory assistance, and Dr. Tom Parker for thoughtful discussion. We thank E. L. DuPont de Nemours and Co. for providing monoclonal antibodies.

REFERENCES

Muscle creatine kinase isoenzyme expression in adult human brain.
R J Hamburg, D L Friedman, E N Olson, T S Ma, M D Cortez, C Goodman, P R Puleo and M B Perryman


Access the most updated version of this article at http://www.jbc.org/content/265/11/6403

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/265/11/6403.full.html#ref-list-1