Regional Creatine Kinase, Adenylate Kinase, and Lactate Dehydrogenase in Normal Canine Brain

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Following acute stroke, creatine kinase and other enzymes are released into the cerebrospinal fluid and blood from injured brain tissue. To determine whether regional differences in brain enzyme activity might exist and therefore affect the amount of enzyme released, we quantified the levels of creatine kinase, adenylate kinase, and lactate dehydrogenase in 12 regions of normal canine brain (n = 4). Adenylate kinase activity varied the least among regions (49 ± 7 units/g), followed by lactate dehydrogenase activity (122 ± 28 units/g). The pattern for both adenylate kinase and lactate dehydrogenase was higher activity in predominantly gray matter areas, lower activity in white matter, and intermediate activity in mixed regions. The distribution of creatine kinase brain isoenzyme and mitochondrial creatine kinase in canine brain was less predictable, showing wider variations among regions (isoenzyme, 462 ± 116 units/g; mitochondrial, 42 ± 20 units/g). Even cerebral gray matter demonstrated substantial regional variations in creatine kinase brain isoenzyme, ranging from 606 units/g in the parietal cortex to 329 units/g in the temporal cortex. We conclude that the content of creatine kinase brain isoenzyme varies more than twofold among areas of brain. This regional variation may be important in the interpretation of creatine kinase brain isoenzyme measurements in cerebrospinal fluid and serum used to assess neurologic injury following stroke.

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cute neurologic injury releases a variety of enzymes into the cerebrospinal fluid (CSF) and blood including creatine kinase (CK), adenylate kinase (AK), and lactate dehydrogenase (LD). The enzyme most frequently studied is the brain isoenzyme of creatine kinase (CK-BB). Global brain injury following cardiac or respiratory arrest is associated with damage to multiple regions of the brain. Measurement of CK-BB activity in the CSF has been evaluated as a method to estimate the extent of brain damage and to predict prognosis following global brain injury. The level of CK-BB in the CSF following transient cardiac arrest correlates well with the degree of brain damage found at autopsy.

More recently, CK-BB levels have been measured in CSF and serum following localized neurologic injury such as acute stroke. Estimation of infarct size using CSF enzyme levels is based on the assumption that all areas of the central nervous system (CNS) contain similar concentrations of the enzyme measured. However, if enzyme levels vary from one anatomic site to another, infarctions of the same size would not necessarily release the same amount of enzyme, potentially making estimation of infarct size less accurate. Previous studies have reported only semiquantitative or quantitatively incomplete descriptions of regional CK, AK, and LD activity in the CNS. In our study, CK, AK, and LD activities were quantified in 12 regions of normal canine brain to determine whether regional differences exist that might potentially limit the use of CSF enzymes in estimating the volume of injured tissue.

Materials and Methods

Whole brains were removed from four large (25 kg), healthy, adult mongrel dogs that had been killed for other experiments that did not affect the brain or cerebral circulation. The only drugs used were anesthetics not known to affect brain enzyme levels. After removal, the brains were dissected and paired samples were taken from both the right and left sides of each region, with the exception of the cervical spinal cord, from which only a single sample consisting of both sides was obtained. Of the regions studied, four consisted entirely of cortical gray matter (frontal cortex, parietal cortex, temporal cortex, and occipital cortex), five were composed of a mixture of gray matter and white matter (hippocampus, cerebellar cortex, midbrain, pons, and cervical spinal cord), and one was composed entirely of white matter (frontal lobe). The gray matter samples from the cerebral cortex were free of white matter; likewise, the white matter samples from the frontal lobe were free of gray matter.
Following removal, the samples were frozen at −70°C. The interval from killing the dog to freezing the individual samples was 40 ± 5 (mean ± SD) minutes. To extract the enzymes, 1 g of sample was rapidly thawed and homogenized in 19 ml of 100 mM ammonium acetate (pH 9.5) containing 1% Triton X-100. Homogenization was carried out using a rotary Teflon pestle and glass mortar for 30–60 seconds at 0°C. Homogenization for up to 5 minutes did not increase the amount of enzyme activity measured in the extracts. Homogenates were centrifuged at 15,000g for 20 minutes at 4°C. To reactivate reversibly oxidized enzymes and to improve the stability of the enzymes, the resulting supernatant was diluted 1:10 and 1:100 in a storage solution consisting of 50 mM Tris (pH 7.4, 25°C) and 20 mM dithiothreitol. All subsequent dilutions were made using the Tris-dithiothreitol solution. It should be noted that there was no difference in the activities of enzyme extracted from fresh tissue compared with frozen tissue. Furthermore, there was no significant difference (p > 0.05) in enzyme activity measured in samples from the same region with postmortem intervals ranging from 30 to 55 minutes. It is estimated that <3% of CK would be lost during this period.15

Total CK and AK activities were measured using a modification of the Rosalki method, which couples ATP produced from creatine phosphate by CK or from ADP by AK to the generation of NADPH, which is measured spectrophotometrically at 340 nm. One unit of CK activity is defined as the amount of enzyme that dephosphorylates 1 μmol creatine phosphate per minute (pH 6.6, 30°C). One unit of AK activity is defined as the amount of enzyme that converts 2 μmol ADP into 1 μmol each of ATP and AMP per minute (pH 6.6, 30°C). Total LD activity was determined by measuring the conversion of pyruvate to l-lactate. One unit of LD activity is defined as the amount of enzyme that converts 1 μmol pyruvate to lactate per minute (pH 7.4, 30°C).

CK isoenzymes were separated electrophoretically using agarose gels and 3-(N-morpholino)-2-hydroxypropanesulfonic acid (MOPS) buffer, pH 7.8 (Corning Medical and Scientific, Palo Alto, California). Standard electrophoresis was performed on all samples for 20 minutes at 90 V. After separation, the activity of each isoenzyme was quantified by measuring the NADPH fluorescence using reagents similar to those described above. The coefficient of variation (CV) for the extraction and measurement of CK-BB, AK, and LD activity was 10%; CV for the measurement of mitochondrial creatine kinase (CK-mt) was 20%. Enzyme activities are expressed as units per gram of wet tissue.

The mean activity and SEM were determined for each region. Differences in activity between paired samples from the left and right sides were analyzed for each enzyme using a repeated-measures analysis of variance (ANOVA). Differences in activity among regions was first analyzed using a one-way ANOVA, which indicated significant (p < 0.01) differences among regions for all enzymes. Differences in activity between individual pairs of regions were then tested for significance using Tukey’s honestly significant difference (HSD) multicomparison test at a significance level of p = 0.05. As there were 12 regions studied, there was a total of 66 individual comparisons for each of the four enzymes. To condense this data, we looked for groups of regions that had essentially the same enzyme activity based on the statistical tests. A summary of this grouping is given in “Results.” A complete set of statistical comparisons among regions using Tukey’s HSD multicomparison test is available on request.

Results

AK, LD, CK-BB, and CK-mt were present in all the brain samples. There was no evidence of creatine kinase isoenzymes that predominate in cardiac and muscle tissue (CK-MB or CK-MM) in any sample. There was no difference in the activity of any enzyme between the paired samples. Therefore, we averaged the values for the left and right sides of each region of each brain. For the four dogs studied, the mean and SEM were calculated from these average values for each region.

Adenylate Kinase Activity

AK activity varied the least from region to region (Figure 1). The overall average AK activity for all 12 regions was 49 units/g (range, 59 units/g in parietal gray matter to 36 units/g in frontal white matter). A one-way ANOVA indicated significant differences in AK activity among regions (p = 0.001). When the individual regions were compared using Tukey’s HSD multicomparison test, AK activity in the different regions fell into three groups. Samples from the four regions of cortical gray matter had significantly higher AK activities than all other regions, but there was no difference in activity among the cortical samples. In contrast, frontal white matter had significantly less AK activity than all other regions. The remaining seven regions (cerebral nuclei and mixed gray and white matter regions) had similar AK activities, significantly less than those in cortical gray matter but more than that in frontal white matter.

Lactate Dehydrogenase Activity

The distribution of LD activity was slightly more complex than that for AK activity but still showed a pattern of higher activity in regions composed predominantly of gray matter and less activity in regions with high white matter content (Figure 1). LD activity averaged 122 units/g overall (range, 158 units/g in parietal gray matter to 74 units/g in frontal white matter). A one-way ANOVA indicated significant differences in LD activity among regions (p = 0.0001). Evaluation of variations in LD activity among regions using Tukey’s HSD multicomparison test indicated that frontal, parietal, and occipital gray matter and the cerebellar cortex had significantly higher activity than all other regions. Intermediate levels of LD activity were found in temporal gray matter, hippocampus,
caudate, and thalamus. The midbrain had significantly lower levels than previously mentioned regions but more than the pons. The pons had higher activity than the cervical spinal cord and frontal white matter, which had similar low LD activities.

Creatine Kinase Activity

The regional distribution of CK-BB and CK-mt did not show the same consistent pattern of high activity in gray matter and low activity in white matter that was found for both AK and LD activity. A one-way ANOVA indicated significant differences in CK-BB and CK-mt activity among regions ($p = 0.0001$ for both). Overall, no pattern was discerned for CK-BB or CK-mt activity based on the type of region sampled.

The caudate nucleus had significantly higher CK-BB activity than all other regions. The next highest activity was in parietal gray matter, followed by a group consisting of frontal gray matter, occipital gray matter, and cerebellar cortex. Intermediate levels of CK-BB activity, significantly lower than those areas listed above, were found in the hippocampus, thalamus, midbrain, and pons. Temporal gray matter, cervical spinal cord, and frontal white matter had CK-BB activities that were significantly lower than all other regions.

The average CK-BB activity for all areas was 462 units/g (range 683 units/g in the caudate to 310 units/g in frontal white matter). CK-mt activity averaged 42 units/g for all areas and showed the largest regional variation, with activities ranging from 83 units/g in the caudate nucleus to 9 units/g in frontal white matter.

While CK-mt activity was always lower than CK-BB activity, the ratio of the activity of these two enzymes showed a pattern similar to that found for AK and LD (Figure 2). Nine regions (frontal gray matter to the midbrain) showed, with a few minor exceptions, no significant difference ($p>0.05$) in the ratio of CK-mt to CK-BB. The pons had a CK-mt: CK-BB ratio lower than these nine regions but higher than the cervical spinal cord and frontal white matter, which had similar ratios.

Discussion

In general, both AK and LD activity followed a pattern consistent with relatively high activity in gray matter, low activity in white matter, and intermediate levels in mixed regions. This pattern was not followed for CK-mt and CK-BB but was followed for the ratio of these two enzymes. The fact that CK-BB varies by more than twofold from region to region may be an important consideration in interpreting CK-BB levels in CSF and serum following localized brain damage.

Bell et al. have presented a model developed in dogs to estimate brain infarct size based on the release and disappearance of CK-BB in serum following experimental strokes induced with Silastic emboli. In their study, strokes of varying sizes were induced in the same area of the brain, eliminating the influence of regional variations from their results. The total amount of CK-BB released from an infarct was estimated from serial serum CK-BB samples. The predicted infarct
size was estimated as the ratio of CK-BB released to total brain CK-BB.

Bell et al\textsuperscript{10} found a good correlation ($r = 0.94$) between predicted and measured infarct size using a morphometric technique. However, for such a model to be clinically useful and for the size of the infarct to be directly proportional to the serum:total ratio, it must be assumed that all regions of the brain contain the same concentration of CK-BB. Our results indicate that the distribution of CK-BB in the brain is not uniform; thus total brain CK-BB used in the model of Bell et al\textsuperscript{10} is not a constant but varies depending on the region damaged. To enhance the accuracy of predictive models, total brain CK-BB would have to be replaced with a value that represents the CK-BB activity of the region that is damaged. Using a single average value would tend to underestimate the size of a stroke in regions with a low CK-BB concentration, such as the temporal cortex, and overestimate the size of a stroke in areas with a high CK-BB concentration, such as the parietal cortex. Other factors that may affect CSF enzyme levels include fractional release of enzymes from various regions and variations in the rate of release.

Variations in regional brain enzyme concentrations must also be taken into account in studies of enzyme release into CSF and blood following localized neurologic injury such as stroke in humans. Whereas gross changes in CSF enzyme levels may be useful in predicting the degree of damage, it may be unwise to attempt direct correlations of infarct size in humans and CSF or serum enzyme levels until more is known about the distribution of these enzymes in human brain and how they are released. For example, it has been demonstrated that lacunar strokes result in little or no increase in CSF enzyme levels compared with cortical strokes.\textsuperscript{21}

Optimally, enzymes used to estimate the size of infarcts should be 1) specific to brain tissue to prevent interference from other sources, 2) of uniform distribution and release throughout the brain, 3) present in relatively high concentration, and 4) stable in CSF and serum to improve the accuracy and precision of clinical assays and their interpretation. None of the enzymes we studied fulfill all these criteria. AK has a relatively uniform distribution in canine brain but is present at only 10\% of the concentration of CK-BB, making it more difficult to measure. In addition, high AK levels can be found in other tissues, making false-positives a potential problem in serum measurements. LD is not a good choice for a number of reasons including moderate variations in activity among regions, lower activity than CK-BB, and lack of specificity. CK-BB is relatively specific for brain and has the highest tissue concentration but shows the widest variation among regions. Overall, CK-BB is probably still the best enzyme for estimation of global brain injury following cardiac or respiratory arrest, in which high levels in CSF are correlated with poor outcome.\textsuperscript{3-4} CSF CK-BB measurements would not appear to be optimal for accurate estimation of infarct size unless regional variations are taken into account.

References

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