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BRAIN RESEARCH

Research Report

N-acetylaspartate synthesis in the brain: Mitochondria vs. microsomes

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ABSTRACT

Several reports during the last three decades have indicated that biosynthesis of N-acetylaspartate (NAA) occurs primarily in the mitochondria. But a recent report by Lu et al. in this journal [2004; 122: 71–78] and subsequent two reports that cited those data suggested a predominant microsomal localization of the NAA biosynthetic enzyme, which is surprising in view of what is known about the biological functions of NAA. Therefore we reinvestigated this issue in rat brain homogenates using a similar fractionation procedure used by Lu et al. but without the loss of enzyme activity that they have encountered. We found that about 70% of the total Asp-NAT activity in the crude supernatant was present in the mitochondrial fraction which is about 5 times more than that in the microsomes. We found similar results in the case of the enzyme from bovine brain. In subsequent studies, we also have found that Asp-NAT activity in the bovine brain is very similar to that in the rat brain in substrate specificity and chromatographic characteristics including the high molecular weight pattern (approx. 670 kD) on size-exclusion HPLC.

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1. Introduction

N-Acetylaspartate (NAA) is an abundant (5–10 mM) amino acid derivative that is found almost exclusively in the nervous system (Tallan et al., 1956). It is widely used clinically as a noninvasive marker of functional integrity of neurons (Clark, 1998). NAA is found to be localized primarily in the neurons (Moffett et al., 1991; Moffett and Namboodiri, 1995; Simmons et al., 1991), and is degraded by the enzyme aspartoacylase (ASPA) which is localized in the oligodendrocytes (Baslow et al., 1999; Kirmani et al., 2002; Klugmann et al., 2003; Madhavarao et al., 2004). Other studies have shown area specific decreases of NAA in the brain in a variety of neuropsychiatric and cognitive disorders (Clark, 1998; Ferguson et al., 2002; Friedman et al., 1998; Jung et al., 1999; Rajanayagam et al., 1996; Ross and Sachdev, 2004; Sibbitt et al., 1997; Tsai and Coyle, 1995). NAA synthesis in mitochondria is catalyzed by the enzyme aspartate-N-acetyltransferase (Asp-NAT; EC 2.3.1.17) which acetylates the amino group of aspartate using acetyl CoA (Goldstein, 1959; Truckenmiller et al., 1985). This enzyme has been found to be restricted to the central nervous system (Benuck and D'Adamo, 1968; Truckenmiller et al., 1985).

Earlier studies on the subcellular localization of Asp-NAT in the brain have indicated that Asp-NAT is localized primarily in the mitochondria (Madhavarao et al., 2003; Patel and Clark, 1979; Truckenmiller et al., 1985). Accumulating evidence in the last decade suggests that NAA is a marker of mitochondrial dysfunction in the brain (Bates et al., 1996; Dautry et al., 2000; Heales et al., 1995; Mathews et al., 1993; Schweinsburg et al., 2005; Signoretti et al., 2001; Signoretti et al., 2004; Vagnozzi et

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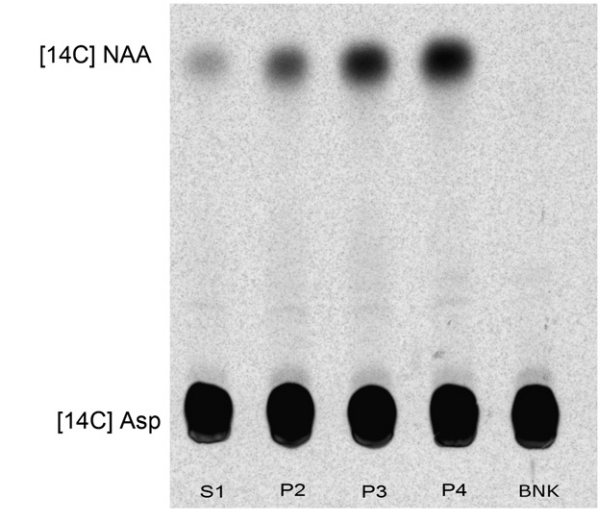
al., 2007, also see the recent review on NAA (Moffett et al., 2007)). However, a recent report by Lu et al. has indicated a bimodal subcellular distribution with a predominant presence in microsomes (Lu et al., 2004). Citing the work of Lu et al., a report on Aralar, an aspartate glutamate carrier protein, which is thought to function in conjunction with the malate–aspartate shuttle, indicated NAA synthesis to occur predominantly outside the mitochondria (Jalil et al., 2005; Satrustegui et al., 2007). It is surprising for the Asp-NAT activity to be predominantly associated with the microsomes in view of what is known about the biosynthesis and functions of NAA (Benuck and D'Adamo, 1968; Chakraborty et al., 2001; Clark, 1998; Madhavarao et al., 2003; Patel and Clark, 1979). Therefore, we reinvestigated this issue using similar methods used by Lu et al. to rule out methodological differences for this discrepant observation. The results showed that Asp-NAT activity is predominantly localized in the mitochondrial fraction in the rat brain. For additional verification, we studied subcellular distribution of Asp-NAT enzyme in the bovine brain and found it to be similar to that in the rat brain. Furthermore, we purified Asp-NAT enzyme from bovine brain and determined its approximate molecular weight to compare it to the rat enzyme to facilitate future studies of this clinically and neurobiologically important enzyme system in the brain.

2. Results

Fig. 1 shows a typical assay in which the radiolabelled product [^{14}C]-NAA is separated from the substrate [^{14}C] L-Asp by TLC. The product [^{14}C]-NAA is quantified with [^{14}C] L-Asp standards and Asp-NAT activity is expressed as n moles of product/h/mg protein. In a typical fractionation experiment, the subcellular distribution of Asp-NAT activity of rat brain using the same method used by Lu et al. is given in Table 1. A comparison of the distribution of Asp-NAT activity in crude mitochondrial versus microsomal fraction from rat and bovine brain using the buffer conditions in our method is given in Table 2. In the first set of experiments (Table 1), we repeated the same procedure used by Lu et al. to relate to their results as closely as possible. We used the same buffer as well as centrifugation conditions to separate each of the subcellular fractions. However, we did not purify the mitochondria further using a density gradient centrifugation as Lu et al. did because of the reasons described in the discussion section. In the second set of experiment (Table 2), the fractionation was done under different buffer conditions (see the Experimental procedures). In both these preparations, the homogenizations of the brain tissue and centrifugation steps for fractionation were exactly same. Since the buffer conditions were different, the assay conditions also became different. In order to replicate the Lu et al. method of assay, we used higher concentrations of the substrates, 2 mM L-Asp and 2 mM acetyl CoA, with an additional 50 mM NaCl. In our method, lower concentrations of the substrates, 1 mM L-Asp and 1 mM acetyl CoA are used. The overall higher activity (compare rat brain enzyme activities in Tables 1 and 2) of the Lu et al. method in our hands is likely to be a result of the higher substrate concentrations. However, in the data reported by Lu et al. {see Table 2 of Lu et al., 2004}, the total activity value was much lower than that which is obtained in the present studies.



Sp activity	Crude Supt(S1)	Crude Mito(P2)	Intermediate(P3)	Microsomes(P4)
(nmoles/hr/mg)	2.6	5.6	10.5	22.4

Fig. 1 – Subcellular fractionation of Asp-NAT activity. Asp-NAT activity in different subcellular fractions of rat brain homogenate is depicted; S1: crude supernatant (Crude Supt) of 800 *g* spin of the homogenate, P2: crude mitochondrial (Crude Mito) pellet, P3: intermediate pellet, P4: microsomal pellet, BNK: without enzyme. Approximately 100 μg of protein from each fraction is included for assay. See Experimental procedures section for detailed methods of fractionation and assays. The picture shows the activity of each fraction as the product spot (on the top area of the picture) which is the [^{14}C]-NAA separated from [^{14}C] Asp (on the bottom area). Specific activity values are given in the table at the bottom.

The lower activity in Lu et al. study cannot be explained by substrate concentrations since they used the higher substrate concentrations as mentioned above. One possibility is that they used a lower amount of brain tissue, obtained from one to two rats as given in their article. We used exactly two rat brains in all the comparison studies. Another possibility is that they used frozen brains and this information is unclear in their report. We have always used fresh brains for these studies. Animal to animal variations also might have contributed to the lower enzyme activity. We have seen batch to batch variations in the total activity in the purification of a related enzyme, aspartoacylase, from rat brain and kidney (Hershfield et al., 2006). Earlier, we also have purified another *N*-acetyltransferase, arylalkylamine *N*-acetyltransferase, from brain and pineal gland of rat and sheep (Namboodiri et al., 1987a,b). We have seen batch to batch variations in those cases as well. In our hands, the percentage distribution of total activity in crude mitochondrial (Crude Mito) and microsomal fraction was consistent in spite of the differences between our method and Lu et al. method. About 70% of the total activity of the crude supernatant was distributed in the crude mitochondrial pellet alone. This is about 5–6 times of the total activity that was found in the microsomal fraction in rat brain preparations; as percentage of the total activity, microsomal enzyme activity