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SHORT COMMUNICATION

A Comparative Study of Methionine Adenosyltransferase Activity and Regional Distribution in Mammalian Spinal Cord

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ABSTRACT. To provide a background for future studies on neurodegenerative changes in the spinal cord, the present study analysed the distribution of the activity of methionine adenosyltransferase (ATP:L-methionine *S*-adenosyltransferase, EC 2.5.1.6, MAT), an enzyme that catalyses the synthesis of the biological methyl group donor *S*-adenosylmethionine (AdoMet), in spinal cords from bovine and pig, and compared the results with those from human spinal cord. The enzyme activity was estimated by a radiochemical method measuring the rate of formation of [^{3}H]AdoMet from L-[methyl-^{3}H]methionine and ATP. The MAT activity (V_{max}) was quite homogeneously distributed between spinal regions and species investigated (19–50 pmol [^{3}H]AdoMet/mg protein/minute), with the highest level found in the male bovine group. The bovine group (both males and females) also presented a 20% higher enzymatic activity in the dorsal horn as compared to the ventral horn and white matter areas. In the pig spinal cord, the highest level of activity was found in the white matter. The lowest affinity for methionine (highest K_m) was found in the human spinal cord. Whole spinal cords of one cat and one rhesus monkey were also analysed and the levels of MAT activity were similar to that of humans and bovine females, respectively. Studies of MAT stability in the rat spinal cord (post-mortem time 0–72 hr) showed a significant decrease in enzyme activity during the interval of 0–8 hr (23°). From this time point on and up to 72 hr (4°), the significant decrease in the activity remained at 60% of the initial value. BIOCHEM PHARMACOL **60**;3:441–445, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. transmethylation; bovine; pig; human; cat; rhesus monkey

AdoMet† is the primary biological methyl group donor of the one-carbon cycle in the cell. It is involved in transmethylation reactions such as the synthesis of proteins, monoamines, phospholipids, neurotransmitters, and nucleic acids [1]. The demethylated product of AdoMet is *S*-adenosylhomocysteine (AdoHcy), which can be recycled back to methionine and AdoMet via the enzyme methionine synthase (MS) (Fig. 1). Moreover, decarboxylated AdoMet participates in the formation of the polyamines spermidine and spermine, considered to be important in both cell regeneration and differentiation [2–5]. The synthesis of AdoMet from methionine and ATP is catalysed by the enzyme MAT. MAT is present in all cells and shows a high degree of conservation between species [6]. Mammalian MAT exists in three isoforms, MAT I, MAT II, and MAT III [7, 8]. MAT I and MAT III are expressed exclusively in the liver. MAT II is also present in the liver, but represents the predominant form of MAT in tissues such as blood, kidney, and the CNS. Values of K_m in the range of 2–10 μM for methionine have been reported for MAT II in human lymphocytes, erythrocytes, brain and spinal cord, rat kidneys and lens, and bovine brain [6, 9–12].

Investigations performed in mice, monkeys, and pigs have provided valuable information on the role of transmethylation mechanisms in neurodegenerative and muscular diseases. Thus, in these species, an association has been found between a defect in the synthesis of methionine and/or MAT activity and the development, for example, of myelopathies, ataxia, peripheral neuropathy, and subacute combined degeneration of the spinal cord [13–15]. Deficiencies in transmethylation reactions have also been reported in patients with demyelination of the CNS, dementia disorders, Parkinson's disease, and schizophrenia [10, 11, 16, 17].

To provide a background for further investigations on transmethylation mechanisms and neurodegenerative changes in the spinal cord, the present study analysed the activity of MAT in bovine, pig, cat, and rhesus monkey spinal cords. The regional distribution of the enzyme through the dorsal, ventral, and white matter regions was also investigated in the spinal cords of bovines and pigs. Additionally, results of MAT activity and distribution in human spinal cord were added from a previous study by our group [12]. The post-mortem stability of MAT activity in the spinal cord was examined in rats.

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† *Abbreviations:* AdoMet, *S*-adenosylmethionine; and MAT, ATP:L-methionine *S*-adenosyltransferase, EC 2.5.1.6.

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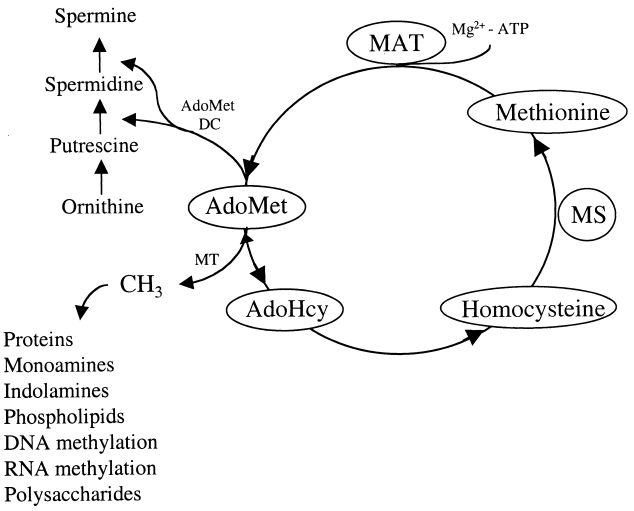

FIG. 1. The one-carbon cycle. MAT, methionine adenosyltransferase; AdoMet, *S*-adenosylmethionine; AdoHcy, *S*-adenosylhomocysteine; MS, methionine synthase; MT, methyltransferase enzymes; AdoMetDC, *S*-adenosylmethionine decarboxylase.

MATERIALS AND METHODS

Chemicals

L-[Methyl-^{3}H]methionine (191 mCi/mmol) was obtained from Dupont NEN Research Products. The ion-exchange resin Bio-Rad AG 50W-X8 was from Bio-Rad Laboratories. Ultra Gold® liquid scintillation fluid was from Beckman Instruments. All other chemicals were from Sigma Chemical Co. and were purchased from Kebo.

Stability of MAT in the Rat Spinal Cord

The post-mortem stability of MAT was measured in spinal cords of 45 male Sprague–Dawley rats (220–250 g) at 0, 2, 4, 8, 16, 24, 36, 48, and 72 hr post-mortem, 5 animals/time interval. The animals received food and water *ad lib*. After decapitation, the spinal cords were left *in situ* at room temperature (23°) for the first 8 hr post-mortem and the remaining time at 4°, before dissection. Tissues were immediately frozen in liquid nitrogen and stored at −75° until analyses. The ethics committee of animal studies in Uppsala (Uppsala djurförsöksetiska nämnd), Sweden approved this study.

Regional Distribution of MAT Activity

Spinal cords from 7 pigs (castrated males) and 13 bovine animals (7 females and 6 males) were obtained from a local slaughterhouse. Tissues were kept on ice until cut into 10-mm thick sections and frozen on a metal plate in liquid nitrogen before storage at −75°. The dorsal horn, ventral horn, and white matter regions were dissected from frozen tissue and kept in liquid nitrogen before homogenised and processed for MAT and protein assays. Spinal cords of one cat and one rhesus monkey were also kept at −75°. The main characteristics of the different animal species and human material are presented in Table 1. The post-mortem delay is defined as the time elapsed between death and freezing of the tissue.

The preparation of homogenates and analyses of MAT activity and proteins were performed according to previously described methods [18–20]. For the MAT assay, tissue extracts (20 μg protein), pH 7.7, were incubated for 60 min at 37° with L-[methyl-^{3}H]methionine, ATP, and unlabelled methionine in the range of 1–60 μM. The apparent values of MAT V_{max} (pmol [^{3}H]AdoMet/mg protein/min) and K_m (μM methionine) were determined by linear regression of the double reciprocal plots.

Statistical Analyses

Values are given as means ± SEM. Comparisons between the groups were performed by factorial ANOVA followed by the Bonferroni/Dunn *post hoc* test.

RESULTS

Stability of MAT in the Rat Spinal Cord

There was a significant decrease in MAT activity during the interval from 0–8 hr in spinal cords from rats maintained at room temperature (23°) after decapitation. From this time point on and up to 72 hr post-mortem (4°), the significant decrease in the activity of MAT remained at the level of 60% of the initial value, [$F = 9.65$; df 8, 34; $P < 0.0001$].

Distribution of MAT Activity in the Spinal Cord

Differences in MAT activity between species were observed for V_{max} [$F = 49.03$; df 4, 65; $P < 0.0001$] and K_m [F =

TABLE 1. Main characteristics of the mammalian species

Species	N	Sex F/M	Age (years)	Post-mortem delay (hr)	Spinal cord level
Bovine	13	7/6	5/2.5	3/4	C8–Th6
Pig	7	M (castrated)	≈0.6	2.5–5	C7–Th9
Cat	1	F	0.5	1.5	C8
Rhesus monkey	1	unknown	adult	6	C
Human*	7	3/4	72.0 ± 2.9 (Mean ± SEM)	26.1 ± 3.1	C3–Th4

N = number of cases, M = male, F = female, C = cervical, Th = thoracic.

*Ekegren *et al.*, 1999. Experimental Neurology **158:** 422–427, published by Academic Press. With the permission of the Editor.

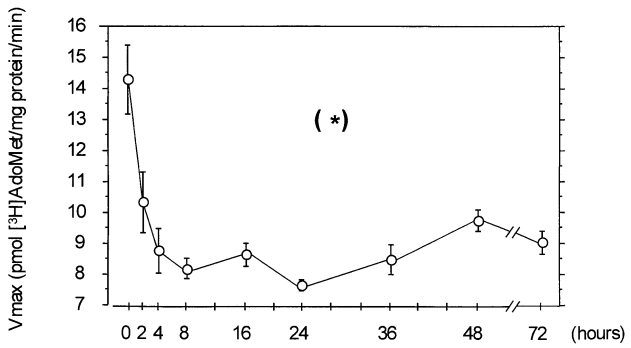

FIG. 2. Effect of post-mortem time on MAT activity in the rat spinal cord. The spinal cords were left *in situ* at 23° for 0–8 hr post-mortem and the remaining time at 4°. N = 5, except at time points 24 and 72 hr (N = 4). All data are means ± SEM.*$P < 0.0001$ (2–72 hr).

121.15; df 4, 65; $P < 0.0001$]. Analyses of the activity (V_{max}) of the enzyme between species showed the highest MAT activity in the male bovine group ($P < 0.0001$ for dorsal and ventral horns in comparison with the corresponding regions of pigs, humans, and bovine females and $P < 0.0001$ for white matter in comparison with the levels of humans and bovine females). The bovine group (both males and females) also presented a 20% higher enzymatic activity in the dorsal horn as compared to the ventral horn and white matter areas. In the pig spinal cord, the highest level of activity was found in the white matter region, $P < 0.005/P < 0.001$ as compared to dorsal horn/ventral horn and $P < 0.0001$ in comparison with human and bovine female white matter areas (Fig. 3A). Concerning the affinity (K_m) of the enzyme, the lowest affinity for methionine (highest K_m) was found in the human spinal cord ($P < 0.0001$ in comparison with all other species and regions). In this group as well, a significant difference in the K_m value between female and male human ventral horn areas was observed when analysed with the Fisher's PLSD *post hoc* test ($P < 0.05$), but not with the Bonnferroni/Dunn test. In the pig spinal cord, a lower affinity for methionine was shown in the white matter as compared to dorsal horn ($P < 0.01$) and ventral horn ($P < 0.0005$) (Fig. 3B). Analyses in whole spinal cords of one cat and one rhesus monkey demonstrated MAT activity levels comparable to those of human and female bovine spinal cords (V_{max}: 21.66/27.23 pmol [^{3}H]AdoMet/mg protein/min and K_m: 2.85/3.55 μM methionine, respectively).

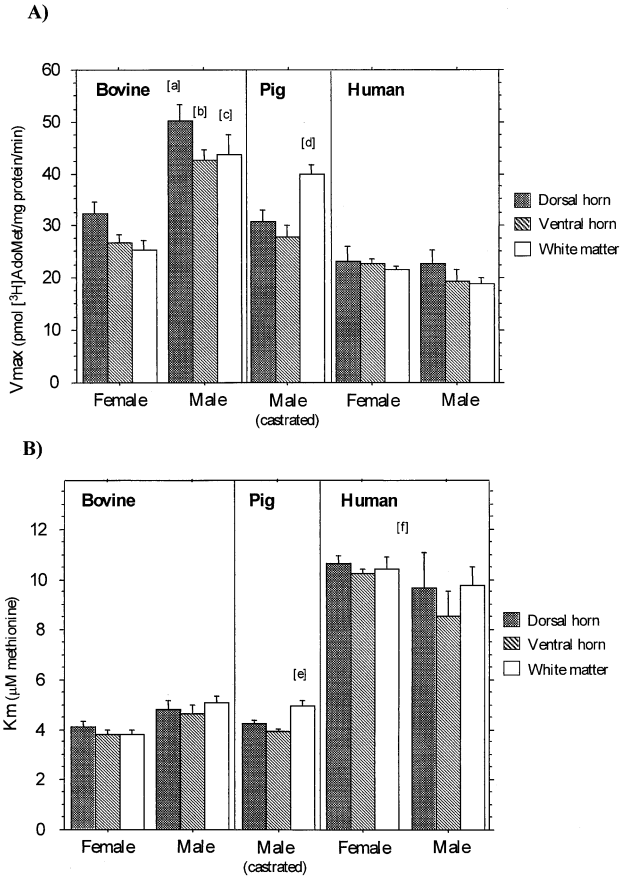

FIG. 3. Regional distribution of MAT activity in spinal cords of bovine (F/M, N = 7/6), pig (N = 7), and human (F/M, N = 3/4) material. All data are means ± SEM. MAT V_{max} (Fig. 3A) and MAT K_m (Fig. 3B) in dorsal horn, ventral horn, and white matter regions. V_{max}: [a]: significantly different in comparison with corresponding regions of pig, human, and bovine female ($P < 0.0001$) [c]: significant difference as compared to white matter regions of human and bovine female ($P < 0.0001$); and [d]: significant difference as compared to pig dorsal ($P < 0.005$) and ventral horn ($P < 0.001$) and white matter of human and bovine female ($P < 0.0001$). K_m: [e]: significant difference as compared to pig dorsal ($P < 0.01$) and ventral horn ($P < 0.0005$) regions [f]: the K_m of human dorsal horn, ventral horn, and white matter is significantly different in comparison to all regions of the other species ($P < 0.0001$).

DISCUSSION

This is to our knowledge the first comparative study of MAT activity in mammalian spinal cord. Furthermore, it also provides information on the post-mortem stability of the enzyme and its regional distribution in spinal cords of bovine and pig. The stability of MAT investigated in the rat spinal cord showed a decrease in enzyme activity during the first 8 hr post-mortem, after which it remained at 60% of the initial value. This is in accordance with a study of MAT stability in the rat brain, which found a steady decrease in activity at room temperature but no significant change at 4° [20]. Extrapolated to autopsy and storage conditions, the present results suggest that MAT should be stable enough for studies in post-mortem tissues. The levels of MAT activity in the spinal cords of bovine, pig, human, one cat, and one rhesus monkey were quite homogeneous, although a higher MAT V_{max} was found in the bovine dorsal horn as compared to the ventral and white matter areas. It could be speculated that this distribution of MAT activity reflects the different amounts of cells in the spinal cord regions, since MAT has been found to be localised almost entirely within the cytoplasmic fraction of the cell [21]. In spite of the low cell content in the white matter

areas, we found rather high MAT activities in this region, probably indicating the importance of transmethylation in myelination reactions in the spinal cord, as shown elsewhere [5, 13, 16, 22]. The significantly higher MAT activity in the white matter of the castrated pigs as compared to grey matter might be connected to an increased lipid metabolism in that area. It has been demonstrated that castrated male pigs have a higher adipose tissue lipogenesis in comparison to normal males and females [23], and AdoMet is known to be involved in the methylation of phospholipids.

The affinity of the enzyme in human spinal cord [≈10 μM methionine] is consistent with findings reported for human brain [20] and bovine brain [9]. However, the MAT affinity in the spinal cords of bovine, pig, cat, and rhesus monkey was much higher as compared to that of the bovine brain [9], although still within the range of MAT II enzyme properties for K_m [2–10 μM methionine]. The MAT activity in the spinal cord of the rhesus monkey was previously examined by Volpe *et al.* [24], but expressed in units/g wet weight of tissue and therefore not comparable to the kinetic analysis of the present study.

Interestingly, the male bovine spinal cord showed a 60% higher activity than that of bovine females. Gender differences in MAT activity were found in erythrocytes of patients with the neurodegenerative disease amyotrophic lateral sclerosis [12]. Furthermore, it has been demonstrated that the treatment of female mice with testosterone induces a significant increase in MAT activity in their kidneys [25].

In conclusion, this comparative study showed a quite homogeneous distribution of MAT activity among the species and regions investigated. The highest enzyme activity was observed in the male bovine group and the lowest affinity for methionine in human spinal cord. The gender difference found in the bovine group might be a subject for further investigations.

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