Our results indicated that the greater part of the Hyp in the serum is not present as free amino acid, and that most of the proteolytic enzymes which can degrade large peptides to amino acids[4,5] do not participate in the postpartum collagen degradation. The increase of both the Hyp concentration and the dry weight of the sediment is consistent with our previous reports[1-3] that most of the uterine collagen degrades during the first two postpartum days.

These results are in accordance with previous observations that the uterine collagenase activity is high during the first two postpartum days[10,11], that the purified uterine collagenase can degrade collagen fibers to small peptides[12], and that a lysosomal proteolytic enzymic activity (cathepsin) is low during the period of rapid degradation of collagen[13].

In summary, uterine collagenase degrades collagen fibers to small peptides and then small peptides are removed by the blood stream.

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Regional pattern and heat-resistance of brain 5′-deoxy-5′-methylthioadenosine phosphorylase

A. Abbruzzese*, G. Della Pietra and R. Porta

Dept of Biochemistry, 1st Medical School, University of Naples, Naples (Italy), 30 September 1985

Summary. The distribution of 5′-deoxy-5′-methylthioadenosine (MTA) phosphorylase in 10 pig brain areas was determined. The observed regional differences of the enzymatic activity seem to reflect more the pattern of brain spermine distribution rather than that of spermidine. Moreover, comparative studies on the heat-resistance of MTA phosphorylase extracted from the whole brain of various species suggest structural differences in the enzyme molecules occurring in the brains of different animals.
Key words. Polyamines; methylthioadenosine; methylthioadenosinephosphorylase; brain; regional distribution.

5′Deoxy-5′-methylthioadenosine (MTA) is a sulfur-containing nucleoside produced stoichiometrically during the synthesis of the polyamines spermidine and spermine, and rapidly cleaved to adenine and 5-methythioribose-1-phosphate by a specific phosphorylase[1]. MTA phosphorylase, an enzyme exhibiting an absolute requirement for inorganic ortho-phosphate, has been partially purified from several sources[2-6]. The finding of the enzyme in most mammalian tissues explains the intracellular free adenine pool and the almost ubiquitous occurrence of the adenine phosphoribosyltransferase activity which converts adenine to purine nucleotides.

Recently, we reported the occurence of MTA phosphorylase in mammalian brain together with its development and subcellular distribution[7]. In the present communication we describe the regional distribution of the enzyme in the pig brain and some interspecies comparative investigations on the heat-resistance of the cerebral enzyme.

Whole fresh brains from pigs, cows and sheep were obtained from a local slaughterhouse. Adult rats (Wistar strain) and mice (Swiss strain) were from Morini, S. Polo d'Enza (RE), Italy, and were housed (07.00–19.00 light cycle followed by a 12 h dark cycle) in stainless steel cages (six animals per cage) for at least 48 h before sacrifice. For the regional distribution studies the brains were dissected in a cold-room over dry ice using a razor blade splint. The whole brains or the dissected cerebral areas were weighed and then homogenized in 5 vols of ice-cold 50 mM potassium phosphate buffer, pH 7.4, containing 0.3 M sucrose and 5 mM dithiothreitol. The supernatants recovered after centrifugation of the homogenates at 104,000 × g for 1 h were used as enzyme sources. MTA phosphorylase activity was determined by a previously described radiometric method[7,8] using as substrate 5′-(methyl-^{14}C)methylthioadenosine prepared by acid hydrolysis[9]. The reaction was linear with time and the amount of protein under experimental conditions used. One unit of enzyme catalyzes the formation of 1 nmol of 5-methylthioribose-1-phosphate in 60 min at 37 °C. Protein was determined by the method of Lowry et al.[10].

MTA phosphorylase distribution in pig brain was quite uniform with only moderate regional differences (table). Frontal cortex, hippocampus, thalamus and hypothalamus (spermine-rich structures) were shown to contain the highest enzymatic activity, whereas the medulla oblongata and the caudate nucleus (sper-

Specific activity and concentration of MTA phosphorylase in different areas of pig brain[a]

Area	N[b]	Specific activity (units/mg of protein)	Concentration (units/g of tissue)
Frontal cortex	4	61.6 ± 3.1	1640 ± 97
Hippocampus	4	55.7 ± 5.1	1566 ± 89
Cerebellar cortex	4	53.7 ± 3.1	1466 ± 91
Thalamus	4	53.4 ± 4.9	1326 ± 54
Hypothalamus	3	52.2 ± 4.5	1696 ± 97
Midbrain	4	44.8 ± 3.7	1521 ± 66
Pons	4	40.5 ± 0.7	1486 ± 79
Caudate nucleus	5	40.4 ± 4.5	1213 ± 59
Substantia nigra	3	36.6 ± 3.5	396 ± 44
Medulla oblongata	4	19.9 ± 0.9	835 ± 62

[a] Values are means ± SEM; [b] Number of animals.

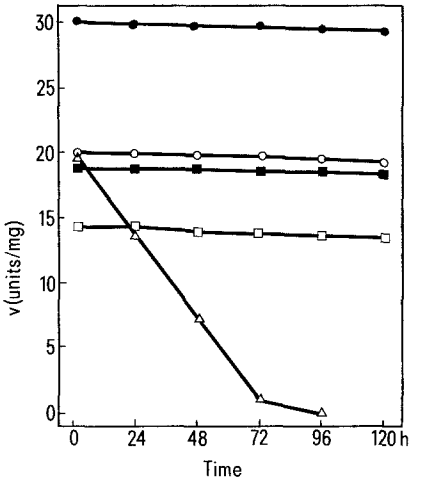

Figure 1. Resistance of brain MTA phosphorylase from various mammals to the storage at 4 °C. Enzymatic activity in the supernatants obtained from cerebral homogenates of pig (●), sheep (○), cow (■), rat (□) and mouse (△) were assayed at different times after extraction.

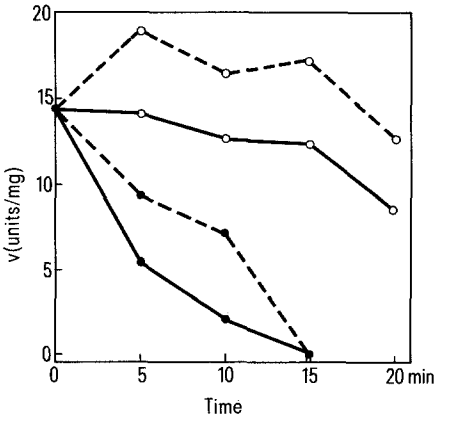

Figure 2. Effect of the heat-treatment on mouse brain MTA phosphorylase activity. Experimental procedures were described in the text. Specific activity of the enzyme occuring in the suspension (–) and in the resulting supernatant at 13,000 × g (---) after different exposure times of the homogenate supernatant at 50 °C (○) or at 60 °C (●).

midine-rich structures) contained the lowest activity. Thus, MTA, which is produced during the biosynthesis of both spermidine and spermine, could be more rapidly metabolized in the cerebral areas where spermine levels are higher. In this respect it would be of interest to correlate the regional distribution of MTA phosphorylase to that of its nucleoside substrate, but, unfortunately, data on the MTA regional pattern are not so far available in literature.

A different set of experiments, carried out to investigate possible differences in the molecular characteristics of MTA phosphorylases occurring in the brain of several mammals, showed a higher sensitivity of the mouse brain enzyme to heat-denaturation. Preliminary observations (fig. 1) indicated a minor resistance at 4 °C of the enzyme extracted from the whole brain of the mouse, relative to that obtained from the brain of cow, sheep, pig and rat. In fact, while the catalytic activity of the mouse enzyme was completely lost after 96 h of cold storage, no changes in the specific activity of the cerebral MTA phosphorylase extracted from the other animals were observed up to 5 days.

Further experiments were carried out to study the effect of temperature on the mouse brain enzymatic activity. Aliquots of supernatant (containing about 0.5 mg of protein) were heated either at 50 °C or at 60 °C in a water bath. At the times indicated in figure 2 the samples were cooled in an ice bath and then assayed for MTA phosphorylase activity. Furthermore, aliquots of the heat-treated samples were centrifuged at 13,000 × g for 15 min and the supernatants were also assayed for enzyme activity and protein concentration. The data shown in figure 2 confirm the lower resistance of the mouse brain enzyme to heat denaturation in comparison to that of the rat brain enzyme. In fact, its activity was reduced to under 50% after 5 min of exposure at 60 °C and was completely lost after 15 min, whereas the rat brain enzyme is known to retain almost all of its catalytic activity following 20 min of the same heat-treatment[7]. Conversely, a more moderate heat exposure (5 min at 50 °C) led to an increase of about 1.3-fold in the specific activity of the enzyme occurring in the supernatant at 13,000 × g with no loss of enzyme total activity.

Experiments devoted to the analysis of other specific properties of the mouse brain enzyme in relation to its molecular characteristics are currently in progress.

*Present address: Enzyme Chemistry Section, Laboratory of Oral Biology and Physiology, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20205, USA.

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