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Regional and Subcellular Distribution of *S*-Adenosylhomocysteine Hydrolase in the Adult Rat Brain

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Abstract: The regional distribution of *S*-adenosylhomocysteine hydrolase was determined in the rat brain. Small variations in enzyme activity between different regions were observed. Highest activity was found in hypothalamus and bulbus olfactorius, the least in pons and medulla. About 70% of the enzyme activity was recovered in the soluble fraction of the tissue homogenate and 25% was localized to the crude mitochondrial fraction. The corresponding values for lactate dehydrogenase were 40% and 50%, respectively. The small amount of enzyme (5%) sedimenting with the nuclear fraction could be explained by contamination of this fraction with soluble proteins and synaptosomes. Further separation of the crude mitochondrial fraction by discontinuous sucrose gradient centrifugation showed that most of the enzyme activity was localized to the synaptosomes, but a substantial amount was found in the top layer of the gradient. The relative specific activity of lactate dehydrogenase in the top layer was less than that of *S*-adenosylhomocysteine hydrolase. No time-dependent leakage of *S*-adenosylhomocysteine hydrolase from the synaptosomes could be demonstrated. After hypoosmotic treatment of the crude mitochondrial fraction and separation of this fraction on a discontinuous sucrose gradient, *S*-adenosylhomocysteine hydrolase and DOPA decarboxylase showed the same distribution in the gradient and were recovered in the cytoplasmic fraction.
Key words: *S*-Adenosylhomocysteine hydrolase—Rat brain.

S-Adenosyl-L-homocysteine hydrolase (EC 3.3.1.1.) catalyses the reversible hydrolysis of *S*-adenosylhomocysteine (Ado-Hcy) to adenosine and homocysteine (de la Haba and Cantoni, 1959). Ado-Hcy, which is formed from *S*-adenosylmethionine upon transmethylation of *S*-adenosylmethionine to cellular acceptors (Cantoni and Scarano, 1954), is a potent inhibitor of methyl transfer reactions (Hurwitz et al., 1964; Zappia et al., 1969; Deguchi and Barchas, 1971; Kerr, 1972; Coward et al., 1972; Pugh et al., 1977). Ado-Hcy may be a regulator of cellular methylation reactions. The cellular level of this metabolite is probably influenced by the enzyme *S*-adenosylhomocysteine hydrolase, which therefore may participate indirectly in the control of cellular methylation reactions (Finkelstein and Harris, 1973; Walker and Duerre, 1975; Schatz et al., 1977; Ueland and Sæbø, 1979).

Few data exist on the subcellular localization of *S*-adenosylhomocysteine hydrolase (Fox and Kelley, 1978). The enzyme activity was mainly recovered in the soluble fraction of rat liver (Finkelstein and Harris, 1973). The activity associated with the nuclear fraction could be explained by contamination with soluble proteins, and the enzyme seems to be localized exclusively to the cytosol (Ueland et al., 1979). The brain enzyme also appeared to be a

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Abbreviations used: Ado-Hcy, *S*-Adenosylhomocysteine; HEPES, *N*-2-Hydroxyethylpiperazine-*N*,*N*'-2-ethanesulphonic acid.

soluble protein, although a significant amount of *S*-adenosylhomocysteine hydrolase activity was associated with the nuclear fraction. Unexpectedly, no activity could be detected in the synaptosomal fraction (Schatz et al., 1979).

The regional and subcellular localization of *S*-adenosylhomocysteine hydrolase in rat brain was the subject of the present study. The distribution of the enzyme in the subcellular fractions was compared with the localization of marker enzymes for the soluble fraction [lactate dehydrogenase] and the mitochondrial fraction [fumarate hydratase (fumarase)]. The possibility of leakage of the enzyme from the synaptosomes was also investigated.

MATERIALS AND METHODS

Animals

Male Wistar rats, weighing 150–250 g, were used.

Chemicals

S-Adenosylhomocysteine, adenosine, inosine, hypoxanthine, adenosine deaminase (type 1 from calf intestinal mucosa) and HEPES were obtained from Sigma Chemical Co. Polyethyleneimine 400 was purchased from Serva, Heidelberg, and cellulose powder (MN-300) from Macherey Nagel Co., F.R.G. Polyethyleneimine-impregnated cellulose thin-layer sheets (0.25 mm) on glass plates were prepared as described by Randerath and Randerath (1967) and were developed in distilled water before use. [8-^{14}C]Adenosine (59 mCi/mmol), L-3,4-dihydroxyphenyl[3-^{14}C]alanine (8.4 mCi/mmol) were from the Radiochemical Centre, Amersham, U.K. *S*-[8-^{14}C]Adenosylhomocysteine (59 mCi/mmol) was synthesized enzymatically and purified as described previously (Ueland and Sæbø, 1979). *S*-Adenosylhomocysteine hydrolase was purified to homogeneity from mouse liver according to a purification scheme published elsewhere (Ueland and Døskeland, 1977).

Preparation of Subcellular Fractions

The method described by Gray and Whittaker (1962) was used. The brains, including cerebellum, were taken out and homogenized in 0.32 M-sucrose (approx. 10% w/v of tissue). The homogenate was separated into crude nuclear (P_1), crude mitochondrial (P_2), microsomal (P_3), and high-speed supernatant (S_3). The P_2 fraction was further separated on a discontinuous sucrose gradient into myelin (A), synaptosomes (B), and mitochondria (C). The intrasynaptosomal localization of the enzymes was studied by resuspending the P_2 fraction in distilled water (5 ml/g original tissue) and separating the components on a discontinuous sucrose gradient (Whittaker et al., 1964). The fractions were cytoplasm (O), vesicles (D), membranes (E, F, G), undisrupted synaptosomes (H), and mitochondria (I).

The leakage of *S*-adenosylhomocysteine hydrolase from synaptosomes was studied by resuspending the P_2 fraction in 5 vol. of 0.32 M-sucrose. The procedure for preparation of the P_2 fraction was shortened to minimize leakage during the preparation (no washing of the P_1 fraction and reducing centrifugation period from 60 to 30 min). The suspension was placed on ice and samples were taken every 30 min. The samples were centrifuged for 30 min at 27,000 *g* and the enzyme activities measured in pellet and supernatant.

Enzyme Assays

All samples were treated with 0.2% Triton X-100 by the addition of a 10% solution, to release occluded enzymes.

S-Adenosylhomocysteine hydrolase activity was determined by a radiochemical method published previously (Ueland and Sæbø, 1979). Briefly, [8-^{14}C]adenosylhomocysteine (20–150 μM) was incubated at 37°C in the presence of enzyme and exogenous adenosine deaminase to trap adenosine formed. The incubation buffer was 15 mM-HEPES, pH 7.0, containing 0.25% bovine serum albumin, 150 mM-KCl, 5 mM-magnesium acetate, and 2 mM-2-mercaptoethanol. The reaction products (inosine, hypoxanthine, and *S*-adenosylhomocysteine) were separated by thin-layer chromatography. There was linearity of enzyme activity versus time and concentration of enzyme, and the activity was determined from the linear part of the curves.

Lactate dehydrogenase was determined at 25°C according to Johnson (1960), and fumarate hydratase (fumarase) at 25°C as described by Racker (1950). DOPA decarboxylase was assayed according to Broch and Fonnum (1972) with the modification described for choline acetyl transferase by Fonnum (1975).

The relative specific activities of the enzymes in the various fractions are expressed as the ratio of the percentage of enzyme activity to the percentage of protein content.

Protein was measured according to Lowry et al. (1950). Triton was not added to these samples.

RESULTS

S-Adenosylhomocysteine hydrolase activity was determined in whole homogenate, P_2 fraction, and S_3 fraction in the absence and presence of Triton X-100. The ratios between the values obtained in the absence and presence of detergent were 0.74, 0.25, and 1.03 in whole homogenate, P_2 fraction, and S_3 fraction, respectively (data not shown). No effect of Triton X-100 on the activity in the S_3 fraction suggested that Triton did not affect the enzyme catalysis.

Regional Distribution of *S*-Adenosylhomocysteine Hydrolase

The enzyme activity was determined in whole homogenate from 13 regions of the brain. The activities were the same when determined at 50 μM and 150 μM of Ado-Hcy, indicating that the enzyme was saturated with respect to substrate. There was a twofold variation between the region with the highest activity, hypothalamus, and that containing

the lowest enzyme level, pons-medulla oblongata (Table 1). The activity of homogeneous *S*-adenosylhomocysteine hydrolase from mouse liver was totally recovered when added to the homogenates. This indicated that the regional variation observed was not caused by the presence of factors suppressing the catalytic activity.

Subcellular Distribution

Approximately 67% of the *S*-adenosylhomocysteine hydrolase activity was recovered in the high-speed supernatant (S_3 fraction) and 25% in the crude mitochondrial fraction (P_2 fraction). The corresponding values for lactate dehydrogenase were 37% and 51%, respectively. The activities in the nuclear and microsomal fractions (P_1 and P_3) were small and did not exceed the amount of lactate dehydrogenase recovered in these fractions (Table 2).

Further separation of the crude mitochondrial fraction showed that most of the *S*-adenosylhomocysteine hydrolase activity was localized in the synaptosomal fraction, B, and the distribution was largely as for lactate dehydrogenase. The relative specific activity of *S*-adenosylhomocysteine hydrolase was, however, higher than that of lactate dehydrogenase in the myelin fraction (A) and correspondingly lower in the synaptosomes (B). The distribution of fumarase was different. This enzyme was mainly localized to the mitochondrial fraction C and partly to the synaptosomes (Table 2).

The crude mitochondrial fraction, P_2, was resuspended in a hypoosmotic medium (distilled water) and then subjected to sucrose gradient centrifugation (Table 3). *S*-Adenosylhomocysteine hydrolase activity followed closely that of DOPA decarboxylase, and was mainly recovered in the soluble (O) fraction. DOPA decarboxylase was used as a marker because of the small degree of binding to membranes (Broch and Fonnum, 1972).

The possibility of leakage of *S*-adenosylhomocysteine hydrolase from the synaptosomes was investigated by centrifugation of the P_2 fraction after various periods of time as described in Materials and Methods. We found that 78% of the *S*-adenosylhomocysteine hydrolase activity and 91% of DOPA decarboxylase activity were recovered in the pellet. The ratio between the activities in the supernatant and pellet remained constant for up to 2 h of incubation (data not shown).

TABLE 1. *Distribution of S-adenosylhomocysteine hydrolase in the rat brain*

Region	Enzyme activity[a] (nmol/min/g protein)	(nmol/min/g wet wt.)
Hypothalamus	463 ± 26	54 ± 5
Bulbus olifactorius	384 ± 27	46 ± 7
Tuberculum olifactorium	348 ± 24	48 ± 2
Thalamus	305 ± 12	39 ± 2
Corpora quadrigemina	287 ± 22	50 ± 6
Cortex, frontal	271 ± 18	33 ± 3
Cortex, occipital	264 ± 12	30 ± 4
Corpus striatum	242 ± 3	34 ± 3
Mesencephalon	239 ± 9	33 ± 2
Cerebellum	236 ± 12	34 ± 4
Hippocampus	235 ± 8	30 ± 2
Capsula interna	228 ± 8	33 ± 3
Pons+ medulla oblongata	200 ± 23	35 ± 5

[a] Means of four experiments ± S.E.M.

DISCUSSION

The finding that *S*-adenosylhomocysteine hydrolase activity was highest in the hypothalamus is in contrast to the observation of Schatz et al. (1977), who reported that the activity was lowest in this region of the brain. This discrepancy could not be explained by the presence of factors affecting the enzyme activity in our enzyme preparation. Schatz and co-workers used the high-speed supernatant of tissue homogenate as enzyme source, whereas in the present work the enzyme activity was measured in whole homogenate in the presence of detergent. The enclosure of *S*-adenosylhomocysteine hydrolase in the synaptosomes and enrichment of these structures in some areas of the brain may offer an explanation for the discrepancy. However, regardless of the reason, the main conclusion is the same: the small regional variations of enzyme activity indicate that there is no obvious reason to emphasize the importance of this enzyme in any region in the brain.

S-Adenosylhomocysteine hydrolase seems to be a soluble enzyme in both liver (Ueland et al., 1979) and brain. The activity associated with the nuclear fraction of brain homogenate could be explained by contamination with synaptosomes and soluble proteins. This statement is based on the observation that a larger portion of lactate dehydrogenase, the cytosol marker, than of *S*-adenosylhomocysteine hydrolase sediments with the nuclear fraction (Table 2).

A substantial part of the activity was recovered in the P_2 fraction (Table 2). This is explained by the localization of the enzyme in the synaptosomes (Table 2), indicating that the enzyme is present in the nerve cells.

Schatz et al. (1979) could not demonstrate any *S*-adenosylhomocysteine hydrolase activity in the synaptosomal fraction. They suggested that the enzyme leaked out of the synaptosomes during the preparation of the fraction. They did not report whether or not detergent was used to release occluded enzyme. The present experiments showed that a considerable amount of *S*-adenosylhomo-

TABLE 2. *Distribution of S-adenosylhomocysteine hydrolase and marker enzymes in homogenate from rat brain*

	Recovery[a] (% of total)	Total activity (μmol/g/min)	Distribution (% of total recovered material)				Relative specific activity			
			P_1	P_2	P_3	S_3	P_1	P_2	P_3	S_3
S-Adenosylhomocysteine hydrolase	109 ± 15	0.021 ± 0.001	5 ± 0.6	25 ± 2	3 ± 1	67 ± 2	0.23	0.49	0.56	2.96
Lactate dehydrogenase	84 ± 7	57 ± 4.8	6 ± 2	51 ± 5	6 ± 0.4	37 ± 7	0.31	1.01	0.90	1.62
Fumarase	125 ± 7	21 ± 2.1	17 ± 3	75 ± 2	3 ± 1	5 ± 2	0.84	1.46	0.56	0.21
Protein	93 ± 7	117 ± 9[b]	20 ± 2	51 ± 2	6 ± 0.3	23 ± 1				

	Recovery[a] (% of P_2)	Distribution in the P_2 fraction (% of total recovered material)			Relative specific activity		
		A	B	C	A	B	C
S-Adenosylhomocysteine hydrolase	80 ± 5	30 ± 3	54 ± 2	16 ± 1	0.81	1.32	0.73
Lactate dehydrogenase	116 ± 42	16 ± 2	65 ± 5	19 ± 4	0.43	1.59	0.86
Fumarase	111 ± 42	3 ± 2	40 ± 3	57 ± 3	0.08	0.98	2.59
Protein	99 ± 5	37 ± 2	41 ± 2	22 ± 1			

Values are means of three experiments ± S.E.M.
[a] ± S.D.
[b] mg/g.

cysteine hydrolase was associated with the P_2 fraction although the relative specific activity was less than that of lactate dehydrogenase. A higher percentage of *S*-adenosylhomocysteine hydrolase than lactate dehydrogenase was recovered in the soluble fraction when the P_2 fraction was further separated by sucrose gradient centrifugation (Table 2), suggesting the possibility of leakage of *S*-adenosylhomocysteine hydrolase. However, data in favour of time-dependent leakage of *S*-adenosylhomocysteine hydrolase from synaptosomes was not obtained.

The recovery of a higher portion of *S*-adenosylhomocysteine hydrolase than lactate dehydrogenase and DOPA decarboxylase in the supernatant obtained by sedimentation of the P_2 fraction could be explained as follows. The enzyme activity in the supernatant may represent contamination with soluble proteins. The glial elements would contribute to the soluble fraction of brain homogenate whereas the synaptosomes are derived from the nerve cells (Gray and Whittaker, 1962). This indicates different distribution of these enzymes in nerve cells versus glial elements. *S*-Adenosylhomocysteine hydrolase activity may be relatively higher in glia cells than nerve cells when compared with soluble enzymes like lactate dehydrogenase and DOPA decarboxylase.

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TABLE 3. *Distribution of enzymes and protein in P_2 fraction after hypo-osmotic treatment*

Fraction	Protein (%)	*S*-Adenosylhomocysteine hydrolase		DOPA decarboxylase	
		%	RSA[a]	%	RSA[a]
O	16 ± 1	58 ± 2	3.62	63 ± 4	3.88
D	5 ± 0.6	7 ± 0.8	1.43	6 ± 0.6	1.16
E	15 ± 1	8 ± 1	0.53	7 ± 2	0.50
F	20 ± 0.4	11 ± 0.7	0.55	7 ± 0.3	0.34
G	13 ± 2	7 ± 0.4	0.49	7 ± 2	0.53
H	11 ± 3	4 ± 1	0.35	8 ± 0.6	0.76
I	20 ± 2	5 ± 0.8	0.28	2 ± 0.5	0.11
Recovery[b] (% of P_2)	98 ± 7	98 ± 16		108 ± 9	

Values are means of four experiments ± S.E.M.
[a] Relative specific activity: % enzyme activity/% protein.
[b] ± S.D.

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