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REGIONAL AND SUBCELLULAR DISTRIBUTION OF CYSTEINE SULFINATE TRANSAMINASE IN RAT NERVOUS SYSTEM

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SUMMARY

The distribution of the cysteine sulfinate transaminase activity in adult and newborn rat central nervous system was studied and compared with the distribution of the glutamate oxaloacetate transaminase activity. The subcellular localization of both enzyme activities was also investigated. These experiments suggest that both enzymes, sometimes considered as identical, are different.

INTRODUCTION

In immature neural tissue, taurine and glutamate present the highest levels compared with other amino acids [1]. But, whereas the concentration of glutamate and other amino-acids increases during early development that of taurine decreases [2,5]. It is well known that the increase in GABA during brain development is paralleled by an increasing activity of glutamate decarboxylase [6], while the decreasing concentration of taurine is accompanied by an increase of the cysteine sulfinate decarboxylase activity (EC. 4.1.1.29) (CSD), this seems apparently to contradict the fact that cysteine sulfinate decarboxylase might be involved in regulation of taurine biosynthesis [7]. These results prompted us to investigate another enzyme cysteine sulfinate transaminase, (CSA-T) which could be responsible for the regulation of the level of the cysteine sulfinate (CSA), substrate of CSD and a key intermediate in taurine biosynthesis.

ABBREVIATIONS

CSD	cysteine sulfinate decarboxylase
CSA-T	cysteine sulfinate transaminase
CSA	cysteine sulfinate
GOT	glutamate oxaloacetate transaminase

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In an attempt to examine the role of cysteine sulfinate transaminase, the distribution of the CSA-T activity was evaluated in some areas of the adult and newborn rat central nervous system. The subcellular localization of the cysteine sulfinate transaminase activity was also investigated. These results are compared with regional and subcellular distribution of the glutamate oxaloacetate transaminase activity (EC. 2.6.1.1.) (GOT) as this enzyme and the cysteine sulfinate transaminase have been sometimes considered identical.

MATERIALS AND METHODS

Animals. Albino rats of the Wistar strain bred in our animal colony were used for the experiments. The rats were killed by decapitation and the brain quickly dissected and stored in liquid nitrogen until assayed for enzyme activity.

Enzyme assay. Brain areas were homogenized in 75 mM buffer Tris-HCl pH : 8.6. The extracts were usually analyzed immediately after homogeneisation. Cysteine sulfinate transaminase was evaluated spectrophotometrically by reading the fluorescence of NADH, formed after two enzymatic steps.

$$\text{Cysteine sulfinic acid} + \alpha\text{-ketoglutarate} \longrightarrow \text{glutamate} + \beta \text{ sulfinyl pyruvate}$$

$$\text{NAD}^+ + \text{Glutamate} \xrightarrow[\text{Glutamate dehydrogenase}]{} \alpha\text{-ketoglutarate} + NH_3 + \text{NADH} + H^+$$

Samples were incubated for 7 min in a mixture containing α -ketoglutarate 7.5 mM, CSA 15 mM, Tris-HCl buffer 75 mM, pH 8.6 in a final volume of 20 µl. The reaction was stopped by addition of 20 µl of 0.1 N HCl, H_2O_2 0.5 % and subsequent heating at 85°C for 3 min.

The tubes contents are quantitatively transferred to fluorometer tubes containing 1 ml of the following mixture (Tris-HCl buffer, pH 8.6 75 mM, ADP 0.1 mM, NAD 0.4 mM). The reaction was started by addition of 5 µl of glutamate dehydrogenase (Boehringer, 10 mg/ml in glycerol).

The fluorescence was read after 30 min at 345 nm excitation, 455 nm emission. Tissue blanks were treated identically removing only the cysteine sulfinic acid. Blank fluorescence were substracted from the fluorescence of samples. The glutamate contents were determined from a series of glutamate standards, treated in the same conditions as for the assay.

The glutamate oxaloacetate transaminase activity was measured with the same procedure described for the cysteine sulfinate transaminase. Aspartate 7,5 mM was introduced instead of cysteine sulfinic acid. In the two determinations, the endogenous glutamate was negligible with regard to the glutamate formed during the first reaction.

Subcellular fractionation.: Subcellular fractions were prepared from adult rat brain by the usual technique [8,9]. Accurate conditions are shown in Table 1 . Protein content was determined by the method of Lowry *et al.* [10]

TABLE 1. Method of subcellular fractionation of rat brain

Fractions and subfractions	Conditions	
Nuclear	900 g, 10 min	
Mitochondrial	11 500 g, 20 min	in 0,32 M sucrose
Microsomal	100 000 g, 60 min	
A) Myelin		0.8 M Sucrose
B) Myelin + nerve endings		1,0 M Sucrose
C) Nerve endings with mitochondria		1.2 M Sucrose
D) Nerve endings with mitochondria	40 000g, 120 min	1.4 M Sucrose
E) Free mitochondria		Pellet

RESULTS

a) Regional distribution. In the newborn brain, the activities of CSA-T and GOT of the different brain areas examined are very similar to each other (Table 2). The mean of activities for the different areas is 1.82 mmoles/h/g wet brain for CSA-T and 1.25 mmoles/h/g wet brain for the GOT. The differences in activities in adult rat brain areas are more marked and the mean through the areas studied are 10,02 mmoles/h/g wet weight for CSA-T and 4.98 mmoles/h/g wet weight for GOT.

The highest activity of CSA-T is observed in the striatum and the minimum in the olfactory bulb, while for GOT the highest value is also found in the striatum, but the lowest value is obtained in the pons medulla.

b) Subcellular fractionation. The results (Table 3a) show that in the primary subcellular fraction the highest specific activity is observed in supernatant for both enzymes (respectively 70,3 mmoles Glu formed/h/g protein for CSA-T and 54,3 mmoles Glu formed/h/g protein for GOT). However, significant differences appear in the distribution of total enzyme activity as 45.6 % of

TABLE 2. Regional distribution of CSA-T and GOT activities

REGION	Specific activity of the cysteine sulfinate transaminase		Specific activity of the glutamate oxaloacetate transaminase	
	mmol glu formed/h/g wet weight			
	New-born	Adult	New-born	Adult
Olfactory bulb	1.99 ± 0.18	7.93 ± 0.62	1.15 ± 0.26	4.41 ± 0.25
Pyriformal cortex	1.63 ± 0.08	11.35 ± 0.63	1.25 ± 0.06	5.20 ± 0.28
Septum		11.19 ± 1.17		5.36 ± 0.18
Striatum		12.36 ± 0.93		6.05 ± 0.16
Amygdala		9.88 ± 0.6		5.63 ± 0.84
Ventro-medial Hypothalamus	1.83 ± 0.11	10.15 ± 1.18	1.30 ± 0.04	5.14 ± 0.38
Lateral Hypothalamus		11.16 ± 0.74		5.51 ± 0.36
Hippocampus		9.69 ± 0.30		4.67 ± 0.30
Cerebellum	1.89 ± 0.17	8.36 ± 0.40	1.25 ± 0.12	4.02 ± 0.06
Pons Medulla	1.78 ± 0.18	8.14 ± 0.47	1.32 ± 0.12	3.84 ± 0.16

the total enzyme activity is found in the supernatant for GOT and only 35.0 % for CSA-T. Differences have also been determined in crude and microsomal fractions. In purified mitochondria (Table 3b) the highest specific activity is obtained for CSA-T whereas GOT presents an activity as high as in whole brain homogenate. Differences are also observed in distribution of the total activity.

c) Effect of Triton. Triton X-100, 0.1 %, was added to the incubation mixture of the enzyme. Results are given in Tables 4a and 4b. Activities of both enzymes are sensitive at different degrees to the presence of Triton in whole brain homogenate, nuclear pellet, crude mitochondria and microsomal fraction, whereas the specific activity in the supernatant remained unchanged.

TABLE 3. Subcellular distribution of CSA-T and GOT activities

TABLE 3a

	CSA-T activity in mmoles/h/g protein	Recovery of total enzyme activity	GOT activity in mmoles/h/g proteins	Recovery of total enzyme activity
Whole brain homogenate	49.0 ± 2.5	100	30.9 ± 3.3	100
Nuclear pellet	43.1 ± 4.1	6.4 ± 0.7	25.8 ± 3.2	5.9 ± 0.6
Crude mitochondria	38.9 ± 1.5	38.3 ± 5.6*	21.5 ± 2.4	28.7 ± 4.2
Microsomal fraction	32.0 ± 3.7	14.4 ± 1.6*	12.6 ± 0.8	11.1 ± 1.3*
Supernatant	70.3 ± 3.8	35.0 ± 3.5*	54.3 ± 6.9	45.6 ± 5.5*

TABLE 3b

	CSA-T activity in mmoles/h/g protein	Recovery of the total crude mitochondrial activity %	GOT activity in mmoles/h g proteins	Recovery of the total crude mitochondrial activity %
Myelin	39.9 ± 2.0	28.7 ± 3.4	24.9 ± 3.5	32.9 ± 43.
Nerve endings and Membrane fragments	23.7 ± 3.5	11.4 ± 1.1*	20.3 ± 2.6	17 ± 2.0*
Nerve endings (C)	15.1 ± 3.5	2.3 ± 0.7	12.5 ± 1.9	2.7 ± 0.2
Nerve endings (D)	11.7 ± 2.4	0.8 ± 0.2	12.6 ± 1.8	2.2 ± 0.3
Purified mitochondria	71.3 ± 7.4	55.3 ± 5.7*	29.7 ± 3.6	45.2 ± 5.1*

-Results represent means ± S.D. of 8 experiments
Pyridoxal phosphate was introduced in the incubation mixture

*$p < 0.05$

DISCUSSION

The regional distribution of CSA-T and GOT in adult rats seems roughly parallel. However, if the ratios of increase between adult and newborn rat CSA-T and GOT activities are considered, marked differences are found ; respectively 7 fold increase for CSA-T and 4 fold for GOT in pyriformal cortex,

TABLE 4. Effect of Triton X-100

TABLE 4a

	CSA-T activity in mmoles/h/g protein	% of the total enzyme activity	GOT activity in mmoles/h/g protein	% of the total enzyme activity
Whole brain homogenate	71.3 ± 7.1	100	39.3 ± 4.1	100
Nuclear Pellet	56.4 ± 6.2	6.9 ± 0.9	31.6 ± 3.9	5.7 ± 0.6
Crude mitochondria	64.5 ± 7.2	42.7 ± 4.4*	30.6 ± 3.1	32.4 ± 5.1*
Microsomal fraction	51.1 ± 5.0	12.8 ± 1.6	20.4 ± 2.3	12.3 ± 1.7
Supernatant	77.8 ± 8.2	33.9 ± 3.8*	52.8 ± 3.5	40.5 ± 4.8*

TABLE 4b

	CSA-T activity in mmoles/h/g protein	% of the total crude mitochondria activity	GOT activity in mmoles/h/g protein	% of the total crude mitochondria activity
Myelin	46.1 ± 4.3	16.9 ± 0.7	26.1 ± 2.8	27.4 ± 3.5
Nerve endings and Membrane fragments	29.2 ± 2.8	8.8 ± 0.9*	19.5 ± 1.7	14.5 ± 1.7*
Nerve endings (C)	15.1 ± 3.5	2.3 ± 0.7	12.5 ± 1.9	2.7 ± 0.2
Nerve endings (D)	11.7 ± 2.4	0.8 ± 0.2	12.6 ± 1.8	2.2 ± 0.3
Purified mitochondria	115.4 ± 10.2	66.0 ± 3.8*	59.7 ± 3.3	58.1 ± 5.3*

Results represent means S.D. of 8 experiments

Pyridoxal phosphate 20 M was introduced in the incubation mixture

*$p < 0.05$

6 fold and 4 fold in hypothalamus, 4.5 fold and 3.5 in cerebellum, 4.5 fold and 3 fold in pons medulla.

Only olfactory bulb present the same ratio of increase for both enzymes

(4 fold). These results are in favour of the existence of two different enzymes as was suggested previously [11].

Significant differences of CSA-T and GOT activities have been found in the localization of the two enzymes after subcellular fractionation 38.3 % for CSA-T and 28.7 % for GOT in crude mitochondria and respectively 21.2 % and 13.0 % in purified mitochondria which indicates that CSA-T is more likely to be a mitochondrial enzyme than GOT in rat brain. It is firmly well established [12,13,14] that there are two isoenzymes of GOT and this situation seems likely to also exist for CSA-T.

Moreover, the specific activity of the CSA-T increases 1.7 times in purified mitochondria compared to the activity of the whole brain homogenate. However, such an increase does not exist for GOT specific activity.

Previously, Kearney *et al.* [15], Perez-Milan *et al.* [16] suggested that CSA-T and GOT were two different enzymes whereas Singer [17] proposed a single enzyme. But no absolute evidence has been given in favour of the first or the second hypothesis and no distinction are made between mitochondrial and soluble enzymes.

The hypothesis of two different soluble enzymes was supplied by the variation in recovery (35.0 % for CSA-T and 45.6 % for GOT), in ammonium sulfate precipitation (unpublished results) and by the differences of activities in brain areas. The hypothesis of two different enzymes might be also deduced from the evidence that Triton X-100 stimulated various modifications of the activities of the two enzymes, respectively an increase of 1.5 for CSA-T and 1.3 for GOT in whole brain homogenate and an increase of 1.6 for CSA-T and 1.3 for GOT in purified mitochondria if the ratios of specific activities with and without Triton are considered , whereas no altered activities are found in the supernatant. Also differences of the measured percentage of the total activity have been shown for both enzymes in purified mitochondria. Our results strongly suggest the presence of two different enzymes in mitochondria as well as in the supernatant. However, these results must be explained with care as

different authors (15-18) have shown that purified GOT can also use CSA as a substrate which renders the discussion of our results more difficult as it seems probable that partially purified CSA-T could also use aspartate as a substrate, with of course different affinity so that purification and further studies on purified enzyme will be necessary to give other support to our hypothesis.

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