Vol. 165, No. 1, 1989 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1989 Pages 372-376

RESPONSE OF HEPATIC ALANINE:GLYOXYLATE AMINOTRANSFERASE 1 TO HORMONE DIFFERS AMONG MAMMALIA

Sueko Hayashi, Haruhiko Sakuraba and Tomoo Noguchi

Department of Biochemistry, Kyushu Dental College,
Kokura, Kitakyushu 803, Japan

Received October 2, 1989

SUMMARY: The subcellular distribution and substrate specificity of hepatic alanine:glyoxylate aminotransferase 1 have been reported to differ among mammalia. In the present study, the response of this enzyme to hormone (glucagon) was found to differ among mammalia.

Alanine:glyoxylate aminotransferase (EC 2.6.1.44) is distributed in livers of a wide range of animal species (1,2). Mammalian liver contains two forms of alanine:glyoxylate aminotransferase, designated AGT 1 and AGT 2, respectively (2). AGT 1 has been reported to be identical with serine: pyruvate aminotransferase (EC 2.6.1.51)(3,4). All of AGT 1 from mammalian liver consist of two identical subunits with a molecular weight of about 40,000 and are immunologically cross-reactive (5). However, the subcellular distribution and substrate specificity of hepatic AGT 1 differ among mammalia (2).

The present report shows that the response of hepatic AGT 1 to hormone (glucagon) also differs among mammalia.

MATERIALS AND METHODS

Animals Male animals were used. Rats (Donryu strain, 100-120 g; Wistar strain, 100-120 g), mice (ddY strain, 15-18 g), chipmunks (40-60 g), Syrian hamsters (Syrian strain, 75-110 g), guinea-pigs (Hartley strain, 250-290 g), rabbits (Nippon white rabbit strain, 700-900 g), dogs (mixed breed, 450-500 g) and cats (mixed breed, 350-400 g) were maintained at about 20 °C in a room with 12 h light/12 h dark cycle. Food and water were available *ad libitum*. Each animal was injected intraperitoneally with glucagon (Sigma) dissolved in 0.9 % (w/v) NaCl, 3 times at 12 h intervals at a dosage of 0.2 mg/100 g body weight. In each case, AGT 1 activity was assayed 4 h after the last injection of glucagon.

0006-291X/89 $1.50

Preparation and Sucrose Density Gradient Centrifugation of Homogenates from Mammalian Liver All procedures were carried out at 0-4°C. Livers were cut into very small pieces with scissors and homogenized by one excursion with 9 volumes of 0.25 M sucrose in 20 mM glycylglycine, pH 7.5, containing 1 mM EDTA in a Potter-Elvehjem homogenizer with a Teflon pestle at 600 rev./min. The homogenate was filtered through two layers of cheese-cloth and centrifuged at 300 x g for 5 min to sediment nuclei and any whole cells. A portion (2.7 ml) of the resulting post nuclear supernatant was layered on a 33-ml linear sucrose gradient (24-54 %, w/v) with 2 ml of 57 % sucrose solution as a cushion in the bottom and centrifuged at 132,400 x g for 70 min with a vertical rotor (RPV 50T) by Hitachi (Tokyo, Japan) 55P-72 centrifuge. After centrifugation, fractions (2.1 ml) were collected from the bottom of the tube.

Enzyme Assays Serine:pyruvate aminotransferase (3), glutamate dehydrogenase (EC 1.4.1.3)(6) and catalase (EC 1.11.1.6)(7) were assayed as previously described. One enzyme unit is defined as the amount of enzyme which catalyses the conversion of 1 μmol of substrate to product/min at 37°C.

RESULTS AND DISCUSSION

Hepatic AGT 1 of rodents (rat and mouse) catalyse transamination between various L-amino acids and pyruvate or glyoxylate, while hepatic AGT 1 from other mammalian species have only alanine: glyoxylate aminotransferase and serine:pyruvate aminotransferase activities (2). In contrast, hepatic AGT 2 are highly specific for L-alanine and glyoxylate in all mammalian species and have no serine:pyruvate aminotransferase activity (2). Therefore, serine: pyruvate aminotransferase activity was determined for the assay of AGT 1.

TABLE I shows effect of glucagon-injection on serine: pyruvate aminotransferase activity of liver homogenates from different mammalian species. In rodents (Donryu rat, Wistar rat, mouse, Syrian hamster and chipmunk), serine:pyruvate aminotransferase activities were elevated remarkably by glucagon-injection as compared with saline-injected controls. In contrast, liver serine:pyruvate aminotransferase activities of other mammalian species (guinea-pig, rabbit, dog and cat) were not changed by glucagon-injection as compared with saline-injected controls.

AGT 1 (serine:pyruvate aminotransferase) has been reported to be located both in the mitochondria and in the peroxisomes in rat liver: in Donryu and Wistar rats, only mitochondrial AGT 1 activity is elevated by glucagon-injection as the result of an increased amount of enzyme but peroxisomal AGT 1 activity is not (2,5,8,9). Studies on glucagon-induction of hepatic AGT 1

TABLE I

Effects of Glucagon-Injection on Serine:Pyruvate Aminotransferase (AGT 1) Activity of Liver Homogenates from Different Mammalian Species

	Serine:pyruvate aminotransferase (μmol/min per g of liver)	
	control	glucagon
Donryu rat	0.43 ± 0.03 (3)	2.85 ± 0.25 (3)*
Wistar rat	0.41 ± 0.03 (3)	2.26 ± 0.24 (3)*
Mouse	0.30 ± 0.02 (5)	1.84 ± 0.17 (3)*
Syrian hamster	0.70 ± 0.06 (3)	3.90 ± 0.34 (3)*
Chipmunk	0.36 ± 0.02 (3)	1.69 ± 0.12 (3)*
Guinea-pig	0.11 ± 0.011 (3)	0.11 ± 0.009 (3)
Rabbit	1.45 ± 0.15 (3)	1.55 ± 0.14 (3)
Dog	3.02 ± 0.03 (3)	3.53 ± 0.26 (3)
Cat	9.30 ± 0.78 (3)	9.40 ± 0.89 (3)

Activity is expressed as the mean ± S.E.M., with the number of observations in parentheses. significance of results: *$P < 0.01$.

were carried out with other rodents (Syrian hamster, mouse and chipmunk). A representative sedimentation in a sucrose density gradient for the post-nuclear supernatant from control Syrian hamster liver is presented in Fig. 1A and that from glucagon-injected Syrian hamster liver in Fig. 1B. The peroxisomes and mitochondria were separated; the peroxisomes, marked by catalase were at a density of about 1.25 g x ml^{-1} and the mitochondria, marked by glutamate dehydrogenase at a density of about 1.19 g x ml^{-1}. About 33 % of serine: pyruvate aminotransferase activity was recovered in the peroxisomal fraction, about 39 % in the mitochondrial fraction and the remainder in the soluble top fraction presumably from broken peroxisomes. With rat (10,11) and human liver (12,13), evidence has been described that AGT 1 activities in the soluble top fraction are from broken peroxisomes. Glucagon-injection to Syrian hamster resulted in about 10-fold increase of the mitochondrial serine:pyruvate aminotransferase activity but did not in the increase of the peroxisomal enzyme activity (Fig. 1B). By sucrose density gradient centrifugation, 50 and 45 % of serine:pyruvate aminotransferase activities of the postnuclear fraction were found to be recovered in the peroxisomal fraction, 35 and 38 % in the mitochondrial fraction and the remainder in the soluble top fraction in mouse and chipmunk liver, respec tively (data not shown). Glucagon-injection resulted in about 13-fold increase in mouse and about 13-fold increase in chipmunk in the mitochondrial serine: pyruvate aminotransferase activity but did not change the peroxisomal enzyme activity (data not shown).

On the other hand, AGT 1 has been reported to be located only in the peroxisomes in guinea-pig, rabbit, human and monkey, and only in the mito-

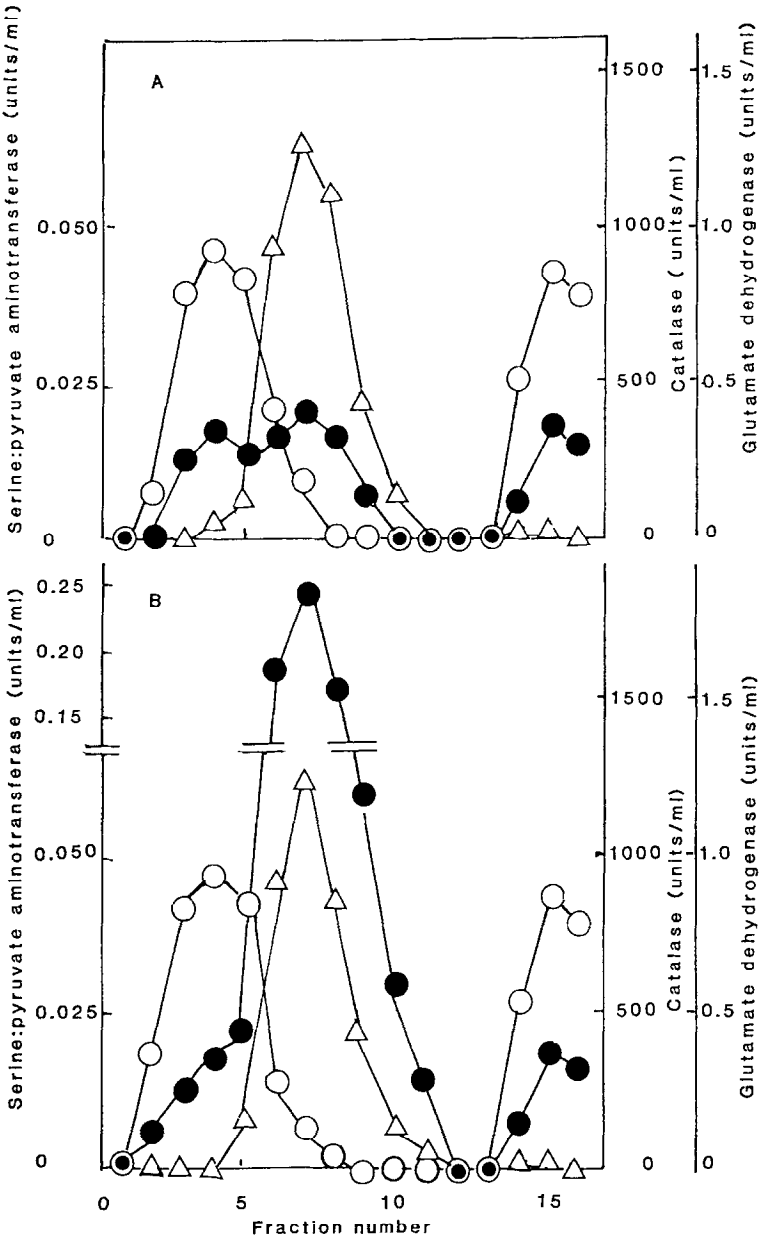

Fig. 1. Subcellular distribution of serine:pyruvate aminotransferase (AGT 1) in livers of control and glucagon-injected Syrian hamsters. The postnuclear fractions were prepared from livers of control and glucagon-injected Syrian hamsters and subjected to sucrose density gradient centrifugation as described in the text. The Figure shows sedimentation profiles of liver serine:pyruvate aminotransferase (AGT 1) activity from control (A) and glucagon-injected (B) Syrian hamsters.
● , serine:pyruvate aminotransferase; ○ , catalase; △ , glutamate dehydrogenase.

chondria in dog and cat (2). Therefore, the present results (Table 1) with animals other than rodents show that peroxisomal AGT 1 of guinea-pig and rabbit liver and mitochondrial AGT 1 of dog and cat liver are not affected by glucagon-injection.

We have reported that the substrate specificity and subcellular distribution of hepatic AGT 1 differ among mammalia (2). The present results show that the response of hepatic AGT 1 to hormone (glucagon) also differs among mammalia, suggesting that liver peroxisomal AGT 1 of primates (human and monkey) are not affected by glucagon-injection.

REFERENCES

1. Rowsell, E.V., Snell, K., Carnie, J.A. and Al-Tai, A.T.(1969) Biochem. J. 115, 1071-1073
2. Noguchi, T. (1987) in Peroxisomes in Biology and Medicine (Fahimi, H.D., and Sies, H., eds) pp. 234-243, Springer-Verlag, Heidelberg
3. Noguchi, T., Okuno, E., Takada, Y., Minatogawa, Y., Okai, K. and Kido, R. (1978) Biochem. J. 169, 113-122
4. Noguchi, T. and Fujiwara, S. (1988) J. Biol. Chem. 263, 182-186
5. Takada, Y. and Noguchi, T. (1982) Comp. Biochem. Physiol. 72B, 597-604
6. Schmidt, E. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H.U., ed) pp. 650-656, Academic Press, New York.
7. Sinha, A.K. (1972) Anal. Biochem. 47, 389-394
8. Noguchi, T., Minatogawa, Y., Takada, Y., Okuno, E. and Kido, R. (1978) Biochem. J. 170, 173-175
9. Oda, T., Yanagisawa, M. and Ichiyama, A.(1982) J. Biochem. 91, 219-232
10. Lazarow, P.B., and de Duve, C. (1973) J. Cell. Biol. 59, 507-524
11. Leighton, F., Coloma, L. and Koenig, C. (1975) J. Cell. Biol. 67, 281-309
12. Noguchi, T. and Takada, Y. (1978) J. Biol. Chem. 253, 7598-7600
13. Noguchi, T. and Takada, Y. (1979) Arch. Biochem. Biophys. 196, 645-647