[原著] Distribution and intracellular localization of metabolic enzyme in several organs of the Habu : Trimeresurus flavoviridis

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Distribution and intracellular localization of metabolic enzyme in several organs of the Habu (*Trimeresurus flavoviridis*)

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Abstract

Lactic dehydrogenase (LDH), glutamic oxaloacetic transaminase (GOT), pyruvic transaminase (GPT) and alkaline phosphatase (ALP) were assayed in several organs and fractionated tissues of the Habu snake (*Trimeresurus flavoviridis*). In spite of relatively lower levels of these enzymes in the snake as compared to human values, LDH in the parenchymal organs of the snake were higher than other enzyme levels. This was considered to be related to the fact that energy utilization in snakes relies heavily on anaerobic pathways. The snake kidney, in addition to having high level of LDH, had both the aerobic enzymes GOT and GPT at levels higher than those in all other tissues examined.

In the serum, enzyme levels were in the same range as those found in humans. Enzyme specific activities in fractionated cells were as follows : LDH was highest in the soluble fraction, GOT and GPT were high in both the mitochondrial and soluble fractions and ALP was significant only in the microsomal fraction.

Introduction

Although venom from the Habu snake (*Trimeresurus flavoviridis*), an inhabitant of the Okinawa Islands (Japan), has often been studied, more general biochemical and physiological information on this snake is largely lacking.

It is generally accepted that metabolic rates differ between reptiles and homeothermal animals and presumably the activities and localizations of the metabolic enzymes differ as well. According to Penny and Kornecki, some human mitochondrial enzymes are localized mainly in the extramitochondrial fraction of turtles[1]. Moberly reported that metabolism ending in *Iguana iguana*, revealing a substantial dependence on an anaerobic pathway[2]. Miller showed lactic dehydrogenase (LDH) (EC;1. 1. 1. 27) levels in the turtle brain to be about twice those in the rat brain[3].

The aerobic metabolic rates in resting lizards are characteristically low, being

approximately one-sixth those of mammals (Bartholomew and Tucker)[4]. Glutamic oxaloacetic transaminase (GOT) (EC; 2. 6. 1. 2) and glutamic pyruvic transaminase (GPT) (EC; 2. 6. 1. 2)are closely related to aerobic metabolism, as the alpha-keto acids produced from their transamination often enter the citric acid cycle.

The present study investigated the localization and metabolic regulation of LDH, GOT, GOT and alkaline phosphatase (ALP) (EC; 3. 1. 3. 1.) which is presumably related to the transport of substances across the cell membrane in the Habu snake.

Materials and Methods

Six adult snakes weighing from 410 to 740g were collected in June and August. Wistar rats (Male, 380-510g) were used as mammalian representatives. Animals were fasted for one day before being killed by decapitation. Blood was collected and then heart, liver, kidney, stomach and intestine of the snakes and liver of rats were removed and washed with physiological saline.

For total activity assay of each enzyme, one gram of tissue sample was cut into pieces with scissors and homogenized in a Potter glass homogenizer with addition of 10 times of 3 mM Tris-HCl buffer (pH 7. 4). Cell membranes were fractured by freeze-thawing three times and spun in a Hitachi preparative ultracentrifuge (Model 65-P) at 105,000g for 30 min. The supernatant was used for the assay.

For cell fractionation, tissue homogenization was carried out by the preceding method and the homogenate was centrifuged at 700g for 10 min. The crude nucleic sediment was washed with buffer and spun at 700g. The resulting supernatant was added to the used buffer from the previous washing and centrifuged at 5,000g for 10 min. The precipitate containing the mitochondrial fraction (Mt) was collected. The resulting supernatant was centrifuged at 105,000g for 30 min to separate the microsomal sediment (Ms) from the soluble fraction (S). Each pellet was resuspended in saline and freeze-thawed at least three times in a solid carbon dioxide and acetone mixture.

After recentrifugation at 105,000g for 20 min, each sample was immediately subdivided into four test tubes (one for each enzyme) and stored in a freezer ($-20°C$) for further use. Serum samples were obtained using standard procedures.

Enzymes were assayed as follows : in LDH, the activity was determined from the amount of NADH produced by the reaction of lactic acid with NAD catalyzed by LDH. (LDH B test Wako, Wako Pure Chemical Industries, LTD). In GOT and GPT, alpha-ketoglutarate and asparatic acid or alanine were used as substrates. The product, pyruvate, was allowed to react with dinitro-phenylhydrazine and the resulting hydrazone was determined spectrophotometrically (Transaminase B tset Wako, Wako Pure Chemical Industries, LTD). ALP, the assay was done according to the method of Kind-king using phenylphosphate as a substrate (ALP test Wako, Wako Pure Chemical Industries, LTD). Spectrophotometric assays were performed on a Hitachi 323 spectrophotometer.

Results

The enzyme levels in the various organs of the snake were, on the whole, higher in the parenchymal organs than in the digestive tracts, except for ALP which had higher levels in the intestine (Table 1). GOT and GPT showed higher activity in the kidney than in any other organ. The levels of GOT and GPT in the kidney were 1.7 and 4.6 times those of liver, respectively. Comparing levels of these enzymes in snake liver to those in rat liver showed the snake LDH to be 1.5 times higher, the levels of GOT and ALP to be approximately half and the levels of GPT to be only 0.07 those of rat.

Table 1 Distribution of enzymes in Habu (*Trimeresurus flavoviridis*)and Rat (Wistar) tissues.

Each enzyme activity is expressed in International Unit (IU) per gram of tissue (wet weight). Values are mean±SD.

	Tissue	n	LDH IU/g	GOT IU/g	GPT IU/g	ALP IU/g	Protein (mg/g)
	Heart	6	40.2±5.5	9.8±0.7	0.9±0.2	1.3±0.3	107.7± 7.8
	Kidney	6	32.5±2.6	22.4±3.2	7.2±0.8	2.0±0.6	115.3±16.8
Habu	Liver	6	39.3±1.7	13.4±3.1	1.6±0.1	1.3±0.2	119.8± 9.7
	Stomach	6	16.0±2.3	4.8±0.4	1.4±0.3	0.2±0.01	105.1± 9.3
	Intestine	6	12.5±3.5	2.8±0.3	1.1±0.3	2.6±0.4	111.1±17.4
Rat	Liver	4	23.5±0.9	25.4±1.6	22.2±1.5	2.4±0.7	125.8± 5.4
	Heart		106.8	75.2	3.4		
Human*	Liver		45.7	68.4	21.2	0.12-0.42	
	Kidney		135.8	43.9	9.2	1.3	

* :Human values are from Yamamura[5] and have been converted to International Units.

Snake serum enzyme levels were in a range similar to the human rather than to the rat levels (Table 2). All human and rat enzyme values examined in this paper were in agreement with the literature of Yamamura, et al., Nagase and Tanaka, and Dixon and Webb[5-7].

Enzyme specific activities in the Mt, Ms and S fractions are shown in Fig. 1. ALP was found mainly in the Ms, LDH and GOT were more abundant in the S ($P<0.001$, each) and GPT had a similar distribution in all cellular subfractions.

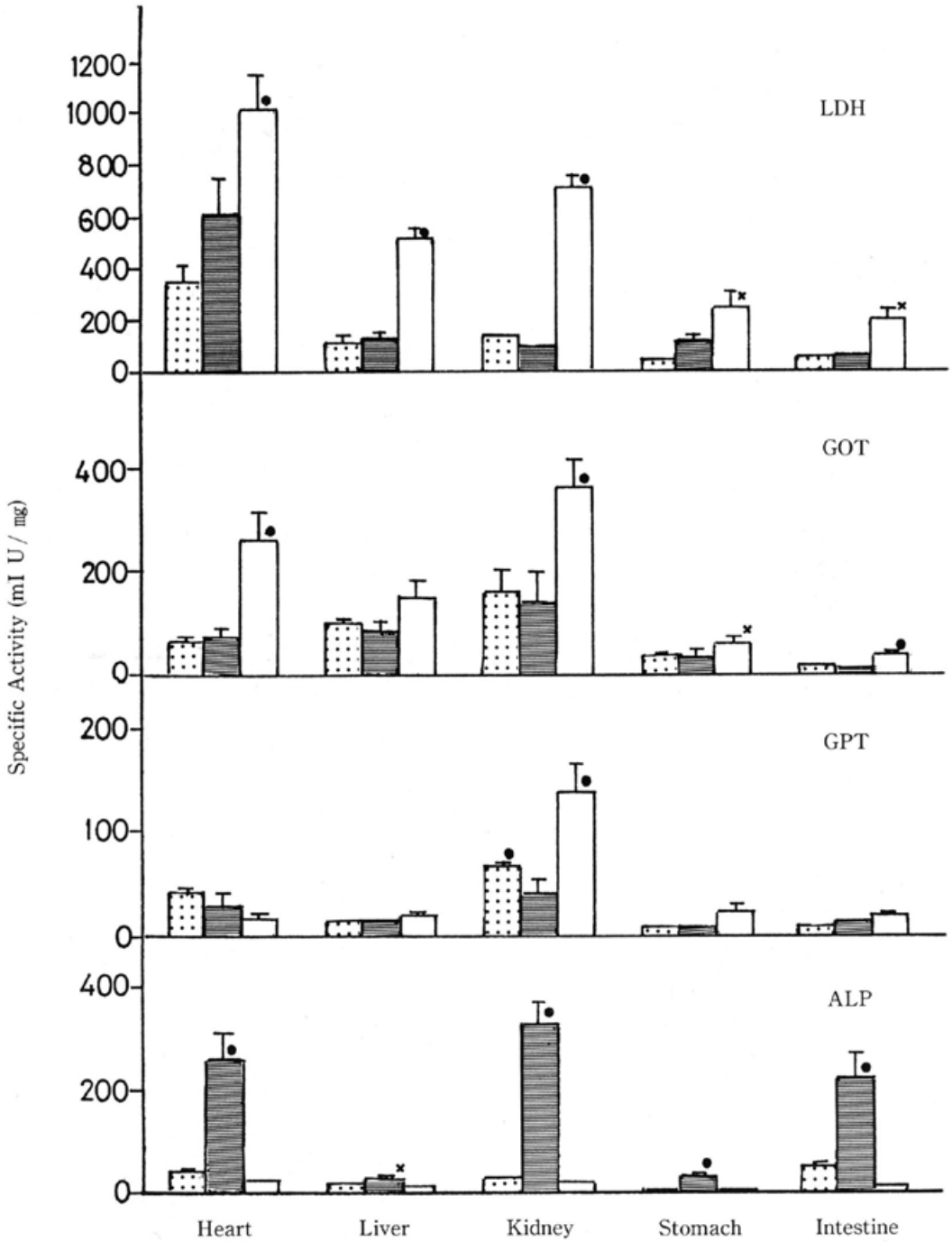

Fig. 1 Enzyme specific activities in cellular fractions of Habu snake (*Trimeresurus flavoviridis*) organs.

Dotted bars, shaded bars and open bars represent Mitochondrial, Microsomal and Soluble fractions, respectively. Vertical lines indicate Standard deviation. ● : $p<0.001$, × : $p<0.01$.

Table 2 Enzyme activities in Habu (*Trimeresurus flavoviridis*), Rat (Wistar) and Hmuan serum. Each activity is expressed in International Unit (IU) per liter of serum. Values are mean ± SD.

	n	LDH	GOT	GPT	ALP	Protein (g)
Habu	5	106.0±6.8	16.7±1.2	8.3±1.4	32.5±5.7	6.7±1.5
Rat	4	80.0±5.1	40.6±6.9	6.9±1.0	46.6±8.9	6.2±1.3
Human	Pooled	103.6	14.8	12.7	39.6	7.5

Discussion

Snake were captured in June and August as it is considered that enzyme activities at this time indicate its characteristic metabolism.

Comparing enzyme activity among several animal organs, *T. flavoviridis* enzywe activity in general was lower than that of human or rat activity (Table 1). The snake cardiac enzymes were approximately one-fourth to one-eighth as active as those of humans, exdept for LDH activity which was about half that of humans and twice that of rats. The average heart weight of the snakes was 1.5g, only 1 / 330 of body weight (this ratio is 1 / 200 in humans) and the heart rate was 38 / min. at 25°C (about half the human rate). In addition to the histochemical differences between the reptilian and the mammalian heart (White[8]), the low level of the cardiac enzyme suggested that the snake heart has only a minor energy metabolism. However, the energy supply to this organ depends mainly on anaerobical energy generated with LDH.

In the liver, GOT, GPT and ALP levels were lower (1/4 to 1/13) than in humans and LDH was about the same level as in humans. Many reports have demonstrated the dependence of reptilian metabolism on LDH. Turtles survive longer than rats in the absence of oxygen and at the same time produce large amounts of lactic acid (Robjn, et. al.[9]). Snakes placed in the above conditions could survive for 33 to 95 minutes (Belkin[10]). The marked dependence on anaerobic metabolism in the reptile was also reported by Moberly[2].

From the present results, it appears that the snake liver, as well as its heart, is adapted for functioning under anaerobic conditions.

LDH activity in the snake kidney was about four times higher than in the rat kidney; furthermore, GOT, GPT and ALP were significantly higher in the snake kidney than in other examined organs. These enzyme levels are closely related to the metabolic characteristics of the kidney. In snakes, 98% of the nitrogenous waste is usually excreted as uric acid in paste-like form, to prevent excess water loss (Khalil[11]).

Consequently, the snake kidney requires a large amount of energy compared with that

required by mammals due to the active transport of electrolytes, organic compounds and the consequent reabsorption of water in the proximal tubules or the excretion of urates.

The enzymes examined in the stomach and intestine were at relatively low levels, except for ALP in the intestine. A high level of ALP may be correlated to intestinal absorption of digested substances related to the characteristic food intake of snakes. Bennet[12] reported that one microscopic field of tissue homogenate contained three to five more mitochondria in the rat than in the lizard. This might be one of the reasons for the lower enzymatic activities in *T. flavoviridis* organs.

Serum enzyme concentrations in the snake were within the normal range for these enzymes in human serum. These levels are difficult to explain because blood enzymes originate from both tissue and blood cells, and the Habu has relatively lower tissue enzyme activity. Furthermore, Na^+ concentration in the snake serum was 143 ± 8.9 *milliequivalents*/1, which is in the same range as in humans (139 ± 5.9). Similarly, Chiodini and Sundberg[13] reported the serum enzymes of *Boa constrictor*, LDH, GOT and GPT to be 139 ± 11, 8 ± 1 and 6 ± 0.7 I. U/1, respectively. It has not yet been established whether a difference exists between humans and snakes in the leakage pf enzymes from tissue into body fluid.

LDH is considered to be one of the cytosol enzymes of humans; similarly, high LDH specific activity was found in the cellular soluble fraction of the snake. However, in the heart, this enzyme was also localized in the Mt and Ms fractions. Because it is believed that the major energy pathway of this organ is anaerobic, the LDH related anaerobical energy in the snake heart might be produced also in the mitochondria and microsomes.

GOT in mammalian cells is localized mainly in the Mt and some in the S fractions (Izumi[14], Roodyn[15]). However, the snake GOT was found to exhibit higher specific activity in S than in other fractions. Kidney GPT also showed a high level of specific activity in the S fraction. ALP was localized in the Ms fraction as has been reported for mammals also. This suggests snake ALP and mammalian ALP may have similar functions.

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