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Threonine metabolism in Japanese quail liver

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Summary. In general, threonine is metabolized by reaction catalyzed by threonine-3-dehydrogenase (TDH), threonine dehydratase (TH) or threonine aldolase (TA). The activities of these three enzymes were compared in the liver of Japanese quails and rats. The animals were fed a standard or threonine rich-diet, or fasted for 3 days. The specific activity of TDH in the liver from quail fed a standard diet was 11 times higher than that in the liver from rats fed a standard diet. The TDH activities in the livers of the fasting and 5% threonine-rich diet groups of quail were 3 and 2 times higher than those in the livers from quail fed the standard diet, respectively. The TH activity in the liver of rats fed a standard diet was 14 times higher than that in the liver of quail fed a standard diet. The TH activity in the rat liver after fasting was 2.3 times higher than that of the standard diet control. The activity of TA in the livers of rat and quail were so low that its role in threonine metabolism in both animals seemed to be negligible. These results suggest that threonine is a ketogenic amino acid in the quail liver, while it is a glucogenic in the rat liver.

Keywords: Threonine metabolism – Quail – D-Lactate – Methylglyoxal – Threonine-3-dehydrogenase – Threonine hydratase

Abbreviations: DCMQ, 6,7-dichloro-2-methylquinoxaline; DCPD, 4,5-dichloro-1,2-phenylenediamine; TDH, threonine-3-dehydrogenase; TH, threonine dehydratase; TA, threonine aldolase; D-LDH, D-lactate dehydrogenase

Introduction

Threonine is the last amino acid discovered in proteins and its metabolism in animals has not been fully resolved. It is, however, generally known that threonine is metabolized through three routes catalyzed by three different enzymes: threonine-3-dehydrogenase (TDH, EC 1.1.1.103), threonine dehyratase (TH, EC 4.2.1.16) and threonine aldolase (TA, EC 4.1.2.5) as shown in Fig. 1. Threonine is converted to 2-amino-3-oxobutyrate in the presence of NAD by TDH. The 2-amino-3-oxobutyrate is either decarboxylated spontaneously to aminoacetone or converted to glycine and acetyl CoA in the presence of CoA by 2-amino-3-oxobutyrate CoA-ligase (glycine C-acetyltransferase, EC 2.3.1.29). When aminoacetone is deaminated, the product, methylglyoxal is converted into D-lactate in the presence of glutathione by glyoxalase I (lactoylglutathione lyase, EC 4.4.1.5) and glyoxalase II (hydroxyacylglutathione hydrolase, EC 3.1.2.6). Cooper and Anderson showed that *E. coli* contained enzymes which convert glucose to pyruvate via methylglyoxal and named this route as methylglyoxal bypass (Cooper and Anderson, 1970). Recently we reported that octopus had the bypass and tissues of some marine invertebrates such as echiuran, cnidarian and mollusk species had high concentrations of D-lactate (Ohmori et al., 1997). We reported that, in cell-free homogenate of *Octopus vulgaris* tentacles, threonine was readily metabolized to D-lactate (Akagi and Ohmori, 2004). The second enzyme TH catalyzes the cleavage reaction of threonine to 2-oxobutyrate and ammonia. The third enzyme TA catalyzes the breakdown of threonine to glycine and acetaldehyde. During our series of investigations on the methylglyoxal bypass which were shortly reviewed in a previous paper (Ohmori et al., 1997), we wanted to know whether birds also have the methylglyoxal bypass. Japanese quails were used in this study and quail's enzyme activities were compared with those of the rat liver. In this report we also discuss the energy metabolism and utilization of end products of threonine in both rats and Japanese quail.

Materials and methods

Chemicals

D-Lactate dehydrogenase (D-LDH) from *Staphylococcus sp* and diaphorase from *Clostridium kluyveri* were kindly supplied by Amano Pharma-

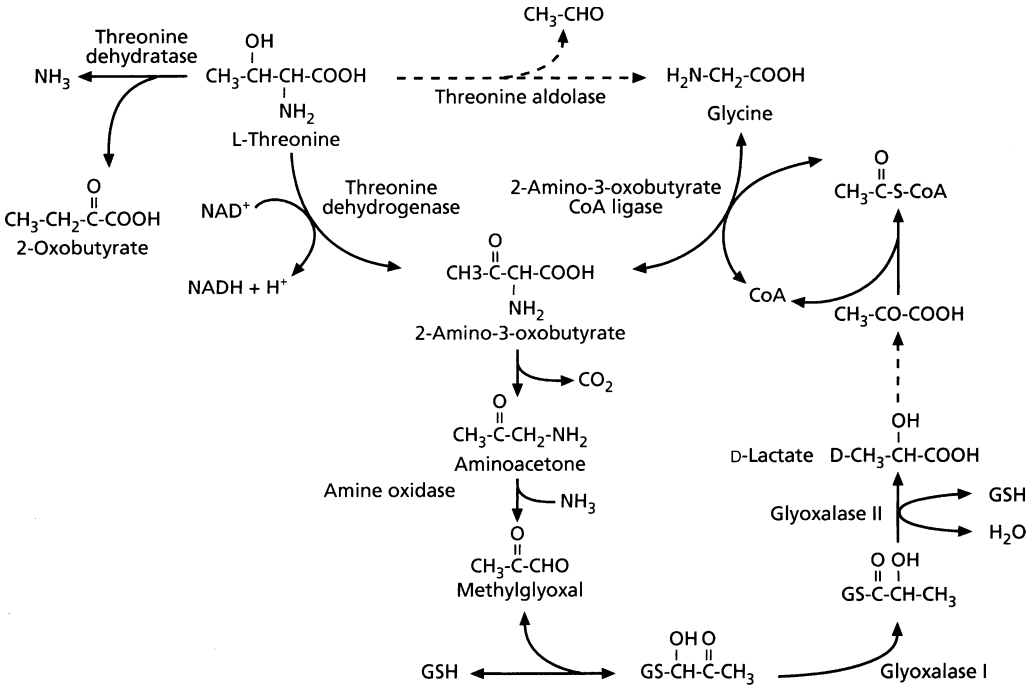

Fig. 1. Threonine metabolism and the methylglyoxal bypass in liver of quail and rat

ceutical (Nagoya, Japan). Lithium D-lactate, S-lactoyl-glutathione, Tris and EDTA-2Na were purchased from Sigma Chemical Co. (St. Louis, Mo., USA). Pyridoxal-5′-phosphate, D,L-6,8-thioctamide, *o*-phenylenediamine and ethyl acetoacetate were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). β-NAD^+, β-NADH, L-lactate dehydrogenase (LDH) from pig heart, aldehyde dehydrogenase (ADH) from yeast were from Oriental Yeast Co. (Tokyo, Japan). 4,5-Dichloro-1,2-phenylenediamine (DCPD) and dimethyl-acetal of methylglyoxal were purchased from Aldrich (Milwaukee, WI, USA). Methylglyoxal was prepared just before use by hydrolysis of the acetal (Kellium et al., 1978). 6,7-Dichloro-2-methyl-quinoxaline (DCMQ) and 2-hydroxy-3-methyl-quinoxaline were prepared in our laboratory. Other chemicals were purchased from Wako Pure Chemicals (Osaka, Japan).

Diet for quail

Standard diet for quail was purchased from Tokai Kigyou Co., Ltd. (Toyohashi, Japan). The diet was manufactured from corn meal, soy bean meal, rice bran and fish meal, and fortified by adding vitamin A, D_3, E, B_1, B_2, B_6, B_{12}, K_3, choline, nicotinic acid, pantothenic acid, folic acid, manganese carbonate, zinc carbonate, ferrous sulfate, cupric sulfate, calcium iodate, ethoxyquin and methionine. The diet consisted of 25.3% crude protein containing 0.65% threonine, 3.6% crude lipid, 49.3% crude carbohydrate and 10.5% ash. We prepared two kinds of threonine-fortified diet. To 100 g of the standard diet 1 g threonine (1% threonine-diet) or 5 g (5% threonine-diet) was mixed. The 1% threonine-diet contained 1.6% threonine in toto and the 5% threonine-diet contained 5.4% threonine in toto.

Animals

Male Japanese quails, *Coturnix coturnix japonica*, were obtained from the Faculty of Agriculture of the Okayama University. The quail population was maintained by random mating. Four week-old quails were housed for 1 week in a controlled temperature (25 ± 2°C) and light (from 6:00 am to 8:00 pm) environment and received water and diet *ad libitum*. For experiments with fasted quail, 5 week-old quails were given only water for 72 h. For threonine-enriched diet experiments, the 5 week-old quails had free access to one of two threonine-added diets for 96 h. At 10:00 am after 72 h for the fasting experiment and 96 h for the threonine-enriched diet experiment animals were anesthetized with pentobarbital (50 mg/kg body weight). After about 5 min, abdomens were opened. Livers were excised and rinsed in an ice-cold physiological saline and homogenized as described below. Five weeks-old male Wistar albino rats were used as reference animals. One group was fed standard rat cake MF (Oriental Yeast) and another group was fasted for 72 h as above.

Instruments

A Shimazu gas chromatograph Model GC-4CMPFE (Shimazu, Kyoto, Japan), equipped with a ^{63}Ni electron-capture detector, was used for the determination of methylglyoxal. The glass column (2 m × 3 mm I.D.) was packed with 1.5% Silicon OV-17 on Shimalite W, 80–100 mesh (Shimazu). The temperature of the detector and injector block was regulated at 270°C, and the column was at 200°C. The flow-rate of carrier nitrogen gas was 50 ml/min. High performance liquid chromatography was used for the determination of D-lactate. The analyses were carried out with a Waters' Chromatograph 5C18-AR equipped with a Gasukuro Kogyo pump (Model 576-1A) and a Gasukuro Kogyo spectro-detector (Model 502T). The mobile phase was 10 mM potassium phosphate (pH 2.1) containing 15% acetonitrile. The column was a 150 mm × 4.6 mm Cosmosil (Nacalai Tesque) and eluted at a flow rate of 1.0 ml/min at 40°C. D-Lactate was determined at 334 nm as 2-methylquinoxanol.

Enzyme activity

The activities of TDH, TH, TA, glyoxalase I and II, and aminoacetone synthase (2-amino-3-oxobutyrate CoA ligase) were determined by the methods of Bird and Nunn (1983); Bird and Nunn (1983), Morris and Peraino (1976); Palekar et al. (1973); Racker (1955); Ray et al. (1991), Urata and Granick (1963); respectively.

Preparation of mitochondrial fraction from quail liver for measuring the TDH activity

Quail liver (1.0 g) was homogenized at 0°C in 9 volumes of a medium containing 0.25 M sucrose and 3 mM potassium phosphate (pH 7.4) using a Potter-Elvehjem homogenizer with a loose-fitting glass pestle at 1,000 rpm. The homogenate was centrifuged at 700 × g and 4°C for 15 min. The supernatant was centrifuged at 5,500 × g and 4°C for 15 min. The pellet was suspended in the homogenizing buffer and used as the enzyme source.

Determination of metabolites concentrations

Methylglyoxal was determined according to the method of Ohmori et al. (1987). Briefly: the 6,000 × g supernatant of the liver homogenate was deproteinized by methanol precipitation. The deproteinized solution was reacted with DCPD. DCMQ formed was determined by gas chromatography with electron capture detection. D-Lactate was determined as follows. D-Lactate was converted to pyruvate by D-LDH, and pyruvate formed was reacted with *o*-phenylenediamine in a one vial-reaction to form 2-methylquinoxanol, which was then determined by HPLC (Ohmori et al., 1988). Glycine was spectrometrically quantitated by our previously reported method (Ohmori et al., 1978). Aminoacetone was determined according to the method of Urata and Granick (1963).

Protein determination

Protein concentrations were determined by the method of Lowry et al., with bovine serum albumin as the standard (Lowry et al., 1951).

Results

Weights of the body and liver of quails fed threonine-enriched diets and intake

Three groups of five week-old male quails were fed a standard diet or threonine-enriched diets for 4 days. The rate of average weight increase was about 9% at the 4th day in all three groups. The average intake at the 4th day of the quails of the 5% threonine-enriched diet group decreased to 97% of the level of the 1st day. The intakes of normal and 1% threonine-enriched diet group showed increases of 13 and 8%, respectively. Liver weights of the normal, the 1% threonine-enriched and the 5% threonine-enriched group at the 4th day of the experiment were 2.1 ± 0.1 g, 2.1 ± 0.2 g, 2.3 ± 0.1 g, respectively, means ± S.E.M., n = 6 to 12. The difference in the average liver weight of the three groups was not significant.

Weights of body and liver of fasted quails

Five week-old male quails were fasted for 3 days. During this time, the average body weight of the control group increased 5%, whereas that of the fasted group decreased to 71% of the starting level. After three days of fasting, the average weight of the liver from a fasted bird was 64% that of the average control group liver; the difference in body weight was about the same (67%).

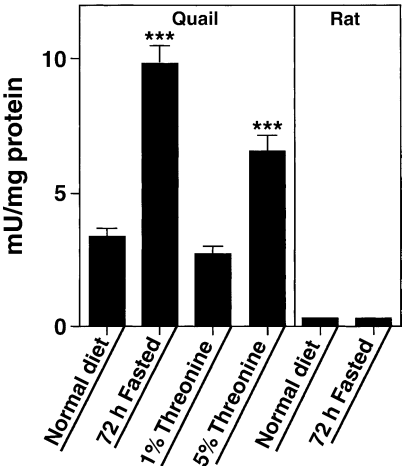

Fig. 2. Specific activities of TDH in liver homogenates of quails and rats which were fed on 1% or 5% threonine-added diets for 4 days or fasted for 72 h. Bars represent ±S.E.M. $^{***}p < 0.001$, n = 12, 8, 6, 6, 3 and 3 for the normal diet, 72 h fasted, 1% threonine-added, and 5% threonine-added groups of quail, and the normal diet and 72 h-fasted groups of rats, respectively

Changes in the activities of liver TDH induced by fasting or by a threonine-enriched diets

Figure 2 shows the liver TDH activity of quails after fasting and intake of threonine-enriched diet, and that of fasted rats. It is clearly shown that the TDH specific activity in the control quail liver was 11 times higher than that of the control rat liver. The specific activity in livers of the fasted and the 5% threonine group of quails were about 3 and 2 times higher than that of the control quail. There is, however, no significant difference between the control and the 1% threonine groups of quail. The results indicated that TDH activity in quail liver increased when quails were fasted or fed with relatively high amounts of threonine. If the TDH activity is expressed as total activity in the quail liver, it increased about 2 and 2.4 times in the fasted and the 5% threonine-fed states, respectively, compared to the control group. As for rats, the TDH specific activity in the liver did not change irrespective of nutritional state.

Changes in the activities of liver TH by fasting or by on threonine-enriched diet

As mentioned above, the specific activity of TDH in the quail liver was very high compared to that in rat liver. Contrary to the activity of TDH, however, the TH activity was much higher in rat liver than that in quail liver regardless of nutritional state (Fig. 3). The specific activity of TH in the normal rat liver was 15 times higher than that of the control quail group. The specific activity of TH in rat liver after fasting was increased by a factor of 2.3 over

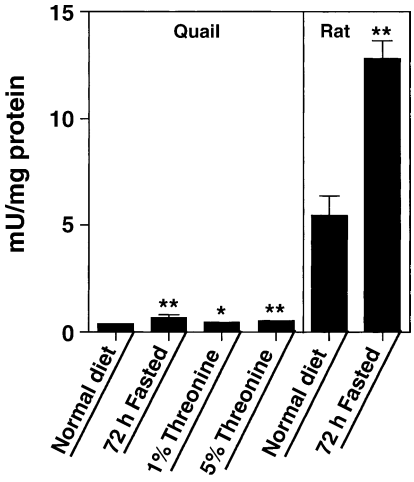

Fig. 3. Specific activities of TH in liver homogenates of quail and rats which were fed on 1% or 5% threonine-added diets for 4 days or fasted for 72 h. Bars represent ±S.E.M. $^{*}p < 0.05$, $^{**}p < 0.01$, n = 12, 6, 6, 6, 3 and 3 for the normal diet, 72 h fasted, 1% threonine-added, and 5% threonine-added groups of quail, and the normal diet and 72 h-fasted groups of rats, respectively

that of normal fed state. Although the specific activities of TH in quail liver was very low, the TH activities in liver of fasted, the 1% threonine and the 5% threonine group were about 2, 1.3 and 1.5 times higher, respectively, than that of the control group of quails.

Changes in the activities of liver TA by fasting or by a threonine-enriched diet

Table 1 shows the activities of TA in quail and rat liver. The TA activity in the liver of the 5% threonine group of quails was slightly higher while the activity in the liver of the fasted rat is lower than that in their respective control liver. The differences between the values for the control and the fasted or fed experiments were statistically not significant. The activity levels of TA in the both livers of rat and quail were about one-50ths of those of TDH and TH.

Concentrations of D-lactate-related metabolites in livers of quails that were fasted or fed on threonine-enriched diets

To study whether threonine is a precursor of D-lactate in quail liver as in octopus, D-lactate-related metabolites in livers were determined in quail fed threonine-enriched diet. The metabolite concentrations in quail liver are shown in Table 2. Three days of fasting led to a 1/3 decrease in methylglyoxal content in whole liver. The concentration of D-lactate in the liver of the normal diet quail was 8.75 nmol per g wet weight, but was not detectable in livers of quails fasted for 3 days. The content of D-lactate in the control quail liver was 8% of the value obtained for livers from rats on a normal diet (Ohmori and Iwamoto, 1988). The total hepatic content of D-lactate in quails fed with the 1% or 5% threonine diet decreased to 57% or 66% of the control group's values, respectively. The hepatic content of pyruvate in quail liver after 3 days fasting was 46% and 31% of the corresponding per gram wet weight and whole liver values determined for the normal diet group, respectively.

The activities of glyoxalase I and II in livers of quails fasted for 72 h or fed on threonine-enriched diet

Glyoxalase I catalyzes conversion of methylglyoxal to S-lactoylglutathione, which is then converted into D-lactate by Glyoxalase II. As seen in Table 3, the specific activity

Table 1. The activity of TA in liver of quails and rats fasted for 3 days or fed on threonine-added diet

Animal	Condition of feeding	(n)	Enzyme activity		
			(a)	(b)	(c)
Quail	Control	(11)	0.10 ± 0.02	7.22 ± 1.51	16.07 ± 3.39
	Fasted	(3)	0.12 ± 0.01	6.21 ± 0.75	11.53 ± 1.83
	1% Threonine	(3)	0.10 ± 0.03	5.79 ± 1.74	13.98 ± 4.46
	5% Threonine	(6)	0.18 ± 0.06	11.07 ± 3.46	25.00 ± 7.88
Rat	Control	(4)	0.12 ± 0.06	6.97 ± 3.23	47.60 ± 22.10
	Fasted	(4)	0.05 ± 0.02	3.26 ± 1.41	13.85 ± 6.05

a mU/mg protein, mU represents nmol/min; *b* U/g wet weight; *c* U/whole liver; *n* number of animals
Five week-old male quails were fed on normal diet, 1% and 5% threonine-added diet for 3 days or fasted for 3 days. The livers were homogenized and homogenates were centrifuged. The reaction mixture (2 ml) consisted of 0.1 M potassium phosphate, 0.05 mM pyridoxal 5′-phosphate, 1.0 mM dithiothreitol, 0.057 mM NADH, 0.7 U alcohol dehydrogenase, 0.1 M threonine and the supernatant of the homogenate. The reaction was recorded at 340 nm and 37°C for 10 min. The control run was carried out without threonine (Palekar et al., 1973). All values are means ± S.E.M.

Table 2. Contents of D-lactate and its related compounds in quail liver

Condition of feeding	Metabolites (means ± S.E.M.)								
	Methylglyoxal			D-Lactate			Pyruvate		
	(n)	(a)	(b)	(n)	(a)	(b)	(n)	(a)	(b)
Control	(5)	1.22 ± 0.16	2.60 ± 0.29	(6)	8.75 ± 2.55	15.94 ± 4.82	(6)	32.50 ± 4.41	74.41 ± 7.39
Fasted	(6)	1.33 ± 0.08	1.72 ± 0.16*	(3)	n.d.	n.d.	(6)	14.96 ± 0.31**	23.00 ± 1.00***
1% Threonine		–	–	(3)	5.18 ± 1.74	9.09 ± 2.87		–	–
5% Threonine		–	–	(6)	4.40 ± 0.79	10.46 ± 1.41		–	–

a nmol/g wet weight; *b* nmol/whole liver; *n.d.* not detected; –: not determined; *n* number of animals
Five week-old male quails were fed on normal diet, 1% or 5% threonine-added diets for 3 days or fasted for 3 days. The livers were homogenized and homogenates were centrifuged. The metabolites in the supernatants were determined. Details are written in ''Materials and methods''. Statistical analysis: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Table 3. The activities of glyoxalase I and II of liver of quails fasted for 3 days or fed on threonine-added diets

Condition of feeding	(n)	Enzyme activity (means ± S.E.M.)					
		Glyoxalase I			Glyoxalase II		
		(a)	(b)	(c)	(a)	(b)	(c)
Control	(11)	0.37 ± 0.05	27.87 ± 3.63	66.41 ± 9.76	0.13 ± 0.01	9.28 ± 1.78	21.31 ± 2.49
Fasted	(5)	0.50 ± 0.05	27.48 ± 3.66	41.92 ± 5.80	0.15 ± 0.02	7.75 ± 1.50	11.94 ± 2.59*
1% Threonine	(4)	0.45 ± 0.07	32.78 ± 4.75	76.99 ± 14.82	0.13 ± 0.01	9.94 ± 1.18	22.70 ± 2.62
5% Threonine	(3)	0.26 ± 0.05	16.01 ± 1.27	37.27 ± 2.43	0.02 ± 0.01***	1.66 ± 0.02***	3.53 ± 0.32**

a U/mg protein, U represents μmol/min; *b* U/g wet weight; *c* U/whole liver; *n* number of animals
Five week-old male quails were fed on normal diet, 1% or 5% threonine-added diets for 3 days or fasted for 3 days. The livers were homogenized and the homogenates were centrifuged. The enzyme activities of glyoxalase I and II in the supernatants were assayed. Details are written in ''Materials and methods''. Statistical analysis: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

glyoxalase I in the liver of fasted quails was 1.4 times higher than that in the control group's livers. The 5% threonine diet led to a decrease in the glyoxalase I activity to 70% that of the control. Total activities of glyoxalase I in the liver of the fasted and the 5% threonine diet groups were 64% and 56%, respectively, of that in the liver of the normal diet group. As for glyoxalase II, which is the rate limiting enzyme of the bypass, the specific activity in the liver of quail fed 5% threonine diet decreased to 15% and total activity decreased to 17% of that determined for the normal group.

The catabolism of threonine by TDH in quail liver mitochondria

TDH is a NAD-dependent mitochondrial enzyme and catalyzes the oxidation of threonine to 2-amino-3-oxobutyrate which spontaneously decomposes to aminoacetone and CO_2 (Aoyama and Motokawa, 1981; Ray and Ray, 1985) (Fig. 1). 2-Amino-3-oxobutyrate CoA-ligase catalyzes the formation reaction of 2-amino-3-oxobutyrate from acetyl-CoA and glycine and the reverse reaction (Fig. 1). The ligase is called also aminoacetone synthase (Ray et al., 1991) and contains pyridoxal phosphate as a coenzyme. The three evidences can be seen from Table 4. a) As for effect of the presence of CoA on the glycine formation from threonine: When CoA, which is a cofactor of 2-amino-3-oxobutyrate CoA-ligase was added to the reaction mixture, more glycine was formed than aminoacetone irrespective of nutritional condition. That is, 3.3 times more threonine was converted into glycine in the presence of CoA in mitochondria of the normal quail liver compared to that in the absence of CoA. b) As for effect of the presence of CoA on the aminoacetone formation from threonine: The conversion of threonine to aminoacetone in the presence of CoA in

Table 4. Formation of aminoacetone and glycine from threonine by threonine dehydrogenase in quail liver mitochondria

Condition of feeding	Reaction condition	Metabolite formed (nmol/ml)	
		Aminoacetone	Glycine
Control	Complete	41.7	106.2
	Complete minus CoA	59.5	31.8
Fasted	Complete	68.1	152.4
	Complete minus CoA	87.2	127.2

Complete reaction mixture contained 0.1 M threonine, 2.5 mM NAD, 0.1 M Tris-HCl (pH 7.4), 0.1 mM CoA and 0.1 ml of the mitochondria suspension in a final volume of 2.0 ml. The reaction was conducted at 37°C for 30 min and terminated by the addition of 1 ml of 15% trichloroacetic acid solution. Aminoacetone and glycine were determined as described under "Materials and methods"

mitochondria of the normal quail liver was only 70% of that produced in the absence of CoA. The threonine metabolism in mitochondria of liver from fasting quail showed the same tendency as that in the control group liver. c) The influence of fasting on threonine degradation: The degradation rate of threonine to glycine and aminoacetone was at least 1.5 times higher in liver mitochondria from fasting quail than in liver mitochondria from quail liver on a normal diet whether the reaction mixtures contained CoA or not. Bird et al. (1984) reported the formation of glycine and aminoacetone from threonine by rat liver mitochondria; they concluded that glycine synthesis predominates and aminoacetone is a minor product. Davis and Austic (1994) reported that when glycine and aminoacetone formation both occurred in isolated hepatic mitochondria from chicken fed on normal diet, the ratio of glycine to aminoacetone formed was 3 to 4, while the ratio for rats was 0.5 to 0.9. Table 4 shows that in the presence of CoA, the ratio of glycine/aminoacetone was 2.2 to 2.5 for liver mitochondria of quails from both test conditions (Table 4). The specific activity of aminoacetone synthase in liver homogenate of quail is shown in Table 5. The activity was higher in the homogenate from the fasting birds than from birds maintained on the standard diet. If expressed on a whole liver basis, the aminoacetone synthase activity of the control group was 1.2 times higher than that of the fasted group.

Discussion

Threonine is ketogenic amino acid in quail and glucogenic amino acid in rat

As mentioned above, threonine is metabolized through three routes by three different enzymes. In Fig. 4 these enzyme activities in the livers of normally fed quail and rats are shown. In the quail liver, 88% of threonine is metabolized by TDH, while in the rat liver nearly all threonine (93%) is metabolized by TH. The TDH activity in quail liver was 11 times higher than that in rat liver. In contrast, the TH activity in the normal rat liver was 15 times higher than that in the normal quail liver (Figs. 2 and 3). When quail were fed the 5% threonine-added diet or were fasted for 72 h, the TDH activity in the liver increased 2 and 3 times, respectively, compared to that of the control group (Fig. 2). On the other hand, the TDH activity in rat liver did not change after fasting (Fig. 2). Though the TH activity in quail liver is relatively low, it increased 1.3, 1.5 and 2 times, when

Table 5. Aminoacetone synthase activity in liver homogenate of quail

Condition of feeding	(n)	Enzyme activity	
		(a)	(b)
Control	(3)	0.17 ± 0.05	108.01 ± 36.66
Fasted	(3)	0.29 ± 0.01	86.96 ± 24.89

a mU/mg protein, mU represents μmol/min; *b* mU/whole liver; *n* number of animals

Glycine (2.5 mM, final), acetyl CoA (0.3 mM, final), Tris-HCl (0.1 M, pH 7.4) and enzyme (0.3 to 1.5 mg protein) were mixed and incubated at 37°C for 30 min. The reaction volume was 2.0 ml. After deproteinization, aminoacetone was reacted with acetylacetone and then further reacted with dimethylaminobenzaldehyde. The absorbance was measured at 552 nm. All values are means ± S.E.M.

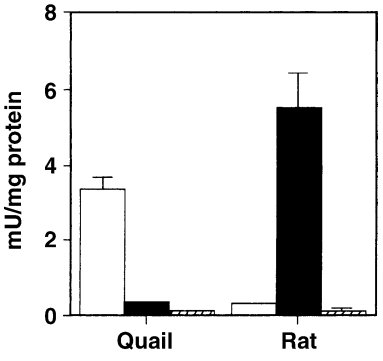

Fig. 4. Comparison of activities of TDH, TH and TA in liver of quail and rat fed on a normal diet. □, Threonine dehydrogenase (TDH); ■, threonine dehydratase (TH); ▨, threonine aldolase (TA). Bars represent ±S.E.M.

the birds were given the 1% threonine-added diet, the 5% threonine-added diet or fasted, respectively (Fig. 3). The TH activity in the rat liver after fasting was 2.3 times higher than that of the normally fed group (Fig. 3).

From Figs. 2 and 4, it can be concluded that threonine is mainly metabolized by TDH in quail liver and converted into acetyl CoA and glycine. Accordingly it can be said that threonine is a ketogenic amino acid in quail. On the other hand, if threonine is metabolized by TH, it is converted into 2-oxobutyrate. 2-Oxobutyrate, needles to say, is converted to propionyl-CoA, which subsequently forms succinyl-CoA. Accordingly threonine plays a role as a glucogenic amino acid in rat, especially in the fasted rats (Figs. 1 and 3). Threonine becomes more ketogenic when quail is fasted or fed on excess amounts of threonine (Fig. 2) and more glucogenic when rat was fasted (Fig. 3). As for TA, its activity was very low in the livers of all experimental groups of quail and rats compared to that of TDH and TH (Fig. 4). The difference between the TA activity of normal and treated animals was not significant (Table 1 and Fig. 4). The physiological meaning of TA and its contribution to the metabolism of threonine can be neglected in livers of both animals (Table 1 and Fig. 4). In 1983, Bird and Nunn reported that the relative activities of TDH, TH and TA were 58, 39 and 3%, respectively, in rat liver of the normally-fed state and 4, 95 and 1% in rat liver of the 72 h-starved state.

Threonine is used effectively for energy production and nitrogen excretion

Here we would like to consider the general energy metabolism in birds. Especially, migratory birds utilize triacylglycerols and ketone bodies as fuel in order to fly for long distances. Q_{O_2} (the oxygen consumption per milligram of tissue) of flight muscle is over 10 times higher than that of conventional muscles or organs of mammals. From this starting point, we think it is fitting that threonine is metabolized via the ketogenic path in quail, especially while fasting. More importantly, since TDH is localized in mitochondria, acetyl CoA formed can directly enter the TCA cycle. Needless to say glycine, another product of the oxidation of threonine by TDH, can be used as energy source. Glycine, however, plays another role in birds. During the synthesis of purines, a glycine molecule is wholly incorporated into a purine ring. Birds excrete nitrogen resulting from the metabolism of nitrogen-containing molecules as uric acid and uric acid is the main end product of purine metabolism in birds. It is clear from this discussion that TDH plays an important role in the excretion of nitrogen. If in quail threonine was metabolized by the TH path as in rat, which is ureoteic, ammonia would be released from threonine. Since birds have not the urea cycle, ammonia cannot be incorporated into urea and excess metabolic nitrogen could not be excreted.

Methylglyoxal bypass

Finally, we will discuss the methylglyoxal bypass. As shown in Table 2, the total amounts of methylglyoxal, D-lactate and pyruvate in quail liver decreased during fasting and during threonine-enriched diet. In particular, D-lactate was not detected in the liver after fasting. Table 3 shows that the total activities of glyoxalase I and II decreased in liver of quail fed the 5% threonine-added diet or fasted for 72 h. Thus, in especially fasting or threonine-enriched diet fed quails, threonine was preferentially metabolized via the ketogenic path and not by the methylglyoxal bypass in the liver (Fig. 1). It is true though, small amounts of aminoacetone must be formed from threonine in both animals, especially in quail as observed above (Figs. 1 and 4). Aminoacetone is deaminated by amine oxidase (EC 1.4.3.4) and converted into methylglyoxal, which, in turn, is isomerized to D-lactate in the presence of glutathione by glyoxalase I and II (the methylglyoxal bypass). If D-LDH were also present in higher animals, the existence of the methylglyoxal bypass would be verified. The presence of D-LDH in higher animals, however, has not been reported, although it is present in octopus muscle (Ohmori et al., 1997). We had reported that livers from fasting rats (Kondoh et al., 1992) and muscle of fasting octopus have significantly higher levels of D-lactate compared to the control tissue (it will be published). We present here a hypothesis that D-lactate may be degraded into formate and acetoaldehyde in higher animals. More recently we reported that in cell-free homogenate of Octopus vulgaris tentacle, threonine was readily metabolized to D-lactate (Akagi and Ohmori, 2004). From the experimental results presented here, however, threonine is not a precursor of D-lactate in Japanese quail. In conclusion we would like to say that TDH is present not for the aminoacetone formation, namely for the methylglyoxal bypass but for glycine and acetyl-CoA formation in quail liver. It has been said that the biological significance of 2-amino-3-oxobutyrate CoA-ligase is not clear. In this report the meaning of the presence of the ligase in quail liver can be explicable.

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