Is methionine transaminated in skeletal muscle?

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Methionine transamination is extensive in rat and chick skeletal-muscle homogenates, but is barely detectable in intact rat, but not chick, skeletal muscles. Branched-chain amino acids essentially block methionine transamination in intact muscles and homogenates from both species. The physiological significance of methionine transamination in skeletal muscle is questioned.

INTRODUCTION

Skeletal muscle is generally considered not to degrade methionine via the trans-sulphuration pathway, owing to the very low activity or absence of the required enzymes [1,2]. An alternative pathway has been proposed which initially involves methionine transamination [3–6]. This proposal is largely based on the detection of methionine transamination in homogenates of skeletal muscle, liver and other tissues [3–5] and in extracts of rat skeletal muscle [6,7]. Recently, it has been reported that [U- ^{14}C]methionine is metabolized to $^{14}\text{CO}_2$ and ^{14}C -labelled tricarboxylic acid-cycle intermediates via a trans-sulphuration-independent pathway in perfused rat hind-quarter preparations [6]. On the basis of studies with muscle homogenates, Benevenga and his associates [4,5] have suggested that the skeletal musculature might be the major site for methionine transamination in animals. However, all of these studies were performed in the absence of amino acids other than methionine [4–7] and at very high methionine concentrations, ranging from 10 mM [4,5] to 20 mM [6,7], which are about 100–200 times physiological plasma concentrations [8]. As a result, the physiological significance of methionine transamination, as shown in skeletal-muscle homogenates and extracts, remains unclear [9]. The present study was therefore conducted to investigate methionine transamination in intact skeletal muscles from rats and chicks in the absence or the presence of amino acids at plasma concentrations.

MATERIALS AND METHODS

Chemicals and animals

L-[1- ^{14}C]Methionine was obtained from American Radiolabeled Chemicals, St. Louis, MO, U.S.A. Its radiochemical purity was determined to be greater than 99% by descending paper chromatography with butan-1-ol/acetic acid/water (2:1:1, by vol.) as the solvent. The source of L-[1- ^{14}C]leucine and all other chemicals was reported previously [10].

Male broiler chicks (9 days old; Hubbard strain, *Gallus domesticus*) and 21-day-old weaned male Buffalo rats were provided free access to food and water. Animals were anaesthetized with halothane before the muscles were removed.

Methionine transamination in skeletal-muscle homogenates of rats and chicks

Rat gastrocnemius (314.2 ± 18.8 mg), diaphragm (122.9 ± 8.4 mg), extensor digitorum longus (EDL) (24.9 ± 2.6 mg) and soleus (20.3 ± 1.6 mg) muscles, and chick extensor digitorum communis (EDC) muscles (26.4 ± 0.8 mg), were homogenized in 0.25 M-sucrose in ice [5] in a Potter–Elvehjem glass tube fitted with a glass pestle. Briefly, individual rat gastrocnemius muscles were homogenized in 2 ml of 0.25 M-sucrose. The rat diaphragm, EDL and soleus muscles from seven rats were pooled by muscle type and homogenized in 8 ml of 0.25 M-sucrose, and each homogenate was distributed equally into four tubes. Two EDC muscles from individual chicks were pooled and homogenized in 2 ml of 0.25 M-sucrose. The homogenates (2 ml) were incubated at 37 °C for 2 h in a final volume of 5 ml in the presence of 10 mM-L-[1- ^{14}C]methionine (11 d.p.m./nmol), 10 mM-pyruvate, 1 mM-ATP, 5 mM-MgCl₂ and 10 mM-phosphate buffer (pH 7.4) saturated with O₂/CO₂ (19:1) as described by Mitchell & Benevenga [5]. BCAA were absent from or present in the incubation media as indicated in Table 1. Incubation media without muscle homogenates were run to correct for background radioactivity values. Incubation was terminated by injection of 0.2 ml of 1.5 M-HClO₄ into the media. $^{14}\text{CO}_2$ from [1- ^{14}C]methionine decarboxylation and from chemical decarboxylation of [1- ^{14}C]oxomethionine by 30% (v/v) H₂O₂ was separately collected in Hyamine hydroxide [10,11]. The net rate of methionine transamination was calculated on the basis of the specific radioactivity of [1- ^{14}C]methionine [11] in the medium.

Methionine transamination in intact rat and chick skeletal muscles

The rat quarter-diaphragm, EDL and soleus muscles and the chick EDC muscles (19.5 ± 0.6 mg) were dissected and incubated in Krebs–Ringer bicarbonate buffer saturated with O₂/CO₂ (19:1) [10,12,13]. The buffer also contained either 0.01 unit of insulin/ml, 2 mM-Hepes, 12 mM-glucose and 0.2 mM-methionine (medium A) or 0.1 unit of insulin/ml, 2 mM-Hepes, 5 mM-glucose and 0.5 mM-methionine (medium B). All muscles were pre-incubated for 30 min as recommended by Goldberg *et al.* [14] in either medium A or medium B, and then incubated for 2 h in fresh media containing L-[1- ^{14}C]methionine

Abbreviations used: EDC, extensor digitorum communis; EDL, extensor digitorum longus; BCAA, branched-chain amino acid; oxomethionine, 2-oxo-4-methiolbutyrate.

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(60 d.p.m./ μ l), with or without the addition of BCAA or all amino acids other than methionine at plasma concentrations [8,15]. In all of these experiments, one muscle was used for the control while the contralateral muscle was used for the treatment. Incubation media without muscles were run to correct for background radioactivity values. At the end of the final incubation period, muscles were removed from the incubation media and placed in 1 ml of ice-cold 2% (w/v) trichloroacetic acid. $^{14}\text{CO}_2$ from methionine decarboxylation and from chemical decarboxylation of $[1-^{14}\text{C}]$ methionine by 30% H_2O_2 was separately collected in Hyamine hydroxide [10,11]. The amount of $^{14}\text{CO}_2$ from chemical decarboxylation of $[1-^{14}\text{C}]$ oxomethionine by 30% H_2O_2 averaged 2540 d.p.m. for chick EDC muscles incubated with 0.2 mM-methionine in the absence of all other amino acids, accounting for approx. 1.2% of the added radioactivity and 24.2% of the total $[1-^{14}\text{C}]$ methionine taken up by the muscle. The rate of methionine degradation was calculated on the basis of the specific radioactivity of intracellular L- $[1-^{14}\text{C}]$ methionine, which was measured essentially as described for that of L- $[1-^{14}\text{C}]$ leucine [10] after correction for the extracellular space [10,12,13]. In a preliminary experiment, the rate of methionine transamination was measured at 30 min intervals during the incubation period in the presence of 0.5 mM-L- $[1-^{14}\text{C}]$ methionine (medium A). Methionine transamination was linear over the 2 h final incubation period ($r = 0.998$, $P < 0.001$; $n = 5$).

Leucine transamination in intact rat and chick skeletal muscle

Intact rat and chick skeletal muscles were dissected and incubated with 0.5 mM-L- $[1-^{14}\text{C}]$ leucine in the ab-

sence of other amino acids (medium B) as described above. $^{14}\text{CO}_2$ released after transamination of L- $[1-^{14}\text{C}]$ leucine was collected in Hyamine hydroxide, and the net leucine transamination rate was calculated from the specific radioactivity of intracellular L- $[1-^{14}\text{C}]$ leucine [10,12,13]. Under these conditions, it was previously demonstrated that the rate of leucine catabolism remains constant throughout the 2 h incubation period [16]. The amount of $^{14}\text{CO}_2$ released after transamination of L- $[1-^{14}\text{C}]$ leucine by chick EDC muscles and rat soleus muscles averaged 13600 d.p.m. and 6600 d.p.m., accounting for approx. 3.6% and 1.7% of the added radioactivity and 82.4% and 77.6% of the total L- $[1-^{14}\text{C}]$ leucine taken up by muscles, respectively.

Statistical analysis

The results, given as means $\pm$ S.E.M., were statistically analysed by the t test as described by Steel & Torrie [17].

RESULTS AND DISCUSSION

Methionine was found to be extensively transaminated in homogenates of rat diaphragm, gastrocnemius, EDL and soleus muscles, and of chick EDC muscles incubated in the presence of 10 mM-methionine in the absence of other amino acids (Table 1). These results are consistent with those reported for rat gastrocnemius muscle homogenates [5] and for rat skeletal-muscle extracts [6,7]. The addition of BCAA markedly inhibited methionine transamination in skeletal-muscle homogenates of rats and chicks (Table 1), suggesting that BCAA transaminase catalyses methionine transamination.

In contrast with muscle homogenates, methionine

Table 1. Methionine transamination in rat and chick skeletal-muscle homogenates

Rat and chick skeletal-muscle homogenates were incubated with 10 mM-L- $[1-^{14}\text{C}]$ methionine either in the absence of other amino acids (Control) or in the presence of 5 mM each of leucine, isoleucine plus valine (BCAA). Results, given as means $\pm$ S.E.M., were statistically analysed by the paired t test for the rat gastrocnemius muscles, and by the non-paired t test for other rat muscles and chick muscles. Treatment means with * and ** are significantly different from control means at $P < 0.05$ and $P < 0.001$ respectively; $n = 7$ for gastrocnemius muscle, $n = 2$ for other rat muscles and $n = 6$ for chick EDC muscles. ND, not detectable.

Muscle	Methionine decarboxylation (nmol/2 h per mg of tissue)	Oxomethionine production (nmol/2 h per mg of tissue)	Net methionine transamination (nmol/2 h per mg of tissue)
Rat			
Gastrocnemius			
Control	0.013 $\pm$ 0.001	0.77 $\pm$ 0.04	0.78 $\pm$ 0.04
+ BCAA	0.010 $\pm$ 0.001**	0.21 $\pm$ 0.04**	0.22 $\pm$ 0.04**
Diaphragm			
Control	0.034 $\pm$ 0.002	1.05 $\pm$ 0.03	1.09 $\pm$ 0.03
+ BCAA	0.024 $\pm$ 0.002	0.35 $\pm$ 0.04*	0.37 $\pm$ 0.04*
EDL			
Control	0.014 $\pm$ 0.001	0.93 $\pm$ 0.02	0.94 $\pm$ 0.02
+ BCAA	0.012 $\pm$ 0.001	0.31 $\pm$ 0.05	0.32 $\pm$ 0.05*
Soleus			
Control	0.017 $\pm$ 0.001	0.87 $\pm$ 0.04	0.89 $\pm$ 0.05
+ BCAA	0.014 $\pm$ 0.001	0.26 $\pm$ 0.03*	0.27 $\pm$ 0.03*
Chick			
EDC			
Control	ND	1.72 $\pm$ 0.18	1.72 $\pm$ 0.18
+ BCAA	ND	0.20 $\pm$ 0.05**	0.20 $\pm$ 0.05**

Table 2. Transamination of methionine and leucine in intact rat and chick skeletal muscles

Intact rat and chick skeletal muscles were incubated with 0.5 mM-L-[1-¹⁴C]methionine (196 d.p.m./nmol) or 0.5 mM-L-[1-¹⁴C]leucine (214 d.p.m./nmol) in the absence of other amino acids as described in the text. Results are given as means $\pm$ S.E.M. for six observations per treatment. ND, not detectable.

Muscle	Methionine		Leucine	
	Intracellular sp. radioactivity (d.p.m./nmol)	Transamination (nmol/2 h per mg of tissue)	Intracellular sp. radioactivity (d.p.m./nmol)	Transamination (nmol/2 h per mg of tissue)
Rat				
Diaphragm	202.3 $\pm$ 7.8	0.036 $\pm$ 0.003	158.8 $\pm$ 6.5	3.87 $\pm$ 0.28
EDL	193.6 $\pm$ 9.5	ND	165.7 $\pm$ 9.1	2.57 $\pm$ 0.19
Soleus	200.2 $\pm$ 8.5	ND	168.5 $\pm$ 7.3	2.02 $\pm$ 0.31
Chick				
EDC	167.5 $\pm$ 7.4	1.15 $\pm$ 0.08	153.1 $\pm$ 6.4	4.55 $\pm$ 0.43

Table 3. Methionine transamination in intact chick EDC muscle

Contralateral EDC muscles held at resting length were incubated in media containing either 0.2 mM- or 0.5 mM-L-[1-¹⁴C]methionine in the absence of other amino acids (Control), or in the presence of either leucine, isoleucine plus valine (BCAA) or all other amino acids (All AA) at normal plasma concentrations. Results, given as means $\pm$ S.E.M. for six observations per treatment, were statistically analysed by the paired *t* test. Treatment means marked with ** are significantly different (*P* < 0.001) from control means obtained from the contralateral muscles. ND, not detectable.

[Methionine]	Addition	Methionine decarboxylation (nmol/2 h per mg of tissue)	Oxomethionine production (nmol/2 h per mg of tissue)	Net methionine transamination (nmol/2 h per mg of tissue)	Intracellular concn. (nmol/mg of tissue)	
					Methionine	BCAA
0.2 mM	Control	0.048 $\pm$ 0.003	0.37 $\pm$ 0.04	0.42 $\pm$ 0.05	0.93 $\pm$ 0.06	0.19 $\pm$ 0.02
	+ BCAA	0.028 $\pm$ 0.003**	ND	0.028 $\pm$ 0.003**	0.42 $\pm$ 0.04**	1.29 $\pm$ 0.06**
	Control + All AA	0.054 $\pm$ 0.003	0.40 $\pm$ 0.04	0.45 $\pm$ 0.05	1.13 $\pm$ 0.08	0.21 $\pm$ 0.02
0.5 mM	+ All AA	0.029 $\pm$ 0.001**	ND	0.029 $\pm$ 0.001**	0.18 $\pm$ 0.0**	1.17 $\pm$ 0.09**
	Control	0.052 $\pm$ 0.005	0.99 $\pm$ 0.04	1.04 $\pm$ 0.04	1.57 $\pm$ 0.13	0.20 $\pm$ 0.01
	+ BCAA	0.034 $\pm$ 0.003**	ND	0.034 $\pm$ 0.003**	0.71 $\pm$ 0.06**	1.35 $\pm$ 0.12**
	Control	0.063 $\pm$ 0.005	1.05 $\pm$ 0.05	1.11 $\pm$ 0.05	1.64 $\pm$ 0.15	0.21 $\pm$ 0.02
	+ All AA	0.025 $\pm$ 0.002**	ND	0.025 $\pm$ 0.002**	0.39 $\pm$ 0.07**	1.13 $\pm$ 0.15**

transamination was not detected in intact rat quarter-diaphragm, EDL and soleus muscles incubated with 0.2 mM-methionine in the presence or absence of plasma concentrations of other amino acids (results not shown). Likewise, methionine transamination was barely detected in intact rat quarter-diaphragms incubated with 0.5 mM-methionine in the absence of other amino acids (Table 2), and was not detected in this tissue in the presence of other amino acids or the BCAAs alone at plasma concentrations (results not shown). Similarly, methionine transamination was not detected in intact rat EDL and soleus muscles incubated with 0.5 mM-methionine in the absence (Table 2) or the presence (results not shown) of plasma concentrations of other amino acids or the branched-chain amino acids alone.

It is unlikely that our failure to detect methionine transamination in intact rat skeletal muscles is due to artifacts involved in muscle incubation, since 0.5 mM-L-[1-¹⁴C]leucine is extensively transaminated in intact rat skeletal muscles incubated under the same conditions

used for methionine-transamination studies (Table 2). Thus our results raise a serious question regarding the physiological significance of methionine transamination, as shown in rat skeletal-muscle homogenates and extracts in which 10 mM- or 20 mM-methionine was the only amino acid provided [4-7].

This question also arises from our measurements of methionine transamination in intact chick skeletal muscles incubated in the absence or the presence of other amino acids. As shown in Tables 2 and 3, methionine is extensively transaminated in intact chick EDC muscles incubated with either 0.2 mM- or 0.5 mM-methionine in the absence of other amino acids. However, methionine transamination is barely detectable in chick muscles incubated in the presence of plasma concentrations of other amino acids or BCAA alone (Table 3). In fact, the presence of plasma concentrations of BCAA is equally as effective in inhibiting methionine transamination as the presence of all plasma amino acids. The inhibitory effect of BCAA may be accounted for in part by a decrease in

methionine uptake (Table 3) and an inhibition of methionine transamination by BCAA (Tables 1 and 3).

It follows that there is a striking difference in methionine transamination between intact rat and chick skeletal muscles incubated in the absence of other amino acids. This species difference may be due to a difference in the activity of the BCAA transaminase for methionine between rats and chicks (Table 1 and 2). Whatever the difference is, our results clearly demonstrate that transamination of methionine is negligible in intact rat and chick skeletal muscle in the presence of plasma concentrations of methionine and other amino acids or the BCAAs alone (Table 3).

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