Glutamine metabolism in skeletal muscles from the broiler chick (*Gallus domesticus*) and the laboratory rat (*Rattus norvegicus*)

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Oxidative decarboxylation of L-[1-¹⁴C]glutamine was studied in isolated chick and rat skeletal muscles incubated in the presence of glucose, insulin and plasma concentrations of amino acids. (1) The rate of oxidative decarboxylation of L-[1-¹⁴C]glutamine was high, and exceeded that of L-[1-¹⁴C]leucine in all muscles. (2) The rate of oxidative decarboxylation of L-[1-¹⁴C]glutamine increased with increasing intracellular concentrations of glutamine. (3) The activities of glutamine aminotransferases K and L were more than 10-fold greater in rat than in chick skeletal muscles. (4) Mitochondrial phosphate-activated glutaminase activity was approx. 10-fold greater in chick than in rat skeletal muscles and increased with increasing glutamine concentrations. (5) An inhibitor of glutaminase, 6-diazo-5-oxo-L-norleucine, inhibited the rate of glutamine decarboxylation in chick, but not in rat, skeletal muscle. These findings suggest that glutamine degradation in skeletal muscle may be substantial and may make an important contribution to the regulation of intramuscular glutamine concentrations. A species difference in the pathways and the subcellular location for the conversion of glutamine into 2-oxoglutarate in rat and chick skeletal muscles is implied by the relative activities of glutamine-degrading enzymes.

INTRODUCTION

Glutamine is the most abundant free amino acid in plasma and skeletal muscle, and is a key precursor for synthesis of compounds such as proteins, ATP, nucleotides and NAD [1,2]. It serves as a major carrier of nitrogen for inter-organ transport, plays a key role in detoxification of ammonia and in maintenance of acid/base balance, and may play a role in regulation of skeletal-muscle protein turnover [2,3]. These important roles of glutamine have prompted extensive studies of its metabolism in a variety of mammalian tissues and cells [2,4]. Studies during the last 10 years have shown not only that glutamine is extensively metabolized in the kidney [5], small intestine [6], reticulocytes [7], certain tumour cells [4], macrophages [8], lymphocytes [9] and cultured cell lines [10], but that it is also an important energy source for these tissues and cells [4–10].

It is generally considered that skeletal muscle is the major source of glutamine during the post-absorptive state [11,12]. As a result, much effort has been directed towards studies of glutamine synthesis in skeletal muscles from experimental animals such as rats [12] and chicks [13]. By contrast, little attention has been paid to glutamine degradation in skeletal muscles, since it has been traditionally considered quantitatively unimportant [3,12,14], on the basis of early observations that glutaminase activity is negligible or absent in rat skeletal muscle [15,16]. The authors are unaware of information about glutamine-metabolizing enzymes in avian skeletal muscle. In view of species differences in amino acid metabolism between chicks and rats, as demonstrated by glutamine metabolism in liver [17] and methionine transamination in skeletal muscle [18,19], the present study was designed to investigate whether glutamine is metabolized in chick skeletal muscle and to relate these observations to parallel observations in the rat.

MATERIALS AND METHODS

Chemicals

L-[1-¹⁴C]Glutamic acid was obtained from American Radio-

labeled Chemicals, St. Louis, MO, U.S.A. L-[1-¹⁴C]Leucine was obtained from ICN Radiochemicals, Montreal, Quebec, Canada. The radiochemical purity of these compounds was determined to be over 99 % by descending paper chromatography [20]. 6-Diazo-5-oxo-L-norleucine and other chemicals were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

L-[1-¹⁴C]Glutamine was prepared from L-[1-¹⁴C]glutamic acid as described by Baverel & Lund [21], except that the glutamic acid concentration in the reaction mixture was 0.45 mM and the source of glutamine synthetase was chick pectoralis muscle or rat liver cytosol obtained by centrifugation of tissue homogenates (1 g of tissue in 4 ml of 0.9 % NaCl) at 15000 g for 10 min at 4 °C. Essentially all the L-[1-¹⁴C]glutamic acid was converted into L-[1-¹⁴C]glutamine, which was separated from any labelled precursor by Dowex anion-exchange chromatography [22]. The radiochemical purity of L-[1-¹⁴C]glutamine was determined to be 98.8 % by paper chromatography [20]. [¹⁴C]Glutamine and [¹⁴C]leucine were purified immediately before use as previously described [22].

Animals

The experiments were carried out in accordance with the guidelines of the Canadian Council of Animal Care; 9-day-old male broiler chicks of the Hubbard strain (115–130 g) and young female Sprague–Dawley rats of the Buffalo strain (60–80 g) were used. Chicks were given a ration containing 23 % (w/w) crude protein, and rats were given a laboratory chow containing 24 % (w/w) crude protein. Water was provided *ad libitum*.

Skeletal-muscle preparation and incubation

Chicks were anaesthetized with halothane, and the extensor digitorum communis (EDC) muscles (19.8 ± 0.4 mg; mean ± S.E.M., *n* = 20) were exposed *in situ* and stainless-steel wire supports were inserted on to their tendons [20]. The tendons were then cut and the muscles preincubated for 30 min in 3.5 ml of Krebs–Ringer bicarbonate buffer saturated with O₂/CO₂ (19:1) [20] and transferred to fresh media of a similar composition

Abbreviations used: EDC, extensor digitorum communis; EDL, extensor digitorum longus.

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Table 1. Oxidative decarboxylation of [1-¹⁴C]glutamine and [1-¹⁴C]leucine in chick and rat skeletal muscles

Isolated skeletal muscles were incubated with 1.0 mM-[1-¹⁴C]glutamine while the contralateral muscles were incubated with 0.5 mM-[1-¹⁴C]leucine in the presence of plasma concentrations of amino acids as described in the text. Means within a column followed by different superscripts are significantly ($P < 0.01$) different, as determined by analysis of variance. The rate of leucine oxidative decarboxylation is significantly less ($P < 0.01$) than that of glutamine in each muscle, as determined by the paired t test ($n = 6$).

Muscle	Glutamine		Leucine	
	Intracellular concn. (nmol/mg of tissue)	Oxidative decarboxylation (nmol/h per mg of tissue)	Intracellular concn. (nmol/mg of tissue)	Oxidative decarboxylation (nmol/h per mg of tissue)
Chick EDC	6.16 ± 0.17 ^a	3.92 ± 0.43 ^a	0.41 ± 0.02 ^a	0.83 ± 0.07 ^a
Rat EDL	8.61 ± 0.26 ^b	3.39 ± 0.14 ^b	0.40 ± 0.03 ^a	1.06 ± 0.05 ^b
Soleus	5.35 ± 0.39 ^c	1.34 ± 0.16 ^c	0.65 ± 0.03 ^b	0.71 ± 0.03 ^c
Diaphragm	2.82 ± 0.20 ^d	3.32 ± 0.32 ^b	0.41 ± 0.01 ^a	1.28 ± 0.09 ^d

Table 2. Effect of glutamine concentration on the oxidative decarboxylation of [1-¹⁴C]glutamine in chick and rat muscles

Oxidative decarboxylation of [1-¹⁴C]glutamine was measured in incubated chick EDC muscle and rat diaphragm as described in the text. Means within a column and within a muscle type followed by different superscripts are significantly different ($P < 0.01$), as determined by one-way analysis of variance ($n = 5$).

Muscle	Medium [glutamine] (mM)	Intracellular [glutamine] (nmol/mg of tissue)	Oxidative decarboxylation of glutamine (nmol/h per mg of tissue)
Chick EDC	0.5	4.16 ± 0.37 ^a	2.35 ± 0.16 ^a
	1.0	6.30 ± 0.29 ^b	3.46 ± 0.07 ^b
	2.0	10.27 ± 0.32 ^c	4.39 ± 0.27 ^c
	4.0	14.12 ± 0.47 ^d	4.98 ± 0.57 ^c
	6.0	17.30 ± 0.51 ^e	6.05 ± 0.34 ^d
	10.0	21.18 ± 0.96 ^f	7.30 ± 0.69 ^e
Rat diaphragm	0.5	1.89 ± 0.22 ^a	2.21 ± 0.13 ^a
	1.0	2.68 ± 0.16 ^b	3.29 ± 0.11 ^b
	4.0	4.85 ± 0.26 ^c	6.11 ± 0.19 ^c
	10.0	9.96 ± 0.44 ^d	8.97 ± 0.48 ^d

and incubated for 2 h. The media contained insulin (0.01 unit/ml), 2 mM-Hepes (pH 7.4), 12 mM-glucose and amino acids at concentrations found in chick plasma [20]. Rat extensor digitorum longus (EDL), soleus and quarter-diaphragm muscles were prepared as outlined previously [23] and incubated in Krebs-Ringer bicarbonate buffer containing insulin (0.10 unit/ml), 2 mM-Hepes (pH 7.4), 5 mM-glucose and amino acids at concentrations found in rat plasma [18].

Measurement of oxidative decarboxylation of glutamine and leucine

Individual EDC muscles from chicks and EDL and soleus muscles from rats were incubated with 1.0 mM-L-[1-¹⁴C]glutamine (150 d.p.m./nmol) while their contralateral muscles were incubated with 0.5 mM-L-[1-¹⁴C]leucine (400 d.p.m./nmol). Quarter-diaphragms from the same rat were incubated with either label. These concentrations are similar to plasma concentrations (mM) *in vivo* of glutamine, which are 1.10 ± 0.08 and 0.71 ± 0.04 ($n = 10$), and of leucine, which are 0.27 ± 0.01

and 0.26 ± 0.01 ($n = 10$) for chicks and rats respectively. At the end of the 2 h final incubation period, muscles were removed from the medium, rinsed well with non-radioactive buffer and placed in 0.75 ml of 1.5 M-HClO₄. Muscle tissue was used for the determination of intracellular specific radioactivity of glutamine or leucine. In preliminary studies we observed that the intracellular specific radioactivities of these amino acids were less than those in the incubation medium; these values were therefore essential for accurate calculation of oxidative-decarboxylation rates (see below). ¹⁴CO₂ in the incubation medium was collected in Hyamine hydroxide after acidifying the medium with 0.2 ml of 1.5 M-HClO₄ [20]. Opening the incubation vials to remove the muscle tissue resulted in decreased recovery of ¹⁴CO₂ in this system ($73 \pm 6\%$; mean $\pm$ S.E.M., $n = 6$). Recovery was determined in a separate experiment using NaH¹⁴CO₃ added to the incubation medium. Calculations of the rates of oxidative decarboxylation of glutamine or leucine were based on the specific radioactivity of intracellular [¹⁴C]glutamine [24] or [¹⁴C]leucine [20], and were corrected for ¹⁴CO₂ recovery. The rates of oxidative decarboxylation of glutamine and leucine were linear over the 2 h final incubation period. The glutaminase inhibitor 6-diazo-5-oxo-L-norleucine was added to the final incubation media as indicated in Table 5. Blanks containing ¹⁴C-labelled amino acids and other components as described above, but in the absence of muscle tissue, were used in each experiment to correct for background.

Preparation of skeletal-muscle mitochondrial and cytosolic fractions

Mitochondria were isolated from chick EDC and pectoralis muscles and rat EDL, gastrocnemius, soleus and diaphragm muscles as described by Lee & Martens [25], except that Nagarse and BSA were not used. The supernatant from the 15000 g centrifugation was used as the cytosolic fraction. The mitochondrial pellet (3.75–4.5 mg of protein) from 1 g of muscle was suspended in 2.0 ml of 0.25 M-sucrose. The mitochondrial protein content was determined by the Bradford method [26].

Assay of glutamine aminotransferases K and L

Glutamine aminotransferase K and L activities were measured as described by Cooper & Meister [27], except that the products of transamination, phenylalanine (glutamine aminotransferase K) or methionine (glutamine aminotransferase L), were quantified by h.p.l.c. [23]. Blanks containing denatured

Table 3. Glutamine aminotransferase K and L activity in rat and chick skeletal-muscle cytosol

Glutamine aminotransferase K activity was assayed in the presence of 10 mM-glutamine and 10–100 μ M-phenylpyruvate, whereas glutamine aminotransferase L activity was assayed in the presence of 10 mM-L-albizzin and 10–100 μ M-4-methylthio-2-oxobutyrate as described in the text. Means for each enzyme followed by different superscripts are significantly different ($P < 0.01$), as determined by analysis of variance. The activity of both aminotransferases in rat muscles is higher ($P < 0.01$) than in chick muscles, and their activities in rat diaphragm are higher ($P < 0.05$) than in the other rat muscles ($n = 3$).

Muscle	Glutamine aminotransferase K activity (nmol of Phe produced/h per mg of tissue) at phenylpyruvate concn. (μ M):				Glutamine aminotransferase L activity (nmol of Met produced/h per mg of tissue) at 4-methylthio-2-oxobutyrate concn. (μ M):			
	10	25	50	100	10	25	50	100
Rat								
EDL	5.74 $\pm 0.09^a$	14.24 $\pm 0.10^b$	14.26 $\pm 0.13^b$	14.52 $\pm 0.29^b$	1.68 $\pm 0.11^a$	3.14 $\pm 0.18^b$	6.41 $\pm 0.56^c$	6.51 $\pm 0.42^c$
Soleus	5.56 $\pm 0.10^a$	12.99 $\pm 0.19^b$	13.32 $\pm 0.11^b$	13.57 $\pm 0.08^b$	1.63 $\pm 0.06^a$	3.97 $\pm 0.07^b$	7.66 $\pm 0.07^c$	7.71 $\pm 0.08^c$
Gastrocnemius	5.43 $\pm 0.11^a$	12.56 $\pm 0.43^b$	13.45 $\pm 0.17^b$	13.21 $\pm 0.10^b$	1.59 $\pm 0.12^a$	3.33 $\pm 0.27^b$	7.98 $\pm 0.21^c$	7.05 $\pm 0.32^c$
Diaphragm	6.87 $\pm 0.07^a$	16.81 $\pm 0.05^b$	17.32 $\pm 0.09^b$	17.67 $\pm 0.24^b$	2.06 $\pm 0.13^a$	4.54 $\pm 0.06^b$	8.43 $\pm 0.13^c$	8.96 $\pm 0.24^c$
Chick								
EDC	0.34 $\pm 0.01^a$	0.40 $\pm 0.02^b$	0.46 $\pm 0.02^b$	0.52 $\pm 0.02^b$	0.19 $\pm 0.01^a$	0.31 $\pm 0.01^b$	0.43 $\pm 0.02^c$	0.56 $\pm 0.02^d$
Pectoralis	0.29 $\pm 0.02^a$	0.37 $\pm 0.03^b$	0.42 $\pm 0.02^b$	0.46 $\pm 0.04^b$	0.16 $\pm 0.01^a$	0.25 $\pm 0.02^b$	0.35 $\pm 0.01^c$	0.46 $\pm 0.01^d$

mitochondrial or cytosolic proteins plus all other reaction components were run in each assay. Phenylalanine and methionine were not detected in blanks of mitochondrial preparations, but they were present in negligible amounts in the blanks of cytosolic preparations. The glutamine aminotransferase K and L reactions were shown to be linear during the 60 min incubation period.

Assay of phosphate-activated glutaminase activity

Phosphate-activated glutaminase activity in the mitochondrial and cytosolic fractions was determined by measuring the conversion of [14 C]glutamine into [14 C]glutamate in the presence of an inhibitor of glutamate oxidation, as described by Smith *et al.* [28]. Blanks with denatured mitochondrial or cytosolic proteins and other reaction components were run in each assay. The mitochondrial phosphate-activated glutaminase reaction was linear during the 60 min incubation period.

Statistical analysis

Results, given as means $\pm$ S.E.M., were analysed statistically by the paired *t* test or by analysis of variance [29], as stated in the Tables.

RESULTS AND DISCUSSION

Glutamine degradation in chick and rat skeletal muscle

It is generally believed that only six amino acids are significantly degraded in skeletal muscle, i.e. alanine, glutamate, aspartate, leucine, isoleucine and valine (e.g. [12]). However, the present study demonstrates that isolated skeletal muscles from both chicks and rats decarboxylate [1- 14 C]glutamine. Indeed, the rate of oxidative decarboxylation of physiological concentrations of glutamine was greater ($P < 0.01$) than that of physiological

concentrations of leucine (Table 1). The rate of oxidative decarboxylation of L-[1- 14 C]glutamine was increased with increasing intramuscular glutamine concentrations (Table 2), demonstrating the considerable capacity of skeletal muscle to convert the glutamine carbon skeleton into citric-acid-cycle intermediates. These findings are in contrast with the common belief, based on reports of negligible glutaminase activity in rat skeletal muscle [15,16], that glutamine degradation in this tissue is negligible [3,12,14].

Role of glutamine aminotransferases K and L in glutamine metabolism in chick and rat skeletal muscle

Cytosolic glutamine aminotransferase K and L activities were found to be more than an order of magnitude lower ($P < 0.01$) in chick than in rat skeletal muscles, as shown in Table 3. The activity of the aminotransferases was negligible in the muscle mitochondrial fractions from both species (results not shown). The activities of glutamine aminotransferases K and L relative to each other were similar in all muscles studied. In rat skeletal muscle, cytosolic glutamine aminotransferases K and L may play a major role in glutamine metabolism, whereas in chick muscle their role is apparently less important. Transamination of glutamine with 2-oxo acids is driven by deamidation of the 2-oxoglutarate produced to 2-oxoglutarate by the relatively high ω -amidase activity found in skeletal muscle [30,31]. The relative importance of the aminotransferase route of glutamine metabolism in chick versus rat skeletal muscles could not be resolved by the use of general aminotransferase inhibitors such as amino-oxyacetic acid. These agents inhibit other important enzymes in glutamine metabolism, such as the glutamate aminotransferases, and would therefore not give interpretable data. For example, treatment of chick or rat skeletal muscles with amino-oxyacetic acid decreased intracellular glutamine concentrations by up to 80% ($n = 7$ for each species; $P < 0.01$). This change in concentration would directly affect the rate of glutamine oxidation in these tissues (Table 2).

Table 4. Phosphate-activated glutaminase activity in chick and rat skeletal muscle

Phosphate-activated glutaminase activity was assayed in the presence of 10 mM-glutamine and 150 mM-phosphate as described in the text. Means within a column followed by different superscripts are significantly different ($P < 0.01$), as determined by analysis of variance. ($n = 8$).

Muscle	Phosphate-activated glutaminase activity (nmol of Glu/h per mg of tissue)
Chick	
EDC	8.04 ± 0.04^a
Pectoralis	14.60 ± 0.10^b
Rat	
EDL	2.25 ± 0.13^c
Gastrocnemius	0.88 ± 0.04^d
Diaphragm	0.73 ± 0.03^e
Soleus	0.34 ± 0.06^f

Table 5. Effect of 6-diazo-5-oxo-L-norleucine (DON) on the degradation of [$1\text{-}^{14}\text{C}$]glutamine in chick EDC muscles

Chick EDC muscles were incubated in the presence of 1.0 mM- [$1\text{-}^{14}\text{C}$]glutamine as described in the text. One muscle was used as the control, while the contralateral muscle was used for the treatment. * Significantly different ($P < 0.01$) from the control (0 mM), as analysed by the paired t test. ($n = 8$).

DON concn. (mM)	Oxidative decarboxylation of glutamine (nmol/2h per mg of tissue)
0	5.62 ± 0.47
1	$4.01 \pm 0.22^*$
0	5.40 ± 0.54
2	$3.36 \pm 0.18^*$
0	5.78 ± 0.49
4	$2.70 \pm 0.21^*$

Role of mitochondrial phosphate-activated glutaminase in glutamine metabolism in chick and rat skeletal muscle

Since glutamine aminotransferases K and L appeared to have a lower activity in chick muscle, we studied phosphate-activated glutaminase activity in chick muscles and compared its activity with that in rat muscles. There was no cytosolic glutaminase activity detected in any of the chick or rat muscles studied in either the presence or the absence of phosphate (results not shown; $n = 8$). Mitochondrial glutaminase activity was negligible in the mitochondrial fractions from both chick EDC and pectoralis muscles incubated in the presence of 10 mM-glutamine and in the absence of additional phosphate (3.3 ± 0.3 to 3.7 ± 0.3 nmol of glutamate/h per mg of mitochondrial protein; $n = 5$). No glutaminase activity was detected in rat muscle mitochondria incubated under these conditions. However, in the presence of 150 mM-phosphate, there was considerable phosphate-activated glutaminase activity in the mitochondrial fraction from chick EDC and pectoralis muscles (ranging from 175.1 ± 10.8 to 359.4 ± 3.6 nmol of glutamate/h per mg of mitochondrial protein; $n = 8$). These results demonstrate that

phosphate-activated glutaminase activity in chick muscle is located in the mitochondrial fraction, as shown previously in the rat small intestine [32], kidney [5], liver [33] and brain [34]. In the presence of 150 mM-phosphate, glutaminase activity in the mitochondrial fractions from rat EDL, gastrocnemius, diaphragm and soleus muscles averaged 42.3 ± 3.0 , 25.8 ± 0.9 , 22.4 ± 0.6 and 18.1 ± 2.8 nmol of glutamate/h per mg of mitochondrial protein respectively ($n = 8$). The total phosphate-activated glutaminase activity in chick muscles was approximately an order of magnitude greater than the activity measured in rat skeletal muscles (Table 4). The low activities of muscle phosphate-activated glutaminase reported in this study for the rat are similar to those previously reported for the rat hindlimb by Ardawi [35,36] ($0.5\text{--}0.8$ nmol of glutamate/h per mg of tissue). The mitochondrial phosphate-activated glutaminase activity in chick EDC and pectoralis muscles increased with increasing glutamine concentrations (results not shown). The apparent $K_{0.5}$ values of glutaminase in the two muscles were 9–10 mM, which is similar to the intracellular concentrations of glutamine in chick muscles incubated with glutamine within its physiological concentration range (Tables 1 and 2). It follows that phosphate-activated glutaminase may play a role in initiating glutamine degradation in chick muscle, as previously demonstrated in mammalian kidneys [5], small intestine [6], brain [34], liver [33], tumour cells [4] and lymphocytes [9].

The role of mitochondrial phosphate-activated glutaminase in glutamine degradation in chick skeletal muscle is further supported by the effect of 6-diazo-5-oxo-L-norleucine, which is known to inhibit glutaminase irreversibly through an interaction with the glutamine-binding site [37,38]. This compound markedly inhibited ($P < 0.01$) mitochondrial phosphate-activated glutaminase activity in chick EDC-muscle mitochondrial preparations by 60, 75, 83 and 83 % at concentrations of 1, 2, 4 and 7 mM respectively. This compound also inhibited oxidative decarboxylation of glutamine in chick muscles (Table 5), but it did not influence oxidative decarboxylation of glutamine in rat EDL muscles (results not shown), supporting the concept of a species difference in the initial step of glutamine degradation in rat and chick skeletal muscle.

A species difference may exist between chicks and rats in terms of the initial stages of glutamine metabolism in their skeletal musculature. In chick muscle, phosphate-activated glutaminase had the highest activity and could be responsible for initiating glutamine degradation, whereas glutamine aminotransferase activity was higher in rat muscle than in chick muscle. The chick muscle glutaminase activity appears to be sufficient to account for the oxidative decarboxylation of glutamine observed in this tissue (Table 1). A difference in the activities of the initial enzymes responsible for glutamine metabolism between these two species has been reported previously by Matsuda *et al.* [17], who showed that hepatic phosphate-activated glutaminase activity is approx. 10-fold greater in the rat than in the chick. Glutamine degradation might proceed by glutamine aminotransferase or glutaminase activities; we identified the presence of and the maximum activities of these enzymes in chick and rat skeletal muscle. The relative physiological importance of these pathways is difficult to establish clearly, since no specific inhibitors of the glutamine aminotransferase are available, and direct measures of the flux through the alternative pathways of glutamine metabolism are not possible in intact tissues.

Intracellular free amino acid concentrations following incubation of rat and chick muscles with different levels of glutamine support the suggested species difference in muscle amino acid metabolism (Table 6). Increased rates of glutamine metabolism in chick muscle are associated with increased ($P < 0.01$) intracellular concentrations of glutamate, aspartate

Table 6. Effect of glutamine addition on intracellular concentrations of glutamate, aspartate and asparagine in rat diaphragm and chick EDC muscles

Isolated rat quarter-diaphragm and chick EDC muscles were incubated as described in the text. Glutamine was added to glutamine-depleted incubation medium at concentrations as shown. Means within a column followed by different superscripts are significantly different ($P < 0.01$), as determined by analysis of variance. ($n = 5$).

Incubation medium [Gln] (mM)	Concn. (nmol/mg of tissue)					
	Rat diaphragm			Chick EDC		
	Glu	Asp	Asn	Glu	Asp	Asn
0.5	1.29 ± 0.26	0.34 ± 0.04	0.16 ± 0.03	1.62 ± 0.08 ^a	0.72 ± 0.03 ^a	0.09 ± 0.01 ^a
1.0	1.11 ± 0.04	0.34 ± 0.03	0.14 ± 0.02	2.11 ± 0.11 ^b	0.86 ± 0.03 ^b	0.12 ± 0.01 ^b
4.0	1.13 ± 0.16	0.38 ± 0.04	0.14 ± 0.02	2.54 ± 0.13 ^c	1.19 ± 0.05 ^c	0.18 ± 0.03 ^c
10.0	1.34 ± 0.21	0.47 ± 0.06	0.14 ± 0.03	2.92 ± 0.15 ^d	1.62 ± 0.10 ^d	0.25 ± 0.04 ^d

and asparagine. In rat muscle the intracellular concentrations of these amino acids did not change with increased rates of glutamine metabolism. The glutamate formed from glutamine in chick muscle may be transaminated with oxo acids by glutamate aminotransferases to form 2-oxoglutarate, which is then converted into CO₂ and C₄ citric-acid-cycle intermediates from which aspartate and asparagine can be produced. The metabolic fate of 2-oxoglutarate produced from glutamine in chick and rat skeletal muscle warrants further investigation.

Physiological significance of glutamine metabolism in skeletal muscle

The net formation and release of an amino acid such as glutamine from the skeletal musculature does not necessarily mean that its synthesis occurs in the absence of its degradation in this tissue. For example, large amounts of alanine are synthesized and released from skeletal muscles obtained from chicks [13] and rats (e.g. [12]); however, alanine is also extensively catabolized in both chick [20] and rat [39,40] muscles. Similarly, glutamine has been shown to be both synthesized and degraded in other rat tissues such as the brain [34] and liver [33]. Since both chick and rat muscles extensively synthesize [12,13] and degrade glutamine (Table 1), intracellular glutamine concentrations as well as the rates of glutamine release from skeletal muscles of both species are determined by the relative rates of these two processes. In our study we observed oxidative decarboxylation of glutamine by muscle tissue ranging from 1 to 4 nmol/h per mg of tissue, in the presence of physiological concentrations of amino acids. These data may be compared with the rate of the opposing process, the net synthesis of glutamine by the same tissue. In incubated muscles from fed chicks, the net glutamine release rate is approx. 0.9–1.2 nmol/h per mg wet wt. of tissue [13]. These values are also similar to those reported by Goldberg & Chang [12] for incubated rat muscle. Comparisons such as these suggest that the oxidative decarboxylation of glutamine may occur at similar rates to net glutamine synthesis. It is important to determine the extent to which glutamine degradation occurs in muscle *in vivo* and to determine the mechanisms by which glutamine degradation is regulated in this tissue under various physiological and pathological states.

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