The effect of glutamine on protein turnover in chick skeletal muscle *in vitro*

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The effect of glutamine on the rates of protein synthesis and degradation was studied in isolated chick extensor digitorum communis muscles incubated in the presence of plasma concentrations of amino acids. Addition of 0.5–15 mM-glutamine increases ($P < 0.01$) intracellular glutamine concentrations by 31–670 %. There is a positive relationship ($r = 0.975$, $P < 0.01$) between intracellular glutamine concentration and the rate of muscle protein synthesis measured by the incorporation of [^3H]phenylalanine. The stimulating effect of 15 mM-glutamine on protein synthesis was decreased from 58 to 19 % in muscles incubated in the absence of tyrosine. The rates of protein degradation, estimated from [^3H]phenylalanine release from muscle proteins prelabelled *in vivo*, decreased ($P < 0.05$) by 15–30 % in the presence of 4–15 mM-glutamine when compared with muscles incubated in the presence of physiological concentrations of glutamine (0.5–1 mM). Glutamine concentrations ranging from 2 to 15 mM appear to have an overall anabolic effect on chick skeletal muscles incubated *in vitro*.

INTRODUCTION

Glutamine is the most abundant free amino acid in human and animal blood plasma and skeletal muscle [1,2]. It plays an important role as a regulator of cellular metabolism, such as inhibition of lipolysis [3], ketogenesis [3,4] and protein degradation [5], and stimulation of both glycogenesis and lipogenesis [4] in the liver. Glutamine has also been shown to inhibit intracellular protein degradation in cultured rat skeletal-muscle cells [6]. Recently, Rennie and his associates have shown that increased intracellular glutamine concentrations increase the rate of protein synthesis in perfused rat skeletal muscles [7]. They also demonstrated, by a ^{15}N -dilution technique, that 15 mM-glutamine inhibited non-myofibrillar-protein degradation in perfused rat skeletal muscles [8]. A positive relationship between intracellular glutamine concentration and the rate of protein synthesis has been reported in skeletal muscles from fasted, injured and endotoxin-treated rats *in vivo* [9]. A role for glutamine in improving whole-body nitrogen balance during catabolic states has recently been demonstrated in patients following major surgery [10].

Garlick & Grant [11], however, have recently reported that intravenous infusion of glutamine has no effect on protein synthesis in skeletal muscles from post-absorptive rats *in vivo*. This lack of an effect [11] may not necessarily negate a role for glutamine in regulating muscle protein synthesis *in vivo*, since a large amount of infused glutamine is actively taken up by post-absorptive rat intestine, where it is the preferred oxidative substrate [12], and thus glutamine may not be directed to skeletal muscle at concentrations sufficient to increase intramuscular glutamine concentrations. It is unfortunate that intramuscular glutamine concentrations were not provided in the study by Garlick & Grant [11].

Preliminary studies from our laboratory have shown that 0.5–2.0 mM-glutamine increases tyrosine release from incubated chick skeletal muscle [13], which has been widely interpreted to reflect the net rate of protein degradation in rat skeletal muscles (e.g. [14–17]). The increased release of tyrosine in these conditions may not necessarily indicate an increased rate of muscle protein degradation, but rather may result from transamination of glutamine with *p*-hydroxyphenylpyruvate, catalysed by glutamine transaminases, which are widely distributed in animal tissues [18]. Also, the absence of tyrosine from the incubation medium in these studies (e.g. [13]) may limit muscle protein synthesis, thereby making the interpretation of these results ambiguous. Thus the role of glutamine in regulation of muscle protein turnover remains to be clarified. In addition, the role of glutamine in regulating skeletal-muscle protein metabolism in avian species has not been reported in the literature. This study was therefore designed to investigate whether physiological and elevated concentrations of glutamine regulate protein synthesis and degradation in chick skeletal muscle in the presence of plasma concentrations of amino acids. Protein degradation in skeletal muscles incubated in the presence of all amino acids was estimated by measuring the release of [^3H]phenylalanine from prelabelled protein *in vivo*. Protein synthesis was estimated by measuring the incorporation of [^3H]phenylalanine into muscle protein.

MATERIALS AND METHODS

Chemicals

L-[2,6- ^3H]Phenylalanine was obtained from ICN Radiochemicals, Montreal, Que., Canada. Amino acids, D-glucose, bovine insulin, *p*-hydroxyphenylpyruvate and all other chemicals were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

Abbreviation used: EDC, extensor digitorum communis.

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Animals

Day-old male broiler chicks (Hubbard strain; *Gallus domesticus*) were obtained from a local hatchery and were provided free access to a broiler starter ration and water. Extensor digitorum communis (EDC) muscles (19.7 ± 0.3 mg; $n = 20$) were removed when the chicks were 10 days old and 120–130 g in weight.

Muscle preparation and incubation

Chicks were anaesthetized with halothane, and the EDC muscles were carefully exposed and inserted into stainless steel supports by means of their tendons [19] to prevent contraction, thereby improving nitrogen balance and energy status of the tissue (e.g. [20]). Muscles were immediately dissected, preincubated for 30 min, and then transferred to fresh media and incubated for 2 h at 37 °C in Krebs–Ringer bicarbonate buffer (pH 7.4) [19], with continuous gassing with O₂/CO₂ (19:1) throughout the incubation period. The buffer also contained insulin (0.01 unit/ml), 12 mM-glucose, 2 mM-Hepes, 1.0 mM-phenylalanine and plasma concentrations of other amino acids [19] except glutamine and tyrosine in some experiments. Glutamine was added to the incubation media as indicated in the Tables.

Measurement of intramuscular amino acids and protein synthesis

Protein synthesis in EDC muscles was estimated by incubating the muscles in the presence of 1.0 mM-L-[2,6-³H]phenylalanine (0.64 mCi/mmol) as previously described [14,20]. At the end of the 2 h incubation period, muscles were rinsed in non-radioactive medium, blotted and homogenized in 2 ml of ice-cold 2% (w/v) trichloroacetic acid. The rate of muscle protein synthesis was estimated as previously described [14,20]. Intramuscular concentrations of amino acids were measured by h.p.l.c. [19], and tyrosine was measured by a fluorimetric method [21]. Intracellular concentrations of amino acids were calculated after correction for the extracellular space determined by incubation of a comparable group of muscles with [³H]inulin for 1 h [22].

Measurement of protein degradation

Individual 9-day-old chicks were injected with 0.45 ml of 0.9% NaCl containing 0.4 mM-L-[2,6-³H]phenylalanine (0.83 Ci/mmol) via the intraperitoneal route. Then, 24 h after injection, the chicks were anaesthetized with halothane, and the EDC muscles were dissected, preincubated twice for 15 min in fresh media and then transferred to fresh media and incubated for 2 h as described above. At the end of the incubation, muscles were removed and homogenized in 2 ml of 2% trichloroacetic acid. The radioactivity in [³H]phenylalanine in the intramuscular free pool and in the media was measured by liquid-scintillation spectrometry [22]. EDC muscle proteins were hydrolysed with 6 M-HCl at 110 °C for 24 h. The hydrolysates were evaporated to dryness and the residue was dissolved in 10 ml of water. A 1 ml portion of the solution was counted for ³H radioactivity, and 0.2 ml was analysed for phenylalanine by h.p.l.c. [19,22]. The rate of protein degradation was calculated by dividing the amount of [³H]phenylalanine radioactivity released to the incubation medium during the final 2 h incubation period by the specific radioactivity of protein-bound [³H]phenylalanine.

Measurement of tyrosine release in muscles incubated in the absence of tyrosine

EDC muscles were incubated in the presence of all amino acids except glutamine and tyrosine. Glutamine was added to incubation media as shown in Table 4. Tyrosine released to the medium and present in the tissue at the end of the 2 h incubation period was measured [21] as an index of the net rate of protein degradation [14–17].

Measurement of glutamine-*p*-hydroxyphenylpyruvate transamination in EDC muscle mitochondrial and cytosolic fractions

Chick EDC muscle mitochondria were isolated as described by Lee & Martens [23], except that Nagarse and bovine serum albumin were not used. The 14000 *g* supernatant was used as the cytosolic fraction. Glutamine-*p*-hydroxyphenylpyruvate transamination in the mitochondrial and cytosolic fractions (0.1 ml) was measured at 37 °C as described by Cooper & Meister [24], except that the transamination product, tyrosine, rather than the formation of α -oxo[¹⁴C]glutaramate from [¹⁴C]-glutamine [24], was measured by a fluorimetric method [21].

Statistical analysis

Results, given as means $\pm$ S.E.M., were analysed by one-way analysis of variance, paired *t* test or simple correlation analysis [25].

RESULTS AND DISCUSSION

Effect of glutamine on intracellular amino acid concentrations

Glutamine is the most abundant free amino acid in chick EDC muscles incubated in the presence of plasma concentrations of amino acids (Table 1), as previously shown for mammalian skeletal muscle [1]. The addition of 0.5–15 mM-glutamine increased ($P < 0.01$) intracellular glutamine concentrations by 31–670% over that measured in the absence of glutamine (Table 1). Intracellular glutamine concentrations approached a plateau as medium glutamine concentration increased above 10 mM. These results suggest that glutamine is rapidly taken up and accumulated by chick skeletal muscle, as previously reported for rat skeletal muscle [7,26].

Glutamine (1–15 mM) increased ($P < 0.05$) the intracellular concentrations of glutamate, aspartate and asparagine (Table 1), probably owing to formation of these amino acids from glutamine, as we have recently found that glutamine is extensively metabolized in chick skeletal muscle [27]. On the other hand, the addition of 2–15 mM-glutamine decreased ($P < 0.05$) intracellular concentrations of alanine and the non-metabolizable amino acids, including serine, glycine, threonine, arginine, tyrosine, methionine and lysine (Table 1), probably resulting from inhibition of their uptake by glutamine [26] and/or from the effect of glutamine on protein turnover in this tissue. The addition of 10–15 mM-glutamine slightly decreased ($P < 0.05$) intracellular concentrations of leucine, isoleucine, valine and phenylalanine.

Effect of glutamine on protein synthesis in the presence of all amino acids

Increasing the incubation-medium concentration of glutamine from 1.0 to 15 mM increased ($P < 0.05$) the

Table 1. Effect of glutamine on intracellular amino acid concentrations in chick skeletal muscle

Chick EDC muscles were incubated in the presence of plasma concentrations of amino acids except glutamine as indicated. Results (nmol/mg of tissue; means $\pm$ S.E.M.; $n = 13$) were analysed by one-way variance analysis: *, **, different from the 0 mM-glutamine group at $P < 0.05$ and $P < 0.01$ respectively; †, ‡, §, different from 0.5–2.0 mM-, 0.5–4.0 mM- and 6 mM-glutamine at $P < 0.05$ respectively.

Amino acid	[Glutamine] in medium (mM)...	Amino acid concn. (nmol/mg)							
		0.0	0.5	1.0	2.0	4.0	6.0	10.0	15.0
Asp		0.66 ±0.05	0.67 ±0.02	0.76* ±0.08	0.79* ±0.03	0.95***† ±0.06	0.92***† ±0.05	1.53***‡§ ±0.11	1.31***‡§ ±0.08
Glu		1.74 ±0.09	1.75 ±0.09	2.10* ±0.13	1.98* ±0.11	2.36***† ±0.07	2.54***† ±0.08	2.61***† ±0.09	2.71***† ±0.12
Asn		0.09 ±0.01	0.10 ±0.01	0.12* ±0.01	0.12* ±0.02	0.16***† ±0.03	0.15***† ±0.01	0.26***‡§ ±0.05	0.24***‡§ ±0.02
Gln		3.32 ±0.21	4.23** ±0.40	6.65** ±0.32	9.95** ±0.52	14.18***† ±0.51	17.11***† ±0.74	22.74***‡§ ±0.90	25.34***‡§ ±0.71
Ser		1.98 ±0.12	1.82 ±0.11	1.74 ±0.13	1.65* ±0.10	1.41*† ±0.05	1.23*† ±0.03	1.16***† ±0.07	0.95***‡§ ±0.05
Gly		2.89 ±0.34	2.78 ±0.38	2.72 ±0.22	2.36* ±0.21	2.49* ±0.28	2.48* ±0.25	2.47* ±0.21	2.38* ±0.17
Thr		1.18 ±0.10	1.23 ±0.10	1.21 ±0.12	1.01* ±0.06	1.02* ±0.06	1.00* ±0.06	1.04* ±0.06	0.82* ±0.04
Arg		0.52 ±0.07	0.48 ±0.08	0.53 ±0.07	0.44* ±0.07	0.37*† ±0.07	0.34***† ±0.04	0.38*† ±0.07	0.34***† ±0.06
Ala		4.05 ±0.20	4.10 ±0.25	3.95 ±0.33	3.53* ±0.31	3.52* ±0.23	3.42* ±0.16	3.21** ±0.18	2.99***† ±0.13
Tyr		0.20 ±0.02	0.19 ±0.02	0.20 ±0.01	0.17* ±0.01	0.17* ±0.01	0.16** ±0.01	0.16** ±0.01	0.14***† ±0.01
Trp		0.18 ±0.02	0.21 ±0.02	0.19 ±0.02	0.18 ±0.01	0.19 ±0.01	0.15*† ±0.05	0.11***† ±0.01	0.12***‡§ ±0.02
Met		0.12 ±0.01	0.11 ±0.01	0.11 ±0.01	0.09* ±0.01	0.09* ±0.02	0.09* ±0.01	0.08** ±0.01	0.08** ±0.01
Val		0.43 ±0.02	0.41 ±0.01	0.42 ±0.03	0.42 ±0.03	0.38 ±0.03	0.40 ±0.02	0.34*† ±0.02	0.35*† ±0.02
Phe		1.23 ±0.03	1.20 ±0.02	1.18 ±0.05	1.19 ±0.06	1.20 ±0.03	1.18 ±0.05	1.13* ±0.03	1.12* ±0.04
Ile		0.21 ±0.03	0.22 ±0.02	0.20 ±0.03	0.21 ±0.03	0.20 ±0.03	0.19 ±0.02	0.17* ±0.02	0.17* ±0.01
Leu		0.29 ±0.02	0.27 ±0.01	0.26 ±0.03	0.26 ±0.03	0.25 ±0.02	0.27 ±0.02	0.24* ±0.02	0.24* ±0.01
Lys		0.54 ±0.10	0.49 ±0.05	0.55 ±0.11	0.43* ±0.08	0.44* ±0.06	0.35* ±0.06	0.28***† ±0.07	0.30***† ±0.06

rate of protein synthesis by 19–58 % over that obtained in the absence of glutamine in EDC muscles (Table 2). There is a positive relationship between intracellular glutamine concentration and the rate of protein synthesis in chick skeletal muscle ($r = 0.975$, $P < 0.01$). The stimulating effect of glutamine on the rate of protein synthesis in chick skeletal muscle is consistent with results obtained with perfused rat skeletal-muscle preparations [7].

The mechanism by which elevated intracellular concentrations of glutamine increase the rate of protein synthesis in skeletal muscle remains unknown. It is unlikely that the rate of protein synthesis is increased simply because of an increased availability of glutamine as a precursor for protein synthesis [7]. Jepson *et al.* [9] suggest that the stimulating effect of glutamine on the rate of protein synthesis in skeletal muscle is not related to glutamine oxidation, since glutamine oxidation is considered to be negligible in this tissue. However, we have recently demonstrated that mitochondrial phosphate-activated

glutaminase activity is high in chick EDC muscles and that glutamine is extensively oxidized in this tissue, even to a greater extent than leucine [27]. Thus it may be possible that the stimulating effect of glutamine on protein synthesis is related to its extensive oxidation in skeletal muscle.

Effect of glutamine on protein degradation in the presence of all amino acids

The intramuscular free [^3H]phenylalanine radioactivity in prelabelled chick muscles before preincubation was 370.0 ± 4.4 d.p.m./mg of tissue ($n = 6$), and was decreased by 94 % after the first 15 min preincubation period. After the second 15 min preincubation period, it was decreased ($P < 0.001$) to 12.5 ± 0.6 d.p.m./mg of tissue ($n = 6$) and remained fairly constant throughout the final 2 h incubation period. These results indicate a rapid exchange between intracellular and extracellular phenylalanine. Since the phenylalanine pool in the incubation medium (3500 nmol) is much larger than the

Table 2. Effect of glutamine on protein synthesis in chick EDC muscles incubated in the presence of plasma concentrations of amino acids

Data were from the same experiment as described in Table 1. Results (means $\pm$ S.E.M.; $n = 13$) were analysed by one-way analysis of variance: *, **, different from 0 mM-glutamine at $P < 0.05$ and $P < 0.01$ respectively; † different from 0.5 mM-glutamine at $P < 0.05$; ‡ different from 1 mM-glutamine at $P < 0.05$; § different from 0.5–2.0 mM-glutamine at $P < 0.01$.

[Glutamine] in incubation medium (mM)	Intracellular sp. radioactivity of [3 H]Phe (d.p.m./nmol)	Protein synthesis (nmol of Phe/2 h per mg of tissue)
0	1481 $\pm$ 42	0.385 $\pm$ 0.013
0.5	1408 $\pm$ 16	0.428 $\pm$ 0.018
1.0	1451 $\pm$ 26	0.459 $\pm$ 0.031*
2.0	1413 $\pm$ 21	0.493 $\pm$ 0.034**
4.0	1432 $\pm$ 15	0.536 $\pm$ 0.021**†
6.0	1343 $\pm$ 32	0.578 $\pm$ 0.027**‡
10.0	1423 $\pm$ 17	0.606 $\pm$ 0.028**§
15.0	1382 $\pm$ 20	0.610 $\pm$ 0.017**§

muscle intracellular pool (about 20 nmol), only a negligible amount of [3 H]phenylalanine arising from muscle protein degradation would be expected to be re-incorporated into muscle protein. Thus the release of [3 H]phenylalanine into the incubation medium should reflect total protein degradation in skeletal muscle.

Table 3 shows the effect of glutamine on protein degradation in chick EDC muscles incubated in the presence of all amino acids including tyrosine. It is noteworthy that glutamine has a biphasic effect on protein degradation in chick skeletal muscle. The addition of 1 mM-glutamine to the incubation medium slightly increases ($P < 0.05$) the rate of protein degradation by 12%, whereas the addition of 0.5 mM-, 2.0 mM-, 4.0 mM- and 6.0 mM-glutamine had no significant effect ($P > 0.05$) on protein degradation as

compared with 0 mM-glutamine. The addition of 10.0–15.0 mM-glutamine significantly inhibited protein degradation by 20–23% as compared with the value obtained at 0 mM-glutamine. The addition of 4–15 mM-glutamine decreased ($P < 0.05$) the rate of muscle protein degradation by 15–30% as compared with the value obtained at 0.5–1.0 mM-glutamine (its normal plasma concentration range).

The inhibition of the rate of protein degradation by elevated concentrations of glutamine in chick EDC muscle incubated in the presence of all amino acids is consistent with the results of MacLennan *et al.* [8], who reported that the infusion of 15 mM-glutamine inhibits protein degradation in perfused rat skeletal muscles. However, the 20% inhibition of the rate of protein degradation in chick EDC muscles by 15 mM-glutamine (Table 3) is much less than the value of 65% reported for rat skeletal muscles perfused with 15 mM-glutamine [8]. This quantitative discrepancy may be due to the different species studied, or the different experimental approaches used for estimating protein degradation. The mechanism by which elevated intracellular glutamine concentrations influence protein degradation in skeletal muscle of rats and chicks remains unknown. Since glutamine is extensively oxidized in skeletal muscle of chicks [27], the inhibiting effect of glutamine on protein degradation may be mediated by the production of ammonia, which is a potent inhibitor of lysosomal proteases [28].

Effect of glutamine on protein synthesis and tyrosine release from EDC muscles incubated in the absence of tyrosine

The rate of protein degradation in preparations of rat skeletal muscles *in vitro* is often estimated by measuring the rate of tyrosine release from muscles incubated in the absence of tyrosine (e.g. [13–17,20]). In some studies, the rates of skeletal-muscle protein degradation and synthesis have been simultaneously estimated in muscles incubated in the absence of tyrosine (e.g. [16]). From the present study it is clear that the presence of plasma concentrations of tyrosine is required to maximize the effect of elevated glutamine concentrations on the rate of protein synthesis

Table 3. Effect of glutamine on protein degradation in chick EDC muscles incubated in the presence of plasma concentrations of amino acids

EDC muscles prelabelled *in vivo* with [3 H]phenylalanine were preincubated twice for 15 min and then incubated in fresh media for 2 h in the presence of plasma concentrations of amino acids except glutamine unless indicated otherwise. Results (means $\pm$ S.E.M.; $n = 8$) were analysed by one-way analysis of variance. Intracellular glutamine concentrations were significantly different ($P < 0.01$) from each other; * different from 0 mM-glutamine at $P < 0.05$; † different from 1.0 mM-glutamine at $P < 0.05$; ‡ different from 0–6.0 mM-glutamine at $P < 0.01$.

[Glutamine] in incubation medium (mM)	Intracellular [glutamine] (nmol/mg of tissue)	Intracellular free [3 H]Phe (d.p.m./mg of tissue)	Protein degradation (nmol of Phe/2 h per mg of tissue)
0	3.15 $\pm$ 0.22	13.7 $\pm$ 0.3	0.381 $\pm$ 0.012
0.5	4.27 $\pm$ 0.43	12.4 $\pm$ 0.3	0.407 $\pm$ 0.010
1.0	6.26 $\pm$ 0.79	11.5 $\pm$ 0.5	0.423 $\pm$ 0.018*
2.0	9.81 $\pm$ 0.66	12.6 $\pm$ 0.2	0.398 $\pm$ 0.016
4.0	13.77 $\pm$ 0.41	12.4 $\pm$ 0.7	0.366 $\pm$ 0.011†
6.0	17.40 $\pm$ 0.49	13.0 $\pm$ 0.2	0.373 $\pm$ 0.025†
10.0	21.42 $\pm$ 0.62	13.6 $\pm$ 0.9	0.295 $\pm$ 0.015‡
15.0	24.64 $\pm$ 1.46	12.3 $\pm$ 0.5	0.303 $\pm$ 0.025‡

Table 4. Effect of glutamine on protein turnover in chick EDC muscles incubated in the absence of tyrosine

Chick EDC muscles were incubated for 2 h after preincubation for 30 min in the presence of chick plasma concentrations of amino acids except tyrosine and glutamine as indicated. Results (means $\pm$ S.E.M.; $n = 10$) were analysed by the paired t test: *, **, different from the control (0 mM-glutamine) at $P < 0.05$ and $P < 0.01$ respectively.

[Glutamine] in incubation medium (mM)	Intracellular [glutamine] (nmol/mg of tissue)	Protein synthesis (nmol of Phe/2 h per mg of tissue)	Free tyrosine (pmol/mg of tissue) in	
			Tissue	Medium
0	3.29 $\pm$ 0.26	0.245 $\pm$ 0.017	49.6 $\pm$ 7.7	29.1 $\pm$ 5.1
0.5	4.43 $\pm$ 0.32**	0.252 $\pm$ 0.016	66.5 $\pm$ 9.2**	37.4 $\pm$ 3.3*
0	3.54 $\pm$ 0.17	0.248 $\pm$ 0.012	55.8 $\pm$ 8.0	27.7 $\pm$ 5.9
1.0	6.68 $\pm$ 0.41**	0.278 $\pm$ 0.014*	78.9 $\pm$ 6.9**	35.8 $\pm$ 3.8*
0	3.31 $\pm$ 0.34	0.233 $\pm$ 0.008	50.8 $\pm$ 7.9	31.3 $\pm$ 4.3
15.0	24.83 $\pm$ 1.36**	0.277 $\pm$ 0.009**	36.7 $\pm$ 8.4**	26.4 $\pm$ 3.2*

Table 5. Effect of *p*-hydroxyphenylpyruvate on tyrosine release and protein synthesis in chick EDC muscles

Chick EDC muscles were incubated for 2 h after preincubation for 30 min in the presence of plasma concentrations of glutamine (1.0 mM) and other amino acids, except that tyrosine was absent. *p*-Hydroxyphenylpyruvate was added to the incubation medium at 0.1 or 1.0 mM. Results (means $\pm$ S.E.M.; $n = 6$) were analysed by the paired t test: *, **, different from the control (0 mM-*p*-hydroxyphenylpyruvate) at $P < 0.05$ and $P < 0.01$ respectively.

[<i>p</i> -Hydroxyphenylpyruvate] in incubation medium (mM)	Protein synthesis (nmol of Phe/2 h per mg of tissue)	Free tyrosine (pmol/mg of tissue) in	
		Tissue	Medium
0	0.220 $\pm$ 0.010	62.1 $\pm$ 4.3	37.2 $\pm$ 4.2
0.1	0.261 $\pm$ 0.016*	68.5 $\pm$ 8.1	36.2 $\pm$ 5.3
0	0.231 $\pm$ 0.012	57.8 $\pm$ 9.2	34.1 $\pm$ 4.8
1.0	0.382 $\pm$ 0.017**	128.3 $\pm$ 7.7**	974.2 $\pm$ 49.5**

in chick skeletal muscles incubated *in vitro*. This observation is supported by the following experimental findings. (1) The basal rate of protein synthesis in EDC muscles incubated in the absence of tyrosine (0.248 nmol of phenylalanine/2 h per mg of tissue; Table 4) is lower ($P < 0.01$) than that in muscles incubated in the presence of tyrosine (0.385 nmol of phenylalanine/2 h per mg of tissue; Table 2). (2) The rate of protein synthesis in the presence of 15 mM-glutamine is increased by 58% in the presence of plasma concentrations of tyrosine (Table 2), but it is only increased by 19% in the absence of tyrosine (Table 4). (3) The addition of *p*-hydroxyphenylpyruvate, the α -oxo acid analogue of tyrosine, increased ($P < 0.01$) tyrosine formation and the rate of protein synthesis in muscles incubated in the absence of tyrosine (Table 5).

The addition of 15 mM-glutamine to the tyrosine-free incubation media decreased ($P < 0.05$) the release and tissue concentrations of tyrosine, which is in contrast with the addition of 0.5 mM- or 1.0 mM-glutamine (Table 4). This decrease in tyrosine release and concentration is probably accounted for by the ability of 15 mM-glutamine simultaneously to increase protein synthesis and to decrease protein degradation. The ability of 0.5 mM- or

1.0 mM-glutamine to increase the tissue concentration ($P < 0.01$) and release ($P < 0.05$) of tyrosine (Table 4) confirms our earlier observations [13], in which we suggested that physiological concentrations of glutamine may increase the rate of protein degradation to a greater extent than protein synthesis in chick skeletal muscle *in vitro*. These results, however, are in contrast with our measurements of [3 H]phenylalanine release from protein and its incorporation into protein, which indicate that the addition of 0.5 mM- or 1.0 mM-glutamine does not increase the rate of muscle protein degradation more than the rate of muscle protein synthesis in the presence of all amino acids (Tables 2 and 3). Possibly the rate of protein degradation exceeds the rate of protein synthesis in skeletal muscles incubated in the absence of tyrosine, whereas in the presence of tyrosine this is not the case. These observations suggest that the validity of measuring tyrosine release as an index of protein degradation in skeletal muscles incubated in the absence of tyrosine should be re-evaluated. An additional complication associated with results obtained in the absence of tyrosine may be that skeletal muscle can use glutamine as an amino donor for providing small amounts of tyrosine.

Is tyrosine synthesized from glutamine in skeletal muscle?

Although it is assumed that tyrosine is not synthesized in skeletal muscle (e.g. [14]), the authors are not aware of information in the literature to confirm this assumption. In the presence of 0.025–0.1 mM-*p*-hydroxyphenylpyruvate, there was no detectable glutamine-*p*-hydroxyphenylpyruvate transamination in either the mitochondrial or cytosolic fractions of chick EDC muscles, even though glutamine concentration ranged from 2.5 to 50 mM. However, glutamine-*p*-hydroxyphenylpyruvate transamination was observed in the cytosolic fraction (0.253 ± 0.041 nmol of tyrosine formed/h per mg wet wt. of tissue) in the presence of 0.5 mM-*p*-hydroxyphenylpyruvate plus 10 mM-glutamine and in both the cytosolic and mitochondrial fractions (0.778 ± 0.022 and 0.079 ± 0.005 nmol of tyrosine formed/h per mg wet wt. of tissue respectively) in the presence of 1.0 mM-*p*-hydroxyphenylpyruvate plus 10 mM-glutamine.

The presence of 1.0 mM-*p*-hydroxyphenylpyruvate resulted in tyrosine formation and increased ($P < 0.01$) rates of protein synthesis in intact chick EDC muscles incubated in the absence of tyrosine (Table 5). It has been reported that plasma concentrations of *p*-hydroxyphenylpyruvate in chicks under normal feeding conditions are much lower than $3 \mu\text{M}$ [29]. Thus, although chick skeletal muscle has the potential for tyrosine synthesis from glutamine and *p*-hydroxyphenylpyruvate, it would not normally produce adequate amounts of tyrosine to complicate data obtained in the present experiments.

Possible role for glutamine in improving nitrogen balance

Although physiological concentrations of glutamine (0.5–1.0 mM) slightly increased the net rate of protein degradation in chick skeletal muscles incubated in the absence of tyrosine (Table 4), elevated concentrations of glutamine appear to have an anabolic effect on protein metabolism in chick skeletal muscles incubated in the presence of plasma amino acids, by increasing protein synthesis and/or decreasing protein degradation (Tables 2 and 3). In this regard, glutamine has a similar effect on skeletal-muscle protein turnover to the branched-chain amino acids, particularly leucine [15,16]. However, unlike leucine, glutamine is rapidly taken up by skeletal muscle and accumulated to a large extent in this tissue (Table 1) [26]. It is noteworthy that Stehle *et al.* [10] have recently shown that the administration of 54 mg of L-alanyl-L-glutamine nitrogen/day per kg body wt. to patients after major injury prevented the post-operative decline in intramuscular glutamine concentrations, and dramatically decreased urinary nitrogen release by over 50%. Possibly administration of glutamine dipeptides to increase intracellular glutamine concentrations would decrease muscle wasting associated with sepsis, injury and other catabolic conditions which are characterized by a dramatic decrease in intracellular free glutamine concentrations in skeletal muscle [1,9].

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