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A positive relationship between protein synthetic rate and intracellular glutamine concentration in perfused rat skeletal muscle

Peter A. MacLennan, R.A. Brown* and Michael J. Rennie

Departments of Physiology and **Mathematics, University of Dundee, Dundee DD1 4HN, Scotland*

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During muscle-protein wasting associated with injury and disease the distribution ratio of free glutamine between muscle and blood falls. In pursuing possible consequences of this, we investigated the relationship between the rate of muscle protein synthesis and intramuscular glutamine concentration, manipulated acutely in the isolated perfused rat hindquarter. Increasing perfusate glutamine from 0.67 to 5.0 mM caused a 200% increase in intracellular glutamine and a 66% increase in protein synthesis in the absence of insulin; in the presence of insulin a 30% increase in intramuscular glutamine was accompanied by an 80% increase in protein synthesis. Analysis of variance of the results confirmed the existence of positive relationships between intramuscular glutamine and protein synthesis in the presence or absence of insulin. Control of the size of the intramuscular free pool of glutamine may be important in determining the muscle protein mass.

Glutamine; Protein synthesis; Insulin; (Muscle, Injury)

1. INTRODUCTION

The skeletal muscle protein mass is subject to wasting in many conditions (e.g. after accidental injury, in cancer, during sepsis) in which there is no myopathy per se. The wasting is accompanied by increased use of muscle-derived amino acids as fuels, as substrates for gluconeogenesis and for anabolic processes such as visceral protein synthesis and wound-healing [1]. Wasting must result from an imbalance between muscle protein breakdown and synthesis, but there is no general agreement on the nature of the imbalance and a variety of different changes have been observed [1].

A characteristic of muscle wasting is an altered profile of distribution ratios of free amino acids between muscle and blood, e.g. lower than normal for glutamine and higher for the branched-chain amino acids (BCAA) [1,2]. In trying to understand these changes we previously investigated the properties of amino acid transport systems of muscle [3–5] which we found to be somewhat different from those of other tissues [6]. In particular we found that glutamine was transported by a single carrier whose properties [3–5] helped explain the normal and abnormal patterns of muscle glutamine distribution. In short, if intramuscular Na were to rise, or blood insulin fall or blood corticosteroids, adrenaline or glucagon rise, or if the muscle were to be denervated or exposed to bacteria or endotoxin, then the characteristics of the glutamine transporter are such as to result in an increase in the net outflow of glutamine and a fall in its intramuscular concentration [7]. Such end results have been observed by us in animal preparations [8–10] and are commonly observed clinically [1,2,7].

Thus, the massive rapid losses of body nitrogen observed after injury or the onset of sepsis may not

Correspondence address: M.J. Rennie, Dept of Physiology, University of Dundee, Dundee DD1 4HN, Scotland

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necessarily be the immediate result of changes in the protein balance of muscle; because the uptake of glutamine by liver appears to be supply driven [11], it may be due to acute stimulation of glutamine transport out of muscle.

However, these observations do not explain why the intramuscular BCAA concentration rises in wasting conditions, i.e. why net BCAA appearance from protein breakdown exceeds removal by protein synthesis and oxidation. It occurred to us that there might be some properties of intramuscular glutamine, which if removed, would have the effect of increasing the net loss of amino acids, either by depressing protein synthesis, or increasing protein breakdown. We have examined the former possibility.

2. MATERIALS AND METHODS

Female, 200 g Wistar rats (Bantin & Kingman, Hull, England) were prepared for perfusion of the complete hindquarter [12], after induction of anaesthesia (Sagatal, 60 mg/kg body wt i.p.). A red-cell free perfusate [Krebs-Henseleit bicarbonate buffer, pH 7.4, containing 6% (w/v) fraction 5 bovine serum albumin (Miles Pentex, Eastbourne, England) and equilibrated with 95% O_2/5% CO_2] was pumped at 5 ml/min without recirculation. The perfusate contained 10 mM glucose and rat-plasma concentrations of all normally occurring amino acids [13], except glutamine and the BCAA, the perfusate concentrations of which were manipulated to alter their intramuscular availability. The BCAA were present throughout at 106 μM (i.e. valine 43 μM, leucine 40 μM and isoleucine 23 μM) which is 25% of their normal plasma concentrations. This was done to limit intramuscular synthesis of glutamine using amino groups transaminated from the BCAA. Glutamine was either absent or present at concentrations between 0.67 and 15 mM (see table and figure legends). Attempts were made in some experiments to ensure low intramuscular glutamine by including the glutamine synthetase inhibitor, methionine sulphoximine (MES) [14] at 4 mM. In some perfusions porcine insulin (100 μU/ml, The Wellcome Foundation, Dartford, England) was present.

Perfusion proceeded under the conditions chosen for 45 min when a flooding dose 2.5 mM, 660 dpm/nmol in the perfusate of tracer L-[^{3}H]phenylalanine was added. After 15 min, superficial muscle from the gastrocnemius and the whole soleus of each leg was taken by freeze-clamping using liquid N_2-cooled tongs. Frozen muscle was processed by standard methods before analysis for free amino acids (glutamine enzymatically [15] and others by amino acid analyser), free and protein-bound phenylalanine specific activity [16], ATP and creatine phosphate [17] and total protein [18]. Intramuscular glutamine concentration was expressed per g of

Table 1

Intramuscular glutamine and protein synthetic rate in rat superficial gastrocnemius muscle during perfusion under the conditions shown

Perfusate conditions	Intramuscular glutamine (μmol/g protein)		Protein synthesis (%/day)	
	− insulin	+ insulin	− insulin	+ insulin
0 mM Gln + MES	7.2 ± 0.6	14.9 ± 2.2	1.0 ± 0.2	2.4 ± 0.4
0 mM Gln	–	13.7 ± 2.0	–	4.5 ± 0.7
0.67 mM Gln	12.2 ± 1.6	22.0 ± 3.1	1.8 ± 0.4	5.6 ± 0.4
2.5 mM Gln	32.0 ± 3.5	26.2 ± 4.1	2.4 ± 0.4	6.0 ± 1.4
5.0 mM Gln	34.5 ± 6.5	29.8 ± 3.5	3.0 ± 0.2	10.1 ± 1.4
15.0 mM Gln	–	32.7 ± 8.4	–	9.7 ± 2.4

See tables 2 and 3 for analyses of variance and assignment of significance. MES, methionine sulphoximine (4 mM); insulin at 100 μU/ml perfusate. Results are means ± SE for 3–4 determinations

muscle protein after correction for perfusate glutamine; extracellular volume was determined with [^{14}C]mannitol in separate experiments as 20 ± 2%.

3. RESULTS

In gastrocnemius muscle, perfusate insulin increased the intramuscular glutamine concentration over that in its absence when perfusate glutamine was zero (+ MES) or 0.67 mM but not at higher concentrations (table 1). MES did not, as had been expected, depress the intramuscular glutamine concentration below that observed in its absence.

Insulin also increased the gastrocnemius protein synthetic rate between 2- and 3-fold over the range of perfusate glutamine concentrations between zero (+ MES) and 5 mM. Stimulation of the protein synthetic rate in perfused rat muscle by the concentration of insulin used, 100 μU/ml, has previously been reported [13] in experiments with a full complement of perfusate amino acids.

The rates of protein synthesis in gastrocnemius muscles perfused with 5 or 15 mM glutamine were markedly higher than those of muscles perfused without glutamine or with 0.67 mM glutamine when insulin was present. In the absence of insulin, the superficial gastrocnemius protein synthetic rate was higher during perfusion with 5 mM glutamine than during perfusion without glutamine or with 0.67 mM glutamine (table 1).

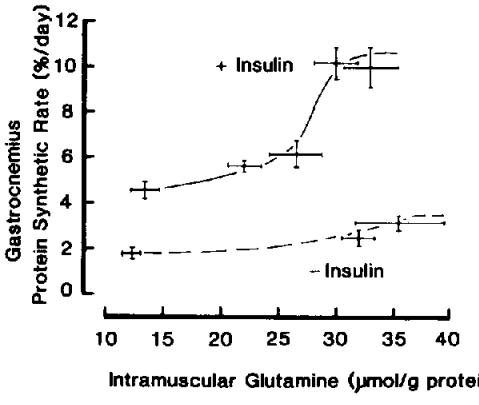

Fig.1. Protein synthesis and intramuscular glutamine in rat gastrocnemius muscle during perfusion with glutamine at 0–15 mM in the presence and absence of insulin (100 μU/ml). All values are means ± SE for n = 3–4 at each point. See table 3 for assignment of significance.

MES inhibited the rate of protein synthesis in superficial gastrocnemius in the presence of insulin (table 1).

There appeared to be positive relationships between protein synthetic rate and intramuscular glutamine concentrations in the presence and absence of insulin (fig.1). The positive effect of glutamine on protein synthesis was much greater in the presence of insulin but appeared to have reached a maximum at intramuscular glutamine above about 25 μmol/g protein. A limit also appeared to have been reached in the extent to which it was possible to increase intramuscular glutamine above 30 μmol/g protein by increasing perfusate glutamine.

Since the effect of insulin itself was to increase intramuscular glutamine, it was difficult to specify an effect of glutamine alone on protein synthesis. Therefore, to clarify whether there were separate effects of insulin and intramuscular glutamine and the extent of any possible positive interaction between them, as well as possible separate effects of MES, analyses of variance [19] were performed using log-transformed data for intramuscular glutamine and protein synthetic rate (tables 2,3).

Analysis of variance of intramuscular glutamine (table 2) gave no evidence that MES had any significant effect. Both insulin and increasing perfusate glutamine had significant positive effects on intramuscular glutamine, and there was a significant positive interaction between them.

There was also strong evidence (table 3) that the gastrocnemius protein synthetic rate was significantly decreased by MES, and was increased by raising perfusate glutamine and by insulin. Again, there was strong evidence of a positive interaction between insulin and perfusate glutamine, in this case to increase protein synthetic rate.

In soleus muscle, increasing the perfusate glutamine concentration and addition of insulin caused a tendency for both intramuscular glutamine and protein synthesis to rise, but the changes were smaller and less clear-cut than in the gastrocnemius (not shown).

Intramuscular ATP and phosphocreatine concentrations in gastrocnemius (means ± SD, 35 ± 7 and 70 ± 8 μmol/g protein, respectively) and soleus (16 ± 6 and 36 ±.9 μmol/g protein, respec-

Table 2

Analysis of variance of log (gastrocnemius glutamine concentration) during perfusion with a variety of different concentrations of glutamine, ± 100 μU/ml insulin and ± MES in the perfusate

Source of variation in intramuscular [Gln]	Sum of squares	Degrees of freedom	Mean square	Variance ratio	p
± insulin	0.45	1	0.45	6.16	0.025
Varying perfusate [Gln]	7.00	3	2.33	31.92	0.001
± MES	0.03	1	0.41	0.41	N.S.
Interaction between insulin and perfusate [Gln]	1.303	3	0.434	16.58	0.005
Residual	1.84	25	0.073	–	–

Table 3

Analysis of variance of log (gastrocnemius protein synthetic rate) during perfusion with a variety of different concentrations of glutamine, ± 100 μU/ml insulin and ± MES in the perfusate

Source of variation in intramuscular [Gln]	Sum of squares	Degrees of freedom	Mean square	Variance ratio	p
± insulin	1.86	1	1.86	13.67	0.005
Varying perfusate [Gln]	5.07	3	1.69	12.50	0.001
± MES	1.80	1	1.80	13.32	0.005
Interaction between insulin and perfusate [Gln]	7.25	3	2.42	16.58	0.001
Residual	3.38	25	0.1351	–	–

tively) appeared to be unaffected by perfusate insulin, glutamine or MES. The values were similar to those we normally find in clamp-frozen muscle of anaesthetised fed rats (not shown).

4. DISCUSSION

It was found to be possible to alter intramuscular glutamine concentrations over a considerable range by varying perfusate glutamine at a fixed low BCAA concentration. This approach revealed that, in gastrocnemius, there was a positive relationship between the protein synthetic rate and muscle glutamine concentration up to the limit of the expansion of the intramuscular glutamine pool. The relationship was steepest over that part of the range of intramuscular glutamine corresponding to the pathophysiological ranges.

It was also apparent that reduction of the perfusate BCAA concentration by 75% in the present experiments did not limit protein synthesis when insulin and high perfusate glutamine concentrations were provided: the protein synthetic rates measured during perfusion with 5 and 15 mM glutamine in the presence of insulin in the present experiments were close to those we commonly observe in fed animals of the same weight in vivo. This casts doubt on the hypothesis of a role for BCAA in controlling protein balance in muscle [20].

What possible mechanism can lie behind the stimulatory effect of glutamine on protein synthesis? It is not dependent on the presence of insulin which, however, apparently has additional positive effects in increasing the responsiveness of protein synthesis to increased intramuscular glutamine.

Glutamine might, in some unknown way, exert a direct stimulatory effect on the protein synthetic process. One clue to the mode of action may be suggested by the effect of MES which depressed protein synthesis but not glutamine in muscle perfused without glutamine. It may be that the inhibitor and glutamine share sufficient homology of structure [14] that MES binds to some site at which glutamine normally causes a stimulation of protein synthesis.

Another possibility is that supply of glutamine is simply rate-limiting for protein synthesis at intramuscular concentrations of less than about 25 μmol/g protein (see fig.1). It is difficult to obtain evidence from the literature for limitation or not of protein synthesis rate by the availability of amino acids. However, in liver from fed and fasted rats, charging of arginyl-tRNA is as great as that of tRNAs for other amino acids, present at 10-times greater concentrations [22], suggesting if arginine charging is typical of tRNA, that the K_m of tRNA ligases is so low that limitation of protein synthesis by amino acid availability is very unlikely. However, this possibility cannot be ruled out until definitive experiments have been performed on muscle.

No other explanation of the relationship between protein synthetic rate and intramuscular glutamine is immediately obvious. Nevertheless, it seems that there does exist some modulating effect on muscle protein synthesis of glutamine, the amino acid whose concentrations and inter-organ fluxes shows the greatest physio- and pathophysiological swings. The nature of the effect and its possible role in controlling alterations of the muscle mass remain to be elucidated.

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