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# Effects of changes in cell volume on the rates of glutamine and alanine release from rat skeletal muscle *in vitro*

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The effect of changes in cell volume on the rates of release of glutamine and alanine from muscle and on the concentrations of these amino acids in muscle were investigated by using an isolated preparation of rat skeletal muscle incubated in the presence of hypo- and hyper-osmotic media. Changes in cell volume were associated with changes in the rates of release of glutamine and alanine from muscle: incubation in hypo-osmotic medium decreased the rates of release of glutamine and alanine, and incubation in hyperosmotic medium increased these rates. These changes were rapidly reversed by a change in osmoticity of the medium. Despite marked changes in cell volume, the concentrations of these amino acids in muscle were maintained. It is suggested that cell volume may play a role in the regulation of amino acid metabolism in skeletal muscle.

## INTRODUCTION

Skeletal muscle can synthesize glutamine, and it contains a high concentration of this amino acid [1,2]. It has been shown that skeletal muscle releases glutamine into the circulation and that the rate of release can be controlled [2,3]. It is also known that the intestine [4], kidney [5] and cells of the immune system [6] utilize glutamine at a high rate, and that much of the glutamine required by these tissues is provided by skeletal muscle. Previous work from our laboratory with isolated incubated muscle has suggested that the outward transport of glutamine across the muscle membrane appears to approach saturation with intracellular glutamine, so that it can be considered to be the flux-generating step for glutamine release from muscle, and therefore it is not surprising that the rate of this transport process can be controlled. This control mechanism may play an important role in maintenance of the plasma glutamine concentration [7,8].

Although it is known that the rate of glutamine release from muscle is changed in a number of physiological and pathological conditions [2,3,9] and by a number of different hormones [2,8,10], the mechanism of control is unclear. It has recently been shown that changes in volume of the liver cell can regulate glutamine synthesis, breakdown and membrane transport [11,12]. Changes in cell volume may also regulate the rate of glycogen synthesis in this tissue [13]. Consequently, it was considered important to investigate the effect of changes in muscle cell volume on the rate of glutamine release from muscle, in an attempt to provide some information on this process.

## MATERIALS AND METHODS

### Materials

All chemicals, enzymes and animals were obtained from sources previously given [3], except for [$^3$H]inulin, which was obtained from Amersham International, Amersham, Bucks., U.K., and sucrose, which was obtained from BDH, Poole, Dorset, U.K.

### Experimental procedure

Soleus muscle preparations were isolated and incubated from male Wistar rats, as previously described [3]. Briefly, this involved a 30 min preincubation period, followed by a 60 min incubation period in Krebs–Henseleit bicarbonate buffer, containing defatted BSA (1.5%, w/v), glucose (5.5 mM) and insulin (10 $\mu$-units/ml). Preincubations were always carried out in iso-osmotic medium (146 mM-$Na^+$). Incubations were carried out in iso-osmotic (146 mM-$Na^+$, control), hypo-osmotic (120 mM-, 95 mM- and 70 mM-$Na^+$) and hyperosmotic (5 mM-, 10 mM- and 50 mM-sucrose) media, in order to induce increases and decreases in cell volume, respectively. To examine the effect of depletion of $Na^+$ ions in the medium, experiments were performed in medium containing 70 mM-$Na^+$, supplemented with sucrose to maintain osmolality. Experiments were also performed to determine if any changes in the rate of glutamine release induced by alterations in cell volume were reversible. This involved preincubation of muscle preparations in iso-osmotic medium, followed by two consecutive incubation periods of 50 min each: the first incubation period in hypo-osmotic medium (70 mM-$Na^+$) and the second in iso-osmotic medium (70 mM-$Na^+$ with added sucrose). To examine whether the changes in osmolarity of the medium produced a constant change in the rate of amino acid release, the time course of glutamine release was determined in different conditions. All muscles were preincubated in iso-osmotic medium and then incubated in either iso-osmotic or hypo-osmotic (70 mM-$Na^+$) medium for 5, 15, 30 or 60 min.

### Analytical techniques

The rates of amino acid release from incubated muscles were determined by enzymic analysis of the incubation medium for concentrations of glutamine [4] and alanine [14], at the end of the incubation period. The amino acid concentrations in muscle were determined in $HClO_4$ extracts of freeze-clamped muscle at the end of the incubation period [3,4,14]. Total muscle water content was measured by dry-weight analysis. The extracellular space was determined in muscles incubated in the presence of the extracellular marker [$^3$H]inulin (0.25 $\mu$Ci/ml). Intracellular space was calculated as the difference between the total and extracellular water contents. The osmolality of the incubation media were determined with a vapour-pressure osmometer (Wescor 5100C).

## RESULTS

Incubation of muscle in hypo-osmotic media caused a marked increase in cell volume, as indicated by intracellular water content: there was an 18% increase in volume in the most hypo-

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**Table 1. Effects of hypo- and hyper-osmotic media on total, intracellular and extracellular water content of incubated soleus-muscle preparations**

Values are means ± S.E.M.; $n > 4$ for all groups. Variation around the mean for the measurement of osmolality was < 2%. The significance of the differences between means is denoted by [a]($P < 0.05$) and [b]($P < 0.01$); nd, not determined.

| Medium composition | Osmolality (mmol/kg) | Muscle water (μl/g) | | |
|---|---|---|---|---|
| | | Total | Extracellular | Intracellular |
| Control | 298 | 788 ± 6 | 271 ± 13 | 517 |
| Hyper-osmotic | | | | |
| 5 mM-sucrose | 303 | nd | nd | nd |
| 10 mM-sucrose | 308 | nd | nd | nd |
| 50 mM-sucrose | 350 | 768 ± 5[a] | 272 ± 10 | 496 |
| Hypo-osmotic | | | | |
| 120 mM-$Na^+$ | 249 | 797 ± 8 | 245 ± 10 | 552 |
| 95 mM-$Na^+$ | 203 | nd | nd | nd |
| 70 mM-$Na^+$ | 158 | 814 ± 4[b] | 202 ± 12[b] | 612 |

**Table 2. Effects of incubation of stripped soleus-muscle preparations in hypo- and hyper-osmotic media on intracellular concentrations of glutamine and alanine**

Values are means ± S.E.M.; $n > 6$ for all groups. Units are nmol/ml of intracellular water.

| Medium composition | Concn. in muscle (nmol/ml) | |
|---|---|---|
| | Glutamine | Alanine |
| Control | 6903 ± 383 | 4340 ± 397 |
| Hyper-osmotic (50 mM-sucrose) | 6976 ± 430 | 4722 ± 322 |
| Hypo-osmotic (70 mM-$Na^+$) | 7136 ± 339 | 3992 ± 156 |

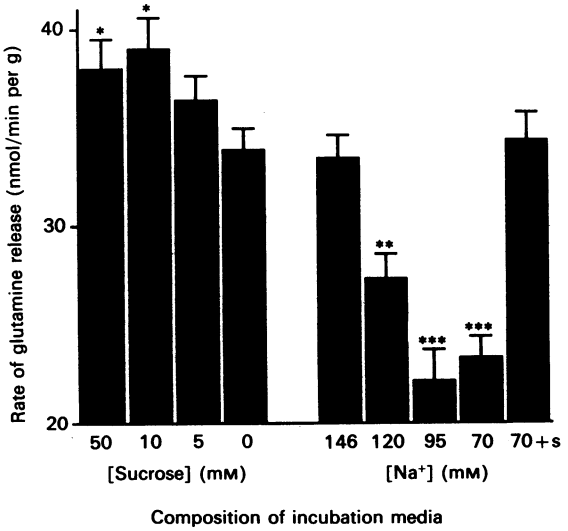


**Fig. 1. Effects of hypo- and hyper-osmotic media on the rate of glutamine release by incubated soleus-muscle preparations**

Values are means ± S.E.M. for between 6 and 24 observations. Medium supplemented with sucrose to maintain iso-osmoticity is indicated by '+s'. The significance of the differences between treated and control means is denoted by *($P < 0.05$), **($P < 0.01$) and ***($P < 0.001$).

**Table 3. Reversibility of changes in the rate of glutamine release from muscle induced by incubation in hypo-osmotic medium**

Values are means ± S.E.M.; $n > 6$ for all groups. The significance of the difference between the first and second incubation periods is denoted by [a]($P < 0.001$), and the significance of the difference between the two first incubations is denoted by [A]($P < 0.001$).

| Rates of glutamine release (nmol/min per g) | |
|---|---|
| First incubation period | Second incubation period |
| Iso-osmotic (146 mM-$Na^+$) | Iso-osmotic (146 mM-$Na^+$) |
| 33.3 ± 1.9 | 37.9 ± 2.7 |
| Hypo-osmotic (70 mM-$Na^+$) | Iso-osmotic (70 mM-$Na^+$ with sucrose) |
| 19.1 ± 1.6[A] | 41.2 ± 3.2[a] |

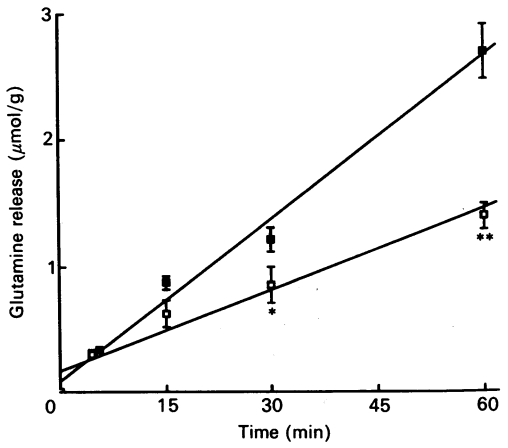


**Fig. 2. Effects of hypo-osmotic medium on the time course of the rate of glutamine release by incubated soleus-muscle preparations**

Values are means ± S.E.M. for 3 preparations, for control (■) and hypo-osmotic (□) media. The significance of the differences between control and hypo-osmotic means is denoted by *($P < 0.05$) and **($P < 0.01$).

osmotic medium used (i.e. 70 mM-$Na^+$). Incubation of muscle in hyperosmotic medium resulted in a small decrease in cell volume (4%) (Table 1).

Incubation of the muscle preparations in hypo- or hyper-osmotic media had no effect on the concentrations of glutamine or alanine in muscle (Table 2). In contrast, the rate of glutamine release from incubated muscle was decreased after an increase in cell volume, and was increased by a decrease in cell volume (Fig. 1). Similarly, the rate of alanine release from muscle (nmol/min per g) was decreased and increased in hypo- and hyper-osmotic media respectively [control 24.0 ± 1.3, $n = 17$; hyperosmotic (50 mM-sucrose) 30.7 ± 2.0, $n = 12$, $P < 0.01$; hypo-osmotic 15.5 ± 1.5, $n = 12$, $P < 0.001$; values are means ± S.E.M.]. Of interest, the rate of glutamine release was negatively correlated with cell volume ($r = -0.799$, 46 degrees of freedom, $P < 0.001$).

The decreases in the rates of glutamine release in hypo-osmotic media were not the result of a decrease in the extracellular concentration of $Na^+$, since the rate of glutamine release was not affected if sucrose was used to maintain the osmoticity of the medium containing decreased concentrations of $Na^+$. The marked decrease in the rate of glutamine release in hypo-osmotic medium was shown to be acutely reversible in experiments involving two consecutive incubation periods in different media: the rate of

glutamine release was decreased during the first incubation period in hypo-osmotic medium (as reported above), but was restored to normal control rates during the second incubation period in iso-osmotic medium (Table 3). The effects of changes in cell volume on the rate of glutamine release are therefore rapid. Indeed, the time courses for the rates of glutamine release from muscle preparations incubated in control and hypo-osmotic media (Fig. 2) showed that the rate of glutamine release was linear and was decreased after 30 min in the presence of hypo-osmotic medium.

## DISCUSSION

We have shown that the rate of glutamine release from isolated incubated rat skeletal muscle is altered markedly after changes in cell volume over a 60 min incubation period and that these changes can be rapidly reversed. These results are similar to those reported for liver: in the perfused rat liver, hyperosmotic medium increased the rate of glutamine release, and hypo-osmotic medium decreased the rate of release and resulted in net uptake of glutamine by the perfused liver [11].

Changes in extracellular osmolarity and in muscle cell volume are associated with a number of physiological and pathological conditions, and some of these conditions are associated with a change in the rate of glutamine release by muscle. For example, prolonged exercise increases the volume of rat soleus muscle [15]; this increase is of a similar magnitude to that induced in the present study by incubation in hypo-osmotic media, and the rate of glutamine release from incubated soleus muscle of the rat after prolonged exercise is decreased [16]. Thus it is suggested that the effects of changes in cell volume reported in the present study involving a preparation of rat skeletal muscle *in vitro* may be important *in vivo* in the control of glutamine transport out of muscle.

The mechanism by which changes in cell volume affect the rate of glutamine release by muscle is not known. Evidence has been obtained to suggest that the rate of glutamine release is independent of the intracellular concentration of glutamine in muscle. The $K_m$ for glutamine release from muscle has not been determined experimentally. However, studies of this process suggest that the $K_m$ may be $< 0.3$ mM and therefore be considerably lower than the intracellular concentration of glutamine [8,17]. Thus the transporter approaches saturation with intracellular glutamine, and consequently any small change in muscle glutamine concentration is unlikely to affect the rate of glutamine release. It is therefore likely that the rate of glutamine release from muscle is controlled by external factors.

We cannot rule out the possibility that changes in cell volume may also alter the rate of uptake of glutamine by skeletal muscle. The uptake of glutamine by rat sarcolemmal vesicles is increased after an increase in extravesicular osmolarity [17]. However, the extracellular glutamine concentration under the present experimental conditions is very low (approx. 0.03 mM at the end of the incubation period), which means that the rate of glutamine uptake is very low ($< 2$ nmol/min per g; [18]). We conclude that changes in the rate of glutamine uptake could not have a significant effect on the current interpretation of the results.

The fact that changes in cell volume induced large changes in the rate of glutamine release, but were without effect on the concentration of glutamine in muscle, suggests that there exists in muscle a mechanism for the regulation of glutamine synthesis, which is designed to maintain the high intracellular concentration of this amino acid. The mechanism is, however, not known.

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