Involvement of integrins and the cytoskeleton in modulation of skeletal muscle glycogen synthesis by changes in cell volume

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Abstract Muscle glycogen synthesis is modulated by physiologically relevant changes in cell volume. We have investigated the possible involvement of integrin-extracellular matrix interactions in this process using primary cultures of rat skeletal muscle subject to hypo- or hyper-osmotic exposure with integrin binding peptide GRGDTP to disrupt integrin actions and the inactive analogue GRGESP as control. Osmotically induced increases (77%) and decreases (34%) in glycogen synthesis (D-[^{14}C]-glucose incorporation into glycogen) were prevented by GRGDTP (but not GRGESP) without affecting glucose transport. Cytoskeletal disruption with cytochalasin D or colchicine had similar effects to GRGDTP. Osmotically induced modulation of muscle glycogen synthesis involves integrin-extracellular matrix interactions and cytoskeletal elements, possibly as components of a cell-volume 'sensing' mechanism.

Key words: Integrin; Glycogen synthesis; Cell volume; Skeletal muscle; Cytoskeleton

1. Introduction

Modulation of metabolic processes in response to physiologically relevant changes of cell volume is now widely recognised to occur in tissues such as liver and skeletal muscle. For example, cell swelling may be induced as an osmotic consequence of increased solute uptake resulting from nutrient supplementation or endocrine stimulation [1–6] and this appears to act as an anabolic signal stimulating processes such as glycogen synthesis. We have shown recently [5] that modulation of glycogen synthesis in skeletal muscle by changes in cell volume involves a mechanism including phosphatidylinositol 3-kinase (PI3-kinase) and p70^{S6} kinase and Krause et al. [7] reported concurrently that these two enzymes exhibit swelling-induced activation in liver cells.

The mechanisms involved in sensing changes of cell volume and in transducing this information to intracellular signalling pathways are poorly understood. Changes in cell volume are likely to alter the degree of 'stretch' imposed on the plasma membrane and a recent study indicates that interactions between integrins and specific extracellular matrix proteins are involved in sensing mechanically induced stretch of skeletal muscle [8]. The cytoplasmic domains of are integrins attached to the cytoskeleton and these linkages may be important elements in a system for cellular mechanotransduction [8–11]. Activation of integrin-β1 (which is expressed in muscle) can stimulate Tyr-phosphorylation of focal adhesion kinase (FAK) with subsequent activation of mitogen-activated protein (MAP) and p70^{S6} kinases via mechanisms involving PI3-kinase [8,12,13]. We have therefore investigated possible involvement of integrins and the cytoskeleton in cell-volume-mediated control of metabolism by assessing the effects of disrupting cytoskeletal elements or integrin-matrix interactions on the modulation of glycogen synthesis in skeletal muscle by osmotically induced changes of cell volume.

2. Materials and methods

Skeletal muscle cells were harvested from thigh muscles of 1-day-old neonatal rats (killed by cervical dislocation) and cultured as described previously [6] in growth medium (25% M199, 65% DMEM) containing 10% foetal calf serum. Myogenesis was promoted by replacement of calf serum with 10% horse serum and 3% chick embryo extract on day 5 of culture [6]. Cell culture media and sera were obtained from Life Technologies (Gibco BRL, Paisley, UK), radiotracers from NEN Research Products (Stevenage, UK) and GRGESP peptide from GENOSYS (Cambridge, UK). All other chemicals were obtained from Sigma (Poole, UK).

All experiments were performed on 10-day-old confluent, multinucleated muscle cells. The experimental medium contained (in mM) 60 NaCl, 4.9 KCl, 2.5 $MgSO_4$, 20 tris(hydroxy methyl)aminomethane hydrochloride and 1 $CaCl_2$, pH 7.4 (170 mosmol/kg). Sucrose was added to give the required osmolalities as outlined below.

Uptake of the non-metabolisable glucose analogue 2-[^{3}H(G)]deoxy-D-glucose (9.25 kBq/well) into muscle was measured after 60 min exposure of cells at 37°C to hypo- (170 mosmol/kg), iso- (300 mosmol/kg) or hyper- (430 mosmol/kg) osmotic medium using D-[1-^{14}C]mannitol (1.9 kBq/well) as an extracellular marker. These osmotic manipulations are known to cause significant (and opposite) alterations in muscle cell volume [6]. Tracer uptake was stopped by rapidly removing the uptake buffer by aspiration and washing the cells with ice cold saline before liquid scintillation counting and protein assay as described previously [6].

Incorporation of D-[U-^{14}C]glucose (37 kBq/well) into glycogen in muscle cells was measured over 60 min in hypo-, iso- and hyper-osmotic media at 37°C. Termination of the reaction was as described for sugar uptake. Labelled glycogen was extracted from the muscle cells by 10% w/v trichloroacetic acid followed by 70% v/v ethanol precipitation [14] with assay for ^{14}C activity as described previously [5].

Uptake of 2-deoxy-D-glucose (0.05 mM) and synthesis of glycogen from glucose (0.05 mM) were also measured during 60 min exposure of muscle cells to cytochalasin D (0.5 μM, to disrupt contractile microfilaments), colchicine (3 μM, to disrupt microtubules) or the integrin binding peptide, GRGDTP (at 25 μg/ml to disrupt integrin-matrix interactions by competitive binding to receptor sites [11]). The inactive peptide GRGESP (also at 25 μg/ml) was used as a control for the active form. Effects of these additions were assessed under hypo-, iso- and hyper-osmotic conditions.

Data are presented as the mean value ± S.E.M. for *n* cell preparations; each experimental measurement in an individual preparation was carried out in triplicate using separate wells of a culture plate.

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Abbreviations: MAP kinase, mitogen-activated protein kinase; PI3-kinase, phosphatidylinositol 3-kinase; FAK, focal adhesion kinase

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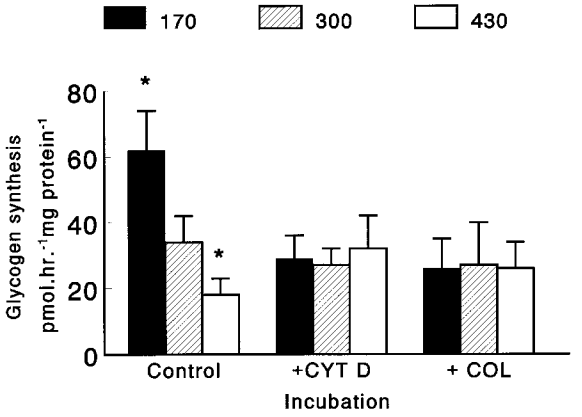

Fig. 1. Effect of cytochalasin D and colchicine on changes in incorporation of glucose into muscle cell glycogen induced by altering external osmolality. 0.05 mM [^{14}C]glucose incorporation into muscle glycogen was measured during 60 min exposure to hypo-, iso- or hyper-osmotic media without or with cytochalasin D (0.5 μM; CYT D) or colchicine (3 μM; COL). Each value represents the mean ± S.E.M. for eight preparations. Statistical significance was measured using paired *t*-test; $*P < 0.05$ from iso-osmotic value for individual treatments.

3. Results

Incorporation of [^{14}C]glucose tracer into glycogen of muscle cells (i.e. glycogen synthesis) was altered in response to cell-volume changes induced by aniso-osmotic exposure; glycogen synthesis was increased (by 77 ± 25%, $P < 0.01$, $n = 12$) in hypo-osmotic medium and decreased (by 35 ± 7%, $P < 0.05$, $n = 12$) in hyper-osmotic medium (Fig. 1) relative to the overall control rate of 40.7 ± 8.5 pmol/h/mg protein observed in cells maintained under iso-osmotic conditions. Cytochalasin D and colchicine treatment abolished these observed changes in glycogen synthesis without affecting the control synthetic rate (Fig. 1) indicating that an intact cytoskeleton (i.e. including microfilaments and microtubules) is important for cell volume-related control of metabolism. Glucose transporter activity in muscle cells was not significantly affected by these experimental manipulations (control values averaged 3.6 nmol 2-deoxy-D-glucose/h/mg protein; Table 1) nor was total [^{14}C]glucose tracer uptake into cells over 60 min (data not shown), confirming that measured changes in tracer incorporation into glycogen reflected changes in synthetic rate rather than in tracer delivery to the sarcoplasm [5].

Changes in incorporation of [^{14}C]glucose into glycogen after hypo- or hyper-osmotic exposure were also blocked by inactivation of integrin-extracellular matrix interactions using the integrin binding peptide GRGDTP, but not by control (inactive) peptide (Fig. 2). Neither peptide had significant independent effects on basal glycogen synthetic rate (Fig. 2), 2-deoxy-D-glucose transport (Table 1) or total glucose (data not shown) uptake under experimental conditions, again indicating that changes in incorporation of [^{14}C]glucose into glycogen reflected alterations in glycogen synthesis.

Under all experimental conditions described here, wortmannin (a PI3-kinase inhibitor) blocked changes in glycogen synthesis resulting from aniso-osmotic exposure [5] and significantly decreased basal glycogen synthesis from glucose to about 40% of values in the absence of inhibitor (data not shown). The latter effect is largely attributable to an inhibitory effect of wortmannin on glucose transport (Table 1 and data not shown).

4. Discussion

The results of this study demonstrate that glycogen synthesis in skeletal muscle is modulated in association with changes in cell volume (independently of changes in glucose uptake) by a mechanism which requires integrin-extracellular matrix interactions and presumably, according to the result of cytochalasin D and colchicine additions, an intact cytoskeleton. PI3-kinase and p70^{S6} kinase are involved in modulation of muscle glycogen synthesis in response to cell-volume change [5] and it has recently been shown that integrin-mediated signal transduction activates the p70^{S6} kinase signalling pathway by a mechanism involving PI3-kinase and FAK [12]. We therefore propose that integrins are directly involved in sensing and/or transducing changes in muscle cell volume with effects on cellular processes such as glycogen synthesis, a view consistent with recent work [11] which has shown that muscle stretch enhances neurotransmitter release from motor nerve terminals by a mechanism which is suppressed by peptides blocking integrin binding and also by certain integrin antibodies. Furthermore, interaction between integrins and specific matrix proteins may be responsible for sensing mechanical stretch in vascular smooth muscle cells [15].

The ability of integrins to interact with specific extracellular matrix proteins whilst being attached to the cytoskeleton may enable these transmembrane proteins to act (possibly in combination with associated proteins including paxillin, tensin, vinculin and talin) as a 'sensor' of mechanical stresses at the

Table 1
Effect of changes in external medium composition on 2 deoxy-D-glucose uptake in muscle cells

Medium composition	0.05 mM 2-deoxy-D-glucose uptake (nmol/h/mg protein)		
	Medium osmolality (mosmol/kg)		
	300	170	430
Control	3.61 ± 0.79	3.43 ± 0.76	3.82 ± 0.85
+wortmannin	1.65 ± 0.37*	1.51 ± 0.38*	1.87 ± 0.35*
+GRGDTP	3.88 ± 0.96	3.73 ± 1.23	3.71 ± 0.92
+GRGESP	4.05 ± 0.81	4.63 ± 0.86	4.23 ± 1.20
+cytochalasin D	3.77 ± 1.15	4.02 ± 1.41	3.47 ± 1.06
+colchicine	3.29 ± 0.47	3.57 ± 1.12	3.31 ± 1.30

0.05 mM 2-[^{3}H]deoxy-D-glucose uptake was measured after 60 min exposure to hypo-, iso- and hyper-osmotic media or to iso-osmotic medium without or with 100 nM wortmannin, 25 μg/ml GRGDTP (active), 25 μg/ml GRGESP (control), 0.5 μM cytochalasin D or 3 μM colchicine. Each value represents the mean ± S.E.M. for nine preparations. Statistical significance was measured using paired *t*-test; $*P < 0.05$ from 300 mosmol/kg control.

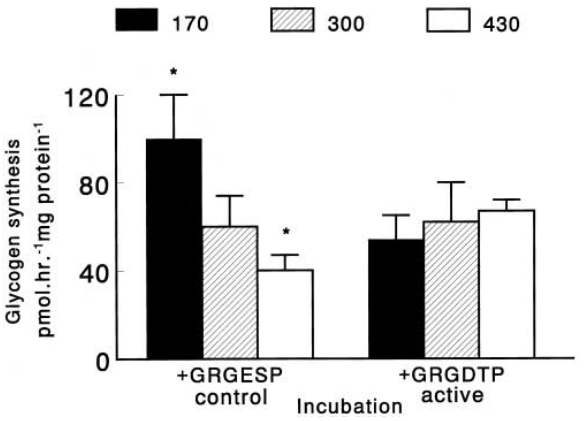

Fig. 2. Effect of the integrin-binding peptide GRGDTP on changes in incorporation of glucose into muscle glycogen induced by altering external osmolality. 0.05 mM [^{14}C]glucose incorporation into glycogen was measured during exposure of muscle cells for 60 min to hypo-, iso- or hyper-osmotic media (170, 300, 430 mosmol/kg respectively) with or without 25 μg/ml GRGDTP (active peptide) or 25 μg/ml GRGESP (control peptide). Each value represents the mean ± S.E.M. for eight preparations. Statistical significance was measured using paired *t*-test; * $P < 0.05$ with respect to glycogen synthesis in iso-osmotic medium for treatment denoted.

cell membrane [9,10] produced e.g. by changes in cell volume and/or stretch. Our previous results indicate [16,17] that PI3-kinase may 'activate' the putative volume sensor in skeletal muscle and indeed PI3-kinase appears to be involved in providing 'positive feedback' to sustain active integrin in certain cell types [8,12,13]. An intact cytoskeleton also appears to be required as a component of the mechanism for signalling or sensing cell-volume change in skeletal muscle (and also in liver [18]). A mechanism including all these elements is proposed in the tensegrity model of cellular mechanotransduction [9,10], in which surface receptors (e.g. integrins) are coupled to intracellular processes via physical associations with the cytoskeleton and changes in tension within the cytoskeletal framework (as induced e.g. by changes in cell volume or shape) may have direct involvement in processes of signal amplification and adaptation.

The intracellular regulatory mechanisms which link cell swelling to glycogen synthase activity remain to be fully elucidated. Stimulation of glycogen synthesis by cell swelling is likely to be due to activation of glycogen synthase (i.e. dephosphorylation via effects on glycogen-associated kinases and/or phosphatases) rather than stimulation of new protein synthesis, since our preliminary data (not shown) indicate that the cell swelling-induced stimulation of glycogen synthesis in muscle cells is insensitive to the protein synthesis inhibitor cycloheximide. Responses to both increased and decreased cell volume involve signal transduction pathways with similarities to those associated with insulin and growth factor signalling (i.e. involvement of the enzymes PI3-kinase and $p70^{S6}$ kinase), but these pathways appear to run in parallel because the anabolic effects of insulin and cell swelling are largely additive (i.e. cell swelling causes increased glycogen synthesis in cells already stimulated by supramaximal insulin concentrations [5]). Further distinctions between these pathways are (i) that muscle glucose transport is insensitive to changes in cell volume (Table 1) and (ii) that disruption of components of the putative integrin-dependent 'volume-signalling' pathway does not affect basal levels of glucose transport in our primary muscle cultures in contrast to the effect of insulin-signalling pathways on muscle glucose transport [5,19]. Insulin may modulate the activity of FAK [20], allowing for feedback/feed-forward interactions between endocrine and integrin signalling mechanisms and their respective stimuli.

It is possible that integrin-dependent signalling mechanisms would be triggered by mechanical stresses resulting from a variety of physiological and pathophysiological events which themselves both involve and modulate metabolism. In particular, the intracellular signalling cascades modulating the stimulation of glycogen synthesis after cell volume increase appear to share common steps with those modulating muscle growth or remodelling after mechanical load [21,22], but the extent of possible interactions and the specific mechanisms which may be involved remain to be elucidated.

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