Mechanical signal transduction in skeletal muscle growth and adaptation

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Tidball, James G. Mechanical signal transduction in skeletal muscle growth and adaptation. J Appl Physiol 98: 1900–1908, 2005; doi:10.1152/japplphysiol.01178.2004.—The adaptability of skeletal muscle to changes in the mechanical environment has been well characterized at the tissue and system levels, but the mechanisms through which mechanical signals are transduced to chemical signals that influence muscle growth and metabolism remain largely unidentified. However, several findings have suggested that mechanical signal transduction in muscle may occur through signaling pathways that are shared with insulin-like growth factor (IGF)-I. The involvement of IGF-I-mediated signaling for mechanical signal transduction in muscle was originally suggested by the observations that muscle releases IGF-I on mechanical stimulation, that IGF-I is a potent agent for promoting muscle growth and affecting phenotype, and that IGF-I can function as an autocrine hormone in muscle. Accumulating evidence shows that at least two signaling pathways downstream of IGF-I binding can influence muscle growth and adaptation. Signaling via the calcineurin/nuclear factor of activated T-cell pathway has been shown to have a powerful influence on promoting the slow/type I phenotype in muscle but can also increase muscle mass. Neural stimulation of muscle can activate this pathway, although whether neural activation of the pathway can occur independent of mechanical activation or independent of IGF-I-mediated signaling remains to be explored. Signaling via the Akt/mammalian target of rapamycin pathway can also increase muscle growth, and recent findings show that activation of this pathway can occur as a response to mechanical stimulation applied directly to muscle cells, independent of signals derived from other cells. In addition, mechanical activation of mammalian target of rapamycin, Akt, and other downstream signals is apparently independent of autocrine factors, which suggests that activation of the mechanical pathway occurs independent of muscle-mediated IGF-I release.

mammalian target of rapamycin; insulin-like growth factor-I; calcineurin; nuclear factor of activated T cells; Akt

The architecture and metabolism of skeletal muscle are highly and subtly sensitive to the mechanical environment. Modifications in the magnitude, frequency, duration, and intensity of mechanical stress can each cause changes in patterns of gene expression in muscle, influence protein synthesis and stability, and affect muscle metabolism to produce adaptations in muscle mass and contractility that reflect the tissue’s recent loading history (50). These relationships between changes in the mechanical environment and changes in muscle structure and physiology suggest that there may be mechanisms within muscle cells through which mechanical signals can be converted to chemicals signals that generate numerous, specific downstream events that determine muscle’s form and function (Fig. 1). However, despite the basic role that mechanical signal transduction serves in modulating muscle growth and adaptation, the processes through which those signals are transduced to chemical messengers that can influence growth and adaptation are only beginning to be understood.

In this review, some of the recent and provocative findings concerning mechanisms through which muscle may respond to changes in the mechanical environment are discussed. A particular emphasis has been placed on pathways that are also activated by insulin-like growth factor (IGF)-I because IGF-I-induced signals have been demonstrated as especially potent in regulating muscle growth and adaptation. In addition, findings that show important similarities between signaling induced by mechanical and IGF-I pathways may overlap at key regulatory steps. However, distinctions between IGF-I-activated pathways and signaling that is responsive to changes in muscle loading have also been clearly demonstrated, suggesting that the pathways may be interactive rather than redundant. The general picture that is emerging from these investigations is that there are multiple processes through which muscle adaptation to mechanical...
loading can occur. However, the current understanding is fragmentary; in some cases, potential downstream signaling events that may be activated by mechanical loading have been identified but await proof that mechanical signals are sufficient for their activation and function in vivo. In other cases, mechanical loading has been shown to generate second messengers with the capacity to affect muscle growth or adaptation, but further investigations to show the physiological effect of those mechanically generated signals have not yet been performed. Nevertheless, important new pieces to the puzzle of how muscle adapts to the mechanical environment have been added by recent research, and they forecast a more thorough understanding of this vital process in the near future.

MECHANICAL STIMULATION OF MULTIPLE, POTENTIAL SIGNALING MOLECULES IN SKELETAL MUSCLE IN VITRO

Early investigations of the response of skeletal muscle cells to mechanical loads applied in vitro showed that the growth and metabolism of muscle cells could be directly influenced by changes in the mechanical environment. Deformation of substrata to which myotubes were adherent stimulated muscle cell growth (59), increased protein synthesis (60), and inhibited proteolysis (60, 61). Together, these findings showed that adaptation of muscle to loading could occur independent of the influences of other tissues or cell types and indicated that mechanical activation of signaling pathways in muscle must occur.

The most direct mechanism for mechanical signal transduction in muscle would involve modulation of ion flux through a force- or deformation-gated ion channel to activate physiologically significant effects. Such direct, mechanical signal transduction is characteristic of specialized senses. For example, mechanoreceptors in the inner ear that are responsible for hearing and balance, and sensory cells in the skin that are responsible for the sensation of touch are both capable of direct and immediate transduction of mechanical signals to chemical messages (23, 27). In each of these models, a transmembrane molecule or molecular complex functions as the mechanoreceptor, and mechanical perturbation applied directly to the mechanoreceptor induces opening of a gated ion channel that modulates signaling within the cell to modify cellular function. Skeletal muscle possesses multiple mechanically sensitive ion channels that could potentially serve this role. Although the molecular identities of many mechanically sensitive ion channels in muscle are not known, electrophysiological observations have shown that mechanical loads influence the activity of Ca\textsuperscript{2+} channels (19, 20), Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels (30), K\textsuperscript{+}/Na\textsuperscript{+}-permeable channels (39), and voltage-sensitive Na\textsuperscript{+} channels (55) that are present in skeletal muscle membranes. Thus mechanical load-induced ion fluxes through any of these channels could feasibly affect signaling that could lead to changes in muscle growth or adaptation, although the possibility has not been tested. However, an early in vitro investigation implicated voltage-sensitive Na\textsuperscript{+} channels in muscle growth that is promoted by mechanical stimulation. Application of a static stretch to skeletal muscle myotubes in vitro significantly increased amino acid transport into the stretched cells, but the transport was prevented by either ouabain or tetrodotoxin (63). Whether the increase in amino acid transport that results from mechanical activation of voltage-sensitive Na\textsuperscript{+} channels can contribute significantly to muscle growth or metabolism remains to be tested.
Mechanical stimulation of muscle cells is also sufficient to generate signaling molecules and growth factors that have the capacity to affect muscle growth or adaptation. Cyclic mechanical loading of myotubes in vitro produced significant increases in numerous second-messenger molecules that can be generated at the cell membrane, including nitric oxide (57), arachidonic acid, diacylglycerol, and prostaglandin E2 (64). Induction of these second messengers introduces rich, unexplored possibilities through which mechanical stimulation can affect muscle growth and adaptation, although there are few experimental findings that directly test this possibility. However, the discovery that mechanical stimulation of muscle cells in vitro increased the release of IGF-I (46) has the most obvious relevance to understanding how mechanical loading can promote muscle growth. Extensive, in vivo evidence has shown that IGF-I is an important regulator of muscle growth in vivo and demonstrated that the experimental manipulation of IGF-I levels in muscle in vivo can cause tremendous increases in muscle mass (3, 4, 10, 28, 32, 37, 70). Furthermore, IGF-I may mediate muscle growth in response to modified muscle loading in response to more than one upstream stimulation. Application of mechanical loads to muscle cells in vitro can cause a rapid increase in IGF-I release within 1 h of increased stimulation (46), which could play a role in rapidly activating signaling pathways that influence muscle’s response to loading. In addition, increased muscle loading in vivo can produce elevated expression of IGF-I within 2–4 days after the increased loading (32, 70), which may mediate later, adaptive responses of muscle to the changing mechanical environment.

ARE CALCINEURIN AND NUCLEAR FACTOR OF ACTIVATED T-CELL COMPONENTS OF A MECHANICAL SIGNAL TRANSDUCTION PATHWAY IN MUSCLE?

The findings that mechanical loading can cause a rapid, transient increase of IGF-I release by muscle cells in vitro (46) and that muscle-derived IGF-I tremendously increased muscle growth in vivo (10) laid the foundations for further exploration of an IGF-I-mediated pathway through which loading could promote muscle growth. Attention soon turned to calcineurin as a possible link between mechanical loading and growth because of several previous findings. First, IGF-I stimulation of muscle fibers can cause increases in cytosolic Ca2+, in part because of increased L-type Ca2+ channel activity (13). In addition, calcineurin is activated by calmodulin that has bound calcium; thus its activity is essentially controlled by changes in cytosolic calcium concentrations. Calcineurin is also highly expressed in muscle at levels 10 times higher than most other tissues. Finally, calcineurin has been implicated in mediating cardiac hypertrophy (35), and its inhibition in vivo prevented muscle regeneration after injury (1).

Knowledge of calcineurin function and regulation that provided the foundation for its study in skeletal muscle was largely based on studies of lymphocytes, in which nuclear factor of activated T cells (NFAT) was demonstrated as an important substrate for the phosphatase activity of calcineurin. NFAT dephosphorylation by calcineurin results in the translocation of NFAT to the nucleus, where it binds the NFAT response element to enhance the expression of specific genes. Several important observations showed that this pathway could be activated in skeletal muscle by IGF-I and that this activation could affect muscle growth and metabolism. First, the hypertrophy of muscle cells in vitro that was induced by IGF-I could be prevented by inhibition of calcineurin activity (36, 53). In addition, overexpression of a calcium-independent form of calcineurin yielded dephosphorylation and nuclear translocation of NFATc1 in muscle cells in vitro (36), which indicated that NFAT was also involved in the calcineurin pathway in skeletal muscle. IGF-I was also implicated in calcineurin activation in vivo. Calcineurin inhibition during rat muscle overload caused by synergist ablation significantly reduced the hypertrophy of muscle and prevented the 20-fold increase in the numbers of slow, myosin heavy chain-I-expressing fibers (slow/type I fibers) that occurred in overloaded muscle in which calcineurin was not inhibited (15). Together, these investigations showed a potentially important regulatory role for calcineurin in muscle growth and adaptation and suggested that mechanical loading and IGF-I could affect muscle through overlapping signaling pathways.

Continuing investigations in which calcineurin expression or activity was perturbed in vivo showed unexpected and not yet understood complexities to the roles of the calcineurin-NFAT pathway in muscle growth and adaptation (Table 1). On one hand, inhibition of calcineurin with cyclosporin A (CsA) significantly reduced the growth of both the slow/type I soleus muscle and fast/type II plantaris muscle in normal, ambulatory rats (5). CsA also slowed the growth of mouse plantaris during overload hypertrophy (15) and reduced or prevented soleus and plantaris growth after a period of unloading and atrophy (34). Similarly, overexpression of a muscle-specific, constitutively active calcineurin caused an increase in soleus muscle mass but produced a decrease in plantaris muscle mass in ambulatory animals (56). Collectively, these observations would support a role for calcineurin activation in promoting muscle growth, particularly in slow/type I soleus muscles. On the other hand, fiber growth in soleus muscle fibers that were regenerating after injection with toxin was not affected by administration of CsA or FK506 (54). Other investigations reported that expression of a muscle-specific, constitutively active calcineurin had no effect on fiber size or muscle mass in soleus or plantaris muscles (41), whereas null mutant mice for the calcineurin-Î± isoform showed an increase in fiber size in soleus muscle, with no change in fiber size in plantaris muscle (45). These latter findings argue against a role for calcineurin activation in promoting muscle growth. To some extent, the apparent conflict of these results may result from differences in dosing with calcineurin inhibitors, where relatively small doses are ineffective at inhibiting growth (33). Similar explanations concerning calcineurin dose dependency may underlie differences in findings on calcineurin-overexpressing animals.

A recent study has contributed valuable new insights concerning the role of calcineurin in muscle growth by generating and analyzing null mutants and muscle-targeted, conditional mutants for specific calcineurin isoforms (44). In one line, the ß isoform of the catalytic subunit of calcineurin (Cnß) was targeted and produced a reduction of ~50% in total calcineurin activity in muscle. In a second line, a conditional, muscle-specific null mutation of the regulatory subunit of calcineurin [CnB1-LoxP(ffe)-MLC-cre mice] produced a >80% reduction in muscle calcineurin activity. Somatic deletion of Cnß
caused a significant reduction in fiber number and muscle mass relative to wild-type mice, but no differences in these parameters were seen in CnB1-LoxP(fl/fl)-MLC-cre mice. A potential explanation for this difference is that CsA treatments would affect calcineurin activity in all muscle cells at all stages of development, whereas activity of calcineurin in the CnB1-LoxP(fl/fl)-MLC-cre mice would be affected only in cells that express myosin light chain 1f. Because myosin light chain 1f expression is initiated after early stages of myogenic cell proliferation and differentiation (29), early myogenic cells in the conditional mutants would be expected to express wild-type levels of calcineurin. However, CnAB-null mutants would be calcineurin deficient throughout myogenesis, which could lead to a reduction in myogenic cells and ultimately a reduction of muscle fiber numbers if myogenic cell proliferation or early differentiation were promoted by calcineurin. There is substantial evidence that calcineurin serves this role in early muscle development. Calcineurin can activate the transcription factors myocyte enhancer factor-2 and MyoD, which leads to the subsequent induction of myogenin and muscle differentiation (22). In addition, inhibition of calcineurin prevents the initiation of early stages of muscle differentiation (21).

Further findings of Molkentin and colleagues (44) show that activation of muscle calcineurin may contribute to muscle fiber

Table 1. Effects of perturbations on calcineurin expression or activity on skeletal muscle size, growth, or phenotype

<table>
<thead>
<tr>
<th>Perturbation</th>
<th>Species</th>
<th>Muscle</th>
<th>Effect</th>
<th>Refs.</th>
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<tr>
<td>CsA treatment</td>
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<td>Soleus</td>
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<td>CsA + overload (synergist ablation)</td>
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<td>Plantaris</td>
<td>CsA prevented mass increase</td>
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<td>Calcineurin overexpression</td>
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<td>Soleus</td>
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<td>Gastrocnemius</td>
<td>No change in fiber cross section</td>
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<td>Tibialis anterior</td>
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<td>No change in mass</td>
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<td>Reduce slow/type I fibers</td>
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<td>Tibialis anterior</td>
<td>No change in mass</td>
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<td>Calcineurin B1 −/− [CnB1-LoxP(fl/fl)-MLC-cre]</td>
<td>Mouse</td>
<td>Soleus</td>
<td>No change in slow/type I</td>
<td>56</td>
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<td>IGF-I-stimulated calcineurin Aβ −/−</td>
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<td>No effect on IGF-I-stimulated mass increase</td>
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<td>Calcineurin Aβ −/− + overload (synergist ablation)</td>
<td>Plantaris</td>
<td>Reduced increase in mass occurring during overload</td>
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<td>Reduced shift to slow/type I occurring during overload</td>
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<td>Plantaris</td>
<td>Increase in muscle mass</td>
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<td>Tibialis anterior</td>
<td>No effect on fiber cross section</td>
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CsA, cyclosporin A; EDL, extensor digitorum lungus.
growth in at least some muscles and under some experimental conditions. CnAβ-null mutants and wild-type mice showed similar increases in muscle mass after IGF-I treatments. However, the significant increase in plantaris muscle mass caused by IGF-I treatments of wild-type mice was not observed in CnB1-LoxP(fl/fl)-MLC-cre mice, although soleus muscle mass increase did occur. Although this finding implicates calcineurin that is expressed specifically in muscle in the adaptive response to IGF-I stimulation, the reason that the somatic mutation of CnAβ does not cause a similar effect has not been established. However, the greater loss of calcineurin activity in muscles of CnAβ-null mutants suggests that the differences may reflect the relative magnitudes of calcineurin activity.

Comparisons of the effects of different perturbations of calcineurin activity on muscle growth during overload may provide some further insights into the interaction between calcineurin and muscle. Growth of plantaris muscles during overload by synergist ablation for 4 wk was reduced by CsA treatments by ~45% (15) with the use of CsA dosages that were sufficient to inhibit calcineurin activity by 65% (17). Similarly, CnAβ-null mutants, in which there was an ~50% reduction in calcineurin activity, showed a 54% reduction in the increase in plantaris mass during 6 wk of overload (44). However, plantaris growth in CnB1-LoxP(fl/fl)-MLC-cre mice, in which there was a >80% reduction in muscle calcineurin activity, showed only a trend of 21% reduction in muscle growth, which was not statistically significant. The greater treatment effect of CsA or CnAβ-null mutation on muscle growth during overload may reflect inhibition of calcineurin during early stages of muscle differentiation, which may produce defects in growth that would not occur in CnB1-LoxP(fl/fl)-MLC-cre mice. However, an additional potential explanation for the differences in treatment effects would be that CsA and CnAβ-null mutation would affect calcineurin activity in nonmuscle cells, including cells that could play a role in regulating muscle growth. For example, CsA can inhibit the expression of cytokines, such as TNF-α in immune cells (11), that may be present in an activated state in several models of muscle growth in which CsA treatments were shown to inhibit muscle growth or regeneration (15, 34). Recent work has shown that null mutation of TNF-α receptor or treatments with function-blocking anti-TNF-α can slow muscle growth and regeneration (67), which suggests the possibility that CsA inhibition of nonmuscle calcineurin may contribute to defects in muscle growth or repair.

Although the relative importance of calcineurin-mediated signaling in promoting muscle growth in response to mechanical stimulation remains murky, a clearer role for calcineurin in signaling the slow/type I muscle phenotype has emerged. Treatment of rodents with CsA or FK506 during compensatory overload (15), during normal amputation (5, 9), or after toxin injection (54) produced a shift toward a fast/type II phenotype. Similarly, fiber switching to a slower phenotype was impaired in CnB1-LoxP(fl/fl)-MLC-cre mice experiencing overload (44), and systemic null mutation of either calcineurin Aα or Aβ produced a reduction in the proportion of slow/type I fibers in healthy, ambulatory animals (45). Likewise, overexpression of calcineurin in muscle produces a shift toward a slower phenotype (41, 56). Together, these findings provide strong support for calcineurin in mediating shifts in muscle phenotype during modified muscle loading. Importantly, many of the genes that are preferentially expressed in slow/type I fibers have binding sites for NFAT that increase expression levels (8, 9).

The apparent role of calcineurin-mediated signaling in promoting the slow/type I phenotype and the regulation of calcineurin activity by changes in cytosolic calcium opens the door to multiple possibilities for affecting muscle adaptation through the calcineurin-NFAT pathway that are independent of IGF-I, including mechanical loading and neuromuscular activity. However, studies of calcium induction of calcineurin activation showed that merely elevating calcium may not be sufficient to produce calcineurin activation, at least in lymphocytes. Transient peaks in cytosolic calcium were insufficient to activate calcineurin in T lymphocytes; instead, prolonged elevations were required for activation and for NFAT dephosphorylation and translocation to the nucleus (14, 18). On the basis of those observations, it was inferred that prolonged increases in cytosolic Ca²⁺ would be similarly required for calcineurin activation in muscle. These prolonged Ca²⁺ transients would more closely resemble transients that occur during activation of slow/type I fibers than the brief, rapid elevations of Ca²⁺ present in activated fast/type II fibers (42). Although the requirement for prolonged elevation of cytosolic Ca²⁺ for activation of muscle calcineurin has not been explicitly tested experimentally, the speculation agrees well with the observation that calcineurin activation mediates increased expression of slow muscle transcripts. Whether calcineurin can be selectively activated as a consequence of muscle stimulation by slow motoneurons was tested in rats subjected to a 3- to 7-day paralysis of the sciatic nerve by chronic superfusion with tetrodotoxin (16). As expected, there was an increase in phosphorylated NFAT in paralyzed soleus muscle that resembled changes in phosphorylation status of NFAT that were attained by calcineurin inhibition with CsA (16). Furthermore, stimulation of denervated soleus muscle to mimic activation by a slow motoneuron increased muscle fiber size, but the effect was blocked by CsA (54).

Whether calcineurin is part of a mechanical signal transduction pathway that modulates skeletal muscle growth or adaptation remains an unanswered question. However, many observations are consistent with the hypothesis. Mechanical loading increases IGF-I release (46), and IGF-I can stimulate Ca²⁺ influx and thereby activate calcineurin. Mechanical activation of stretch-sensitive Ca²⁺ channels in muscle may also provide an additional route for calcineurin activation, independent of IGF-I release. Finally, experimental manipulations that cause increased mechanical loads on muscle can produce increases in muscle mass that can be attenuated by reductions in calcineurin activity (15, 34, 44). However, many central questions remain concerning the role of calcineurin in mechanical signal transduction in skeletal muscle. Is mechanical loading sufficient to activate this pathway for muscle growth, independent of neural activation of the muscle? Conversely, does neural activation of the calcineurin-mediated shift to the slow/type I phenotype depend on the associated mechanical load placed on the muscle? Can calcineurin activation by neural stimulation or mechanical stimulation produce muscle adaptation or growth that is independent of IGF-I-mediated signaling? Genetically mod-
ARE Akt AND MAMMALIAN TARGET OF RAPAMYCIN COMPONENTS OF A MECHANICAL SIGNAL TRANSDUCTION PATHWAY IN MUSCLE?

Contemporaneous with studies examining the potential role of calcineurin and NFAT in mediating IGF-I-stimulated muscle growth and adaptation, other investigators explored the Akt/mammalian target of rapamycin (mTOR) pathways in the same context. Akt is a serine-threonine kinase, also called protein kinase B, that can be activated as a result of IGF-I stimulation. IGF-I binding to its receptor activates the kinase activity of the receptor, which then recruits the insulin response substrate-1, causing activation of phosphatidyl-inositol-3 kinase (PI3K) to phosphorylate Akt. Once phosphorylated, Akt can act on a broad spectrum of substrates that can influence cell survival and proliferation and protein synthesis (65). Phosphorylation of mTOR by Akt leads to mTOR activation (40, 52) and the subsequent activation of p70S6K (47). This latter event has great potential importance for the promotion of muscle growth by the IGF-I/Akt/mTOR pathway, because p70S6K is a potent stimulator of protein synthesis that can be activated by increases in muscle contraction (2).

Activation of the Akt/mTOR pathway may also promote muscle growth by inhibiting glycogen synthase kinase (GSK)-3β. GSK-3β is a serine/threonine kinase that can block translation that is initiated by eukaryotic initiation factor-2B (24) and may thereby reduce protein synthesis. GSK-3β activity can be inhibited by Akt phosphorylation (12), which may provide a mechanism for Akt to promote muscle growth through inhibition of the negative regulator GSK-3β. GSK-3β inhibition appears to be a significant mechanism for increasing muscle growth in vitro. Inhibition of GSK-3β induced myotube hypertrophy in vitro (66), and expression of a dominant negative form of GSK-3β in myotubes in vitro was sufficient to inhibit myotube hypertrophy that was stimulated by IGF-I (47).

Numerous observations support the likely importance of the Akt/mTOR/p70S6K pathway as promoting muscle growth. Akt1 phosphorylation and expression are elevated during muscle hypertrophy and reduced during atrophy (6), and expression of a dominant negative form of Akt1 blocks IGF-I-induced hypertrophy of myotubes in vitro (47). In addition, blocking mTOR function with rapamycin inhibits myotube hypertrophy in vitro (47) and muscle growth in vivo (6, 43). Furthermore, rapamycin treatments that prevented muscle growth in vivo did not inhibit Akt phosphorylation but reduced p70S6K activation (6), which is consistent with inhibition of mTOR downstream of Akt activation but upstream of p70S6K activation.

Can mechanical signals similarly activate the akt/mTOR pathway to promote muscle growth? Initial findings appeared to conflict in showing either a positive relationship or no relationship between increased muscle contraction and increased activation of components of the Akt/mTOR signaling pathway. For example, whereas some investigations showed significant elevations of Akt phosphorylation in vivo with increased muscle activation (38, 48, 58), others showed no significant activation of Akt (31, 68, 69) or observed significant activation of Akt but not mTOR (7). However, much of the variability in findings may partially reflect differences in muscle fiber type that was assessed. Muscle contraction that was induced in situ by motoneuron stimulation produced large increases in Akt phosphorylation in fast/type II muscles (3.3- to 13-fold increases), whereas relatively little change in the Akt activation occurred in the slow/type I soleus muscle (1.6-fold increase) (49). In addition, Akt activation after increased muscle use in vivo is transiently and briefly elevated after resistance exercise (7), although treadmill running caused prolonged Akt activation (48). This indicates that the time of sampling and type of muscle activity may also contribute to the apparent discrepancies of findings concerning activation of Akt by contraction.

Although studies of the relationship between muscle contraction and Akt activation were sufficient to show that contraction could induce a significant, transient activation of Akt in at least some muscles, multiple interpretations were available to explain how the effects were mediated. Because all models that involved increased muscle contraction employed increased neural activation in vivo or application of stimulating electrodes in vitro, it was feasible that nerve-derived factors could induce the effect. In addition, Akt activation can be stimulated by circulating factors so that increases in blood circulation during contraction could possibly affect the delivery of exogenous agents to promote activation. Furthermore, autocrine stimulation by IGF-I release that may be induced by contraction could also contribute to increased activation. However, several investigators have recently provided the first findings to show that the direct application of mechanical loads to muscle is sufficient to increase activation of Akt and other downstream signaling molecules. Passive stretches applied to isolated, fast/type II extensor digitorum longus muscle from rat doubled Akt activation, although the same manipulation produced no change in Akt activation in isolated, slow/type I soleus muscle (48). Interestingly, increased contraction of the same muscles in vivo during treadmill running produced significant increases in Akt activation in both muscles (48), which may indicate that systemic, circulating factors may be required for Akt activation in soleus. Although these findings were sufficient to show that Akt can be activated by passive loading of muscle and suggested that downstream events such as mTOR activation may be subsequently activated in response to mechanical loading, mTOR-mediated signaling can also be mechanically activated in the absence of Akt1 signaling. Passive loads applied to mouse extensor digitorum longus muscles can activate mTOR-dependent signaling in which PI3K is inhibited or in which there is a null mutation for Akt1 (26). These findings demonstrate that mechanical signaling leading to mTOR activation, and potentially to muscle growth, occurs through a pathway that overlaps with IGF-I-mediated signaling but differs in its independence of PI3K/Akt activation.

A recent study has provided further new insights into the mechanism through which mechanical signals can also directly affect Akt-mediated signaling involved in muscle growth via pathways that overlap IGF-I-activated pathways but are non-identical. Esser and colleagues (25) tested whether application of cyclic, uniaxial, or multiaxial strains to C2C12 myotubes in vitro could affect phosphorylation of Akt or p70S6K and observed interesting and unexpected complexity in signal activa-
tion by direct mechanical loads. Although either strain field produced significant activation of Akt, only multiaxial strains yielded increased p70S6k activation. These observations show that Akt activation by mechanical loading is not sufficient for p70S6k activation and indicate that an event downstream of Akt activation requires activation by multiaxial loading or that multiaxial loading activates p70S6k activation through an Akt-independent pathway. In addition, the results of this interesting work showed that Akt/p70S6k activation in mechanically loaded skeletal muscle can occur independent of autocrine or paracrine signaling because conditioned media from myotubes that had been subjected to multiaxial loading did not induce p70S6k or Akt activation in unstrained myotubes. Thus mechanical stimulation of IGF-I release is not necessary for mechanical activation of Akt/p70S6k signaling in skeletal muscle.

The clear evidence that mechanical loading is sufficient for Akt, mTOR, and p70S6k activation raises the central question concerning how the mechanical signals are transduced to affect enzyme activity. A potential regulatory interface between mechanical loading and the regulation of Akt function has been suggested by the work of Sawada and Sheetz (51) in which they demonstrated that the application of mechanical strains to fibroblasts in vitro induced tremendous increases in Akt binding to the cytoskeleton. These observations suggested that mechanical strains induced changes in the conformation of cytoskeletal proteins that made an Akt binding site available. Whether Akt binding to the cytoskeleton affected its activity is unknown and remains an intriguing possibility. However, the observation that disruption of the actin cytoskeleton with cytochalasin D prevented strain-induced activation of p70S6k without reducing Akt activation (25) is consistent with the possibility that Akt activation of downstream events may be modulated by positive allosteric interactions with the actin cytoskeleton.

SUMMARY

Identification of the pathways through which IGF-I can influence muscle growth and adaptation has provided a framework for examining how mechanical signals can also regulate these processes in muscle. In particular, IGF-I can promote the slow/type I muscle phenotype and stimulate muscle growth through a calcineurin-NFAT-mediated pathway, and recent findings suggest that modifications in muscle loading may also affect muscle growth and adaptation through an overlapping pathway. However, whether the increase in muscle growth that involves signaling through calcineurin during increased muscle loading reflects a direct response of muscle to mechanical loading or is an indirect response that is mediated by other cell types has not been tested explicitly. Extensive data also support an important involvement of calcineurin-NFAT in promoting the slow/type I phenotype in skeletal muscle and show that muscle stimulation with a slow motoneuron pattern of activation is sufficient to cause a shift to the slow muscle phenotype via the calcineurin-NFAT pathway in vivo. However, whether slow activation patterns are required for that shift remains unknown, and whether mechanical stimulation in the absence of neural stimulation can produce activation of the calcineurin-NFAT pathway is unexplored.

Mechanical stimulation of muscle growth through activation of the Akt/mTOR pathway has become a clearly established mechanism through which the mechanical environment can directly influence muscle growth or adaptation. However, many of the most fundamental and potentially interesting studies remain. Although mechanical stimuli are sufficient for Akt and p70S6k activation in myotubes in vitro, is activation sufficient to promote growth? Are mechanical signals sufficient to produce activation of the Akt/mTOR pathway in vivo? Does the association of Akt with the cytoskeleton that is induced by mechanical loading influence mechanical signal transduction through this pathway? How are Akt associations with the cytoskeleton mediated? Further insights into these questions will likely emerge soon from continuing studies at the interface of mechanical and chemical signaling in skeletal muscle.

REFERENCES


