Effect of Contraction on Mitogen-activated Protein Kinase Signal Transduction in Skeletal Muscle

INVOLVEMENT OF THE MITOGEN- AND STRESS-ACTIVATED PROTEIN KINASE 1*

(Received for publication, July 16, 1999, and in revised form, October 6, 1999)

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Growing evidence suggests that activation of mitogen-activated protein kinase (MAPK) signal transduction mediates changes in muscle gene expression in response to exercise. Nevertheless, little is known about upstream or downstream regulation of MAPK in response to muscle contraction. Here we show that ex vivo muscle contraction stimulates extracellular signal-regulated kinase 1 and 2 (ERK1/2), and p38 MAPK phosphorylation. Phosphorylation of ERK1/2 or p38 MAPK was unaffected by protein kinase C inhibition (GF109203X), suggesting that protein kinase C is not involved in mediating contraction-induced MAPK signaling. Contraction-stimulated phosphorylation of ERK1/2 and p38 MAPK was completely inhibited by pretreatment with PD98059 (MAPK kinase inhibitor) and SB203580 (p38 MAPK inhibitor), respectively. Muscle contraction also activated MAPK and MAPKAP-K2 most closely reflects ERK and p38 MAPK activation, as endothelin-1 activation of p38 MAPK in rat ventricular myocytes is inhibited by phorbol ester downregulation of PKC or by treatment with a PKC inhibitor GF109203X (18). However, the role of PKC in the exercise-induced activation of ERK1/2 or p38 MAPK signaling pathways has not been determined.

Activated MAPK interacts with a number of downstream targets, including the p90 ribosomal S6 kinase (p90^Rsk^) (19) and MAPK-activated protein kinase-2 (MAPKAP-K2) (20, 21), which are activated by ERK1/2 and p38 MAPK, respectively. Additionally, the recently identified mitogen- and stress-activated protein kinase 1 (MSK1) is activated by a mechanism involving an interaction with either ERK or p38 MAPK (22). To date, the role of MSK1 in exercise-induced signal transduction is unknown.

The aim of this study was to first assess whether PKC is involved in MAPK activation in response to muscle contraction. Second, we investigated the effect of ex vivo muscle contraction on MAPK signaling to the downstream targets p90^Rsk^, MAPKAP-K2, and MSK1. Here we show that contractile activity activates p90^Rsk^ and MAPKAP-K2. Activation of p90^Rsk^ and MAPKAP-K2 by muscle contraction most closely reflects ERK and p38 MAPK activity, respectively. Additionally, we show that

1 The abbreviations used are: MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; PKC, protein kinase C; MEK, MAPK kinase; MAPKAP-K2, MAPK-activated protein kinase 2; MSK1, mitogen- and stress-activated protein kinase 1; KHB, Krebs Henseleit buffer; TPA, 12-O-tetradecanoylphorbol 13-acetate; PAGE, polyacrylamide gel electrophoresis.

2 MSK1, mitogen- and stress-activated protein kinase 1; KHB, Krebs Henseleit buffer; TPA, 12-O-tetradecanoylphorbol 13-acetate; PAGE, polyacrylamide gel electrophoresis.

*l This work was supported by Grants 12669, 12679, 9517, and 12211 from the Swedish Medical Research Council and by funds from the Thuringia Foundation, the Magnus Bergwalls Foundation, the ToreNilsons Foundation, the Novo Nordisk Foundation, the Marcus and Amalia Wallenberg Foundation, the Harald and Greta Jeannssons Foundation, the Swedish Diabetes Association, the Foundation of Scientific Studies of Diabetology, and the Swedish Society for Medical Research. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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contractile activity stimulates the recently identified MSK1. The use of an ex vivo model of muscle contraction demonstrates that local effects of muscle contraction lead to the activation of the MAPK signaling cascade, independent of systemic influence.

**EXPERIMENTAL PROCEDURES**

**Animals**—Male Wistar rats (90–105 g) were purchased from B & K Universal (Sollentuna, Sweden) and housed in the animal facility at the Karolinska Institute for 1 week prior to use. Rats were maintained on a 12-h light-dark cycle and allowed free access to water and standard rodent chow. Animals were studied in the overnight fasted state. The animal ethical committee of the Karolinska Institute approved all procedures.

**Materials**—[3H]-O-Methylglucose and [14C]mannitol were from American Radiolabeled Chemicals (St. Louis, MO). Human insulin (Actrapid) was from Novo Nordisk (Bagsvaerd, Denmark). Polyclonal phospho-ERK1/2, phospho-p38MAPK, and p38MAPK antibodies were from New England Biolabs (Beverly, MA). Monoclonal ERK1 and ERK2 antibodies were from Transduction Laboratories (Lexington, KY). Antibodies were prepared as described for p90Rsk (23), MAPKAP-K2 (24), and MSK1 (22). Horseradish peroxidase-conjugated goat anti-rabbit and anti-mouse immunoglobulin G were from Bio-Rad. Chemiluminescence cassettes from Amersham Pharmacia Biotech. PD98059, and GF109203X were from Cal-Biochem (La Jolla, CA). All other reagents were of analytical grade (Sigma).

**Muscle Incubations**—All incubation media were prepared from a pregassed (95% O2, 5% CO2) stock of Krebs Henseleit buffer (KHB) supplemented with 5 mM HEPES and 0.1% bovine serum albumin (RIA grade). Rats were anesthetized with sodium pentobarbital (5 mg/100 g body weight), and epitrochlearis muscles were carefully dissected out and incubated in a shaking water bath (30 °C) for 60 min in 2 ml KHB containing 5 mM glucose and 15 mM mannitol. When pharmacological inhibitors were used, an additional 30-min incubation was employed to pre-expose the muscle to the inhibitor. Once added, inhibitors remained present for the duration of the experiment. Final concentration of dimethyl sulfoxide was adjusted to 0.1% for each group (with or without inhibitor) in the experiments using GF109203X or 0.35% in experiments using PD98059 and SB203580. In some experiments muscles were incubated for 20 min in 12-O-tetradecanoylphorbol 13-acetate (TPA) as described in the figure legends.

**Ex Vivo Muscle Contraction**—Following preincubation, epitrochlearis muscles were placed inside a temperature controlled (30 °C) stimulator and immersed in 4 ml of KHB identical to preincubation condition. Each muscle was positioned between two platinum electrodes with the distal tendon mounted to the bottom of the chamber. The proximal tendon was connected to a jeweler’s chain, which was fixed to an isometric force transducer (Harvard Apparatus, Inc., South Natick, MA). Resting tension was adjusted to 0.5 g. The isometric tension development during the contraction protocol was recorded using a compact 2-channel Student Oscillograph (Harvard Apparatus, Inc., South Natick, MA). Isometric muscle contraction was achieved via electrical stimulation. Muscles were stimulated at a frequency of 100 Hz (pulse duration, 0.2 ms; amplitude, 10 V) delivered at a rate of one 0.2-s contraction every 2 or 0.2-s s or one 0.1-s contraction every 60 s (10/60 s) for 10 min. The pulses were generated by a Tektronix TM 503 Power Module (Beaverton, OR) and amplified on a 4-Chanel Power Amplifier (Somedic, Inc., Sollentuna, Sweden). Basal muscles were treated as described above minus electrical stimulation. Muscles were then frozen immediately between aluminum tongs cooled to the temperature of liquid nitrogen or further incubated for the assessment of glucose transport activity. Additional experiments were performed examining the effects of electrical stimulation with various voltages or time points as described in the figure legends.

**Glucose Transport Activity**—Muscles were transferred to vials containing glucose free KHB without or with the addition of 120 mM insulin and incubated for 10 min. Muscles were then transferred to KHB containing 8 mM [3H]-O-methylglucose (438 Bq/μmol) and 12 mM [14C]mannitol (42 μCi/μmol) without or with insulin, and glucose transport activity was assessed as described by Wallberg-Henriksson et al. (25). Glucose transport activity was assessed for 20 (basal) or 12 (insulin or contraction) min. Glucose transport activity is expressed as μmol/mg of intracellular water.

**Glycogen Analysis**—Glycogen content was determined fluorometrically on HCl extracts as described by Lowery and Passonneau (26). Results are expressed as μmol glucose/g wet weight.

**Muscle Homogenization**—Epitrochlearis muscles were homogenized in ice in 0.6 ml of buffer A (50 mM Tris-HCl, pH 7.5, 0.1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 10 mM β-glycerophosphate, 5 mM sodium pyrophosphate, 0.1% 2-mercaptoethanol, and 100 mM sodium chloride). Homogenates were rotated for 60 min at 4 °C and subjected to centrifugation (13,000 g for 10 min at 4 °C). Supernatants were removed and protein determined, and protein concentration was determined using a commercially available kit based on the Bradford method (Bio-Rad).

**Western Blot Analysis**—Aliquots of muscle homogenates containing 20 μg of protein were suspended in Laemmli buffer. Proteins were separated by SDS-PAGE (10% resolving gel), transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA) with 5% nonfat milk. Membranes were incubated in primary antibody overnight at 4 °C as described in the figure legends. Membranes were washed in TBST (10 mM Tris, 140 mM NaCl, 0.02% Tween 20, pH 7.6), incubated with appropriate secondary antibody, and washed in TBS again. Proteins were visualized by enhanced chemiluminescence and quantified by densitometry.

**Kinase Activity of p90Rsk, MAPKAP-K2, and MSK1**—Aliquots of muscle homogenates (100 μg of protein for p90Rsk, 300 μg of protein for MAPKAP-K2 or MSK1) were immunoprecipitated for 60 min at 4 °C with the appropriate antibody previously equilibrated with protein G-Sepharose in buffer A. Immunoprecipitates were washed three times in buffer A containing 0.5 mM NaCl and two times in buffer B (50 mM Tris-HCl, pH 7.5, 0.03% Brij-35, 0.1 mM EGTA, 0.1% 2-mercaptoethanol). Samples were resuspended in 50 μl of kinase buffer (50 mM Tris-HCl, pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 17 μM cAMP-dependent protein kinase inhibitor peptide, 16.7 mM Mg(Ac)2, 50 μM peptide substrate (cross-tide for p90Rsk or MSK1, KKLNRTLSVA peptide for MAPKAP-K2), and 2 μCi of [γ-32P]ATP) and incubated for 10 min at 30 °C. The reactions were terminated on ice by addition of sample buffer (125 mM Tris, 6 mM urea, pH 6.8). Reaction products were resolved on a 40% acrylamide gel, and 32P incorporation into peptide substrate was analyzed by exposing the gels to a phosphorimager (Bio-Rad).

**Statistics**—Data are presented as the means ± S.E. Differences were determined using a one-way analysis of variation with a subsequent Fisher’s LSD post hoc analysis. Significance was accepted at p < 0.05.

**RESULTS**

**Glucose Transport Activity and Muscle Glycogen Content**—To test the efficacy of two different electrical stimulation protocols with respect to altering muscle metabolism, glucose transport and muscle glycogen content were analyzed following either insulin exposure or ex vivo muscle contraction. Muscle contraction was delivered at a rate of one 0.2-s contraction every 2 s or one 0.1-s contraction every 60 s for 10 min. Glucose transport activity increased 7-fold (p < 0.001) relative to the resting condition (120 nM) (Table I). Ex vivo contraction resulted in a similar 5–6-fold (p < 0.001) increase in 3-O-methylglucose transport activity (Table I), with no statistical difference observed between the two contraction protocols. Muscle glycogen utilization was also similar between the two contraction protocols, with glycogen stores reduced > 60% (p <
Phosphorylation—Following 60-min recovery period, muscles presence of 5 were incubated for an additional 30 min in the absence or activation was sustained at 20 min (Fig. 2, phosphorylation was observed after 10 min of muscle contraction

Muscle contraction upon p38MAPK signaling was observed similar to the results noted for ERK1/2, the greatest effect of p38MAPK was not altered by muscle contraction (Fig. 1). When muscles were pre-exposed to GF109203X for 30 min. Thereafter, muscles were incubated without or contraction-induced phosphorylation of ERK1/2 and p38MAPK was only detectable (Fig. 1, D). After 10 min of muscle contraction via electrical stimulation, phospho-p38MAPK immunoreactivity was markedly increased (Fig. 1, p < 0.005). Protein expression of p38MAPK was not altered by muscle contraction (Fig. 1E). Similar to the results noted for ERK1/2, the greatest effect of muscle contraction upon p38MAPK signaling was observed when impulses were delivered for 0.2 s at a rate of one contraction every 2 s for 10 min, therefore in subsequent experiments we utilized this protocol. To assess whether muscle contraction or electrical stimulation directly account for increased MAPK phosphorylation, epitrochlearis muscles were stimulated with 0 (basal), 1, 2, or 10 V for 10 min. Tension development was undetectable (<0.02 g) when 1 V was applied. In contrast, initial tension development was 17 ± 3 and 25 ± 3 g of tension in response to stimulation with 2 and 10 V, respectively. Increased phosphorylation of ERK1/2 and p38MAPK was only detectable in contracting muscles (Fig. 2, A and B). Furthermore, contraction-induced phosphorylation of ERK1/2 and p38MAPK occurred in a time-dependent manner. Maximal phosphorylation was observed after 10 min of muscle contraction and activation was sustained at 20 min (Fig. 2, C and D).

Effect of Protein Kinase C Inhibition on ERK1/2 and p38 Phosphorylation—Following 60-min recovery period, muscles were incubated for an additional 30 min in the absence or presence of 5 μM GF109203X (bisindolylmaleimide I), a highly selective PKC inhibitor (27). When muscles were pre-exposed to GF109203X, the 2-fold increase in TPA stimulated (5 μM) ERK phosphorylation was reduced 50% (Fig. 3A). TPA did not increase p38MAPK phosphorylation (data not shown). To determine whether PKC mediates MAPK phosphorylation in response to muscle contraction, muscles were incubated in the absence or presence of 5 μM GF109203X. Thereafter, muscles were incubated in KHB (basal) or forced to contract via electrical stimulation as described in Fig. 3. Exposure to GF109203X did not alter contraction-induced ERK1/2 phosphorylation (Fig. 3B). Similarly GF109203X was without effect upon p38MAPK phosphorylation in response to muscle contraction (Fig. 3C).

Effect of PD98059 and SB203580 on contraction-stimulated ERK1/2 and p38MAPK phosphorylation. A and B, rat epitrochlearis muscles were incubated under basal conditions (nonstimulated) or electrically stimulated with 1, 2, or 10 V for a total of 10 min. Electrical stimulation was delivered for 0.2 s every 2 s for 10 min. C and D, muscles were incubated in KHB (basal; nonstimulated) or forced to contract via electrical stimulation for 2, 5, 10, or 20 min at a rate of one 0.2-s contraction every 2 s. Thereafter, ERK1/2 or p38MAPK phosphorylation was determined by Western blot analysis as described in Fig. 1.
Contraction-induced MAPK Signaling

**TABLE II**

| Condition | Initial tension development | Time to 50% fatigue |
|-----------|-----------------------------|-------------------|
| No inhibitor | $22 \pm 1 (9)$ | $107 \pm 7 (9)$ |
| PD98059 | $22 \pm 3 (5)$ | $102 \pm 12 (5)$ |
| SB203580 | $23 \pm 2 (8)$ | $107 \pm 8 (8)$ |
| PD98059 + SB203580 | $24 \pm 2 (5)$ | $102 \pm 3 (5)$ |

Muscles were incubated for 30 min in KHB with or without the addition of 50 μM PD98059 or 10 μM SB203580. Thereafter, muscles were forced to contract via electrical stimulation at a rate of one 0.2-s contraction every 2 s for 10 min. Tension development was measured with an isotonic force transducer as described under “Experimental Procedures.” Values are presented as the means ± S.E. Number of muscles is shown in parentheses (n).

**Fig. 4. Effect of PD98059 and SB203580 on contraction-stimulated ERK1/2 and p38MAPK phosphorylation.** Rat epitrochlearis muscles were incubated in the absence (−) or presence (+) of 50 μM PD98059, 10 μM SB203580, or a combination of both inhibitors for 30 min. Thereafter, muscles were incubated in KHB (basal; nonstimulated) or forced to contract via electrical stimulation for 10 min at a rate of one 0.2-s contraction every 2 s. Muscle proteins were separated by SDS-PAGE, and phospho-ERK1/2 and phospho-p38MAPK were detected via Western blot analysis using phospho-specific antibodies as described in Fig. 1 A, ERK1 (open bars) and ERK2 (closed bars) phosphorylation quantified by densitometry. Representative autoradiograph is presented, and order of sample loading is identical to histogram order. A representative autoradiograph is presented, and order of sample loading is identical to histogram order. Results are presented as the means ± S.E. for 5–9 muscles/group.

combination of PD98059 and SB203580. The utilities of PD98059 (23, 28) and SB203580 (20, 29) as respective inhibitors of MEK1 and p38MAPK have previously been described. After initial exposure to the inhibitor(s), ex vivo muscle contraction was evoked for 10 min in identical media. Ex vivo muscle contraction led to 3.2- (p < 0.001) and 2.0-fold (p < 0.001) increases in ERK1 and ERK2 phosphorylation, respectively (Fig. 4A). Addition of 50 μM PD98059 reduced ERK1/2 activity 80–95% (p < 0.001) in contracting muscles. Basal phosphorylation of ERK1/2 was also decreased 70% by prior exposure to PD98059 (data not shown). Exposure of muscles to the p38MAPK inhibitor SB203580 (10 μM) reduced contraction-induced phosphorylation of ERK1/2 25% (p < 0.05). Nevertheless, ERK1/2 phosphorylation was still significantly increased above basal levels (p < 0.05). Contraction-induced p38MAPK phosphorylation was not significantly reduced by 50 μM PD98059 (Fig. 4B). In contrast, SB203580 reduced p38 phosphorylation 90% (p < 0.001). Alterations in contraction-stimulated MAPK signal transduction cannot be accounted for by an inhibition of muscle contractile activity, because initial tension development and time to 50% fatigue were not altered by PD98059 or SB203580 (Table II).

p90Rsk (MAPKAP-K1) and MAPKAP-K2 Activity Is Increased by Muscle Contraction—Ex vivo muscle contraction increased p90Rsk activity 5-fold (Fig. 5A, p < 0.001). The contraction-induced increase in p90Rsk activity was completely prevented by the addition of 50 μM PD98059. Similar to the results noted above for ERK1/2 phosphorylation, SB203580 led to a 48% reduction in contraction-stimulated p90Rsk activity (p < 0.001). Nevertheless, contraction-induced p90Rsk activity was elevated 2.5-fold (p < 0.001) with respect to basal levels after SB203580 exposure. MAPKAP-K2 activity was increased 10-fold by muscle contraction (Fig. 5B, p < 0.005). This effect was unaffected by PD98059 but completely inhibited by the presence of SB203580, suggesting that p38MAPK mediates the activation of MAPKAP-K2 in response to muscle contraction.

Muscle Contraction Activates MSK1 Activity—Recently, MSK1 has been shown to be activated by both ERK and p38MAPK pathways. Ex vivo muscle contraction led to a 5-fold increase in MSK1 activity (Fig. 6, p < 0.001). This effect was completely inhibited by the addition of either PD98059 or SB203580, suggesting that muscle contraction stimulates MSK1 by both ERK and p38MAPK mediated pathways.

**DISCUSSION**

Exercise and contractile activity stimulate ERK (3–7), c-Jun N-terminal kinase (3, 5, 8), and p38MAPK (6). We have utilized an ex vivo system to achieve muscle contraction to examine the direct role of muscle contraction upon stimulation of MAPK signal transduction. This system allows us to study the effects of muscle contraction on signal transduction independent of systemic factors or influence of surrounding tissue. Contractile activity increased muscle glucose transport 5–6-fold, and decreased muscle glycogen stores > 60%, demonstrating a profound effect of contractile activity on carbohydrate metabolism in skeletal muscle. This system of muscle contraction greatly influenced signaling by the MAPK pathways. Here we show that ex vivo muscle contraction, void of any systemic factors, is sufficient for ERK1/2 and p38MAPK phosphorylation. Increased phosphorylation of ERK1/2 and p38MAPK occurred only at voltages that elicit measurable muscle contraction. This suggests that muscle contraction rather than electrical stimulation is directly responsible for initiating MAPK signaling in skeletal muscle. Furthermore, we show that ex vivo contraction activates the downstream MAPK targets p90Rsk, MAPKAP-K2, and MSK1.

Classic growth factor-mediated activation of ERK1/2 involves the Ras/Raf/MEK signaling cascade (9–11). Activated MEK1/2 phosphorylates and activates ERK1/2. Exercise/contraction-induced ERK1/2 signaling appears to involve similar signal transduction (4, 7). However, the mechanism by which muscle contraction initiates the Ras-Raf association remains undetermined (7). PKC activation is also known to lead to Ras...
activation and thus stimulate MAPK activity (12). Additionally, PKC can mediate p38MAPK activation by an unidentified mechanism (18). Muscle contraction has been suggested to activate PKC in response to electrical stimulation (30, 31). Sciatic nerve stimulation in rat hind limb is associated with a rapid (2–5 min) shift of PKC activity, as measured by 32P incorporation to histone IIIS, from the cytosol to the membrane fraction (30, 31). Therefore, we determined whether PKC mediates MAPK signal transduction in response to ex vivo muscle contraction. Pre-exposure of rat epitrochlearis muscles to the PKC inhibitor GF109203X did not alter ERK1/2 or p38MAPK phosphorylation in contracting skeletal muscle. These results suggest that GF109203X-sensitive PKC isoforms (i.e. conventional and novel) are not involved in contraction-induced MAPK kinase signaling. The mechanism by which muscle contraction initiates Ras-Raf association (7) remains elusive.

The uses of the specific inhibitors PD98059 and SB203580 have been powerful tools in the delineation of downstream MAPK signaling in response to numerous stimuli. PD98059 (23) and SB203580 (20) have been reported to be highly selective inhibitors of MEK1 and p38MAPK activity, respectively, with very little inhibition reported on other members of the MAPK family. We show that PD98059 completely inhibited contraction-stimulated ERK1/2 phosphorylation, with ERK2 phosphorylation reduced to 50% of basal activity. This indicates that MEK1 is the primary kinase involved in ERK1/2 phosphorylation in response to muscle contraction. Unexpectedly, SB203580 also inhibited ERK1/2 phosphorylation in contracting muscle, although to a lesser degree than observed with PD98059. Contraction-stimulated ERK1/2 phosphorylation was reduced by 25% by SB203580; however, ERK1/2 phosphorylation was still significantly elevated over basal levels. The activity of p90Rsk activity in contracting skeletal muscle paralleled ERK phosphorylation. Muscle contraction induced a 5-fold increase in p90Rsk activity. This effect was completely abolished in the presence of PD98059, indicating that muscle contraction simulates p90Rsk activity via an ERK dependent mechanism. Similar to our findings for ERK phosphorylation, exposure of skeletal muscle to SB203580 resulted in an intermediate degree of inhibition on p90Rsk activity. To our knowledge, these results are the first to show an inhibitory effect of SB203580 on the ERK signaling pathway. Previously, SB203580 has been reported to be without inhibitory effect on MEK1, ERK2, or p90Rsk activity (20). Our results could suggest that there is cross-talk between the activation of the p38MAPK pathway and the ERK pathway, which may be promoting the activation of ERK in skeletal muscle. Although we observe no inhibition of force development in contracting muscles exposed to SB203580, the possibility remains that SB203580 inhibits unidentified signaling molecules involved in the coupling of muscle contraction to the MAPK signaling pathway.

In the present study, we show that p38MAPK phosphorylation is dramatically increased in direct response to ex vivo muscle contraction. Exposure to PD98059 did not prevent contraction-induced phosphorylation of p38MAPK. In contrast, p38MAPK phosphorylation was reduced by 90% in muscle exposed to SB203580. Similar to the observed effects of contraction on p38MAPK activation, MAPKAP-K2 activity was increased 10-fold in response to muscle contraction. This response was also unaffected by PD98059 but completely inhibited in the presence of SB203580. The mechanism of SB203580 inhibition is through binding to the ATP pocket of p38MAPK (32, 33), therefore the finding that SB203580 inhibited p38MAPK phosphorylation was unexpected. However, this is not the first report to show SB203580-induced inhibition of p38MAPK phosphorylation, because SB203580 exposure to ischemic cardiomyocytes also leads to reduced p38MAPK phosphorylation (34). Interestingly, in epitrochlearis muscles exposed to osmotic shock (KHB supplemented with 600 mM mannitol) for 20 min we do not observe an inhibition of p38MAPK phosphorylation by SB203580 exposure. Therefore, SB203580 may inhibit contraction-

*J. W. Ryder, A. Krook, and J. R. Zierath unpublished results.
MAPK signal transduction coupling, with the greatest effects elicited upon the p38MAPK cascade. Nevertheless, p38MAPK inhibition is evident from the complete ablation of contraction-induced MAPKAP-K2 activity.

Recently a new MAPK target protein, MSK1, has been identified (22). Here we provide evidence that contractile activity activates MSK1 in skeletal muscle. Thus, muscle contraction is the first identified stimulus of MSK1 activity in primary tissue. In 293 cells, MSK1 is activated in an ERK-dependent manner by stimulation with TPA or epidermal growth factor (22). Interestingly, ultraviolet light, arsenite, or H2O2 also activate MSK1 through p38MAPK. These results suggest that either ERK or p38MAPK activity are sufficient for activation of MSK1 (22). Accordingly, because muscle contraction leads to the activation of ERK1/2 and p38MAPK kinase, simultaneous inhibition of ERK and p38MAPK activity would be expected to fully inhibit MSK1 activity in response to muscle contraction. Surprisingly, PD98059 and SB203580 independently and completely inhibited contraction-induced MSK1 activity. Inhibition of MSK1 occurred despite the observation that contraction-stimulated MAPKAP-K2 and p90Rsk activities were significantly increased following PD98059 and SB203580 exposure, respectively. Thus, both ERK and p38MAPK activation appear to be required for MSK1 activation in skeletal muscle in response to muscle contraction.

MAPK activation plays an important role in the regulation of gene transcription. Here we provide evidence that muscle contraction stimulates activation of ERK and p38MAPK pathways, separate from any endocrine or paracrine influence. This activation appears to occur independently of PKC. Moreover, we show that contraction-induced p90Rsk and MAPKAP-K2 activation occur largely via separate MAPK signaling pathways. Importantly, we provide evidence demonstrating that MSK1 is directly activated by ex vivo muscle contraction and that this activation requires the simultaneous activation of both the ERK and p38MAPK pathways.

REFERENCES

1. Ren, J.-M., Semenkovich, C. F., Gulve, E. A., and Holloszy, J. O. (1994) J. Biol. Chem. 269, 14396–14401
2. Hjeltnes, N., Galuska, D., Bjørnholm, M., Akenes, A., Lannem, A., Zierath, J. R., and Wallberg-Henriksson, H. (1998) FASEB J. 12, 1701–1712
3. Goodyear, L. J., Chang, P., Sherwood, D. J., Dufresne, S. D., and Moller, D. E. (1996) Am. J. Physiol. 271, E403–E408
4. Aronson, D., Violan, M. A., Dufresne, S. D., Zangen, D., Fielding, R. A., and Goodyear, L. J. (1997) J. Clin. Invest. 99, 1251–1257
5. Aronson, D., Dufresne, S. D., and Goodyear, L. J. (1997) J. Biol. Chem. 272, 25636–25640
6. Wu, Y., Xiang, X. J., Krook, A., Chibalin, A. V., Bjørnholm, M., Talley, M., Roth, R. A., Henriksen, J., Wallberg-Henriksson, H., and Zierath, J. R. (1998) FASEB J. 12, 1379–1389
7. Sherwood, D. J., Dufresne, S. D., Markus, J. F., Chestham, B., Möller, D. E., Järv, D., and Goodyear, L. J. (1999) Am. J. Physiol. 276, E570–E578
8. Aronson, D., Boppard, M. D., Dufresne, S. D., Fielding, R. A., and Goodyear, L. J. (1998) Biochem. Biophys. Res. Commun. 251, 106–110
9. Cohen, P. (1997) Trends Cell Biol. 7, 353–361
10. Ray, R. B., and Sturgil, T. W. (1998) J. Biol. Chem. 263, 12721–12727
11. Davis, R. J., (1993) J. Biol. Chem. 268, 14553–14556
12. van Biesen, T., Hawes, B. E., Raymond, J. R., Luttrell, L. M., Koch, W. A., and Richter, E. A., Cleland P. J. F., Rattigan, S., and Clark, M. G., (1988) FASEB J., 12, 25636–25640
13. Schonwasser, D. C., Marais, R. M., Marshall, C. J., and Parker, P. J. (1998) Mol. Cell. Biol. 18, 790–798
14. Skolnik, E. Y., Batzer, A., Li, N., Lee, C. H. (1993) Science 260, 1953–1955
15. Prechney, N. W., Rawlingson, L., Gueden, P., Jones, E., Cowley, H., Susa, A., and Saklatvala, J. (1994) Cell 78, 1039–1049
16. Price, M. A., Cruzalegui, F. H., and Treisman, R. (1996) EMBO J. 15, 6552–6563
17. Han, J., Lee, J.-D., Bibbs, L., and Ulevitch, R. J. (1994) Science 265, 808–811
18. Clerk, A., Michael, A., and Sugden, P. H. (1998) J. Cell Biol. 142, 523–535
19. Zhao, Y., Bjerkbak, C., and Möller, D. E. (1996) J. Biol. Chem. 271, 29773–29779
20. Cuenda, A., Rouse, J., Doza, Y. N., Meier, R., Cohen, P., Gallagher, T. F., Young, P., and Lee, J. (1995) FEBBS Lett. 364, 229–233
21. Bayart, R., Cuenda, A., Vanden Berghe, W., Piasance, S., Lee, J. C., Horensen, G., Cohen, P., and Fiers, W. (1996) J. Biol. Chem. 271, 1266–1269
22. Rechley, D., Clifton, A. D., Lucoq, J. M., and Alessi, D. R. (1996) EMBO J. 17, 4426–4441
23. Alessi, D. R., Cuenda, A., Cohen, P., Dudley, D. T., and Saltiel, A. R. (1995) J. Biol. Chem. 270, 27489–27494
24. Clifton, A. D., Young, P. R., and Cohen, P. (1996) FEBBS Lett. 392, 209–214
25. Wallberg-Henriksson, H., Zetan, N., and Henriksson, J. (1987) J. Biol. Chem. 262, 7665–7671
26. Lowery, O. H., and Passonneau, J. V. (1972) A Flexible System of Enzymatic Analysis, pp. 189–193, Academic Press, New York
27. Toullec, D., Panietti, P., Coste, H., Bellevuegne, P., Grand-Parret, T., Akana, M., Baudet, V., Boissin, P., Boursier, E., and Lorillie, E. P. (1991) J. Biol. Chem. 266, 15771–15781
28. Dudley, D. T., Pang, L., Decker, S. J., Bridges, A. J., and Saltiel, A. R. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 7666–7669
29. Lee, J. Laydon, J. T., McDonnell, P. C., Gallagher, T. F., Kumar, S., Green, D., McNulty, D., Blumenthal, M. J., Heys, J. R., Landvetter, S. W., Stricker, J. E., McLaughlin, M. M., Siemens, I. R., Fisher, S. M., Livi, G. P., White, J. R., Adams, J. L., and Young, P. R. (1994) Nature 372, 738–746
30. Richter, E. A., Cleland P. F. J., Rattigan, S., and Clark, M. G. (1988) FEBBS Lett. 217, 232–236
31. Cleland, P. F. J., Appleby, G. F., Rattigan, S., and Clark, M. G. (1989) J. Biol. Chem. 264, 17704–17711
32. Tong, L., Pav, S., White, D. M., Rogers, S., Crane, K. M., Cwyn, C. L., Brown, M. L, and Pargellis, C. A. (1997) Nat. Struct. Biol. 4, 311–316
33. Young, P. R., McLaughlin, M. M., Kumar, S., Kassis, S., Doyle, M. L., McNulty, D., Gallagher, T. F., Fisher, S., McDonnell, P. C., Cerr, S. A., Huddleston, M. J., Seibel, G., Porter, T. G., Livi, G. P., Adams, J. L., and Lee, J. C. (1997) J. Biol. Chem. 272, 12116–12121
34. Armstrong, S. C., Delacey, M., and Ganote, C. E. (1999) J. Mol. Cell. Cardiol. 31, 555–567