

Acute exercise activates nuclear factor (NF)- κ B signaling pathway in rat skeletal muscle

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ABSTRACT Two studies were performed to investigate the effects of an acute bout of physical exercise on the nuclear protein κ B (NF- κ B) signaling pathway in rat skeletal muscle. In Study 1, a group of rats ($n=6$) was run on the treadmill at 25 m/min, 5% grade, for 1 h or until exhaustion (Ex), and compared with a second group ($n=6$) injected with two doses of pyrrolidine dithiocarbamate (PDTC, 100 mg/kg, i.p.) 24 and 1 h prior to the acute exercise bout. Three additional groups of rats ($n=6$) were injected with either 8 mg/kg (i.p.) of lipopolysaccharide (LPS), 1 mmol/kg (i.p.) *t*-butylhydroperoxide (tBHP), or saline (C) and killed at resting condition. Ex rats showed higher levels of NF- κ B binding and P50 protein content in muscle nuclear extracts compared with C rats. Cytosolic I κ B α and I κ B kinase (IKK) contents were decreased, whereas phospho-I κ B α and phospho-IKK contents were increased, comparing Ex vs. C. The exercise-induced activation of NF- κ B signaling cascade was partially abolished by PDTC treatment. LPS, but not tBHP, treatment mimicked and exaggerated the effects observed in Ex rats. In Study 2, the time course of exercise-induced NF- κ B activation was examined. Highest levels of NF- κ B binding were observed at 2 h postexercise. Decreased cytosolic I κ B α and increased phospho-I κ B α content were found 0–1 h postexercise whereas P65 reached peak levels at 2–4 h. These data suggest that the NF- κ B signaling pathway can be activated in a redox-sensitive manner during muscular contraction, presumably due to increased oxidant production. The cascade of intracellular events may be the overture to elevated gene expression of manganese superoxide dismutase reported earlier (*Pflugers Arch.* 442, 426–434, 2001).—Ji, L. L., Gomez-Cabrera, M.-C., Steinhafel, N., Vina, J. Acute exercise activates nuclear factor (NF) κ B signaling pathway in rat skeletal muscle. *FASEB J.* 18, 1499–1506 (2004)

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IT HAS BEEN SHOWN over the past two decades that unaccustomed exercise can increase the generation of reactive oxygen species (ROS) in biological tissues that increase metabolic rate and oxygen consumption, such as skeletal muscle and myocardium (1–4). During and after heavy exercise, ROS production is increased in

several cellular sources such as mitochondrial respiratory chain, xanthine oxidase, NADPH oxidase, and activated phagocytes, challenging the endogenous antioxidant defense system and causing oxidative stress and damage (5, 6). However, animals frequently exposed to exercise (chronic training) have shown less oxidative damage, largely attributed to the up-regulation of endogenous antioxidant enzymes such as mitochondrial superoxide dismutase (MnSOD), glutathione peroxidase (GPX), and γ -glutamylcysteine synthetase (GCS) (7–10). Since the adaptive properties result from the cumulative effects of repeated exercise bouts, the initial signal for the stimulation leading to the long-term modulation must occur after each individual exercise bout. However, the nature of this important biological adaptation is still largely unknown.

Several oxidative stress-sensitive signaling pathways are operational in mammalian systems and play an important role in maintaining cellular oxidant-antioxidant balance (11). One of the most important signaling pathways that could be activated by ROS is nuclear protein κ B (NF- κ B) (12, 13). The NF- κ B/Rel transcription factors are present in the cytoplasm in an inactive state, bound with the inhibitory I κ B subunit proteins. NF- κ B is activated by a variety of external stimulants, such as H₂O₂, proinflammatory cytokines [tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), IL-6], lipopolysaccharide (LPS), and phorbol esters, via phosphorylation of I κ B α at Ser-32 and -36 by I κ B kinase (IKK). Phosphorylation of I κ B α sets the stage for the dissociation and nuclear translocation of the active P50/P65 complex, which binds the corresponding DNA sequence of the target genes, including MnSOD and GCS (14).

We recently reported that an acute bout of exercise in rats increased NF- κ B binding to its corresponding DNA binding domain of MnSOD gene, which was accompanied by an elevation of MnSOD mRNA level and protein content (15). Since MnSOD is a well-known antioxidant enzyme that modulates its gene expression in response to cellular pro-oxidative and antioxidant status (16, 17), we speculate that NF- κ B

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activation by ROS generated in contracting muscle may be the underlying mechanism for training adaptation and increased expression of antioxidant enzymes. Our overall hypothesis, therefore, is that ROS are directly involved in the signal transduction driving increased expression of antioxidant genes through NF- κ B activation in response to acute and chronic exercise. We first aimed to demonstrate that an acute bout of exercise could activate the NF- κ B signaling cascades in rat skeletal muscle. Our working hypotheses were 1) nuclear protein binding to a consensus sequence of NF- κ B will be increased in the nuclear extract of skeletal muscle from exercised rats; 2) cytosolic I κ B content will be decreased whereas phospho-I κ B (p-I κ B) content will be increased, as a result of exercise; 3) exercise will lead to NF- κ B nuclear translocation, increasing P50/P65 concentration in muscle nuclear extraction; and 4) exercise will cause activation of IKK in rat skeletal muscle.

MATERIALS AND METHODS

Animals

All animal use protocols were approved by the University of Wisconsin-Madison Research Animal Resource Center. Female Sprague-Dawley rats (age, 4 months; body wt, 220–280 g) were housed individually in animal facilities at the University of Wisconsin-Madison in temperature-controlled rooms (22°C) using a reverse 12 h light/dark cycle (7:00–19:00 dark, 19:00–7:00 light). Animals were fed a chow diet and tap water ad libitum.

Experimental design

All rats were initially acclimated to running on a motor-driven treadmill at 15 m/min, 0% grade for 15 min per day for 2 wk. This protocol accustomed the rats to the locomotion involved in the final exercise experiments without resulting in training adaptation in skeletal muscle. Two studies were conducted. In the first, 30 rats were randomly divided into five groups. The first group ran on the treadmill at 25 m/min, 5% grade, for 1 h or until exhaustion and were killed immediately. The workload was ~75% of the rats' maximal aerobic capacity (VO_{2max}) (18). Exhaustion was determined as the point when an animal was unable to right itself when placed on its side. A second group of rats was injected (i.p.) with two doses of pyrrolidine dithiocarbamate (PDTC, 100 mg/kg) carried in 1 mL saline 24 h and 1 h before the acute exercise bout as described above. A third group was injected with 8 mg/kg (i.p.) of LPS and rested for 1 h before killing. A fourth group was injected with 1 mmol/kg (i.p.) *t*-butylhydroperoxide (tBHP) and rested for 1 h before killing. A fifth group of rats was injected with 1 mL of saline and rested for 1 h before killing as control. In the second study, 28 rats were acclimated to treadmill running for 2 wk as described previously. On the day of the experiment, five groups of rats ($n=4$ each) were randomly assigned to an exercise protocol similar to that used in Study 1, i.e., running on treadmill at 25 m/min, 5% grade for 1 h or until exhaustion, and killed either immediately (0 h) or 1, 2, 4, 24 or 48 h after cessation of exercise ($n=4$). Another group of four rats was killed at rest as controls.

Tissue preparation

All animals were killed by decapitation. After exsanguination, the deep portion of the vastus lateralis (DVL) and gastrocnemius muscle was immediately excised, freeze-clamped between aluminum tongs precooled with liquid nitrogen, and stored at –80°C. One part of each muscle was weighed and submerged in ice-cold 0.1 M K₂HPO₄-KH₂PO₄ (wt:vol of 1 g:10 mL), minced, and homogenized at 0–4°C with a motor-driven Potter-Elvehjem Teflon glass homogenizer. Homogenates were subjected to a centrifugation at 9000 *g* for 20 s. Cell debris and connective tissues in the pellets were discarded and the supernatants were stored at –80°C until further enzyme assay. Another portion of the frozen muscle sample was used for nuclear extraction (see below).

Gel electromobility shift analysis (EMSA)

Nuclear extracts were prepared by the method of Dignam et al. (19) with modifications (15). The following single-stranded oligonucleotides were obtained from a labeling kit from Promega (Madison, WI, USA).



Double-stranded oligonucleotides were subjected to labeling with terminal transferase, which adds a single digoxigenin-11-ddUTP (DIG) moiety to the 3' end of the oligonucleotides, using the DIG Gel Shift Kit (Roche Applied Science, Mannheim, Germany). The oligonucleotide (3.85 pmol/ μ L) was mixed with labeling buffer (1M potassium cacodylate, 0.125 M Tris-HCl, 1.25 mg/mL bovine serum albumin, pH 6.6), CoCl₂ solution, DIG-11-ddUTP solution, and 1 μ L terminal transferase. The mixture was incubated for 15 min at 37°C and placed on ice, then precipitated with 2 μ L of 4 M LiCl and 60 μ L of chilled ethanol. After incubation for 30 min at 70°C, it was centrifuged for 15 min at 4°C and 12,000 rpm. The resultant pellet was washed three times with 500 μ L of 70% chilled ethanol, dried under vacuum, and dissolved in TEN buffer (10 mM Tris-HCl, 1 mM EDTA, 0.1 M NaCl, pH 8.0).

The labeled DNA fragment containing the sequence of interest was mixed on ice with muscle nuclear extract (40 μ g of protein) and binding buffer (100 mM HEPES, pH 7.6, 5 mM EDTA, 50 mM (NH₄)₂ SO₄, 5 mM DTT, Tween 20, 1% (w/v), 150 mM KCl). Unspecific binding was blocked by using 1 μ g of poly [d(I-C)] and 1 μ g of poly L-lysine. The mixture was incubated for 30 min at room temperature, then transferred onto a native polyacrylamide (8%) gel and subjected to gel electrophoresis. After electrophoretic separation, the oligonucleotide-protein complexes were blotted by electroblotting onto nylon membranes, positively charged (Amersham Pharmacia Biotech, Dublin, Ireland). The digoxigenin-labeled probes were subsequently detected by an enzyme immunoassay using anti-digoxigenin Fab fragments conjugated with alkaline phosphatase (AP) (Roche Applied Science) and the chemiluminescent substrate CSPD (Roche Applied Science). The chemiluminescent signals were recorded by exposure to X-ray film for 40–60 min.

Western blot analysis

After homogenization, the muscle samples were immediately boiled for 10 min to inactivate proteases and phosphatases, electrophoresed in SDS-10 or 12.5% polyacrylamide gels, electroblotted (Bio-Rad, Hercules, CA, USA) onto an immo-

bilon-P nylon membrane (GIBCO, Grand Island, NY, USA), then incubated with appropriate IgG fractions in PBS containing 5% nonfat dry milk (Carnation, Wilkes-Barre, PA, USA), 0.2% Tween 20 (Sigma, St. Louis, MO, USA), and 0.02% sodium azide (Sigma). Antibodies against I κ B α , p-I κ B α , IKK α , phospho-IKK α (p-IKK α), P50, P65, and α -actin were purchased from Cell Signaling Technology Inc. (Beverly, MA, USA). After overnight incubation, the blots were washed three times with a wash buffer (PBS-0.2% Tween 20) for 30 min each time at room temperature, then incubated for 2 h with a secondary horseradish peroxidase (HRP)-conjugated donkey anti-rabbit antibody (Amersham, Arlington Heights, IL, USA), HRP-conjugated protein A (Amersham), or HRP-conjugated donkey anti-mouse antibody (Oncogene Science, Uniondale, NY, USA). The blots were washed three times as above and developed using the enhanced chemiluminescence (ECL) procedure as specified by the manufacturer (Amersham). Autoradiographic signals were assessed using a Bio-Rad scanning densitometer.

Statistical analysis

All data were subjected to one-way ANOVA using SYSTAT (version 5.03; Evanston, IL, USA). For post hoc analysis, a Fisher's least significant difference test was performed to test for significant differences between means. $P < 0.05$ was accepted as significant.

RESULTS

Study 1

Figure 1a depicts the EMSA for NF- κ B binding in the various groups of rats. **Figure 1b** is a graphic expression

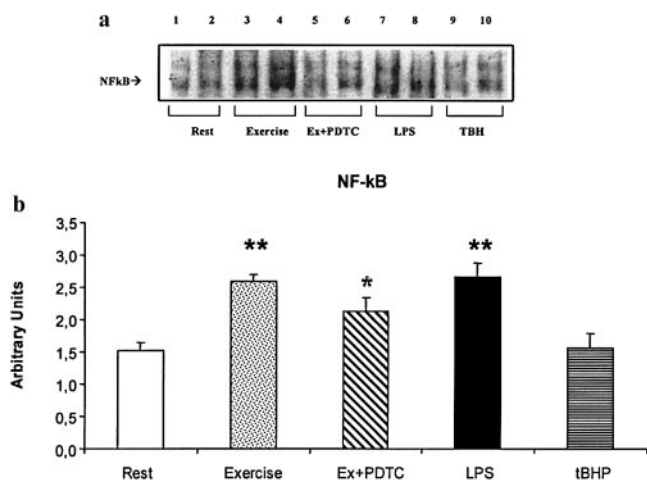


Figure 1. *a*) EMSA sample using NF- κ B probe end-labeled with digoxigenin-11-ddUTP, shown with two muscle samples randomly selected from each treatment group ($n=6$). Lanes 1 and 2: rest. Lanes 3 and 4: ran on treadmill at 25 m/min, 5% grade for 1 h or until exhaustion. Lanes 5 and 6: exercised and injected with pyrrolidine dithiocarbamate (PDTC, 100 mg/kg, i.p.) 24 and 1 h before killing. Lanes 7 and 8: injected with lipopolysaccharide (LPS, 8 mg/kg, i.p.) 1 h before killing. Lanes 9 and 10: injected with t-butylhydroperoxide (tBHP, 1 mmol/kg, i.p.) 1 h before killing. *b*) Mean \pm SE ($n=6$) of NF- κ B binding intensity derived from densitometry, * $P < 0.05$; ** $P < 0.01$, vs. rest.

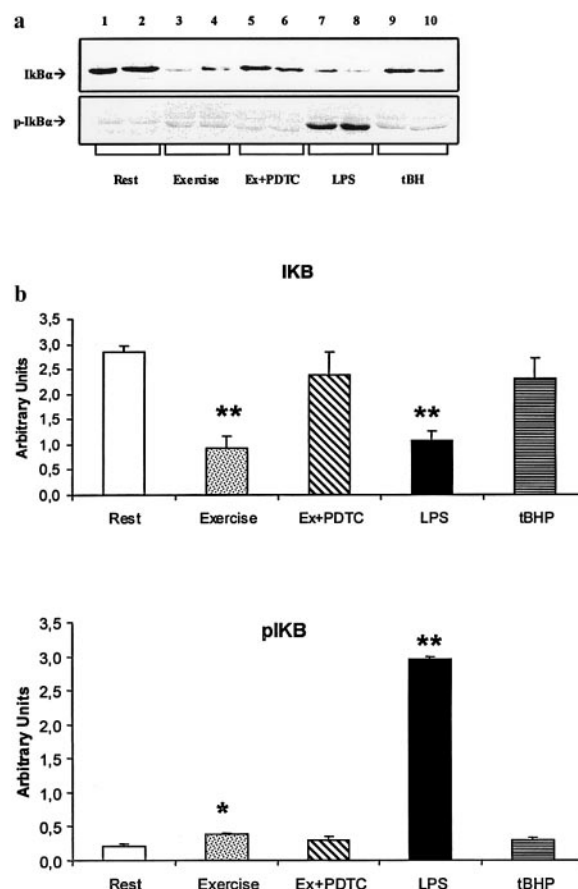


Figure 2. *a*) Western blot analysis of I κ B α and phospho-I κ B α in the cytosolic fraction of DVL muscle. Two muscle samples were randomly selected from each treatment group ($n=6$). Lane descriptions are identical to those in Fig. 1. Assay was performed per product instructions from Cell Signaling Technology, Inc. *b*) Mean \pm SE ($n=6$) of I κ B α and phospho-I κ B α contents derived from densitometry. * $P < 0.05$; ** $P < 0.01$, vs. rest.

of the intensity (mean \pm SE) of NF- κ B binding. An acute bout of exercise markedly increased NF- κ B binding in DVL muscle ($P < 0.01$). Administration of PDTC attenuated the exercise effect, although Ex+PDTC rats still had higher levels of NF- κ B binding than rested rats ($P < 0.05$). Rats treated with LPS 1 h before killing showed a higher level of NF- κ B binding than the control rats ($P < 0.01$); no effect was found in the tBHP-treated rats.

We examined whether increased NF- κ B binding was associated with phosphorylation and degradation of I κ B α . At rest, most of the I κ B α protein was in the nonphosphorylated form and little was present in the phosphorylated form (**Fig. 2a**, lanes 1 and 2). DVL muscle from exercised rats showed a marked reduction of I κ B α ($P < 0.01$) and a corresponding increase in p-I κ B α ($P < 0.05$) (lanes 3 and 4). This exercise effect was almost abolished by treatment with PDTC (lanes 5 and 6). LPS-treated rats demonstrated a 10-fold ($P < 0.01$) increase in p-I κ B α content, with a dramatic reduction of I κ B α ($P < 0.01$) (lanes 7 and 8). In the

tBHP-treated rats, although there appeared to be a decrease of I κ B α and an increase in p-I κ B, these changes were not significant (lanes 9 and 10).

Since I κ B phosphorylation is controlled by IKK in most cells, we measured the content of IKK α and its active form p-IKK α in rat DVL muscle. Ex rats showed a marked reduction of IKK α ($P < 0.05$) and an increase in p-IKK α ($P < 0.05$) (Fig. 3). Ex+PDTC showed little change in IKK α content but a significant increase in p-IKK α content ($P < 0.05$) compared with rested control rats. A large decrease in IKK α ($P < 0.01$) and a 5-fold increased in p-IKK α ($P < 0.01$) was found in LPS-treated rats. tBHP-treated rats showed no change in either IKK α or p-IKK α . The content of α -actin, a housekeeping protein marker in muscle, was not altered in the various treatment groups of rats (Fig. 3).

To determine whether oxidative-stress induced I κ B α phosphorylation was associated with a nuclear translocation of P50/P65 subunits, we measured P50 content in the DVL muscle nuclear extracts, as shown in Fig. 4. Ex and LPS groups of rats both showed dramatic increases in P50 levels compared with rest controls

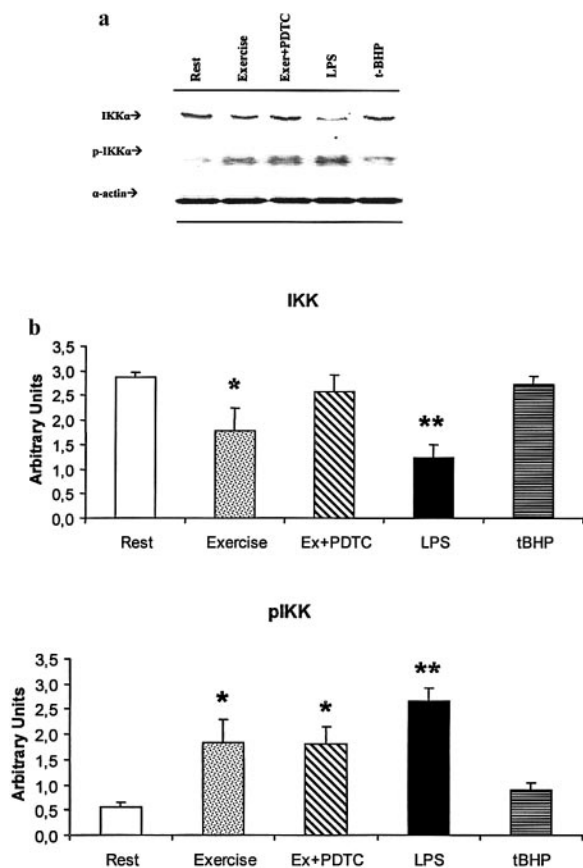


Figure 3. *a)* Western blot analysis of IKK α , phospho-IKK α and α -actin in the cytosolic fraction of DVL muscle ($n=6$). Experimental groups were treated the same as described in Fig. 1. Assay was performed for at least 3 times per product instructions from Cell Signaling Technology, Inc. *b)* Mean \pm SE ($n=6$) of IKK α and phospho-IKK α contents derived from densitometry. * $P < 0.05$; ** $P < 0.01$, vs. rest.

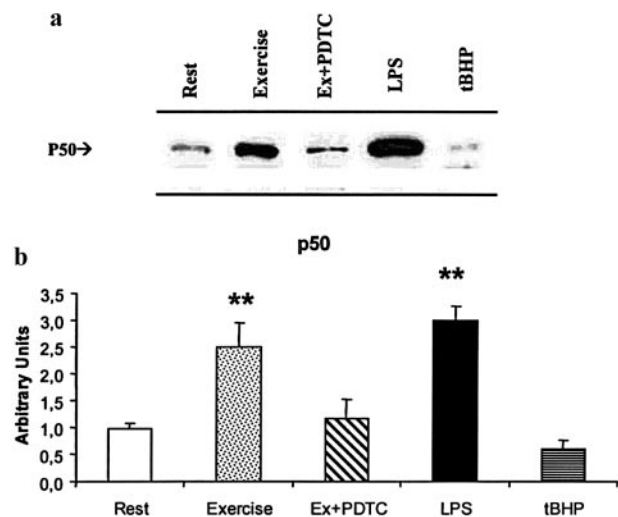


Figure 4. *a)* Western blot analysis of P50 in the nuclear extraction of DVL muscle ($n=6$). Experimental groups were treated the same as described in Fig. 1. Assay was performed for at least 3 times per product instructions from Cell Signaling Technology. *b)* Mean \pm SE ($n=6$) of P50 content derived from densitometry. ** $P < 0.01$, vs. rest.

($P < 0.01$), whereas Ex+PDTC and tBHP treatments did not elicit any significant effect.

Study 2

In the time course study, rats were subjected to an acute bout of treadmill running for 1 h and killed at various times up to 48 h postexercise. As shown in Fig. 5a, increased NF- κ B binding was noticed in the gastrocnemius muscle immediately (0 h) after exercise; a marked increase was revealed thereafter, which reached 9-fold higher at 2 h, then gradually returned to resting level at 48 h. Figure 5b is a graphic expression of the time course of NF- κ B activation by exercise. A significantly greater level of NF- κ B binding was found at 0 ($P < 0.05$), 1 ($P < 0.05$), 2 ($P < 0.01$), 4 ($P < 0.05$), and 24 h ($P < 0.05$) after exercise compared with resting level.

In Fig. 6a, a marked reduction of cytosolic I κ B α and corresponding increase in p-I κ B α was clearly demonstrated at 0 h. After 4 h, I κ B α and p-I κ B α returned to pre-exercise levels, with no noticeable p-I κ B α in muscle extract at 24 and 48 h. At 24 and 48 h, I κ B α showed a marked increase above resting levels, presumably reflecting increased de novo expression of this inhibitory subunit of NF- κ B complex.

P65 content in muscle nuclear extract showed a gradual increase after exercise, with a maximal level revealed at 4 h (Fig. 6b). P65 content returned to resting level at 24 and 48 h postexercise.

DISCUSSION

Oxidative stress (or redox)-sensitive signaling pathways use ROS to transfer signals from the cytosol to the

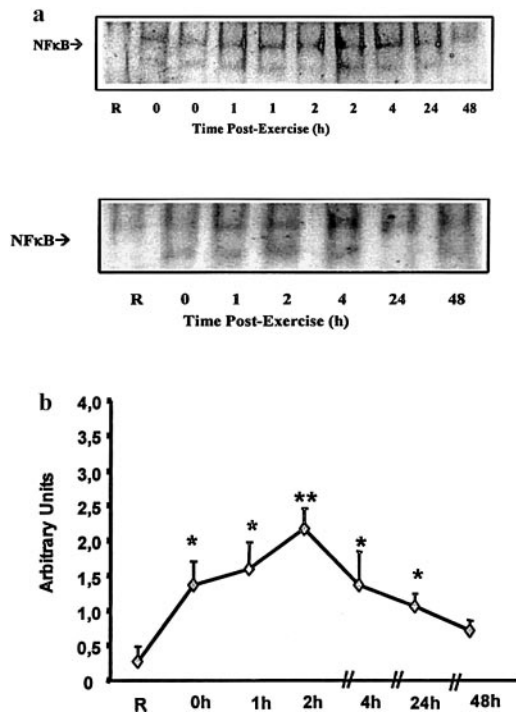


Figure 5. *a*) Representative EMSA samples using NF- κ B probe end-labeled with digoxigenin-11-ddUTP. Muscle nuclear extracts were from pooled gastrocnemius muscle of rats at rest (R) or 0, 1, 2, 4, 24, and 48 h after an acute bout of treadmill run at 25 m/min, 5% grade for 1 h or until exhaustion. *b*) Time course of NF- κ B binding intensity derived from densitometry. Each point represents mean \pm SE ($n=4$). * $P < 0.05$; ** $P < 0.01$, vs. rest.

nucleus to stimulate growth, differentiation, proliferation, and apoptosis (20). Among the main pathways are NF- κ B, MAPK signaling cascade, the phosphoinositide 3-kinase (PI₃K)/Akt pathway, p53 activation, and the heat shock response. Although these pathways are all important in regulating homeostasis of oxidant-antioxidant balance, NF- κ B and MAPK signaling pathways are considered the most critical for the cells to cope with oxidative stress (16). NF- κ B is a polymeric transcription factor composed of members of the Rel family. In mammals, these proteins include p50 (NF- κ B1), p65 (RelA), p52 (NF- κ B2), RelB, c-Rel, p105, and p100 (21, 22). NF- κ B is activated by a variety of external stimulants, including H₂O₂, proinflammatory cytokines, LPS, UV irradiation, viral infection, and phorbol esters. These signals can lead to elevation of intracellular ROS, which may serve as proximal messengers to activate key upstream kinases of the NF- κ B cascade. The double-stranded, RNA-activated protein kinase (PKR) and protein kinase C (PKC ζ) are believed to be involved in activating IKK (23, 24). Recently, MAPK/ERK kinase (MEK1) was shown to be a required enzyme connecting a Bcl-2 mediated activation of IKK β (25). Phosphorylation of two critical serine residues (Ser-32 and Ser-36 in I κ B α , Ser-19 and Ser-23 in I κ B β) by IKK primes I κ B for ubiquitination and proteolytic degradation by the 26S proteasomes (26). I κ B dissociation unleashes P50/

P65 to translocate into the nucleus and binds DNA motifs (consensus: 5'-GGG-puNNPuPuCC-3') of the gene targets.

In the current study, we demonstrated that contractile activity could activate NF- κ B signaling pathway in rat skeletal muscle. Although we previously reported elevated NF- κ B binding in rat DVL muscle during postexercise (15), this study was the first to reveal that increased NF- κ B binding was accompanied by a cascade of events in the exercised muscle, including enhanced I κ B α phosphorylation, decreased I κ B α content in the cytosol, and increased nuclear P50 content. Furthermore, our data showed an elevated p-I κ B α level immediately after exercise whereas NF- κ B binding peaked \sim 2 h postexercise. These findings about the time sequence demonstrate that phosphorylation of I κ B α took place during muscular contraction, followed by P50 translocation and binding to the NF- κ B motif in the muscle nucleus. The cytosolic content of I κ B α was elevated markedly after the initial decline and was 3- and 5-fold higher than resting levels at 24 and 48 h, respectively. These changes probably reflected a well-known negative feedback control mechanism, because I κ B α as a strong sequester of P50/P65 is also a gene target of NF- κ B signaling. High levels of I κ B α probably shut down the NF- κ B signal cascade and prevent unwanted overexpression of target genes (13). While nonphosphorylated I κ B α content in the DVL muscle showed a large decline in exercised rats of Study 1, the increase in p-I κ B α was not sufficiently robust to explain the magnitude of decrease in I κ B α content. This discrepancy might be due to alternative sites of I κ B α phosphorylation that were not detected by the antibody used in our Western analysis. For example, tyrosine kinase activity appears to be necessary for the activation

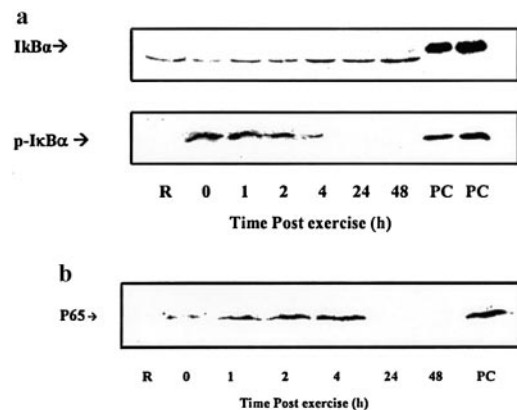


Figure 6. *a*) Western blot analysis of I κ B α and phospho-I κ B α in the cytosolic fraction of gastrocnemius muscle pooled from each treatment group ($n=4$). Time course description is identical to that in Fig. 5. Assay was performed per product instructions from Cell Signaling Technology, Inc. R, rested. PC, positive control with TNF- α -treated HeLa cell samples. *b*) Western blot analysis of P65 in the nuclear extraction of rat gastrocnemius muscles pooled from each treatment group ($n=4$). R, rested. PC, positive control in TNF- α -treated HeLa cell samples.

of NF- κ B, involving tyrosine phosphorylation of I κ B α (27). This pathway, however, does not lead to ubiquitination and proteosomal degradation of I κ B.

Physical exercise can elicit a variety of physiological events that could potentially activate NF- κ B pathway. During heavy muscular contraction with relatively sufficient oxygen supply, O₂^{•-} production is increased through complexes I and III of the mitochondrial electron transport chain (1, 28). O₂^{•-} may be dismutated by MnSOD to H₂O₂, which diffuses outside of mitochondria. H₂O₂ has been shown to activate IKK, which might explain the increased I κ B α phosphorylation in our study (24). This scenario was also supported by our data that IKK was activated (i.e., phosphorylated) in the exercised muscle. We did not measure muscle ROS generation in the current study, but our previous work with rats exercising on treadmill at similar speed and grade showed a 38% increase in ROS above resting levels (28). The majority of the ROS was presumably H₂O₂ under the experimental conditions. In addition to mitochondria, several other sources might also contribute to ROS generation during this type of exercise, such as xanthine oxidase in the cytosol and endothelial cells, catalase in the peroxisomes, membrane lipooxygenase, and NADPH oxidase (5, 6). These enzymes can generate O₂^{•-} and H₂O₂, especially under the influence of proinflammatory cytokines (TNF- α , IL-1, and IL-6), which increase their infiltration and de novo expression from neutrophils and/or damaged muscle tissues in an autocrine fashion (29–31). Their expression also depends on the NF- κ B signaling pathway (14).

IKK activation by muscle contraction was not as robust as expected. We speculate this might be partially explained by the specificity of IKK isoforms present in rat skeletal muscle. IKK α catalyzes the phosphorylation of Ser-32 and Ser-36 in I κ B α whereas IKK β phosphorylates Ser-19 and Ser-23 (26). The antibody used in the current study could detect both IKK α (85 kDa) and IKK β (87 kDa) when the kinase was activated by phosphorylation at Ser-180 or Ser-181, respectively. In our experiments, the major band detected was identified as the 85 kDa IKK α . Regula et al. (25) showed in ventricular myocytes that IKK β was the primary enzyme to activate NF- κ B pathway due to phosphorylation of Ser-181 by MEKK-1. Since acute exercise has been reported to increase Raf-1/MEKK activity in human muscle (32), this mechanism is speculated to play a role in the current experimental model. However, IKK can be activated by other kinases such as PKR and PKC ζ , depending on the cell type (23, 24). NF- κ B-induced kinase is known to phosphorylate and activate IKK (33). The predominate form that could confer an exercise-induced activation of IKK in skeletal muscle remains to be identified.

The physiological role of NF- κ B pathway activation during muscle contraction is still elusive. We hypothesized that the signaling cascade might target genes that could be quickly activated transcriptionally to counter

the increased ROS production, such as MnSOD, nitric oxide synthase, and GCS, all of which have mandatory NF- κ B binding sequences on their promoter region (16, 34). We previously reported an increase in MnSOD mRNA abundance, along with elevated NF- κ B and AP-1 binding, in rat skeletal muscle shortly after an acute bout of treadmill running, followed by increased MnSOD protein content 48 h later (15). This antioxidant enzyme-inducing function of NF- κ B may be especially important in tissues like skeletal muscle and myocardium, where intrinsic antioxidant capacity is relatively low. Sufficient MnSOD activity is considered critical for protecting mitochondria from the cytotoxic effects of •OH formed via Haber-Weiss reaction, which elicit oxidative damage to membrane lipid, amino acids, mtDNA, and enzymes (35). However, our current data did not provide a direct link between NF- κ B signaling cascade and other important intracellular events, such as MnSOD gene expression in the skeletal muscle. Another possible function of NF- κ B activation may be related to apoptosis. In response to heavy muscle contraction, apoptotic activity has been reported to increase, perhaps to rid damaged muscle fibers, soft tissue cells, and nonfunctional immune cells in order to facilitate homeostatic turnover (36). However, NF- κ B activation is also known to inhibit selective apoptotic pathways, leading to caspase 3 inhibition. Thus, the relationship between NF- κ B activation and apoptosis would be an important area for understanding homeostatic regulation in response to muscle contraction (37). Our future effort will concentrate on the physiological relevance of NF- κ B activation as well as the potential mechanism governing this important signaling pathway.

In an attempt to gain some insight into such mechanisms, we investigated the effect of PDTC, a well-known antioxidant inhibitor of NF- κ B. PDTC treatment was found to partially block exercise-induced signaling cascade. PDTC-treated exercised rats showed lower NF- κ B binding in the muscle nuclear extraction, whereas exercise-induced I κ B α phosphorylation and degradation, as well as elevation of P50 content, were almost abolished by PDTC treatment. However, PDTC did not affect IKK activation prominently. The mechanisms involved in PDTC inhibition of NF- κ B are not entirely clear. As an antioxidant, PDTC is capable of influencing the redox-sensitive steps of NF- κ B pathway in the cell, like glutathione (GSH) and N-acetyl-cysteine (NAC) (38). However, some studies suggest that PDTC might directly interact with NF- κ B motifs in target genes (39). Based on our previous findings, GSH did not prevent exercise-induced activation of MnSOD gene expression (40). Thus, PDTC may inhibit NF- κ B signaling pathway by a specific mechanism independent of its influence on the muscle redox status. Our findings that PDTC partially inhibited exercise-activated NF- κ B could potentially be a useful tool to probe whether some well-known training adaptations, such as

antioxidant enzyme induction (MnSOD) are mediated by this signaling pathway.

We have applied two well-known oxidative stress agents, LPS and tBHP, as in vivo positive controls. LPS dramatically increased P50 concentration and enhanced NF- κ B binding in the muscle nuclear extraction, accompanied by phosphorylation and degradation of I κ B α . This classic endotoxin stimulates TNF- α secretion by macrophages, lymphocytes, and fibroblasts, which in turn greatly increases ROS production via respiratory burst by neutrophils and perhaps mitochondria (41). The resulting activation of NF- κ B pathway by LPS exaggerates exercise effects in the treated resting muscles. In contrast, tBHP at a dose of 1 mmol/kg elicited no significant effect on any components in the NF- κ B signaling pathway. Although tBHP is a potent oxidant, it is possible that the i.p. injected tBHP was effectively removed by hepatic and plasma antioxidant systems, especially GPX and GSH sulfurtransferase. Any tBHP entering muscle cells might have been weakened by cytosolic GPX, resulting in a concentration too low to stimulate the kinases required for NF- κ B activation.

In summary, we demonstrated that an acute bout of exercise could activate the NF- κ B pathway in rat skeletal muscle. This activation was almost abolished by PDTC treatment prior to exercise. The series of cascade events observed were likely the overture to muscle adaptive responses, especially for the elevated gene expression of MnSOD we reported earlier (15). The upstream activators and the physiological function of the exercise activation of NF- κ B pathway remain to be examined. FJ

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