Caspase activation contributes to endotoxin-induced diaphragm weakness

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Supinski, Gerald S., and Leigh A. Callahan. Caspase activation contributes to endotoxin-induced diaphragm weakness. J Appl Physiol 100: 1770–1777, 2006. First published February 16, 2006; doi:10.1152/japplphysiol.01288.2005.—Infections produce significant respiratory muscle weakness, but the mechanisms by which infection-induced muscle force remain incompletely understood. Recent work suggests that caspase 3 releases actin and myosin from the contractile protein lattice, so we postulated that infections may reduce skeletal muscle force by activating caspase 3. The present experiments were designed to test this hypothesis by determining (1) diaphragm caspase 3 activation in the diaphragm after endotoxin and (2) the effect of a broad-spectrum caspase inhibitor, Z-Val-Ala-Asp(OCH3)-fluoromethylketone (zVAD-fmk), and a selective caspase 3 inhibitor, N-acetyl-ASP-Glu-Val-Asp-OH (DEVD-CHO), on endotoxin-induced diaphragm weakness. Caspase 3 activation was assessed by measuring caspase protein levels and by measuring cleavage of a fluorogenic substrate. Diaphragm force was measured in response to electrical stimulation (1–150 Hz). Caspase-mediated spectrin degradation was assessed by Western blotting. Parameters were compared in mice given saline, endotoxin (12 mg/kg ip), endotoxin plus zVAD-fmk (3 mg/kg iv), zVAD-fmk alone, or endotoxin plus DEVD-CHO (3 mg/kg iv). Endotoxin increased diaphragm active caspase 3 protein (P < 0.003), increased caspase 3 activity (P < 0.002), increased diaphragm spectrin degradation (P < 0.001), and reduced diaphragm force (P < 0.001). Administration of zVAD-fmk or DEVD-CHO prevented endotoxin-induced weakness (e.g., force in response to 150-Hz stimulation was 23.8 ± 0.8 N/cm², respectively, for control, endotoxin, endotoxin plus zVAD-fmk, endotoxin plus DEVD-CHO, and zVAD-fmk alone treated groups, P < 0.001). Caspase inhibitors also prevented spectrin degradation. In conclusion, endotoxin administration elicits significant diaphragm caspase 3 activation and caspase-mediated diaphragmatic weakness.

Recent work indicates that critically ill patients have profound levels of respiratory muscle weakness, with pressure-generating capacity of these muscles reduced to 20–30% of that observed in normal healthy individuals (14, 26). Many of these patients have respiratory failure that is either precipitated or complicated by viral and bacterial infections, and, as a result, infection-induced muscle dysfunction may be a major contributor to weakness in these patients (11, 19). In keeping with this possibility, previous studies in both humans and animals indicate that even minor infections can induce significant reductions in respiratory muscle strength (2, 5, 11, 16, 19). In addition, more severe infections have been shown to produce marked reductions in respiratory muscle strength, even inducing death by respiratory failure (13).

METHODS

Experimental protocols. Adult male mice (ICR strain, initial weight ~30 g) were used for experimentation. Animals were given food and water ad libitum and housed in university animal facilities. Approval for this work was granted by the Medical College of Georgia Institutional Animal Care and Use Committee. Three groups of experiments were performed. The first set of experiments determined whether animals killed 24 h after endotoxin...
administration had evidence of diaphragmatic caspase activation. For these experiments, we studied five control mice injected intraperitoneally with saline (0.3 ml) and five mice injected intraperitoneally with endotoxin (12 mg/kg, Escherichia coli lipopolysaccharide, Sigma Chemical, St. Louis, MO). All animals were killed at 24 h after injections, diaphragms were removed, and determination was made of diaphragm caspase activity, active caspase 3 levels, myosin levels, and actin levels. In a second set of experiments, we examined the time course of caspase activation and its relationship to endotoxin-induced reductions in diaphragm force. We studied seven control mice and mice killed at 6, 12, 24, and 48 h (n = 4, 4, 6, and 4, respectively) after intraperitoneal injection of endotoxin (12 mg/kg). At the time of death, diaphragms were removed, and measurements made of the diaphragm force-frequency relationship and diaphragm active caspase 3 protein levels. We then conducted a third series of experiments to determine whether administration of a broad-spectrum caspase inhibitor, zVAD-fmk (Biomol International, Plymouth Meeting, PA) could prevent endotoxin-induced diaphragm weakness. We compared data from 1) control, saline-injected mice (0.3 ml ip), n = 4; 2) endotoxin-injected mice (12 mg/kg ip), n = 4; 3) mice given both endotoxin (12 mg/kg ip) and zVAD-fmk (3 mg/kg administered via tail vein), n = 5; and 4) mice given zVAD-fmk alone (3 mg/kg iv), n = 4. We also tested an additional group of animals treated with both endotoxin (12 mg/kg ip) and DEVD-CHO (Biomol International, 3 mg/kg administered via tail vein), n = 4, to determine whether this caspase 3 specific inhibitor produced an effect equivalent to that of zVAD-fmk. Injections of zVAD-fmk and DEVD-CHO were made immediately after endotoxin administration (i.e., within a minute). Animals were killed 24 h after injections, and determination was made of the diaphragm force-frequency relationship. Diaphragm α-spectrin degradation product levels were measured for saline-treated controls, zVAD-fmk alone, endotoxin-treated, and zVAD-fmk plus endotoxin-treated groups. zVAD-fmk was chosen for these experiments because this agent does not appear to alter systemic cytokine levels during infection (10, 15), does not have nonspecific antioxidant effects (28), does not inhibit calpain activity (unpublished data from our laboratory), and has not been reported to produce other nonspecific systemic effects (10, 15, 28). The dose chosen is based on previous experimental work with this agent (15). DEVD-CHO was chosen because this latter agent is a more specific caspase 3 inhibitor.

In all experiments, animals were given saline (60 mg·kg⁻¹·day⁻¹) subcutaneously to maintain fluid volume status. All animals were sedated with pentobarbital sodium (50 mg/kg ip) just before death.

Assessment of caspase activity levels. A modified BIOMOL assay (Biomol International) was used to determine caspase activity for muscle homogenates. For this assay, homogenate (100 µg of protein) was added to assay buffer and fluorescent substrate [N-acetyl-Asp-Glu-Val-Asp-AMC (7-amino-4-methylcoumarin), Ac-DEVD-AMC]. A baseline fluorescent measurement of AMC was performed and repeated after 1.0 h of incubation at 30°C. AMC and caspase standards were used to quantitate activity levels. DEVD-CHO, a caspase 3 inhibitor, was added to duplicate samples to verify inhibition of signals by this compound.

Caspase, spectrin, actin, and myosin protein levels. Western blotting was employed to measure diaphragm levels of caspase 3, myosin, actin, and spectrin degradation products. For these determinations, muscle samples were diluted with an equal volume of loading buffer (126 mM Tris-HCl, 20% glycerol, 4% SDS, 1% 2-mercaptoethanol, 0.005% bromphenol blue, pH 6.8) and loaded onto Tris-glycine polyacrylamide gels, and proteins were separated by electrophoresis (Novex Minicell II, Carlsbad, CA). Proteins were then transferred to polyvinylidene fluoride membranes and incubated overnight at 4°C with antibodies to targeted proteins (anti-myosin, anti-actin, anti-caspase 3 from Santa Cruz Biotechnology, Santa Cruz, CA, and anti-α-spectrin degradation product from Chemicon International, Temecula, CA). Subsequently, membranes were incubated with horseradish peroxidase-conjugated secondary antibodies and antibody binding was detected using enhanced chemiluminescence (NEL Science Products, Boston, MA). Gel densitometry was performed using a Microtek scanner (Carson, CA) and UN-SCAN-IT software (Silk Scientific, Orem, UT). For caspase 3 and α-spectrin Western blots, membranes were stripped and reprobed with primary antibodies to α-tubulin (Santa Cruz Biotechnology) to verify equal loading among lanes. We chose α-tubulin for this normalization because previous experiments indicate that this protein is not altered in skeletal muscle by sepsis (8). Densities of the α-tubulin bands were determined by using a Microtek scanner and were used to normalize caspase 3 and α-spectrin band densities.

Measurement of force generation. For determination of force generation, left hemidiaphragms were excised, placed in a dissecting dish, and diaphragm strips excised. Strips were then mounted in organ baths containing Krebs-Henseleit solution (22°C, 50 mg/l curare, pH 7.40; 135 mM NaCl, 5 mM KCl, 11.1 mM dextrose, 2.5 mM CaCl₂, 1 mM MgSO₄, 14.9 mM NaHCO₃, 1 mM NaHPO₄, 50 units/l insulin, 95% O₂-5% CO₂). One end of each strip was tied to the organ bath base and the other was attached to a force transducer (Scientific Instruments, Heidelberg, Germany). Platinum mesh field electrodes were used to deliver supramaximal currents by using a constant current amplifier driven by a Grass S48 stimulator. After a 15-min equilibration period, muscle length was adjusted to optimal length and strips were sequentially stimulated with trains of 1, 10, 20, 50, 100, and 150 Hz stimuli (train duration 800 ms, 30 s between adjacent trains). Cross-sectional area was calculated as muscle strip weight times muscle density (1.06) divided by muscle length. Muscle specific force was calculated as raw force divided by cross-sectional area.

Statistical analysis. ANOVA testing was used for comparison of protein levels and forces across experimental groups. Post hoc testing using Tukey’s test was used to determine differences between individual groups. A P value of less than 0.05 was taken as indicating statistical significance. Data are presented as means ± SE.

RESULTS

Endotoxin and diaphragm protein levels. In our first group of experiments, we compared parameters in diaphragms from control animals and animals killed at 24 h after injection with a single dose of endotoxin. We found that there was no significant difference for diaphragm wet weights between control and endotoxin-treated animals, which averaged, respectively, 53.7 ± 2.8 and 51.2 ± 3.0 mg (not significant). In addition, the wet diaphragm weight-to-total body weight ratio was similar between controls (1.77 ± 0.03 mg/g) and endotoxin-treated animals (1.71 ± 0.03 mg/g, not significant). Protein per milligram wet weight of muscle is presented in Fig. 1 and was also similar between control and endotoxin-treated animals. We also used Western blotting to determine whether there were obvious alterations in diaphragm myosin or actin levels and found that these were similar for control and 24-h-endotoxin-treated animals (Fig. 1). Finally, we performed Coomassie-stained SDS-PAGE gels for diaphragm proteins and observed no obvious alterations in major band patterns between diaphragm samples for control and 24-h endotoxin-treated animals (Fig. 1). Although this analysis does not exclude the possibility that endotoxin administration may induce reductions in selected proteins or chemical side chain modification (e.g., phosphorylation) of contractile elements, it does indicate that wholesale, obvious reductions in diaphragm protein concentrations are not produced within the first 24 h after endotoxin administration.

Endotoxin and diaphragm caspase activation. We next examined diaphragm samples obtained 24 h after endotoxin...
administration for evidence of caspase activation. Western blot assessment of caspase proteins revealed similar procaspase 3 concentrations for diaphragm samples from control and 24-h endotoxin-treated animals, as shown in Fig. 2. In contrast, cleaved active caspase 3 protein levels were extremely low for control diaphragm samples and were markedly increased for diaphragm samples from 24-h endotoxin-treated animals (Fig. 2) \((P < 0.003)\). On average, active caspase protein levels for samples from 24-h endotoxin-treated animals were 615% of levels for control animals \((P < 0.003)\).

Caspase activity of diaphragm homogenates, assessed using a fluorogenic substrate, paralleled caspase protein levels, as shown in Fig. 3. Specifically, caspase assay activity for homogenates from 24-h endotoxin-treated animals was increased to 315% of activity levels for samples from control animals \((P < 0.002)\). The specificity of this fluorogenic assay as an indication of caspase activity was confirmed by the finding that addition of DEVD-CHO, a chemical caspase 3 inhibitor, markedly reduced fluorogenic substrate cleavage and eliminated a difference between control and endotoxin-treated sample cleavage rates (Fig. 3).

**Time course of caspase activation and alterations in diaphragm force generation.** If caspase activation is pathophysiologically linked to endotoxin-related reductions in muscle force generation, then one would expect increases in caspase activity to be followed by reductions in muscle force. In our second set of experiments, therefore, we measured diaphragm force and caspase activity at several time points after endotoxin administration (i.e., 6, 12, 24, and 48 h). As shown in Fig. 4, we found that diaphragm caspase activity increased progressively after endotoxin administration, rising to 257% of control levels by 12 h, to 331% at 24 h, and to 525% at 48 h \((P < 0.003 \text{ for last comparison})\). Increases in caspase activity were associated with reductions in diaphragm specific force generation (Fig. 4), which decreased successively to 86, 51, and 49% of control levels at 12, 24, and 48 h, respectively, after endotoxin \((P < 0.001 \text{ for comparison of control muscle specific force generation in response to 150-Hz electrical stimulation to muscle specific force at 24 and 48 h})\).

**Response to a broad-spectrum caspase inhibitor, zVAD-fmk.** In a third set of experiments, we determined whether it was possible to prevent endotoxin-induced reductions in diaphragm force generation by administration of a broad-spectrum, cell-permeant, irreversible caspase inhibitor \((zVAD-fmk)\). We found that zVAD-fmk largely prevented endotoxin-induced reductions in diaphragm specific force generation, as shown in Fig. 5. For example, endotoxin administration (24 h) evoked a reduction in diaphragm specific force generation in response to
150 Hz from a control value of 23.8 ± 1.4 to 12.1 ± 1.3 N/cm² ($P < 0.001$), and administration of zVAD-fmk to endotoxin-treated animals increased 150-Hz specific force generation to 23.5 ± 0.8 N/cm² ($P < 0.001$ compared with the endotoxin alone group). Forces generated by muscles from animals given zVAD-fmk alone were similar to values for control animals. The effects of DEVD-CHO (Fig. 6) were similar to the effects of zVAD-fmk, with DEVD-CHO increasing 150-Hz specific force generation to 22.7 ± 1.3 N/cm² ($P < 0.001$ compared with the endotoxin alone group).

Endotoxin administration also induced a marked increase in diaphragm levels of several degradation products of the cytoskeletal protein α-spectrin (also known as α-fodrin). As shown in Fig. 7, endotoxin evoked increases in diaphragm levels of both 150- and 120-kDa spectrin degradation products. Administration of zVAD-fmk to endotoxin-treated animals largely ablated formation of both 150- and 120-kDa spectrin degradation products, as shown both for representative Western blots and for group mean data in Fig. 7. On average, 150- and 120-kDa spectrin degradation protein levels were increased to 173 and 219% of control levels, respectively, for endotoxin-treated animals ($P < 0.001$ for both comparisons).

ZVAD-fmk administration to endotoxin-treated animals reduced diaphragm sample 150- and 120-kDa spectrin degradation levels to values close to control values (101 and 91% of control, respectively, $P < 0.001$ for comparison of both levels to values for the endotoxin-treated group).

**DISCUSSION**

**Infection and respiratory muscle weakness.** Respiratory muscle weakness is currently a major untreatable clinical problem for critically ill patients (14, 26). Recent work indicates that diaphragmatic strength in these patients is a fraction of that observed in normal individuals, predisposing patients to respiratory failure and prolonged ICU stays. This is best illustrated by studies by Laghi et al. (14) and Watson et al. (26), who found that the average twitch transdiaphragmatic pressure generated by mechanically ventilated patients averages only 20–30% of the levels seen in normal control subjects (14, 26). The factors responsible for inducing this severity of diaphragm weakness in patients remain unclear, albeit many of these patients are infected and several studies indicate that patients with infections and other causes of the systemic
inflammatory response syndrome have a high incidence (60–85%) of neuromuscular dysfunction (10). In keeping with these human studies, numerous animal studies indicate that models of systemic infection and/or inflammation (i.e., sepsis, peritonitis, pneumonia) rapidly induce large reductions in diaphragm function (2, 5, 13, 16).

The potential mechanisms by which infection and other forms of systemic inflammation alter respiratory and limb skeletal muscle function have been the focus of intensive

Fig. 3. Caspase activity assayed by cleavage of an artificial fluorogenic substrate. Samples from endotoxin (24 h)-treated animals had significantly higher active caspase activity than control samples ($P < 0.002$). In vitro addition of $N$-acetyl-Asp-Glu-Val-Asp-al (DEVD-CHO), a caspase 3 inhibitor, reduced measured activity in all samples. *Statistical difference compared with controls.

Fig. 5. Mean diaphragm force-frequency curves comparing control animals, $Z$-Val-Ala-Asp(OCH$_3$)-fluoromethylketone ($z$VAD-fmk)-treated animals, endotoxin-treated animals (24 h), and animals given both $z$VAD-fmk and endotoxin. Endotoxin administration reduced force generation compared with controls ($P < 0.005$ for 10, 20, 50, 100, and 150 Hz, $P < 0.01$ for 1-Hz data). Administration of $z$VAD-fmk prevented endotoxin-induced reductions in muscle force generation ($P < 0.001$ for 50, 100, and 150 Hz, $P < 0.05$ for 1, 10, and 20 Hz). *Statistical difference compared with controls.

Fig. 6. Mean diaphragm force-frequency curves comparing control animals, endotoxin-treated animals (24 h), animals given both $z$VAD-fmk and endotoxin, and animals given DEVD-CHO and endotoxin. DEVD-CHO was equivalent to $z$VAD-fmk in preventing endotoxin-induced reductions in diaphragm force (not significant for comparison of DEVD-CHO plus endotoxin and $z$VAD-fmk plus endotoxin group forces at all frequencies). *Statistical difference compared with controls.

Fig. 4. Comparison of the time course of alterations in caspase activity and diaphragm force (in response to 150-Hz stimulation) after endotoxin administration. Caspase activity levels increased over the 48 h after endotoxin administration ($P < 0.001$ for comparison of levels at 48 h to control). Force began to fall at the 12-h time point and reached its lowest level at 48 h ($P < 0.001$ for comparison of control to 48 h force). *Statistical difference compared with controls.

Fig. 6. Mean diaphragm force-frequency curves comparing control animals, endotoxin-treated animals (24 h), animals given both $z$VAD-fmk and endotoxin, and animals given DEVD-CHO and endotoxin. DEVD-CHO was equivalent to $z$VAD-fmk in preventing endotoxin-induced reductions in diaphragm force (not significant for comparison of DEVD-CHO plus endotoxin and $z$VAD-fmk plus endotoxin group forces at all frequencies). *Statistical difference compared with controls.
investigation. Numerous studies have shown that several components of the proteosome proteolytic degradation system (atrogen, other E-3 ligases, the 20S proteosome subunit, etc.) are upregulated in skeletal muscle in both infected patients and animal models of systemic inflammation (3, 21, 27). This has led to the thinking that infection or inflammation induces generalized skeletal muscle protein degradation in skeletal muscle and that proteosome-mediated protein loss is responsible, in turn, for reductions in skeletal muscle function.

The findings in the present study, however, indicate that loss of diaphragm force-generating capacity after endotoxin administration cannot be accounted for by such a process. In fact, by 24 h after administration of endotoxin, diaphragm specific force had fallen by 50% without any reduction in diaphragm muscle mass or diaphragm protein content, obvious depletion of major diaphragm protein bands, or specific loss of muscle myosin or actin stores. Our in vivo findings are in keeping with the observations by Reid et al. (20), who previously demonstrated that incubation of diaphragm muscle in cytokine-containing physiological solutions results in large reductions in muscle force generation without overt reductions in muscle protein stores. This previous in vitro work also demonstrated that cytokines reduce skeletal muscle force generation by altering contractile protein function rather than by altering contractile protein activation (i.e., cellular calcium cycling). Taking the present findings together with these previous data, it would appear that inflammatory stimuli may induce early reductions in diaphragm muscle force generation by a process that reduces contractile protein and whole muscle force-generating capacity in the absence of generalized muscle protein depletion.

Caspase activation in skeletal muscle. There are several theoretical processes that could account for reductions in muscle specific force generation in the absence of generalized protein loss. For one thing, Du et al. (7) have shown that some syndromes (diabetes, uremia) associated with the development of generalized muscle weakness have activation of caspase in skeletal muscle and caspase-mediated actomyosin cleavage. Caspase is thought to be an extremely selective proteolytic enzyme, causing discrete cleavage of a relatively small number of proteins at very selective sites (18). As a result, caspase-mediated cleavage has the potential for causing myofibrillar disruption in the absence of generalized protein loss. In keeping with this possibility, activated caspase has been shown to selectively damage cardiac contractile proteins in several disease states, reducing cardiac contractile protein force generation in the process (4, 22).

There has been no previous demonstration, however, of a role for caspase activation in skeletal muscle in an animal model of infection or inflammation. Our data provide the first evidence of caspase activation in a skeletal muscle in such a model, revealing a marked increase in diaphragm active...
caspase 3 protein levels and caspase 3 catalytic activity after a single sublethal dose of endotoxin. We found that caspase activation occurred within 12 h after endotoxin administration, preceding reductions in diaphragm specific force-generating capacity.

We also found a marked increase in diaphragm levels of α-spectrin degradation products after endotoxin administration. α-Spectrin (also called α-fodrin) is a large cytoskeletal support protein, thought to play an important role in maintaining cellular integrity. Degradation of α-spectrin in neural tissue has been associated with cell injury and death (18) whereas loss of α-spectrin in cardiac tissue has been shown to result in marked cardiac dysfunction (24). α-Spectrin degradation is known to occur in response to activation of caspase, with caspase-mediated α-spectrin cleavage resulting in production of both 120- and 150-kDa molecular weight degradation products (25). Of interest, another proteolytic enzyme, calpain, also degrades α-spectrin and generates 150-kDa spectrin products, but this latter enzyme does not generate a 120-kDa spectrin degradation product (17, 18). As a result, generation of the 120-kDa spectrin degradation product can be employed as an index of caspase-mediated structural protein degradation. Our observation that endotoxin administration increases formation of 120-kDa spectrin degradation products in the diaphragm is therefore consistent with caspase-mediated α-spectrin cleavage and is not seen after activation of calpain alone.

More importantly, we also found that administration of a broad-spectrum, cell-permeant, irreversible caspase inhibitor (zVAD-fmk) prevented both endotoxin-induced spectrin degradation and endotoxin-induced reductions in diaphragm specific force generation. We also examined the effects of a specific caspase 3 inhibitor (DEVD-CHO), and this latter agent also prevented the effect of endotoxin to reduce muscle force, producing an effect equivalent to that observed in response to zVAD-fmk alone. This is the first demonstration of which we are aware suggesting that inhibition of active caspase can improve skeletal muscle force-generating capacity in an animal model of disease. Because caspase inhibitors were administered intravenously in the present study, it is possible that some systemic effect of caspase inhibition (e.g., to alter the systemic inflammatory response) may have indirectly influenced the evolution of diaphragm dysfunction in our animal model. We think this possibility unlikely, however, because previous studies have shown that administration of caspase inhibitors in animal models of inflammation (i.e., endotoxin administration and cecal ligation-perforation induced sepsis) does not reduce levels of TNF-α, IL-1β, or other cytokines (10, 15). In fact, caspase inhibitor administration has previously been shown to blunt lymphocyte apoptosis in animal models of sepsis, potentiating lymphocyte cytokine generation and maintenance of an inflammatory state (10). This argues that zVAD-fmk improved diaphragm function after endotoxin administration in the present study by altering the response of this muscle to cytokines and other inflammatory mediators. As a result, we believe that the most likely explanation for our findings is that diaphragmatic caspase activation after endotoxin administration is pathophysiologically linked to reductions in diaphragm specific force generation in this animal model of inflammation.

Potential implications. There is increasing evidence that the systemic inflammatory response syndrome and other inflammatory states induce caspase activation in numerous organs. Past studies have reported caspase activation in lymphocytes, gut epithelium, pulmonary epithelium, liver parenchymal cells, cardiac myocytes, and renal parenchymal cells in various animal models of infection and inflammation (1, 9, 10, 12, 15, 25, 28). In most of these tissues, caspase activation is thought to alter organ function by inducing apoptosis and cell death. In the heart, however, recent work suggests that activation of caspase more commonly results in cellular dysfunction rather than cell death (4, 22). This is thought to be so because cardiac contractile proteins are relatively susceptible to caspase-mediated degradation. In addition, because cardiac cells are multinucleated, activation of apoptotic programs in individual nuclei often may not be sufficient for cell death. As a result, caspase activation in the heart can result in some nuclear dropout and loss of force generation without necessarily inducing cell death. It is reasonable to postulate that caspase activation in skeletal muscle, also multinucleated with specific domains for each nucleus, may often result in cellular remodeling rather than myocyte death.

The present work extends these earlier studies by demonstrating that inflammation can also potentiate activate caspase 3 in the diaphragm and that caspase 3 activation may be linked to inflammation-induced reductions in diaphragm force generation. Our data support the notion that, as in the heart, caspase activation in skeletal muscle may result primarily in weakness. Although the present experiment employed a commonly utilized animal model of inflammation to examine the relationships between caspase activation and diaphragm weakness, additional work is required to determine whether caspase activation in the diaphragm and other skeletal muscles also occurs in patients with infections and other inflammatory conditions. If so, determination of the precise downstream targets of caspase in skeletal muscle may lead to the identification of markers that can be used to detect caspase-mediated skeletal muscle disruption in patients. Moreover, it may also prove possible to prevent skeletal muscle weakness in these patients through therapeutic administration of inhibitors of skeletal muscle caspase or its immediate upstream activators.

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REFERENCES


