Trichostatin A, a histone deacetylase inhibitor, modulates unloaded-induced skeletal muscle atrophy

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First published June 25, 2015; doi:10.1152/japplphysiol.01031.2014.—Skeletal muscle atrophy is commonly associated with immobilization, ageing, and catabolic diseases such as diabetes and cancer cachexia. Epigenetic regulation of gene expression resulting from chromatin remodeling through histone acetylation has been implicated in muscle disuse. The present work was designed to test the hypothesis that treatment with trichostatin A (TSA), a histone deacetylase inhibitor, would partly counteract unloaded-induced muscle atrophy. Soleus muscle atrophy (∼38%) induced by 14 days of rat hindlimb suspension was reduced to only 25% under TSA treatment. TSA partly prevented the loss of type I and IIa fiber size and reversed the transitions of slow-twitch to fast-twitch fibers in soleus muscle. Unloading or TSA treatment did not affect myostatin gene expression and follistatin protein. Soleus protein carbonyl content remained unchanged, whereas the decrease in glutathione vs. glutathione disulfide ratio and the increase in catalase activity (biomarkers of oxidative stress) observed after unloading were abolished by TSA treatment. The autophagy-lysosome pathway (Bnip3 and microtubule-associated protein 1 light chain 3 proteins, Atg5, Gabarapl1, Ulk1, and cathepsin B and L mRNAs) was not activated by unloading or TSA treatment. However, TSA suppressed the rise in muscle-specific RING finger protein 1 (MuRF1) caused by unloading without affecting the forkhead box (Foxo3) transcription factor. Prevention of muscle atrophy by TSA might be due to the regulation of the skeletal muscle atrophy-related MuRF1 gene. Our findings suggest that TSA may provide a novel avenue to treat unloaded-induced muscle atrophy.

hypokinesia; histone acetylation; oxidative stress; proteolysis

DISUSE MUSCLE ATROPHY is a well-known consequence of prolonged bedrest, spaceflight, and many diseases such as cancer cachexia, sepsis, diabetes, chronic heart failure, obstructive pulmonary disease, or ageing. The loss in skeletal muscle mass is primarily due to an increase in myofibril degradation rate although a decrease in protein synthesis may also contribute to muscle atrophy (38, 44). Two main proteolytic pathways are involved in muscle wasting, the autophagy-lysosome system and the ATP-ubiquitin-dependent proteasome pathway (9, 22, 44). The mammalian forkhead members of the class O (Foxo) transcription factors are implicated in the regulation of muscle cell size through the transcriptional regulation of a number of key atrophy-associated genes, including two critical muscle-specific ubiquitine ligases, muscle-specific RING finger protein 1 (MuRF1) and muscle atrogin1/muscle atrophy F-box (MAFbx) (43). The insulin-signaling pathway induces Akt-mediated phosphorylation of Foxo, causing sequestration of Foxo proteins in the cytoplasm and inhibition of Foxo-dependent gene regulation (11, 56). The subcellular localization to the nucleus and the transactivation activity of Foxo proteins is regulated by protein-protein interactions, as well as posttranslational modifications such as phosphorylation, ubiquitination, and acetylation/deacetylation (2).

The interactions between histone acetyltransferases (HATs) and histone deacetylases (HDACs) play important roles in the epigenetic regulation of gene expression (15, 17, 19, 33, 46, 48). In denervated muscle, the class IIa HDAC, HDAC4, is required to activate muscle atrophy (15, 17, 52). A HDAC-Dach2-mygogenin signaling pathway promotes neurogenic muscle atrophy by inducing the E3 ubiquitin ligases MuRF1 and atrogin1/MAFbx (17, 33, 52). HATs and HDACs also regulate the activity of transcription factors, including the Foxo family of transcription factors, p300/cAMP response element binding-binding protein (CBP) is a HAT whose function has been investigated in several catabolic conditions (7, 46). p300/CBP is sufficient to repress the physiological activation of Foxo3 and its nuclear translocation in response to muscle disuse in vivo or in C2C12 cells during nutrient deprivation or dexamethasone treatment (46). A cascade of posttranslational modifications has also been shown to modulate activity and localization of Foxo3 in denervated muscles (7). During the first days of denervation, Foxo3 is translocated in the nucleus, where it is acetylated by p300/CBP. Acetylation of lysine 262 with acetyl group is a critical step for Foxo3 relocation in the cytosol, where the E3 ligase Mdm2 ubiquitinates Foxo3, leading to its degradation via proteasome. Oxidative stress also promotes the interaction of Foxo with p300/CBP (19), which is in agreement with the role of oxidative stress during muscle atrophy (10, 41, 47). More recently, class I HDACs have been identified as the specific proteins regulating Foxo3a deacetylation (6). Specifically, HDAC1 is sufficient to increase Foxo transcriptional activity of atrophy-target genes [cathepsin L, microtubule-associated protein 1 light chain 3 (LC3), MAFbx, and MuRF1] and induce muscle atrophy in vivo. Furthermore, inhibition of class I HDACs (with MS-275) prevents the decrease in specific force occurring in soleus muscle after 10 days of cast immobilization (6).

These experiments indicate that inhibiting HDAC could be a potent pharmacological strategy for muscle sparing during reduced activity. Among HDAC inhibitors, trichostatin A (TSA) is an antifungal antibiotic, which inhibits class I and II
HDACs. Previous studies suggest that inhibitors of nuclear deacetylases such as TSA may favor the recruitment and fusion of myoblasts into preformed myotubes (26). Follistatin, a protein that interacts with members of the transforming growth factor-β (TGF-β) family, is known to counter the activity of myostatin, a negative regulator of skeletal muscle mass (28). Follistatin seems to be an essential mediator of HDAC inhibitor-induced increase of muscle size and satellite cell recruitment. TSA treatment also promotes structural and functional recovery of dystrophic muscles, increasing myofiber size in dystrophin-deficient MDX, a mouse model for Duchenne muscular dystrophy, through an upregulation of follistatin expression in satellite cells (32). Furthermore, TSA ameliorated muscle atrophy and neuromuscular junction denervation in a mouse model of amyotrophic lateral sclerosis (55) and enhanced myofiber size, motor behavior, and survival in a mouse model of spinal muscular atrophy when administered after disease onset (5).

The aim of the present study was therefore to test the potential muscle beneficial effect of the well-known HDAC inhibitor TSA on muscle atrophy in a physiological model, the rat hindlimb unloading model. This model has been used extensively to study the response of muscle to disuse as well as to spaceflight. We specifically investigated some of the events involved in the growth of soleus muscle mass. We hypothesized that TSA may modulate the myostatin/follistatin axis and muscle oxidative stress and regulate the autophagy and proteasome pathways.

### Table 1. Oligonucleotide primers used for PCR analysis

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Fig. 1. Levels of acetyl-histone H3 protein (A), myostatin mRNA (B), and follistatin protein (C) in soleus muscle after unloading and trichostatin A (TSA) treatment. C, DMSO-treated control rats; C+TSA, TSA-treated control rats; H, DMSO-treated hindlimb-unloaded rats; H+TSA, TSA-treated and unloaded rats. Values are means ± SE for 5–6 animals. ¥P < 0.05 vs. C + TSA rats.
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Table 3. Cross-sectional areas of fibers in soleus muscle

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Type I</th>
<th>Type I/IIa</th>
<th>Type IIa</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>2,457 ± 150</td>
<td>1,652 ± 81</td>
<td>2,092 ± 139</td>
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<tr>
<td>C + TSA</td>
<td>2,623 ± 93</td>
<td>1,899 ± 112</td>
<td>2,170 ± 191</td>
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<tr>
<td>H</td>
<td>1,455 ± 116*</td>
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<td>2,023 ± 161†‡</td>
<td>1,502 ± 177†‡</td>
<td>1,727 ± 201†‡</td>
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Values are means ± SE for 7 animals. *P < 0.05, significantly different from C rats; †P < 0.05, significantly different from C + TSA rats; ‡P < 0.05, significantly different from H rats.

Materials and Methods

Animal care and protocol. Thirty-two pathogen-free female Wistar rats, weighing 200 g (3 mo) from Charles River, were housed in a temperature-controlled room (24 °C) with a 12-h:12-h light/dark cycle. After 1 wk of acclimation, rats were assigned randomly to one of four experimental conditions. Two groups of eight rats received daily intraperitoneal injections of TSA (dissolved in DMSO at a dose of 600 µg/kg body wt per day) for 14 days. Half of them were suspended for 14 days in individual cages using Morye’s tail-suspension model (20). Briefly, adhesive tape was wrapped around the tail and connected to a pulley by a plastic bar. Rats were able to move in 360° of the arc with their forelimbs, thus allowing exercise and access to food and water, although the hindlimbs were non-load bearing. Other rats were kept as controls. It has been previously demonstrated that the dose of TSA used here increased muscle histone H3 acetylation (6, 32). TSA is an HDAC inhibitor with IC50 of 0.4 nM. TSA inhibits class I and II HDACs. HDAC8 is the only known member of the HDAC family that is not affected by TSA.

Two other groups of eight rats received vehicle (DMSO) injections for 14 days, and half of them were suspended for 14 days. This protocol generated four groups of rats: DMSO-treated control rats (C), TSA-treated control rats (C + TSA), DMSO-treated hindlimb-unloaded rats (H), and TSA-treated and unloaded rats (H + TSA). TSA was purchased from Selleck Chemicals. By the end of the different protocols, rats were anesthetized (intraperitoneal injection of 80 mg/kg ketamine and 16 mg/kg xylazine). Soleus and plantaris muscles were excised, weighed, frozen in isopentane chilled with liquid nitrogen, and stored at −80°C until analysis. All procedures were approved by the Institutional Animal Care and Use Committee of University Lyon 1 and carried out under authorization BH2011-41 following guidelines issued by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes.

Histochemical analysis. To determine the effect of trichostatin on fiber cross-sectional areas, serial transverse sections (10 µm) from the midbelly region of each muscle were cut on a microtome at −20°C and stained for myosin ATPase activity as previously described (20). A preincubation was carried out in acid buffer (acetic acid 50 mM) with 25 mM CaCl2 at differing pH values (4.35, 4.40, 4.45) for 4 min at 25°C. The ATPase reaction was then performed at pH 9.4 with 18 mM CaCl2 and 2.7 mM ATP at 37°C for 20 min. On the basis of observed difference in pH lability of the myosin ATPase activity of the isomyosins in the different fibers, muscle fibers were classified into two major types (I, Ia) and hybrid type I/Ia fibers. Fiber type was assayed as the number of fibers of each type relative to the total number of fibers. The fiber cross-sectional areas were measured by use of an image analyzer (AxioVision Imaging System).

Table 4. Percentage distribution of fibers in soleus muscle

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Type I</th>
<th>Type I/IIa</th>
<th>Type IIa</th>
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<tr>
<td>C</td>
<td>92.5 ± 4.2</td>
<td>4.4 ± 2.4</td>
<td>3.1 ± 1.8</td>
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<tr>
<td>C + TSA</td>
<td>94.6 ± 3.0</td>
<td>3.9 ± 2.2</td>
<td>1.5 ± 0.9</td>
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<tr>
<td>H</td>
<td>74.4 ± 1.4*</td>
<td>11.6 ± 1.4*</td>
<td>14.1 ± 1.2*</td>
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<tr>
<td>H + TSA</td>
<td>90.6 ± 3.7†</td>
<td>5.2 ± 2.1†</td>
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Values are means ± SE for 7 animals. *P < 0.05, significantly different from C rats; †P < 0.05, significantly different from C + TSA rats.
RT-qPCR and Western blot analyses. Total RNA was extracted from 20–30 mg of soleus muscle using the RNeasy fibrous tissue mini kit (Qiagen) following the manufacturer’s instructions. cDNA was generated from 400 ng of RNA using iScript cDNA synthesis kit (Bio-Rad). The selected forward and reverse primer sequences are listed in Table 1. Real-time PCR was performed in a 20-μl final volume and optimized concentrations for each primer using the SsoFast EvaGreen Super mix (Bio-Rad) and a CFX96 Real-Time PCR Detection System, C1000 Thermal Cycler (Bio-Rad). Hypoxanthin guanine phosphoribosyl transferase was used to normalize the expression levels of genes of interest as previously described (37).

The same soleus muscles used for RT-qPCR analysis were used for Western blotting. Frozen tissue samples were disrupted in ice-cold buffer (50 mM Tris·HCl, 2.5% SDS, 10 mM dithiothreitol, 5% glycerol, and protease inhibitor cocktail) (Roche Applied Science) and incubated at 100°C for 5 min. After centrifugation (10,000 g, 10 min, 4°C), the supernatants were removed for protein quantification and then stored at −80°C. Equal amounts of protein were separated by SDS-PAGE and transferred onto polyvinylidene difluoride membranes (Millipore). To evaluate successful equal protein loading and transfer, membranes were incubated in Ponceau S solution. Blots were incubated overnight at 4°C with antibodies against acetyl-histone H3 (1:1,000, Cell Signaling Technology), follistatin (1:750), MAFbx and MuRF1 (1:1,000, ECM Biosciences), Bnip3 (1:1,000, Cell Signaling Technology), LC3b (1:800, Sigma-Aldrich), and Foxo3a (1:1,000, Cell Signaling Technology). Incubation with corresponding IgG-peroxidase-conjugated goat anti-rabbit (1:8,000, Sigma-Aldrich) was performed for chemiluminescence detection (ECL Prime Western Blotting Detection Reagent, GE Healthcare). Specific bands were detected using the ChemidocTM MP Imaging System (Bio-Rad).

Statistical analysis. All data reported are means ± SE. A two-way ANOVA (with group and treatment as main factors) was used for intergroup comparisons. The Fisher paired least-significant difference was used to identify specific means differences. Values were considered statistically different when P < 0.05.

RESULTS

TSA treatment increased the level of acetyl-histone H3 (a class I HDAC target) in control and unloaded rats (Fig. 1A).

Effect of TSA on muscle mass, fiber size, and type. Body mass was similar across groups whatever the treatment, unloading or TSA (Table 2). Absolute and relative soleus and plantaris muscle masses are shown in Table 2. After 14 days of

Fig. 2. TSA treatment reverses the transitions of slow-twitch to fast-twitch fibers in soleus muscle. Type I (A) and IIa (B) myosin heavy chain (MHC) mRNA. Values are means ± SE for 5–6 animals. *P < 0.05 vs. C rats; §P < 0.05 vs. C + TSA rats.

Fig. 3. Protein carbonyl content (A), reduced glutathione (GSH) to oxidized glutathione (GSSG) ratio (GSH/GSSG, B). Activity of superoxide dismutase (SOD) (C) and catalase (D). Values are means ± SE for 7 animals. *P < 0.05 vs. C; #P < 0.05 vs. H rats.
unloading, atrophy of the soleus, a slow oxidative muscle (−38%), was greater than for the plantaris, a fast glycolytic muscle (−12%). TSA treatment resulted in a complete recovery of plantaris muscle mass, whereas soleus muscle atrophy was reduced to only 25%. Accordingly, the decrease in soleus muscle fiber size was also partially prevented under TSA treatment. Type I, I/IIa, and IIa fiber sizes were decreased by 41, 35, and 39%, respectively, after unloading, whereas type I, I/IIa, and IIa fiber cross-sectional areas were only reduced by 20% with TSA (Table 3). We focused our study on soleus muscle, as this muscle is preferentially atrophied after hindlimb unloading. Interestingly enough, a protective effect of TSA was observed on the percentage of distribution of fibers (Table 4). Unloading was associated with a reduction in the percentage of type I fibers by 20% in favor of an increase in type I/IIa and IIa fibers. TSA fully prevented the slow-to-fast contractile phenotype transition. Accordingly, myosin heavy chain I (MHC-I) mRNA was significantly decreased after unloading. The increase in MHC-IIa mRNA did not reach statistical significance but was reversed by TSA (Fig. 2).

**Myostatin/follistatin axis.** Follistatin protein and myostatin mRNA levels were similar in control and DMSO-treated hindlimb-unloaded rats (Fig. 1). No obvious effect of TSA was observed on myostatin mRNA (Fig. 1B). Although there was a trend toward reduced protein level of follistatin in response to TSA treatment, the differences between TSA-treated control and TSA-treated unloaded rats was at the limit of statistical significance (*P* = 0.05, Fig. 1C).

**Muscle oxidative stress.** We next investigated cell oxidative stress, as several lines of evidence link muscle-derived reactive oxygen species (ROS) to muscle atrophy via regulation of proteolysis. No change in protein carbonyl content was observed (Fig. 3A). As shown in Fig. 3, unloading resulted in a decrease (−30%) of GSH/GSSG ratio, an effect that was prevented by TSA treatment. Hindlimb unloading induced a large increase in the antioxidant activity of SOD (+32%) and catalase (+35%) (Fig. 3, C and D). SOD remained still enhanced, whereas catalase activity returned to the normal range after TSA treatment (Fig. 3, C and D).

**The autophagy/lysosome and ubiquitin-proteasome pathways.** The autophagic/lysosomal and proteasomal pathways might play a key role in soleus skeletal muscle wasting. Foxo3, the forkhead box O transcription factor controlling these two major proteolytic pathways, remained similar at the transcript and protein level after unloading or TSA treatment (Fig. 4). No significant changes were detected in LC3b, Atg5, Gabarapl1, and Ulk1 mRNA or in LC3 II/LC3 I ratio. (Figs. 5 and 6). The decreased soleus expression of Bnip3 in TSA-treated and unloaded rats was not confirmed at the protein levels (Fig. 5). TSA treatment reduced the upregulation of cathepsin L mRNA, but this transcript was induced less than twofold by 14 days of hindlimb suspension, suggesting that unloading and TSA have minimal effect on autophagy (Fig. 6). No statistical change occurred in basal MAFbx protein level in various treatments, but the increase in MuRF1 protein level was reversed after TSA treatment (Fig. 7).

**DISCUSSION**

In the present study, we show that soleus muscle atrophy (−38%) induced by 14 days of rat hindlimb suspension was reduced to only 25% under TSA treatment. We further demonstrate that TSA promotes a partial prevention of the decrease in fiber size in type I, I/IIa, and IIa fibers.

The present data are consistent with recent works showing that TSA promotes a complete prevention of the fast-twitch plantaris muscle fiber atrophy in response to 3 days of nutrient deprivation (6) and that TSA inhibits fiber atrophy in *mdx* mice, a model of Duchenne muscular dystrophy (32). TSA inhibits class I and II HDACs, and we have shown that TSA treatment increased the level of acetyl-histone H3 (a class I HDAC target). TSA-induced partial prevention of soleus muscle atrophy could be mediated by the regulation of histone acetylation.

Although we observed a significant decrease in MHC-I mRNA level with unloading in presence of TSA, our histological data clearly indicate that TSA completely prevents the slow-to-fast fiber transition induced by unloading. However, the discrepancy between mRNA data (a decrease in MHC-I mRNA level) and histological data (an unchanged percentage of type I fiber) suggests that a decrease in the percentage of distribution of type I fiber in TSA-treated animals may have occurred if the unloading process has been pursued. Pandorf et al. thus (36) assessed acetylation of histone H3 at genomic regions of the second intron of each of the MHC genes. Relative histone H3 acetylation corresponded with the transcriptional activity of the MHC genes under steady-state transcriptional states, in the slow-twitch soleus and fast-twitch plantaris muscles. Furthermore, 7 days of hindlimb unloading induced a decrease in type I MHC mRNA gene expression.
correlated with histone 3 deacetylation at the type I MHC gene in soleus muscle (36). Therefore, TSA might regulate the transcriptional activity of MHC genes through the acetylation state of histone H3. Moreover, a previous study indicates that the ubiquitination and degradation of class II HDACs by the proteasome promote the formation of slow oxidative fibers through the activation of the myocyte enhancer factor 2 transcription factor (40). Interestingly, Cohen et al. (18) have just reported that pan-HDAC inhibitor treatment causes a reduction of MHC II expression and an induction of MHC I in C2C12 myotubes.

TSA has recently been shown to increase muscle size and satellite cell recruitment by stimulating the expression of follistatin in regenerating muscle of mdx mice (32). Follistatin is a protein that interacts and inhibits members of the TGF-β family, including activins and myostatin, a negative regulator of skeletal muscle mass (31). HDAC inhibitors also stimulate follistatin expression in injured muscles injected with cardiotoxin (26). By contrast, follistatin expression was decreased in a mouse model of spinal muscular atrophy treated with TSA (5). Therefore, TSA-induced follistatin expression might be related to the capacity of muscle regeneration and satellite cell activation in the context of regenerating muscle (mdx mice and cardiotoxin injection) (26, 32).

Myostatin, a member of the TGF-β family, has also been implicated in skeletal muscle atrophy given muscle type specificity and atrophy models. After 1 day of hindlimb unloading, an increase in myostatin gene expression was not associated with the slow soleus muscle atrophy but was increased during atrophy of fast muscles (14). On the contrary, Murphy et al. (35) showed that PG-354 antibody-directed myostatin inhibition attenuated soleus atrophy and the loss of muscle function within the first 2 wk of unilateral limb casting. Our current findings suggest that modulation of the myostatin/follistatin axis does not appear involved in soleus muscle wasting induced by hindlimb unloading and that TSA has no beneficial effect even if we are aware that these are preliminary results. However, a complex TGF-β network contributes to the regulation of muscle growth (45). Interestingly, recent work suggests that the bone morphometric protein-Smad1/5/8 signaling axis is a positive regulator of muscle mass that operates in competition with the myostatin pathway (45).

Among potential signaling pathways underlying muscle atrophy, oxidative stress might contribute to muscle wasting even if it is still debated whether cellular redox imbalance would be a key “trigger” or a byproduct for skeletal muscle plasticity (10, 27, 47). A complex cytoprotective system that includes enzymes scavenging ROS is recruited against free radical damage. Protein carbonyl content did not change either after unloading or TSA treatment. Muscle unloading increases SOD and catalase activities as previously observed (4, 47) to maintain muscle homeostasis in the face of a higher rate of ROS production. Previously, Cannavino et al. (12) showed no change in soleus protein carbonylation, whereas an increase in superoxide content in mRNA and protein levels of SOD1 was observed after 7 days of unloading. Moreover the same authors reported in a fast-twitch muscle (gastrocnemius) no increase of protein carbonylation and no H2O2 accumulation but an increase in SOD1 and catalase mRNA (13). They suggested that antioxidant defenses reacted to redox imbalance and that prob-

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Fig. 5. Unloading and TSA treatment have no effect on the process of autophagy. Microtubule-associated protein 1 light chain 3b (LC3b) mRNA (A) and LC3II/LC3 I ratio (B). Bnip3 mRNA (C) and protein levels (D). Values are means ± SE for 5–6 animals. *P < 0.05 vs. C rats; §P < 0.05 vs. C + TSA rats.

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ably protein carbonylation was a late phenomenon. The lower ratio of GSH vs. GSSG and the increased catalase activity returned to normal range after TSA treatment, suggesting that a reduction in oxidative stress could be involved in muscle sparing through a downregulation of the NF-κB and Foxo signaling pathways. The mechanism underlying muscle ROS and reactive nitrogen species production after hindlimb suspension is not clear but might involve NO production. Suzuki et al. (50) showed that, after 14 days of hindlimb suspension, neuronal nitric oxide synthase dissociated from α-syntrophin-generating NO, which then induced proteolysis via regulation of Foxo transcription factors. In our study, SOD activity remained elevated in unloaded soleus muscle even after TSA treatment. A possible explanation for these findings might be that TSA mediates transcriptional activation of Mn-SOD via an inhibitory effect of TSA on HDAC1 activity resulting in a rapid acetylation of histones H3 and H4 around the proximal promoter region of Mn-SOD (29). Very little data are available concerning the links between HDACs themselves and oxidative stress. Rahman et al. (42) reported that oxidative stress inhibited HDAC activity and activated NF-κB in lung epithelial cells. Oxidative stress also increased complex formation between the coactivator CBP/p300 and the p65 subunit of NF-κB, suggesting a further role of oxidative stress in chromatin remodeling. Thus oxidative stress might regulate both chromatin remodeling and signal transduction. Further studies

Fig. 6. Gabarapl1 (A), Ulk1 (B), cathepsin B (C), and cathepsin L (D) mRNA. Values are means ± SE for 5–6 animals. * P < 0.05 vs. C rats.

Fig. 7. TSA rescues muscle-specific RING finger protein 1 (MuRF1) protein level. Protein levels of MuRF1 (A) and muscle atrophy F-box (MAFbx) (B). Values are means ± SE for 5–6 animals. *P < 0.05 vs. C rats.
may help to shed some light on links between HDACs and muscle oxidative stress.

Hindlimb unloading induces a rapid decrease in protein synthesis rate followed by a slower transient increase in protein breakdown (53). The autophagy-lysosome and ubiquitin-proteasome pathways are involved in muscle atrophy and coordinately activated via Foxo3 (8–9, 47, 51, 56). The role of HDACs in the regulation of autophagy has been well investigated in skeletal muscle. Beharry et al. (6) reported that muscle atrophy induced by HDAC1 was linked to the induction of several atrogenes such as LC3, MuRF1, and MAFbx, which required Foxo3 deacetylation. Moresi et al. (34) also showed that HDAC1 and 2 control skeletal muscle homeostasis and regulate autophagy flux in mice. However, the regulation of autophagy-lysosome pathway has been less documented in the model of hindlimb suspension. An increased ratio of LC3II/I, but no change in Beclin-1 protein, has been already reported (4, 30). In the present study, several markers of autophagy-lysosome pathways, LC3b, Atg5, Bnip3, Gabarapl1, Ulk1, and cathepsins B and L, were analyzed. Briefly, LC3b is involved in the expansion and closure of the autophagosome membrane, whereas Atg5 contributes to the conjugation of LC3b-I to phosphatidylethanolamine to generate LC3b lipidation (LC3b-II). LC3 also interacts with Bnip3 primarily localized at the membrane of mitochondria and targets mitochondria for removal by autophagosomes (24). No change occurred in Atg5 mRNA level and in Bnip3 protein level after 14 days of muscle unloading, whereas the lipidated form of LC3b (LC3b-II) was slightly increased. Cathepsin B and L transcripts were upregulated at day 14 in accordance with their increased activities previously reported at day 9 of hindlimb suspension (51). TSA treatment reduced the upregulation of cathepsin L mRNA, but this transcript was induced less than twofold by unloading. Taken together, our data suggest that unloading and TSA treatment have no or little effect on the process of autophagy.

The ubiquitin-proteasome pathway is mainly responsible for the unloading-induced soleus muscle wasting via the regulation of the expression of muscle-specific ubiquitin ligases (E3), MAFbx, and MuRF1 (9, 22, 49). MuRF1 plays a critical role in the selective loss of myosin light chains 1 and 2 and MHCs during muscle atrophy (16), but muscle actin is also a target of MuRF1 (39). However, work from Gomez et al. (23) demonstrated that MuRF1 might control the degradation of contractile proteins but also other components of the ubiquitin proteasome system such as deubiquitinating enzymes, ubiquitin ligases, and ubiquitin-specific peptidases in derenatured MuRF1-null mice. MuRF1 protein level was elevated at day 14, suggesting a different time course in the activation of MAFbx and MuRF1 proteins. During muscle atrophy, the expression of these two atrogenes was regulated by Foxo and NF-κB transcriptions factors (25, 43). The transactivation of the Foxo transcription factors is modulated by different posttranslational modifications such as acetylation (7, 46). Increased p300/CBP HAT activity repressed Foxo 3 activity and prevented its nuclear localization in response to catabolic stimuli such as nutrient deprivation or dexamethasone treatment (46). Moreover, a recent study showed that class I HDACs are activators of Foxo during muscle atrophy, suggesting that HDAC inhibitors would counteract muscle disuse via a downregulation of Foxo target genes involved in autophagy and proteasome (6). In accordance with these findings, our data show that the rise in MuRF1 protein at day 14 of muscle unloading was abolished after treatment with the HDAC inhibitor TSA, but transcript and protein levels of Foxo3a remained unchanged. However, MuRF1 might be a direct or indirect target of Foxo. A recent study shows that NF-κB but not Foxo sites in the MuRF1 promoter are required for MuRF1 transactivation after 5 days of hindlimb unloading (54).

The present data show that HDAC activity is required for unloaded muscle disuse. TSA abolished the slow-to-fast fiber type shift and partially prevented soleus muscle atrophy. Muscle unloading and TSA treatment have little effect on the autophagic/lysosomal pathway, whereas the increased level of MuRF1 is reversed by TSA. Our findings suggest that TSA may provide a possible avenue to treat hindlimb-unloaded-induced muscle atrophy.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: S.D.-A., J.C., D.G.F., and D.D. performed experiments; S.D.-A., J.C., and D.D. analyzed data; D.G.F. and D.D. interpreted results of experiments; D.G.F. and D.D. drafted manuscript; D.G.F. and D.D. edited and revised manuscript; D.G.F. and D.D. approved final version of manuscript; D.D. conception and design of research; D.D. prepared figures.

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