Activation of the CRF 2 receptor modulates skeletal muscle mass under physiological and pathological conditions

Richard T. Hinkle,1 Elizabeth Donnelly,1 David B. Cody,1 Steven Samuelsson,1 Jana S. Lange,1 Mary Beth Bauer,1 Mark Tarnopolsky,2 Russell J. Sheldon,1 Sarah C. Coste,3 Eric Tobar,3 Mary P. Stenzel-Poore,2 and Robert J. Isfort1

1Procter & Gamble Pharmaceuticals, Mason, Ohio 45040-9317; 2McMaster University, Hamilton, Ontario, Canada L8N 3Z5; and 3Oregon Health Sciences University, Portland, Oregon 97201

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CRF, also known as corticotropin-releasing hormone (CRH), and its functionally and structurally related analogs are known to have many physiological functions, including the coordination of the endocrine, autonomic, behavioral, and immune responses to stress. These include, for example, increased secretion of epinephrine, norepinephrine, mineralocorticoids, corticosteroids, somatostatin, dynorphin, β-endorphin, ACTH, and glucose and decreased secretion of gonadotropin-releasing hormone, luteinizing hormone, follicle-stimulating hormone, and melatonin; increased arousal and emotional reactivity, including increased locomotion, sniffing, grooming, and rearing; decreased sexual behavior and reproductive potential; decreased feeding and gastrointestinal functionality; increased heart rate and mean arterial blood pressure by central administration or decreased heart rate and mean arterial blood pressure by peripheral administration; neuronal activity modulation; and altered immune system activity (11, 13, 19, 43). CRF and related functional/structural analogs mediate their effects through CRFRs via both neuronal (as putative neurotransmitters) and neuroendocrine pathways. There are two CRFRs identified to date (CRF1R and CRF2R), which belong to the G protein-coupled receptor (GPCR) seven transmembrane receptor class. Both the rodent and human CRF1R and CRF2R (with multiple splice variants for each receptor) have been cloned, with unique distribution patterns observed for each receptor (10, 13, 14, 40). Gene ablation of both the CRF1R and CRF2R has recently been reported, with CRF1R knockout mice demonstrating impaired stress response and reduced anxiety-like behavior, whereas CRF2R knockout mice demonstrated an impaired food intake reduction after stimulation with urocortin I, lack of vasodilation after stimulation with urocortin I, a normal stress response, and postnatal hypervascularization (3, 4, 12, 33, 49, 52). The CRF1R and CRF2R are positively coupled to Gαs, which, upon agonist activation, activates adenylyl cyclase, catalyzing the formation of cAMP. cAMP, as a second messenger, has pleiotropic effects including, for example, the activation of protein kinase A and phospholipase C, increase in intracellular Ca2+ concentration, and mitogen-activated protein kinase induction (14, 22, 34, 46, 54). Additional G protein coupling has been described for CRFRs, including coupling to Gqα, resulting in an increase in inositol trisphosphate upon receptor stimulation (54). The specificity of coupling is apparently dependent on the particular tissue investigated. The CRFRs can be distinguished pharmacologically through the use of receptor-selective agonists and antagonists (10, 30, 40). These selective agonists and antagonists, along with the CRFR knockout mice, have been useful in determining which CRF receptor mediates specific biological responses (10, 30, 39, 40, 44).

Skeletal muscle is a plastic tissue that readily adapts to changes in physiological demand for work and met
CRF2R localization. Mouse and rat skeletal muscle samples were obtained by removing the medial gastrocnemius muscle and immediately placing the sample in optimal cutting temperature (OCT) compound (Tissue-Tek no. 4583; Sakura Finetek, Torrance, CA). Human vastus lateralis muscle biopsies from healthy young adults, already frozen in OCT, were obtained from Dr. Mark Tarnopolsky (McMaster University, Hamilton, ON, Canada). Human muscle biopsies were obtained with the approval of the McMaster University Ethics Committee. Tissues were placed in liquid nitrogen-cooled isopentane and held at −70°C. Two anti-CRFR antibodies from Santa Cruz Biotechnology (Santa Cruz, CA) were used throughout this study. The CRFR (C-20, sc-1757) antibody reacts with both CRFR1 and CRFR2, whereas the CRFR2 (N-20, sc-1826) antibody is specific for CRFR2. Anti-CRFR1 and anti-CRFR2 (RDI-CRFR1CabG, RDI-RTCRFR2NabG) antibodies (Research Diagnostics, Flanders, NJ) were used to validate results obtained with the Santa Cruz products. The specificity of each CRFR antibody was established by running preadsorption controls. Each antibody was preincubated for 4 h with a 5,000-fold molar excess of its corresponding peptide or an irrelevant peptide, the antigen-antibody mixture was thoroughly washed, and the mix was used in place of primary antibody in the normal immunocytochemistry protocol. Preadsorbed samples were processed in parallel with normal CRFR and binding protein antisera to compare positive staining with staining after preadsorption. Immunohistochemical analysis was performed by cutting 7-μm frozen sections (Leica 3050 Cryostat), attaching these sections to slides, and processing the sections for indirect labeling. Once the sections were attached, the slides were warmed to room temperature, sections were circled with a PAP pen, and the sections were hydrated in phosphate-buffered saline (PBS). Sections were then permeabilized in PBS-0.1% Triton X-100 for 30 min and blocked with 2% BSA-10% normal donkey serum in PBS at room temperature. Anti-primary antibody was incubated directly after blocking solution was aspirated, and labeling was for 1 h at room temperature in PBS-bovine serum albumin (BSA) buffer. The solution was aspirated, and the slide was washed 3× for 10 min in PBS. Fluorescein-conjugated secondary antibodies were used at 5 μg/ml with 0.5 μM diamidino-phenylindole dihydrochloride, and sections were labeled for 1 h at room temperature in PBS-bovine serum albumin (BSA) buffer. The solution was aspirated, and the slide was washed 3× for 10 min in PBS. Fluorescein-conjugated secondary antibodies were used at 5 μg/ml with 0.5 μM diamidino-phenylindole dihydrochloride, and sections were labeled for 1 h at room temperature in PBS-BSA. Sections were washed 2× for 10 min in PBS followed by a final wash in 0.1 M Tris buffer, pH 8.5. Sections were covered with coverslips in PPD glycerol (10% 1 M Tris, pH 8.5, 90% glycerol, and 1 mg/ml P-phenylenediamine) so no bubbles were generated, sealed with fingernail polish, dried, cleaned with distilled water, and stored at −20°C. Tissue sections were examined with a Nikon Microphot FXA equipped with epifluorescence illumination. Images were captured with a Spot digital camera (Diagnostic Instruments) using Metamorph 4.6 imaging software (Universal Imaging).

Tissue bath experiments. Mice were anesthetized with isoflurane, and the tibialis anterior and medial gastrocnemius muscles were removed, pinned to rubber strips attached to glass coverslips, and placed in oxygenated Krebs-Ringer solution in Radnoti High-Tech Tissue Organ Bath. The Krebs-Ringer solution was maintained at 30°C and continuously aerated with 95% O2-5% CO2 gas. The muscles were incubated in the oxygenated Krebs-Ringer solution for 15 min, after which 25 mM theophylline was added. Muscles were incubated for an additional 15 min in this solution before test compounds were added, after which the muscles were incubated for an additional 1 h. The muscles were then removed from the bath, weighed, and snap-frozen in liquid N2. Frozen muscles were ground to a fine powder with a mortar and pestle cooled and maintained in dry ice. cAMP

Abolish need. Control of these processes is complex and believed to involve multiple unknown mediators. Skeletal muscle atrophy can be initiated by a variety of stimuli, including starvation, disuse, denervation/nerve damage, high levels of glucocorticoids, sepsis, cachexia, chronic pulmonary obstructive disease, congestive heart failure, neurodegenerative disease, and muscular dystrophy (1, 2, 5, 6, 8, 15-18, 20, 24, 29, 31, 35, 41, 42, 47, 48, 50, 51, 53). Although the initiating event is different for the different types of skeletal muscle atrophy, several common biochemical changes occur in the affected skeletal muscle fiber, including a decrease in protein synthesis and an increase in protein degradation, and changes in both contractile and metabolic enzyme protein isoforms characteristic of a slow (highly oxidative metabolism/slow contractile protein isoforms) to fast (highly glycolytic metabolism/fast contractile protein isoforms) fiber switch (7, 9, 17, 26, 29). Additional changes in skeletal muscle that occur include the loss of vasculature and remodeling of the extracellular matrix (27, 28, 38). Atrophy and hypertrophy are conserved processes across mammalian species. This is probably due to the fact that muscles serve the same physiological functions in all mammals, including movement and the storage of amino acids/energy. Multiple studies have demonstrated that the same basic molecular, cellular, and physiological processes occur during atrophy in both rodents and humans.

Although it has been observed that the CRF2R is expressed in skeletal muscle (21, 32, 37), the role of the CRF2R in skeletal muscle biology is unknown. We hypothesize that the CRF2R, a G protein-coupled receptor that signals in a manner similar to the β2-adrenergic receptor in skeletal muscle, may have a similar function to the β2-adrenergic receptor in modulating skeletal muscle mass and function. Therefore, we have initiated the studies outlined in this report to determine the effects of activation of the CRF2R on skeletal muscle mass and force production.

METHODS

Materials. The nonselective CRFR agonist sauavagine was purchased from Bachem (King of Prussia, PA). The CRF2R-selective agonist urocortin II was synthesized at Procter & Gamble Pharmaceuticals essentially as described previously (25, 45). Dexamethasone and clenbuterol were purchased from Sigma (St. Louis, MO). Six-week-old male and female Sprague-Dawley rats (Charles River, Raleigh, NC), C57Bi6 mice (Charles River), CRFR1 knockout mice (52), CRF2R knockout mice (12), and their wild-type littermates (Oregon Health Sciences University, Portland, OR) were singly housed and acclimatized to the conditions of the facility (27, 28, 38). Animals were subjected to standard conditions of humidity, temperature, and a 12:12-h light-dark cycle. All studies described in this report were conducted in compliance with the US Animal Welfare Act and rules and regulations of the State of Ohio Departments of Health and were approved by the local Institutional Animal Care and Use Committee.

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was extracted by the addition of 1 ml of acidic EtOH to the muscle powder, after which insoluble material was removed by centrifugation. The supernatant was then dried using a Speed-Vac, and cAMP was measured using an Amersham Enzyme Immunoassay system according to the manufacturer’s instructions (Amersham, Piscataway, NJ). Each cAMP measurement and all muscle incubations and stimulations were performed in triplicate.

Sciatic nerve denervation atrophy model. Mice were anesthetized with isoflurane, the upper right leg was shaved and disinfected, and a 1-cm incision was made in the skin of the upper right leg to expose the sciatic nerve. The right sciatic nerve was isolated and lifted out with a surgical hook, a 3- to 5-mm segment was removed, and the incision was closed with surgical staples. The test materials were administered either by subcutaneous injection in the midscapular region or by continuous infusion via implantation of an osmotic minipump (Alza, Palo Alto, CA) in the midscapular region. Ten days after denervation, animals were euthanized by carbon dioxide asphyxiation followed by cervical dislocation. The tibialis anterior and medial gastrocnemius muscles were dissected from both the denervated (right) and nondenervated (left) legs. The muscles, cleaned of tendons and connective tissue, were weighed and the mass was recorded.

Glucocorticoid-induced atrophy model. Glucocorticoid-induced atrophy was achieved by including dexamethasone (6 mg/l) in the drinking water for a total dose of 1.2 mg kg⁻¹ day⁻¹. The test materials were administered by continuous infusion via implantation of an osmotic minipump (Alza) in the midscapular region. Nine days after the initiation of dexamethasone dosing, mice were euthanized by carbon dioxide asphyxiation followed by cervical dislocation. The tibialis anterior and medial gastrocnemius muscles were removed and processed as described above.

Leg casting disuse atrophy model. Mice were anesthetized with isoflurane, and the lower right leg was casted from the knee to the toes with heat-activated casting material (Vet Lite, Kruuse, Marslev, Denmark). The test materials were administered by subcutaneous injection in the midscapular region. Ten days after casting, animals were euthanized by carbon dioxide asphyxiation followed by cervical dislocation, and the tibialis anterior and medial gastrocnemius muscles were removed and processed as described above.

Muscle functional analysis. Ten days after application of casts, mice were anesthetized with isoflurane. The casts were removed, the right legs were shaved, and the extensor digitorum longus and soleus muscles were exposed. Silk sutures (5-0) were tied to the proximal and distal tendons of the extensor digitorum longus and soleus muscles, and the muscles were removed, tendon-to-tendon, from the casted mouse leg. The muscles were placed into a Plexiglas chamber filled with Ringer solution (in mM: 137 sodium chloride, 24 sodium bicarbonate, 11 glucose, 5 potassium chloride, 1 magnesium sulfate, 1 sodium phosphate, and 0.025 tubocurarine, all at pH 7.4) and oxygenated with 95% O₂-5% CO₂ constantly bubbled with 95% O₂-5% CO₂ maintained at 25°C. Muscles were aligned horizontally between a servomotor lever arm (model 305B-LR; Cambridge Technology, Watertown, MA) and the stainless steel hook of a force transducer (model BG-50; Kulite Semiconductor Products, Leonia, NJ) and were field stimulated by pulses transmitted between two platinum electrodes placed longitudinally on either side of the muscle. Square wave pulses (0.2 ms duration) generated by a personal computer with a Labview board (model PCI-MIO-16E-4; Labview, Austin, TX) were amplified (Acurus Power Amplifier model A250, Dobbs Ferry, NY) to increase and sustain current intensity to a level sufficient to produce a maximum isometric tetanic contraction. Stimulation voltage and muscle length were adjusted to obtain maximum isometric twitch force. Maximum tetanic force production was determined from the plateau of the frequency-force relationship. After the force analysis, the muscles were weighed.

Fig. 1. Immunohistochemical localization of the corticotropin-releasing factor 2 receptor (CRF2R, or CRF-RII) in mouse, rat, and human skeletal muscle. Skeletal muscle samples were prepared, and immunohistochemical analysis was performed as described in METHODS. C-20, carboxy-terminal CRFR-selective antibody; N-20, amino-terminal CRF2R-selective antibody; S6, human muscle sample; Mouse, mouse muscle sample; Rat, rat muscle sample.
pinned to wax at resting length, and placed in 10% neutral buffered formalin for processing for muscle fiber cross-sectional area analysis.

Muscle fiber cross-sectional area analysis. Muscle fiber cross-sectional area analysis was performed in skeletal muscle preparations at resting length fixed with 10% neutral buffered formalin solution. After paraffin embedding, cross sections were cut from the center of the soleus and extensor digitorum longus muscles in triplicate. Sections were stained with Picro-sirius red (F3B, C.I. 35782), which stains endomysium collagen red, resulting in clearly delineated and easily digitalized muscle fibers (muscle fibers stain light yellow). Digitized images of the stained samples were acquired using a SPOT RT camera and the SPOT Advanced Imaging Software (Universal Imaging, Downingtown, PA) from the center one-third of each section. Automated segmentation and muscle fiber cross-sectional area measurements, based on differential staining of the endomysium and myofibers, was performed using custom software developed at Procter & Gamble Pharmaceuticals. Aphelion 3.2 software (Amerinex Applied Imaging, Amherst, MA) was used to manually edit the processed images to ensure accurate measurement of only myofiber cross-sectional area.

Statistical analysis. Statistical analysis of the data was performed using an analysis of covariance model, with treatment effect and starting weight as the covariates. Pairwise comparisons for all end points were generated using least square means (SAS, Cary, NC), adjusted for unequal sample sizes and starting weight.

RESULTS

Expression and activation of CRF2R in skeletal muscle. To confirm that CRF2R protein is expressed in skeletal muscle, immunocytochemical analysis of human, rat, and mouse skeletal muscle was performed. As can be seen in Fig. 1, two different antibodies to two different epitopes in the CRF2R (carboxy-terminus epitope C-20 and amino-terminus epitope N-20) were utilized to stain human, rat, and mouse skeletal muscle. Similar perimyocyte staining was observed in all of the muscle samples from all three species. Staining was specific, since blocking peptides specific for either the C-20 or N-20 antibodies blocked the staining, whereas nonspecific peptides did not (data not shown).

The observation of CRF2R protein expression in skeletal muscle supports previous reports describing expression of CRF2R mRNA in skeletal muscle [Heldwein et al. (21); Lovenberg et al. (37); Kishimoto et al. (32)].

![Graph A](image1.png)  
**Fig. 2.** Functional coupling of the CRF2R in mouse skeletal muscle in vitro. Mouse tibialis anterior and medial gastrocnemius muscles were incubated and stimulated as described in METHODS. A: cAMP levels (fmol cAMP/mg muscle) in tibialis anterior and medial gastrocnemius muscles after stimulation with 1.0, 0.1, 0.01, and 0.001 μM sauvagine and human urocortin II. Saline, saline control (no theophylline); theophylline, muscle incubated in 25 mM theophylline without compound (theophylline control); sauvagine, muscles incubated with sauvagine in the presence of theophylline; urocortin II, muscles incubated with human urocortin II in the presence of theophylline. B: cAMP levels (fmol cAMP/mg muscle) in normal and casting-induced atrophied tibialis anterior and medial gastrocnemius muscles after stimulation with 10.0 μM sauvagine. Saline, saline control (no theophylline); theophylline, muscle incubated with 25 mM theophylline without sauvagine (theophylline control); sauvagine, muscles incubated with 10 μM sauvagine in the presence of theophylline.
Next, we evaluated the functionality of the CRF2R expressed in skeletal muscle. Organ bath analysis of mouse tibialis anterior and medial gastrocnemius muscles was undertaken by stimulating muscle with CRF2R ligands and evaluating cAMP second messenger levels. As can be seen in Fig. 2, stimulation of tibialis anterior and medial gastrocnemius muscles with either sauvagine or urocortin II resulted in an increase in cAMP levels in both the tibialis anterior and medial gastrocnemius muscles (Fig. 2A). Both compounds were approximately equipotent with regard to cAMP activation. To evaluate whether the effect of sauvagine to stimulate cAMP production in skeletal muscle in organ baths was altered if the muscle was in a normal or atrophied state when removed from the animal, 10-day-casting-induced atrophied muscle and the contralateral normal muscle were removed from mice and placed in the organ bath. As can be seen in Fig. 2B, both atrophied and normal muscle responded with an increase in intracellular cAMP levels to stimulation with sauvagine.

![Figure 3](http://ajpendo.physiology.org/)

Fig. 3. Effect of the nonselective CRFR agonist sauvagine on denervation-induced tibialis anterior and medial gastrocnemius muscle atrophy in wild-type, CRF1R, and CRF2R knockout mice. A and B: continuous infusion dosing of wild-type and CRF2R knockout mice with right leg sciatic nerve damage with either 0.3 or 1.0 mg·kg⁻¹·day⁻¹ sauvagine or 3.0 mg·kg⁻¹·day⁻¹ clenbuterol. Denervation resulted in an ~25% loss of muscle mass (water normal vs. water denervated) in wild-type mice and an ~30% loss of muscle mass in the CRF2R knockout mice. C and D: continuous infusion dosing of wild-type and CRF1R knockout mice with right leg sciatic nerve damage with either 0.3 or 1.0 mg·kg⁻¹·day⁻¹ sauvagine or 3.0 mg·kg⁻¹·day⁻¹ clenbuterol. Denervation resulted in an ~20% loss of muscle mass (water normal vs. water denervated) in wild-type and CRF1R knockout mice. *Statistically significant (P < 0.05) result.
CRF2R mediates skeletal muscle anti-atrophy effects of nonselective CRFR agonists. Studies were undertaken to evaluate the effect of CRFR agonist treatment on skeletal muscle mass in rodents. At the time of these investigations, only nonselective CRFR agonists were known, including urocortin I, sauvagine, CRH, and urotensin I. For our investigations, we utilized sauvagine to treat mice with lower leg atrophy resulting from the removal of a segment of the sciatic nerve, dexamethasone treatment, or casting, since sauvagine demonstrated pharmacological and physiochemical properties compatible with our experimental paradigm. To define the role of the two CRFRs in maintenance of skeletal muscle mass after sauvagine treatment in mice, CRF1R and CRF2R knockout mice were utilized. As can be seen in Fig. 3, A-D, removal of a segment of the sciatic nerve from the right leg resulted in a 20–30% loss of tibialis anterior and medial gastrocnemius muscle mass in CRF1R and CRF2R knockout mice and their respective wild-type littermates. Treatment of the wild-type mice with sauvagine resulted in reversal of muscle mass loss in the tibialis anterior, but not the medial gastrocnemius muscles, of the C57Bl6 wild-type mice (background strain of the CRF2R knockout mice; Fig. 3, A and B) but not the C57Bl6 or CD-1/129 strain of wild-type mice (background strain of the CRF1R knockout mice; Fig. 3, C and D). CRF2R knockout mice did not demonstrate an increase in denervated tibialis anterior or medial gastrocnemius muscle mass after sauvagine treatment; instead, sauvagine treatment resulted in increased tibialis anterior and medial gastrocnemius muscle mass loss in CRF2R knockout mice (Fig. 3, A and B). In contrast, treatment with sauvagine did result in an inhibition of tibialis anterior and medial gastrocnemius muscle mass loss after sciatic nerve removal in CRF1R knockout mice (Fig. 3, C and D). Treatment of CRF1R and CRF2R knockout mice and the corresponding wild-type mice with the β2-adrenergic receptor agonist clenbuterol, a compound which has been shown to inhibit skeletal muscle mass loss after sciatic nerve damage (20), resulted in an increase in denervated tibialis anterior and medial gastrocnemius muscle mass (Fig. 3, A–D), demonstrating that the effects observed with sauvagine were the result of CRFR deletion and not a general loss of muscle responsiveness to CRFR agonists in the knockout animals.

In an effort to further understand the effects of sauvagine on skeletal muscle mass, two additional skeletal muscle atrophy models were utilized, corticosteroid-induced atrophy and casting-induced atrophy. For the corticosteroid atrophy model, mice were given dexamethasone in the drinking water for 9 days. This treatment resulted in an ∼20% loss of tibialis anterior and medial gastrocnemius muscle mass. Treatment with sauvagine decreased the loss of tibialis anterior and medial gastrocnemius muscle mass resulting from dexamethasone treatment (Fig. 4, A and B). Casting of the lower right leg of mice for 10 days results in an ∼20–30% loss of extensor digitorum longus and soleus muscle mass. Treatment with sauvagine decreased the casting-induced loss of extensor digitorum longus and soleus muscle mass (Fig. 5, A and B). For comparison, clenbuterol decreased casting-induced extensor digitorum longus muscle mass loss but not casting-induced soleus muscle mass loss. The soleus and extensor digitorum longus muscles were then utilized in an in vitro function assay to evaluate the effect of sauvagine on force maintenance. As can be seen in Fig. 5, C and D, casting of the lower right leg resulted in an ∼30–45% drop in extensor digitorum longus and soleus muscle absolute force. Treatment with sauvagine decreased the loss of absolute force observed after casting in both the soleus and extensor digitorum longus muscles. Treatment with clenbuterol decreased the loss of force in the extensor digitorum longus but not in the soleus muscle. Finally, muscle fiber cross-sectional area measurements were performed on extensor digitorum longus and soleus muscles. Casting results in an ∼30–35% loss of extensor digitorum longus and soleus muscle fiber cross-sectional area (Fig. 5, E and F). Sauvagine treatment decreased the casting-induced loss in muscle fiber cross-sectional area in both the extensor digitorum longus and soleus muscles; clen-
buterol treatment decreased casting-induced extensor digitorum longus but not soleus muscle fiber cross-sectional area loss (Fig. 5, E and F).

The CRF2R-selective agonist urocortin II inhibits skeletal muscle atrophy. Recently two CRF2R-selective peptides, urocortin II and urocortin III, have been described (25, 36, 45). These peptides demonstrate an almost absolute selectivity for the CRF2R, with urocortin II being the more potent peptide with regard to CRF2R activation. To validate the antiatrophy role of the CRF2R, mice undergoing casting-induced atrophy were treated with urocortin II. As can be seen in Fig. 6, casting results in an ~20–25% loss in tibialis anterior and medial gastrocnemius muscle mass. Treatment with urocortin II completely inhibited the casting-induced loss of tibialis anterior muscle mass (Fig. 6A) and partially inhibited the loss of medial gastrocnemius muscle mass (Fig. 6B). In addition, urocortin II treatment resulted in an increase in noncasted tibialis anterior and medial gastrocnemius muscle mass (Fig. 6, A and B).

**DISCUSSION**

In this report we demonstrate, for the first time, that activation of the CRF2R decreases skeletal muscle...
CRF2R MODULATES MUSCLE MASS

The CRF2R appears to function to moderate the effects of activation of the CRF1R (4, 12, 33). Thus the CRF2R present in skeletal muscle may function to moderate the wasting effects of cortisol/corticosterone production resulting from CRF1R/hypothalamo-pituitary-adrenal (HPA) axis activation. When wild-type and knockout mice undergoing denervation-induced atrophy were treated with the nonselective CRFR agonist sauvagine, muscle mass increased, decreased, or did not change. The effect on muscle mass was muscle type, atrophy model, and mouse strain specific, because sauvagine treatment increased tibialis anterior muscle mass in C57Bl6 and CRF1R knockout mice undergoing denervation-induced atrophy, had no effect on the tibialis anterior muscle mass of C57Bl6/CD-1/129 mice, and decreased tibialis anterior muscle mass in CRF2R knockout mice; it increased medial gastrocnemius muscle mass in CRF1R knockout mice, decreased medial gastrocnemius muscle mass in CRF2R knockout mice, and did not affect medial gastrocnemius mass in wild-type mice; and it increased medial gastrocnemius, tibialis anterior, soleus, and extensor digitorum longus muscles in mice undergoing corticosteroid and casting-induced muscle atrophy. Finally, treatment with urocortin II not only increased casting-induced atrophying tibialis anterior and medial gastrocnemius muscle mass but also increased normal tibialis anterior and medial gastrocnemius muscle mass. Together, these data suggest that activation of the CRF2R in skeletal muscle functions to increase skeletal muscle mass in normal and atrophying skeletal muscle; however, under certain conditions and with certain muscles, nonselective CRFR agonists cause muscle loss. The underlying mechanism that explains these observations is at present unknown, although one possibility is that, under certain conditions, activation of the CRF1R results in skeletal muscle mass loss in certain sensitive muscles, possibly via HPA activation and corticosteroid production. This observation is supported by the finding that sauvagine treatment of CRF1R knockout mice undergoing denervation-induced atrophy resulted in increased tibialis anterior and medial gastrocnemius muscle mass, whereas sauvagine treatment in wild-type mice with an active CRF1R did not increase skeletal muscle mass. Also, sauvagine treatment resulted in increased tibialis anterior and medial gastrocnemius muscle mass loss in CRF2R knockout mice compared with wild-type mice. Thus activation of the CRF1R under conditions of an inactive CRF2R results in loss of skeletal muscle mass; conversely, activation of the CRF2R under conditions of an inactive CRF1R results in increased skeletal muscle mass; finally, activation of both the CRF1R and CRF2R has minor effects on skeletal muscle mass. Additional experimentation will be required to better understand this phenomenon.

The observation that activation of the CRF2R by the selective agonist urocortin II results in the prevention of skeletal muscle mass loss in atrophying muscle and increased skeletal muscle mass in nonatrophy skeletal muscle. We have demonstrated this by utilizing CRF1R and CRF2R knockout mice to dissect the skeletal muscle effects of a nonselective CRFR agonist and by utilizing a CRF2R-selective agonist. In particular, we have demonstrated that activation of the CRF2R inhibits skeletal muscle mass and function loss resulting from nerve damage, corticosteroid use, and casting-induced disuse. In addition, we have observed that treatment of mice with the CRF2R selective agonist urocortin II resulted in increased normal skeletal muscle mass. Together, these data indicate that the CRF2R modulates skeletal muscle mass.

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mass loss in atrophying skeletal muscle and increases nonatrophy skeletal muscle mass. We have demonstrated this by utilizing CRF1R and CRF2R knockout mice to dissect the skeletal muscle effects of a nonselective CRFR agonist and by utilizing a CRF2R-selective agonist. In particular, we have demonstrated that activation of the CRF2R inhibits skeletal muscle mass and function loss resulting from nerve damage, corticosteroid use, and casting-induced disuse. In addition, we have observed that treatment of mice with the CRF2R selective agonist urocortin II resulted in increased normal skeletal muscle mass. Together, these data indicate that the CRF2R modulates skeletal muscle mass.

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and knockout mice undergoing denervation-induced atrophy were treated with the nonselective CRFR agonist sauvagine, muscle mass increased, decreased, or did not change. The effect on muscle mass was muscle type, atrophy model, and mouse strain specific, because sauvagine treatment increased tibialis anterior muscle mass in C57Bl6 and CRF1R knockout mice undergoing denervation-induced atrophy, had no effect on the tibialis anterior muscle mass of C57Bl6/CD-1/129 mice, and decreased tibialis anterior muscle mass in CRF2R knockout mice; it increased medial gastrocnemius muscle mass in CRF1R knockout mice, decreased medial gastrocnemius muscle mass in CRF2R knockout mice, and did not affect medial gastrocnemius mass in wild-type mice; and it increased medial gastrocnemius, tibialis anterior, soleus, and extensor digitorum longus muscles in mice undergoing corticosteroid and casting-induced muscle atrophy. Finally, treatment with urocortin II not only increased casting-induced atrophying tibialis anterior and medial gastrocnemius muscle mass but also increased normal tibialis anterior and medial gastrocnemius muscle mass. Together, these data suggest that activation of the CRF2R in skeletal muscle functions to increase skeletal muscle mass in normal and atrophying skeletal muscle; however, under certain conditions and with certain muscles, nonselective CRFR agonists cause muscle loss. The underlying mechanism that explains these observations is at present unknown, although one possibility is that, under certain conditions, activation of the CRF1R results in skeletal muscle mass loss in certain sensitive muscles, possibly via HPA activation and corticosteroid production. This observation is supported by the finding that sauvagine treatment of CRF1R knockout mice undergoing denervation-induced atrophy resulted in increased tibialis anterior and medial gastrocnemius muscle mass, whereas sauvagine treatment in wild-type mice with an active CRF1R did not increase skeletal muscle mass. Also, sauvagine treatment resulted in increased tibialis anterior and medial gastrocnemius muscle mass loss in CRF2R knockout mice compared with wild-type mice. Thus activation of the CRF1R under conditions of an inactive CRF2R results in loss of skeletal muscle mass; conversely, activation of the CRF2R under conditions of an inactive CRF1R results in increased skeletal muscle mass; finally, activation of both the CRF1R and CRF2R has minor effects on skeletal muscle mass. Additional experimentation will be required to better understand this phenomenon.

The observation that activation of the CRF2R by the selective agonist urocortin II results in the prevention of skeletal muscle mass loss in atrophying muscle and increased skeletal muscle mass in nonatrophy skeletal muscle. We have demonstrated this by utilizing CRF1R and CRF2R knockout mice to dissect the skeletal muscle effects of a nonselective CRFR agonist and by utilizing a CRF2R-selective agonist. In particular, we have demonstrated that activation of the CRF2R inhibits skeletal muscle mass and function loss resulting from nerve damage, corticosteroid use, and casting-induced disuse. In addition, we have observed that treatment of mice with the CRF2R selective agonist urocortin II resulted in increased normal skeletal muscle mass. Together, these data indicate that the CRF2R modulates skeletal muscle mass.

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investigations will be required to fully understand the role of the CRF2R in skeletal muscle biology. These observations and the results we present in this report suggest that CRF2R agonists may have clinical utility for the treatment of muscle-wasting diseases, including cachexia associated with acquired immunodeficiency syndrome and cancer; muscle atrophy associated with congestive heart failure and chronic obstructive pulmonary disease; age-associated muscle loss or sarcopenia; and acute skeletal muscle atrophy resulting from disuse due to immobilization, nerve damage, corticosteroid use, or autoimmune disease. In addition, because activation of the CRF2R results in skeletal muscle hypertrophy, CRF2R agonists may have utility in treating muscle weakness or frailty observed in the elderly, improving muscle function in individuals afflicted with muscular dystrophies by maximizing the effectiveness of the remaining functional muscle, and by maintaining muscle mass during periods of exposure to low gravity, such as that experienced in space. Further experimentation will be required to validate the potential of CRF2R agonists for the treatment of skeletal wasting diseases.

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