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Urocortin II Treatment Reduces Skeletal Muscle Mass and Function Loss During Atrophy and Increases Nonatrophying Skeletal Muscle Mass and Function

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Two corticotropin-releasing factor 2 receptor (CRF2R)-selective peptides have been recently described, urocortin II (also known as stresscopin-related peptide) and urocortin III (stresscopin). We have used urocortin II to evaluate the effects of activation of the CRF2R on skeletal muscle-related physiological processes. Administration of urocortin II to mice prevented the loss of skeletal muscle mass resulting from disuse due to casting, corticosteroid treatment, and nerve damage. In addition, urocortin II treatment prevented the loss of skeletal muscle force and myocyte cross-sectional area that accompanied muscle mass losses resulting from disuse due to casting. Finally, we observed increased skeletal muscle mass and force in normal muscles when mice are treated with urocortin II. These results were confirmed using two additional CRF2R agonists, urocortin I and sauvagine. Thus, activation of the CRF2R modulates skeletal muscle mass in both normal and atrophying muscle. Therefore, CRF2R-selective agonists may find utility in the treatment of skeletal muscle wasting diseases including age-related muscle loss or sarcopenia. (*Endocrinology* 144: 4939–4946, 2003)

THE CORTICOTROPIN-RELEASING factor (CRF) peptide family is highly conserved throughout evolution, with closely related homologs found in fish, amphibians, birds, and mammals (including humans) (1–3). These peptides fall into three distinct families based on CRF receptor (CRFR) selectivity including the CRF1R-selective peptides of the CRH subfamily, the CRF2R-selective peptides of the urocortin II and urocortin III subfamilies, and the CRFR-nonselective peptides of the urocortin I, urotensin I, and sauvagine subfamilies (1–3). The CRF family of peptides have many physiologically important functions including coordination of the adaptive stress response (3–8). In particular, peptides that activate the CRF1R appear to function to activate the stress response, in part through the activation of the hypothalamic-pituitary-adrenal (HPA) axis (9, 10). Peptides that activate the CRF2R appear to function to dampen the stress response by activating CRF2Rs in tissues modified by activation of the HPA axis (3, 11–17). In addition to the systemic role of these peptides in modulating the adaptive stress response, these peptides may function as neuroendocrine factors, acting at both peripheral and central sites, to modulate neuronally mediated physiological responses (2, 3, 14–25).

Our interest in skeletal muscle has led us to investigate the role of the CRF2R in the physiological processes of atrophy and hypertrophy. We have focused on the CRF2R because it is the CRFR expressed in skeletal muscle (5, 26–28) and we have recently demonstrated using CRFR knockout mice that activation of the CRF2R modulates skeletal muscle mass (29). In addition, the CRF2R-selective peptides urocortin II and urocortin III are expressed in skeletal muscle (14–16). In an effort to further characterize the role of the CRF2R in modulation of skeletal muscle mass and to understand the potential role of the CRF2R-selective peptides in mediating the skeletal muscle atrophy/hypertrophy effect, we have used the CRF2R-selective peptide urocortin II to activate the CRF2R under physiological conditions that lead to skeletal muscle atrophy including disuse due to casting, corticosteroid administration, and nerve damage. In this report, we demonstrate that urocortin II administration inhibits the loss of skeletal muscle mass and function resulting from these conditions. In addition, we observed that urocortin II administration causes hypertrophy of normal skeletal muscle.

Abbreviations: CHO-K1, Chinese hamster ovary-K1; CRF, corticotropin-releasing factor; CRFR, CRF receptor; CRF1R or CRF2R, CRF 1 or 2 receptor; EDL, extensor digitorum longus; HPA, hypothalamic-pituitary-adrenal.

Materials and Methods

Materials

The CRF2R-selective agonist human urocortin II was synthesized at Procter & Gamble Pharmaceuticals (Mason, OH) as described previously (14–16). Human urocortin I and sauvagine were purchased from Bachem (Bachem Biosciences Inc., King of Prussia, PA). Dexamethasone was purchased from Sigma (St. Louis, MO). Male C57BL6 mice (Charles River, Raleigh, NC) were single-housed and acclimatized to the conditions of the facility for approximately 1 wk before use. Mice had access to lab chow and water *ad libitum*. Animals were subjected to standard conditions of humidity; temperature; and a 12-h light, 12-h dark cycle. All studies described in this report were conducted in compliance with the United States Animal Welfare Act and rules and regulations of the State of Ohio Department of Health and approved by the local Institutional Use and Animal Care Committee.

In vitro CRFR activity assay

CRF1R and CRF2R activation after peptide exposure was performed as described previously (15, 16). Briefly, CRF1R and CRF2R containing CHO-K1 cells (Euroscreen s.a., Brussels, Belgium) were plated into 96-well plates at 20,000 cells/well 24 h before use. Just before assay, the media were removed, cells washed with PBS, and cells incubated in

serum-free DMEM. Compound was added at the indicated concentrations and the cells were incubated at 37 C for 30 min. After incubation with the test compound, the media were aspirated off the cells, the cells lysed in lysis buffer (Amersham, Inc., Piscataway, NJ) and the cAMP levels were quantitated using an RIA kit (Amersham) and following the manufacturer's recommended procedures. Potencies and EC_{50} calculations were performed using GraphPad Prism software (GraphPad, San Diego, CA). To determine the EC_{50}, we analyze the data using a nonlinear regression, sigmoidal dose-response (variable slope) equation. All experiments were performed in triplicate with at duplicate analysis of each dose of each compound.

Tissue bath experiments

Mice were anesthetized with isoflurane, the tibialis anterior and medial gastrocnemius muscle were removed, pinned to rubber strips attached to glass coverslips, and placed in oxygenated Krebs-Ringers solution in Radnoti High-Tech Tissue Organ Bath. The Krebs-Ringers solution was maintained at 30 C and continually aerated with 95% O_2/5% CO_2 gas. The muscles were incubated in the oxygenated Krebs-Ringers 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 an additional 1 h. The muscles were then removed from the bath, weighed, and snap frozen in liquid nitrogen. Frozen muscles were ground to a fine powder using a mortar and pestle cooled and maintained in dry ice. cAMP was extracted by the addition of 1 ml of acidic ethanol 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). Each cAMP measurement was performed in triplicate with all muscle incubations/stimulations repeated three independent times.

Sciatic nerve damage atrophy model

Eight mice per treatment group 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 material was administered by sc injection in the midscapular region of the back. Nine 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 recorded.

Glucocorticoid-induced atrophy model

Glucocorticoid-induced atrophy was achieved by including dexamethasone (6 mg/liter) in the drinking water for a total dose of 1.2 mg/kg·d. The test materials were administered by sc injection in the midscapular region of the back to eight mice per treatment group. 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 from both legs were dissected, the muscles were cleaned of tendons and connective tissue, weighed, and the mass recorded (data given as combined mass of both the right and left tibialis anterior and both the right and left medial gastrocnemius muscles).

Leg casting disuse atrophy model

Eight mice per treatment group 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 Inc., Marslev, Denmark). The test materials were administered by sc injection in the midscapular region of the back. Nine days after casting, animals were euthanized by carbon dioxide asphyxiation followed by cervical dislocation. The cast was removed, and the tibialis anterior and medial gastrocnemius muscles were dissected from both legs; the muscles were cleaned of tendons and connective tissue and weighed, and the mass was recorded.

Muscle functional analysis

Ten days after application of casts or in noncasted animals, eight mice per treatment group were anesthetized using isoflurane. The casts were

TABLE 1. CRF2R and CRF1R selectivity of human urocortin II, human urocortin I, and sauvagine

Peptide	CHO-K1 rCRF2R EC_{50} (nM)	CHO-K1 hCRF2R EC_{50} (nM)	CHO-K1 hCRF1R EC_{50} (nM)
Human urocortin II	0.15	0.10	>1000
Human urocortin I	0.03	0.01	0.12
Sauvagine	0.11	0.11	2.20

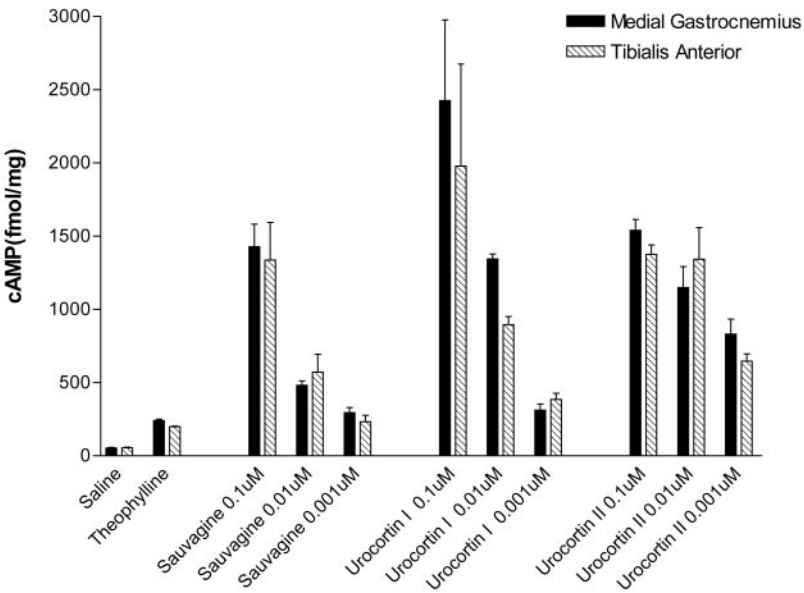

FIG. 1. Functional coupling of the CRF2R in mouse skeletal muscle *in vitro*. Mouse tibialis anterior and medial gastrocnemius muscles were incubated and stimulated as described in *Materials and Methods* section. cAMP levels (femtomoles cAMP/milligram muscle) in tibialis anterior and medial gastrocnemius muscles after stimulation with 0.1, 0.01, and 0.001 μM sauvagine, human urocortin I, 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 I, muscle incubated with human urocortin I in the presence of theophylline; Urocortin II, muscles incubated with human urocortin II in the presence of theophylline. All analyses were performed in triplicate.

removed, the right legs shaved, and the extensor digitorum longus (EDL) and soleus muscles were exposed. Silk sutures (5–0) were tied to the proximal and distal tendons of the EDL and soleus muscles, and they were removed. The muscles were then immediately placed into a bath with oxygenated Ringer's solution (95% O_2 and 5% CO_2), tied to a force transducer and a fixed post within the bath and stimulated with single and trains of electrical pulses (1 Hz and 10–200 Hz, respectively) to assess the muscles ability to generate force. After force generation analysis, the muscles were removed from the baths, blotted dry, and weighed.

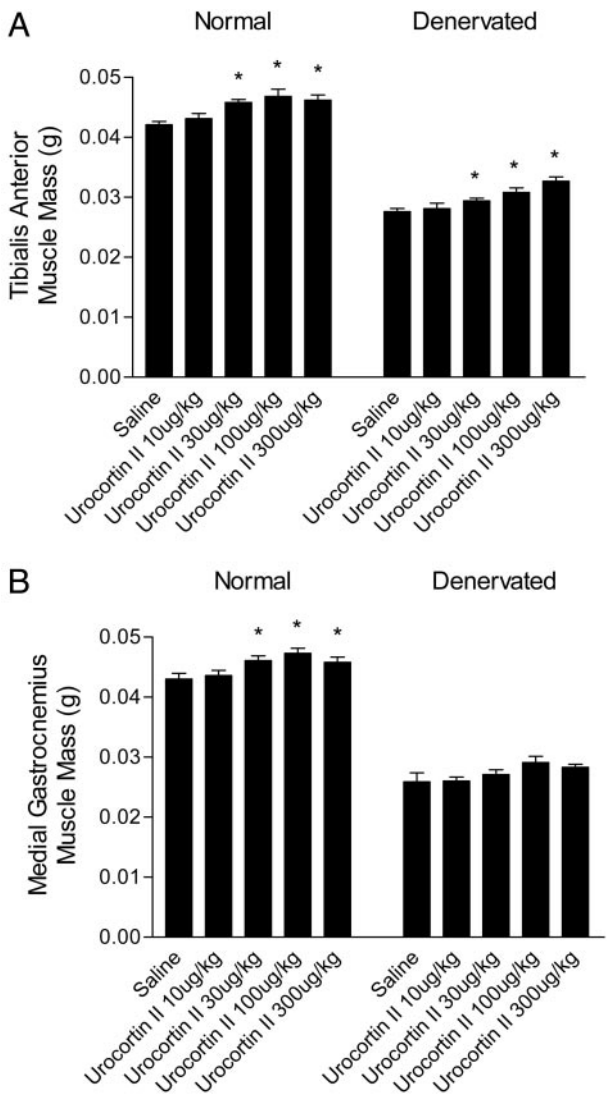

FIG. 2. Urocortin II treatment inhibits denervation-induced tibialis anterior muscle mass loss and results in increased nonatrophying tibialis anterior and medial gastrocnemius muscle mass in C57BL6 mice. A, Once daily sc injection dosing of mice with right leg sciatic nerve damage with 10, 30, 100, and 300 μg/kg·d of urocortin II resulted in a statistically significant ($P < 0.05$) inhibition of denervation-induced tibialis anterior mass loss at the 30, 100, and 300 μg/kg·d urocortin II doses compared with saline control (n = 8 mice per treatment group). Urocortin II treatment at the 30, 100, 300 μg/kg·d doses resulted in statistically significant increases in normal (left leg) tibialis anterior muscle mass. B, Dosing of mice as described in panel A above did not result in statistically significant inhibition of denervation-induced medial gastrocnemius muscle mass loss but did cause statistically significant increase in normal (left leg) medial gastrocnemius muscle mass at the 30, 100, 300 μg/kg·d urocortin II doses. Sciatic nerve damage resulted in an approximate 30% loss of tibialis anterior and medial gastrocnemius muscle mass (saline-treated left leg *vs.* saline-treated right leg muscle). *, Statistically significant ($P < 0.05$) result.

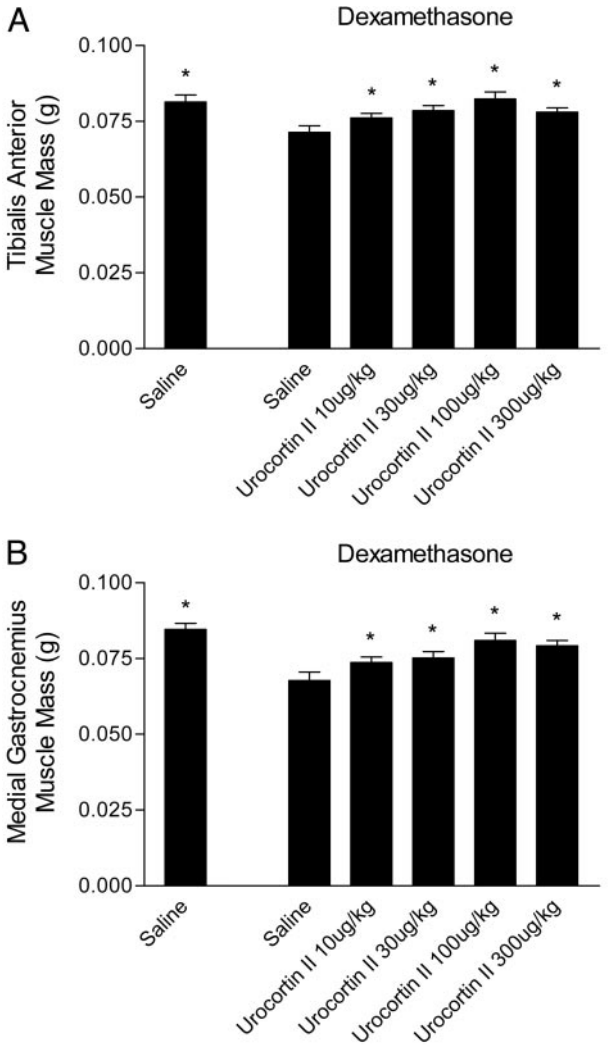

FIG. 3. Urocortin II treatment inhibits corticosteroid-induced tibialis anterior and medial gastrocnemius muscle mass loss in C57BL6 mice. A, Once daily sc injection dosing of mice receiving 1.2 mg/kg·d of dexamethasone in the drinking water with 10, 30, 100, and 300 μg/kg·d of urocortin II (n = 8 mice per treatment group) resulted in a statistically significant ($P < 0.05$) inhibition of tibialis anterior muscle mass loss at all urocortin II doses tested compared with saline control. B, Dosing of mice as described in panel A above resulted in statistically significant ($P < 0.05$) inhibition of medial gastrocnemius muscle mass loss at all urocortin II doses compared with saline control. Dexamethasone treatment resulted in an approximate 20% loss of tibialis anterior and medial gastrocnemius muscle mass (saline treatment *vs.* saline + D treatment). *, Statistically significant ($P < 0.05$) result. Note: Data given as combined mass of both the right and left tibialis anterior and both the right and left medial gastrocnemius muscles.

Muscle fiber cross-sectional area analysis

Muscle fiber cross-sectional area analysis was performed on 10% neutral buffered formalin-fixed resting length skeletal muscle preparations. After paraffin embedding, cross-sections were cut from the center of the soleus and EDL muscles in triplicate. Sections were stained with Picro-sirius Red (Sirius red F3B, C.I. 35782) that stains endomysium

collagen red resulting in clearly delineated and easily digitalized muscle fibers (muscle fibers stain light yellow). Digitalized images of the stained samples were acquired using a SPOT RT camera and the SPOT Advanced Imaging Software (Universal Imaging Corp., 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 Institute, Cary, NC), adjusted for unequal sample sizes and starting weight.

Results

CRF2R ligand activation of CRF2R in cells and skeletal muscle

Three corticotropin peptide family members that activate the CRF2R were evaluated for potency and selectivity using Chinese hamster ovary-K1 (CHO-K1) cells containing either the CRF1R or the CRF2R. Full dose-response analysis was performed to determine an EC_{50} at each receptor type. As can be seen in Table 1, human urocortin II demonstrated potent and selective activation of both the rat and human CRF2R but did not activate the CRF1R. Human urocortin I and sauvagine demonstrated potent activation of the rat and human CRF2R and the human CRF1R.

To evaluate the potential for urocortin II, urocortin I and sauvagine to activate the CRF2R present in skeletal muscle, skeletal muscle organ baths were employed. Figure 1 demonstrates that sauvagine, urocortin I, and urocortin II are approximately equally potent at activating the CRF2R, the only CRFR expressed in skeletal muscle (5, 26–28), in both tibialis anterior and medial gastrocnemius muscles.

Effect of urocortin II treatment on sciatic nerve damage-induced skeletal muscle atrophy

Studies were undertaken to evaluate the effects of urocortin II treatment on nerve damage-induced skeletal muscle atrophy. For these studies, a segment of the right sciatic nerve is removed, effectively eliminating innervation to the lower right leg muscles. The animals were followed for 9 d after nerve removal, with or without urocortin II treatment, and the mass of the tibialis anterior and medial gastrocnemius muscles from either the nerve-damaged leg or contralateral normal leg was measured. As shown in Fig. 2, A and B, removal of a segment of the right sciatic nerve resulted in an approximate 30% loss of tibialis anterior and medial gastrocnemius muscle mass (compare saline left leg muscle to saline right leg muscle). Treatment with urocortin II resulted in a dose-dependent increase in denervated tibialis anterior (Fig. 2A) but not medial gastrocnemius (Fig. 2B) muscle mass. In addition, treatment with urocortin II resulted in a dose-dependent increase in normal tibialis anterior and medial gastrocnemius muscle mass (Fig. 2, A and B).

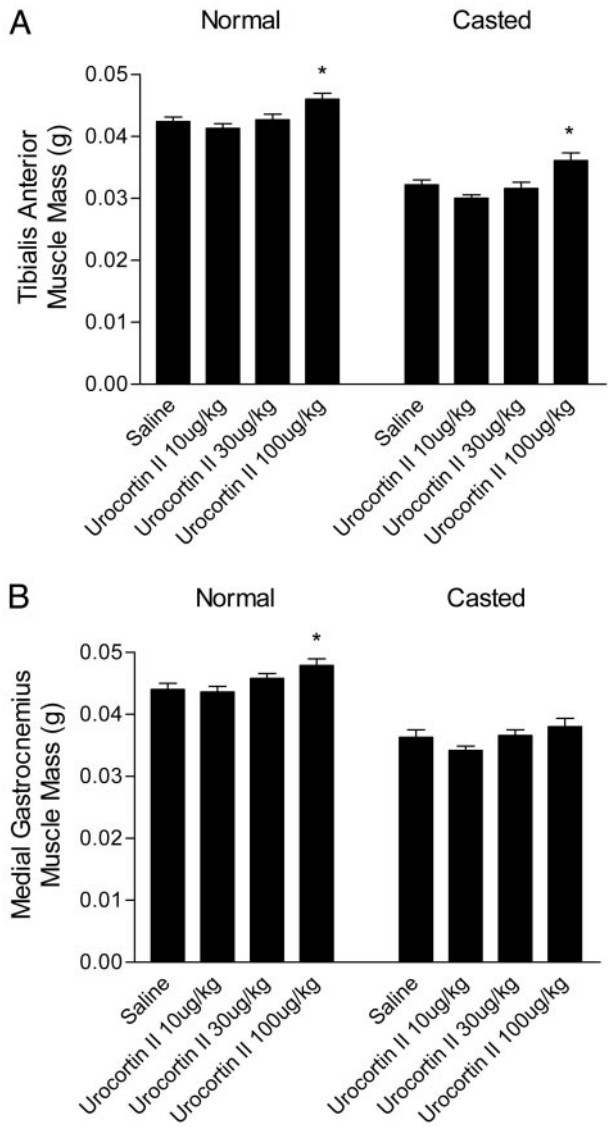

FIG. 4. Urocortin II treatment inhibits casting-induced tibialis anterior muscle mass loss and increases nonatrophying tibialis anterior and medial gastrocnemius muscle mass in C57BL6 mice. A, Once daily sc injection treatment of mice with their lower right leg casted with 10, 30, and 100 μg/kg·d urocortin II resulted in a statistically significant ($P < 0.05$) inhibition of casting-induced tibialis anterior muscle mass loss at the 100 μg/kg·d dose compared with saline control (n = 8 mice per treatment group). Urocortin II treatment at the 100 μg/kg·d dose resulted in statistically significant increase in normal (uncasted left leg) tibialis anterior muscle mass (Fig. 4B). Dosing of mice as described in Fig. 4A above did not result in statistically significant inhibition of casting-induced medial muscle mass loss but did result in a statistically significant increase in normal (uncasted left leg) medial gastrocnemius muscle mass. Casting resulted in an approximate 20% loss in tibialis anterior and medial gastrocnemius muscle mass (saline-treated left leg *vs.* saline-treated right leg muscle). *, Statistically significant ($P < 0.05$) result.

Effect of urocortin II treatment on corticosteroid-induced skeletal muscle atrophy

A second model of skeletal muscle atrophy, corticosteroid-induced atrophy, was used to evaluate the effect of urocortin II to modulate corticosteroid-induced skeletal muscle mass

loss. In this model, mice are administered dexamethasone in the drinking water at a daily dose that results in an approximate 20% loss of muscle mass after 9 d (compare saline to saline + dexamethasone muscles). As shown in Fig. 3, A and B, treatment of mice undergoing dexamethasone-induced skeletal muscle atrophy with urocortin II resulted in a dose-dependent inhibition of tibialis anterior and medial gastrocnemius muscle mass loss (Fig. 3, A and B).

Effect of urocortin II treatment on casting-induced skeletal muscle atrophy

A third model of skeletal muscle atrophy, casting-induced atrophy was used to evaluate urocortin II effects. This model consisted of casting the lower right leg of mice for 10 d. At the end of 10 d, skeletal muscle mass is measured. Casting-induced immobilization results in an approximate 20% loss of muscle mass after 10 d (compare saline-treated casted leg muscles to saline-treated normal leg muscles). As shown in Fig. 4, A and B, treatment with urocortin II decreased casting-induced skeletal muscle mass loss in the tibialis anterior but not the medial gastrocnemius muscle. In addition, urocortin II caused an increase in normal tibialis anterior and medial gastrocnemius muscle mass.

Effect of urocortin II treatment on casting-induced atrophied and normal skeletal muscle mass and function

To evaluate the effect of urocortin II treatment on skeletal muscle mass and function, urocortin II treated and untreated, atrophied and normal, soleus and EDL muscles were used in an *in vitro* function analysis. As can be seen in Fig. 5, A and B, casting results in an approximate 20% loss in EDL and soleus muscle mass after 10 d (compare naïve muscle to saline-treated muscle). Urocortin II treatment decreased casting-induced loss of EDL (Fig. 5A) but not soleus (Fig. 5B) muscle mass. Casting results in an approximate 25% loss in EDL and soleus absolute force (compare naïve muscle to

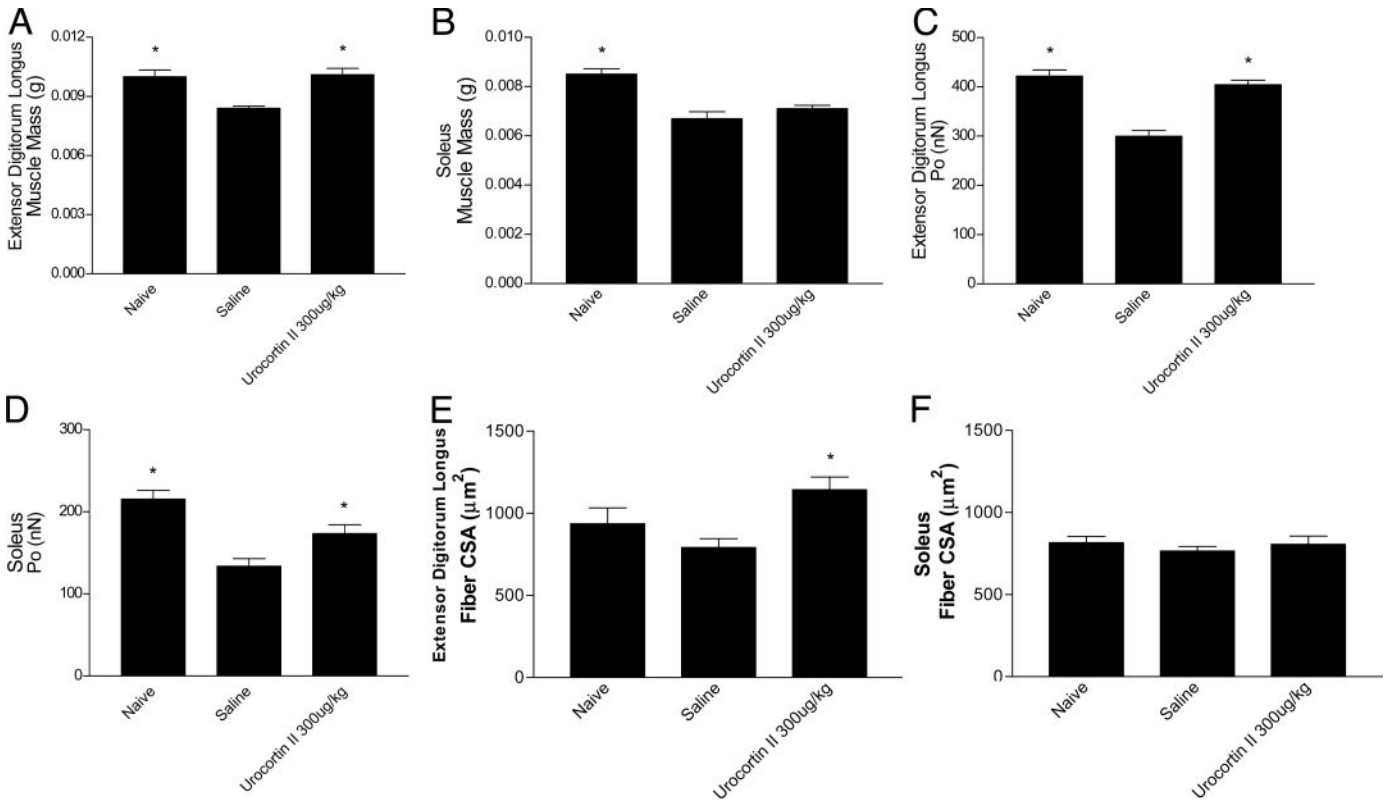

FIG. 5. Once daily sc injection dosing of C57BL6 mice with 300 μg/kg·d of urocortin II inhibits casting-induced EDL muscle mass loss, casting-induced EDL and soleus muscle force loss and casting-induced EDL muscle myocyte cross-sectional area loss. A, Treatment of lower right leg casted mice with 300 μg/kg·d urocortin II resulted in a statistically significant ($P < 0.05$) inhibition of casting-induced EDL muscle mass loss compared with saline control (n = 8 mice per treatment group). B, Treatment of lower right leg casted mice with 300 μg/kg·d urocortin II did not result in a statistically significant ($P < 0.05$) inhibition of casting-induced soleus muscle mass loss compared with saline control (n = 8 mice per treatment group). C, Treatment of lower right leg casted mice with 300 μg/kg·d urocortin II resulted in a statistically significant ($P < 0.05$) inhibition of casting-induced EDL muscle force loss compared with saline control (n = 8 mice per treatment group). D, Treatment of lower right leg casted mice with 300 μg/kg·d urocortin II resulted in a statistically significant ($P < 0.05$) inhibition of casting-induced soleus muscle force loss compared with saline control (n = 8 mice per treatment group). E, Treatment of lower right leg casted mice with 300 μg/kg·d urocortin II resulted in a statistically significant ($P < 0.05$) inhibition of casting-induced EDL muscle myocyte cross-sectional area loss compared with saline control (n = 8 mice per treatment group). F, Treatment of lower right leg casted mice with 300 μg/kg·d urocortin II did not result in a statistically significant ($P < 0.05$) inhibition of casting-induced soleus muscle myocyte cross-sectional area loss compared with saline control (n = 8 mice per treatment group). Casting resulted in an approximate 20% loss in EDL and soleus muscle mass, an approximate 25% loss in EDL and soleus muscle force, and a 20% loss in EDL and 10% loss in soleus myocyte cross-sectional area (naive *vs.* saline-treated muscles). *, Statistically significant ($P < 0.05$) result.

saline-treated muscle); urocortin II treatments decreased casting-induced loss of EDL (Fig. 5C) and soleus (Fig. 5D) absolute force. Finally, an analysis of myocyte cross-sectional area demonstrated that casting resulted in an approximate 20% decrease in EDL myocyte cross-sectional area and a 10% decrease in soleus myocyte cross-sectional area (not statistically significant) (compare naïve muscle to saline-treated muscle); urocortin II treatment decreased casting-induced loss of EDL (Fig. 5E) and soleus (Fig. 5F) myocyte cross-sectional area, although the soleus effect was not statistically significant.

Treatment of noncasted mice with urocortin II resulted in increased EDL and soleus muscle mass (Fig. 6A) and increased EDL and soleus absolute force (Fig. 6B).

Effect of additional CRF2R agonists on skeletal muscle mass and function

Two additional CRF2R agonists were evaluated for their effect on skeletal muscle mass. Sauvagine, a frog peptide that is equipotent for the CRF1R and CRF2R, was evaluated in the mouse casting model. As can be seen in Fig. 7, A and B, sauvagine treatment decreased casting-induced loss of tibialis anterior and medial gastrocnemius muscle mass. In addition, sauvagine treatment increased normal tibialis anterior and medial gastrocnemiusm muscle mass. Urocortin I, a human peptide that is equipotent for the CRF1R and CRF2R, was evaluated in the mouse casting atrophy model. As can be seen in Fig. 7, C and D, urocortin I treatment decreased casting-induced loss of tibialis anterior and medial gastrocnemius muscle mass.

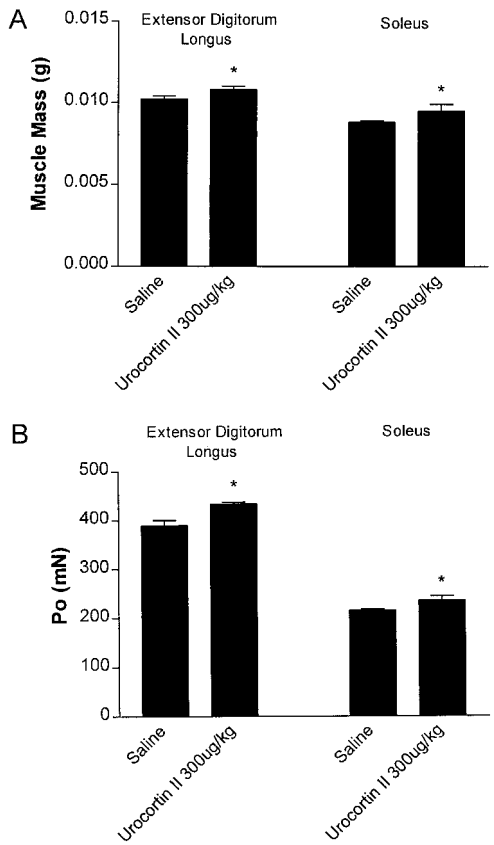

FIG. 6. Once daily sc injection dosing of C57BL6 mice with 300 μg/kg·d of urocortin II increases normal EDL and soleus muscle mass and force. A, Treatment of mice with 300 μg/kg·d urocortin II resulted in a statistically significant ($P < 0.05$) increase in EDL and soleus muscle mass compared with saline control (n = 8 mice per treatment group). B, Treatment of mice with 300 μg/kg·d urocortin II resulted in a statistically significant ($P < 0.05$) increase in EDL and soleus muscle force compared with saline control (n = 8 mice per treatment group). *, Statistically significant ($P < 0.05$) result.

Discussion

In this report, we demonstrate that the CRF2R-selective ligand, urocortin II, is able to inhibit nerve damage, corticosteroid administration, and casting immobilization-induced loss of skeletal muscle mass, function and myocyte cross-sectional area. In addition, urocortin II treatment increases normal muscle mass and function. Urocortin II treatment inhibits nerve damage-induced, corticosteroid-induced, and casting immobilization-induced muscle mass loss best in fast-twitch muscle, whereas it had less of an effect on slow-twitch muscle. In contrast, urocortin II treatment results in equivalent increase in normal fast twitch and normal slow twitch muscle mass, suggesting that atrophy somehow modulates the CRF2R present on slow twitch atrophying muscle so that it is less responsive to urocortin II treatment. Also, we observe increased skeletal muscle mass in atrophying and normal muscle after treatment with one additional CRF2R agonist, sauvagine; a second CRF2R agonist, urocortin I, was effective in increasing atrophying skeletal muscle mass but not normal skeletal muscle mass. These results suggest differences in how these different CRF2R agonists modulate the CRF2R present on atrophying or normal fast and slow twitch muscle types. Finally, we observe that direct application of urocortin II, urocortin I and sauvagine to fast- and slow-twitch skeletal muscle results in increased muscle cAMP levels, demonstrating that the CRF2R (the only CRFR present in skeletal muscle) is functional and able to respond to agonist stimulation.

What is the role of the CRF2R in skeletal muscle physiology? The observation that the CRF2R and two CRF2R-selective peptides, urocortin II and urocortin III, are found in skeletal muscle indicates that this system may have a physiological role in modulation of skeletal muscle mass. One possible function of this system is to moderate the skeletal muscle-wasting effects of corticosteroids. It is possible that this system functions to respond to the induction of the stress response by maintaining skeletal muscle mass in the face of the elevated corticosteroid levels that result from activation of the CRF1R and the HPA axis. It is interesting that urocortin II treatment was most effective in reducing tibialis anterior and medial gastrocnemius muscle wasting resulting from corticosteroid administration, in contrast to the observation that urocortin II treatment reduced tibial anterior muscle but not medial gastrocnemius muscle wasting resulting from casting and nerve damage. In support of this hypothesis is the observation that glucocorticoids up-regulate the expression of the mouse urocortin II gene via increased gene expression after activation of GRE sequences in the urocortin II gene promoter (17).

A second possible function of the CRF2R system in skeletal

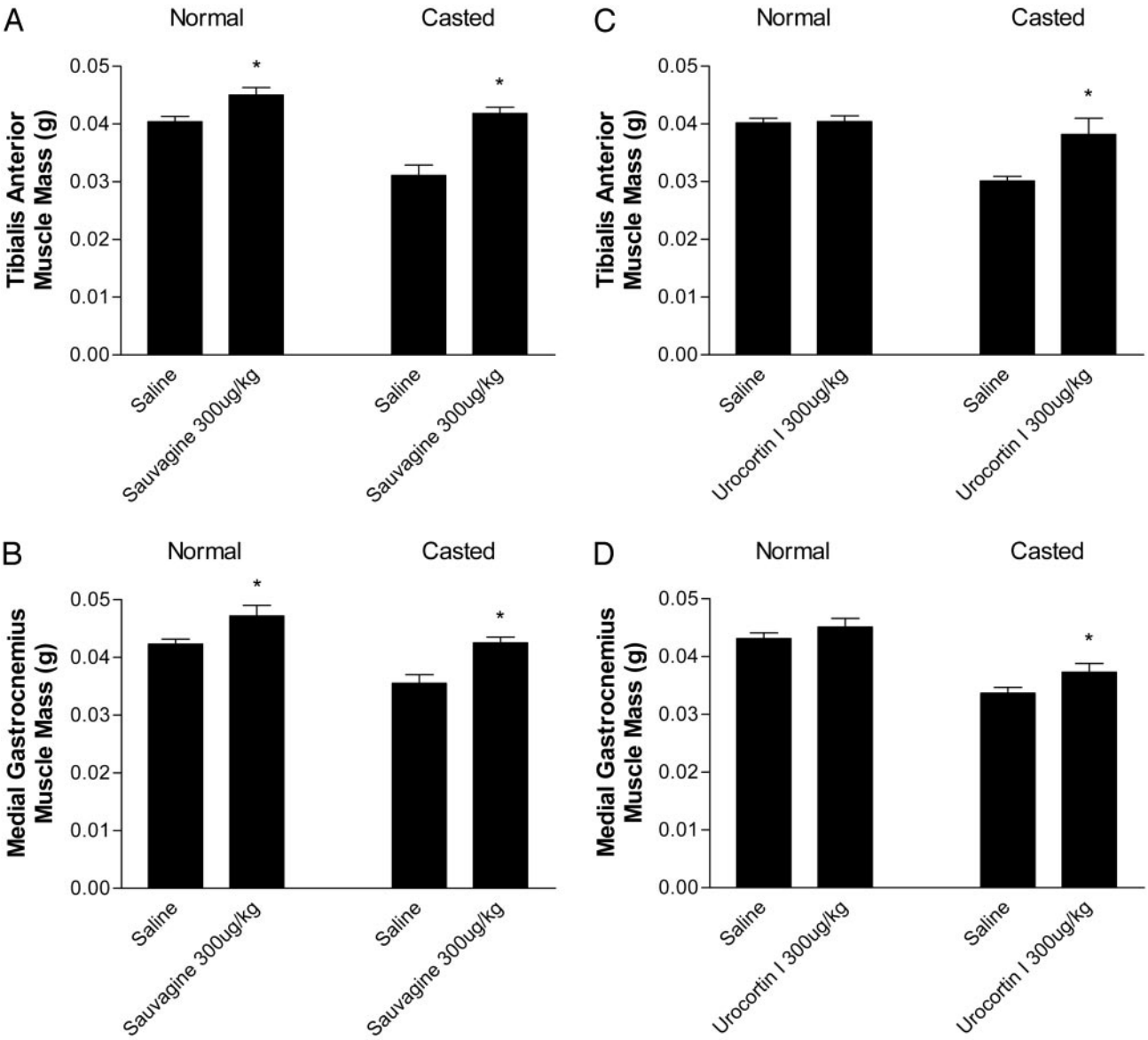

FIG. 7. Sauvagine and urocortin I treatment inhibits casting-induced tibialis anterior and medial gastrocnemius muscle mass loss, whereas sauvagine treatment increases nonatrophying tibialis anterior and medial gastrocnemius muscle mass in C57BL6 mice. Once daily sc injection treatment of lower right leg casted mice with 300 μg/kg·d sauvagine resulted in a statistically significant ($P < 0.05$) inhibition of casting-induced tibialis anterior (A) and medial gastrocnemius (B) muscle mass loss compared with saline control (n = 8 mice per treatment group). Sauvagine treatment resulted in statistically significant increase in normal (uncasted left leg) tibialis anterior (A) and medial gastrocnemius (B) muscle mass. Once daily sc injection treatment of lower right leg casted mice with 300 μg/kg·d urocortin I resulted in a statistically significant ($P < 0.05$) inhibition of casting-induced tibialis anterior (C) and medial gastrocnemius (D) muscle mass loss compared with saline control (n = 8 mice per treatment group). Urocortin I treatment did not result in statistically significant increase in normal (uncasted left leg) tibialis anterior (C) and medial gastrocnemius (D) muscle mass. *, Statistically significant ($P < 0.05$) result.

muscle may be to regulate skeletal muscle mass/function through a neuronally mediated mechanisms as follows. First, the CRF2R is present and functional in myocytes; stimulation of motoneurons result in the release of CRF2R agonist from the motoneurons that directly activates myocyte CRF2R, resulting in increased myocyte mass. Second, CRF2R on motoneurons are stimulated by CRF2R agonists released by myocytes, resulting in motoneuron release of trophic factors that increases myocyte mass. Thirdly, CRF2R on innervating nerves are stimulated by CRF2R agonists released by the pressure sensory organs of the muscle, Golgi tendon organ or muscle spindle organs, in response to muscle contraction resulting in the release of trophic substances from motoneurons. Whereas direct evidence for any of these potential mechanisms is at present lacking, several pieces of evidence provide insight into how the CRF2R functions in skeletal muscle. For example, it is known that members of the CRH family of ligands function as neurohumoral factors (2, 3, 18–25). It has been observed that peripheral CRFR agonistic peptides activate CRFR on neurons in the colon, bronchi, stomach, airway smooth muscle, ileum, and duodenum (18–23). The effect of CRFR activation in these tissues is either

directly mediated by stimulation of the CRFR on the nerves that innervate that tissue or by potentiating the action of other neurohumeral factors. These data support the model that CRFR on nerves function to modulate neuronal release of neurohumeral factors and neuronal activity in smooth muscle; no data currently exist to support this concept in skeletal muscle. Additional experimentation will be required to fully understand the mechanism by which activation of the CRF2R in skeletal muscle by the CRF2R-selective agonist inhibits atrophy and induces hypertrophy in skeletal muscle.

The observation that activation of the CRF2R with the CRF2R-selective agonist (urocortin II) and nonselective agonists (sauvagine and urocortin I), results in the modulation of skeletal muscle mass suggests that CRF2R agonists may have clinical utility for the treatment of muscle wasting diseases including cachexia associated with AIDS 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, and autoimmune disease. In addition, because activation of the CRF2R results in skeletal muscle hypertrophy, CRF2R agonists may have utility in the treatment of muscle weakness or frailty observed in the elderly; for improving muscle function in individuals afflicted with muscular dystrophies by maximizing the effectiveness of the remaining functional muscle; and for preventing or maintaining muscle mass during periods of exposure to low gravity, such as that experienced in space. Thus CRF2R agonists may have a multitude of uses in the treatment of skeletal muscle wasting phenomena.

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