Published in final edited form as:

Pain. 2016 November ; 157(11): 2561–2570. doi:10.1097/j.pain.0000000000000677.

Modulation of Musculoskeletal Hyperalgesia by Brown Adipose Tissue Activity in Mice

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Abstract

Cold exposure and a variety of types of mild stress increase pain in patients suffering from painful disorders such as fibromyalgia syndrome. Acutely, stress induces thermogenesis by increasing sympathetic activation of beta-3 (β_3) adrenergic receptors in brown adipose tissue. Chronic stress leads to the hypertrophy of brown adipose, a phenomenon termed adaptive thermogenesis. Based on the innervation of skeletal muscle by collaterals of nerves projecting to brown adipose, we theorized an association between brown adipose tissue activity and musculoskeletal hyperalgesia and tested this hypothesis in mice. Exposure to a cold swim or injection of BRL37344 (β_3 adrenergic agonist) each enhanced musculoskeletal hyperalgesia, as indicated by morphine-sensitive decreases in grip force responses, whereas SR59230A (β_3 adrenergic antagonist) attenuated swim-induced hyperalgesia. Chemical ablation of interscapular brown adipose, using Rose Bengal, attenuated the development of hyperalgesia in response to either swim stress or BRL37344. In addition, elimination of the gene expressing uncoupling protein-1 (UCP1), the enzyme responsible for thermogenesis, prevented musculoskeletal hyperalgesia in response to either a swim or BRL37344, as documented in UCP1-knock out (UCP1-KO) mice compared to wild type controls. Together these data provide a convergence of evidence suggesting that activation of brown adipose contributes to stress-induced musculoskeletal hyperalgesia.

Keywords

Hyperalgesia; Stress; Pain; Brown adipose tissue; UCP-1; β_3 -adrenergic receptor

1. Introduction

Many painful conditions are exacerbated by stress in animals [46] and humans [5; 20; 53]. For example, musculoskeletal hyperalgesia associated with fibromyalgia syndrome is

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The authors report no conflict of interest with any known agency.

worsened by stress or cold. Sympathetic nervous system activity is high in these patients [46] and they exhibit a hyperalgesic response to norepinephrine [33], suggesting stress-induced hyperalgesia may be supported by sympathetic tone.

Acute stress enhances sympathetic tone with an associated release of catecholamines stimulating adrenergic receptors [16; 40]. Sympathetic nerves stimulate brown adipose [23; 28; 45] via beta-3 (β_3) adrenergic receptors, a subtype located in only a handful of locations in the body [7; 11]. While the majority of β_3 adrenergic receptors are located in brown adipose, they are absent in skeletal muscle [7; 11; 27]. Exposure to acute cold [7] or even psychological stress [23] stimulates thermogenesis by activation of brown adipose. Although described as early as 1551 [7], until recently it was assumed that brown adipose was unimportant in humans. Newer studies emphasize the physiologic importance of brown adipose even in adults [7].

Non-shivering thermogenesis is mediated by uncoupling protein-1 (UCP1), a protein highly concentrated in mitochondria of brown adipocytes [7; 11; 34; 36]. Chronic exposure to cold or stress increases brown adipose tissue mass and upregulates uncoupling protein-1 (UCP1) synthesis [34], a process called adaptive thermogenesis. Nerves projecting to brown adipose also project collaterals to regions surrounding tender points characteristic of fibromyalgia, primarily in supraclavicular regions, but also in supra-axial, peri-renal and subcutaneous areas [47]. These patients have lower than normal body temperatures in skin above tender points [21; 29] whereas transient relief of musculoskeletal pain is provided by heat [31].

Based on the literature [29], we hypothesized that stress-induced activation of brown adipose contributes to musculoskeletal hyperalgesia. To test this, we examined decreases in grip force as a measure of musculoskeletal hyperalgesia in mice [19], after a forced swim, a stress frequently used to induce hyperalgesia in rodents [2; 13; 19; 37; 44; 49]. Acute stress typically causes antinociception or has no effect on thermal and tactile mechanical nociception; hyperalgesia develops only after chronic stress. Grip force is unique as even single exposures to a forced swim cause muscle hyperalgesia, as indicated by a morphine-sensitive decrease in grip force responses [19]. Based on this, we examined whether ablation of interscapular brown adipose alters musculoskeletal hyperalgesia. We further postulated that β_3 adrenergic activity in brown adipose recapitulates musculoskeletal hyperalgesic effects of swim stress and β_3 adrenergic antagonism blocks it. We tested this by the ability of BRL37344 (a β_3 adrenergic receptor agonist) to mimic stress-induced hyperalgesia and by the tendency for either SR59230A (a β_3 adrenergic receptor antagonist) or ablation of brown adipose to inhibit hyperalgesia. Because UCP1 is the enzyme responsible for thermogenesis, we compared abilities of stress and of BRL37344 to induce musculoskeletal hyperalgesia in wild type (WT) mice to those whose gene responsible for expression of UCP1 was deleted (UCP1-knock-out, UCP1-KO). Together these data suggest that activation of brown adipose contributes to musculoskeletal hyperalgesia.

2. Materials and Methods

2.1 Animals

Adult female and male Swiss Webster mice (Harland Sprague Dawley INC, Indianapolis, MN) weighed 20-25 g. UCP-1 knock out mice (Ucp^{tm1Kz}) and their wild type littermate controls C57BL/6J (The Jackson Laboratory, Bar Harbor, ME) were all female weighing 13-15 g. All mice were housed 3 to 5 mice per cage and allowed to acclimate for at least one week prior to use. Free access to food and water was allowed during acclimation and the room was maintained at a constant temperature of 23°C on a 12-h light-dark cycle. The estrus cycle of the female mice was not taken into account when comparing results. Male and female Swiss Webster mice did not differ in the degree of hyperalgesia induced by a forced swim or by an injection of BRL37344. All procedures were performed according to the guidelines of the International Association for the Study of Pain (IASP), the University of Minnesota Animal Care and Use Committee, and the Committee of Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication NIH 78-23, revised 1995) and approved by the University of Minnesota Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternative in vivo techniques, when possible.

2.2 Drugs and chemicals

(R*, R*)-[4-[2-[[2-(3-Chlorophenyl)-2-hydroxyethyl]amino]propyl]phenoxy]acetic acid sodium hydrate (BRL37344), a β_3 adrenergic agonist, was obtained from Tocris Bioscience (Bristol, United Kingdom). The primary metabolite of BRL37344 induces increased energy expenditure in brown adipose tissue of mice [8]. BRL37344 dissolved in Nanopure water (pH 4.0-4.5) and administered subcutaneously (s.c.) at 1 mg/kg [39] in Swiss Webster mice, a dose previously found to be effective in rats. When the anti-hyperalgesic effect of morphine was tested in mice injected with BRL37344, optimal hyperalgesia was first induced with BRL37344 using increasing daily doses of 1, 3 and 10 mg/kg. Based on preliminary studies in this strain, C57BL/6J mice were injected with 10 mg/kg of BRL37344. The selectivity of the effect of BRL37344 for β_3 rather than β_1 adrenergic receptors was determined in preliminary studies by its lack of effect at doses of 1, 3 or 10 mg/kg on heart rate, a parameter that is increased by β_1 adrenergic receptor activity. The dependence of forced swim-induced hyperalgesia on β_3 receptor activity was tested using 2 mg/kg of hydrochloride SR59230A hydrochloride, a β_3 adrenergic receptor antagonist at low doses [38] and α_1 adrenergic receptor antagonist at high doses [30], using a dose of 2 mg/kg s.c. that has been used previously [32] based on its ability to inhibit β_1 more than α_1 adrenergic activity [3]. Morphine sulfate, an opioid receptor agonist, was purchased from Mallinckrodt (St. Louis, MO). Morphine was diluted in saline (pH 5.0) and injected intraperitoneally (i.p.) at a dose (10 mg/kg) that had strong antinociceptive effects in preliminary studies employing the thermal tail flick assay in water maintained at either 49°C.

2.3 Partial ablation of brown adipose tissue

Rose Bengal dye was obtained from Sigma-Aldrich (St. Louis, MO) and injected at a dose of 10 mg/kg into the vascular system by an intracardiac stick while mice were anesthetized using isoflurane. Because of the small volume of interscapular brown adipose tissue, male mice were used as they were larger than females at similar ages. The area of the interscapular brown adipose tissue was exposed, isolated and exposed to an intense light source for 10 min using a Fiber Optic Illuminator from Cole-Parmer (Vernon Hills, IL). The high intensity lamp (150 watt quartz halogen lamp with fiber optic diameter of 0.3 mm and light intensity set at 50%) is reported to induce the generation of Rose Bengal free radicals that cause a local degeneration of tissue resulting in a partial ablation of the illuminated tissue [22; 55]. To induce similar conditions but without tissue ablation, sham mice were injected with dye and the brown adipose tissue isolated surgically, but not exposed to the high intensity illumination.

2.4 Forced swim

A forced swim was used as a form of acute stress as this has been previously found to influence mechanical nociception [41-43; 49; 50]. Each mouse was placed, individually, in a 2-L beaker (diameter: 15 cm, height: 20 cm) containing 18 cm of water maintained at 26°C. This depth was sufficient that mice could not touch their feet or tail to the bottom. Grip force was evaluated after a 15-min swim, previously found to induce an optimal stress-induced hyperalgesia [2], and at various time-intervals thereafter to establish the time-course of the resulting hyperalgesia. Forced swims were repeated daily and the grip force values after each swim were routinely compared to other control groups or, where indicated, to their baseline measurements.

2.5 Grip force assay

Forelimb grip force was measured using a force transducer as previously described in rats [25] and mice [24]. The grip force apparatus consists of a force transducer connected to a wire mesh grid ($12 \times 7 \text{ cm}^2$ outer diameter with a 0.5 cm^2 wire grid) approximately 30 cm above the bench top. During testing, each mouse was held by the base of its tail and gently passed in a horizontal direction over the wire grid until it grasped the grid with its forepaws. The force transducer recorded the force exerted by the forelimbs of each mouse when pulling on the grid. Grip force data are expressed in terms of grams (g). Two grip force measurements were obtained at each time-point and the average of these measurements used to represent each mouse's forelimb grip force at a particular time. Mice were familiarized with the grip force measurement procedure for three days prior to the experiment. On the third day, grip force measurements obtained prior to each intervention were used as baseline values for each animal.

2.6 Weighing of BAT

In a subset of mice, both experimental animals and controls were sacrificed after manipulations and their interscapular brown adipose depot removed and weighed.

2.7 Tail Flick Assay

While animals were manually restrained, the tail was submerged in a water bath maintained at a temperature of 49°C. The withdrawal latency was defined as the time for the animal to withdraw its tail from the water. To avoid tissue damage, a cut-off time of 15 sec was used.

2.8 Statistical Analyses

Although we consistently saw decreases in grip force values in response to a swim or injection of BRL37344, there was variability from one experiment to another with respect to their baseline values. This may be due to differences in mouse size on arrival, sex, and perhaps their history of stress, even prior to or during delivery. While baseline values within each experiment were very consistent, we still randomized mice with respect to grip force prior to each experiment to ensure that our baseline values not only did not differ between groups, to minimize variability within groups, and to blind observers. This allowed us to compare the effects of swim or drug treatment within experiments, but was still not optimal for comparing effects between experiments. The most direct way to depict data from multiple experiments, such as that in figure 1, was to compare differences in the degree of hyperalgesia produced by interventions compared to their own baseline values, which were equivalent within each group. This normalization of data allowed us to summarize and compare the effects of swim and of drug treatment.

Data involving comparisons of more than two groups were analyzed using one-way ANOVA followed by the Newman-Keuls post hoc analysis to determine differences between treatment groups tested at the same time. Differences in body temperature and grip force at specific time-intervals after drug injection were compared to the appropriate pre-injection control values using a paired Student's t-test or by one-way ANOVA followed by Newman-Keuls post hoc analysis. In all analyses, differences were considered significant if the probability that it occurred because of chance alone was less than 5% ($P < 0.05$).

3. Results

3.1 Musculoskeletal hyperalgesia induced by swim stress and its reversal by either ablation of interscapular brown adipose or by elimination of the UCP1 gene

The average decrease in grip force responses of mice subjected to a 15-min swim at 26°C was greater than in unstressed control mice tested on the same day (Figure 1), consistent with our previous study [1]. To determine whether activation of brown adipose tissue is necessary for the transient musculoskeletal hyperalgesia observed after a forced swim, interscapular brown adipose of 12 mice was partially ablated using injection of Rose Bengal, as described in methods [22; 55]. Two weeks later, when exposed to the forced swim, sham-operated mice whose interscapular brown adipose was not ablated exhibited greater decreases in grip force responses immediately after the swim than previously ablated mice (Figure 1). In contrast to Swiss Webster mice, WT C57BL/6J mice were somewhat less sensitive to the hyperalgesic effect of the forced swim. Baseline grip force responses of wild type C57BL/6J mice did not differ from that of UCP1 knockout mice (WT: mean=127.6±1.8 g; KO=125.9±2.6 g, n=40/group, two-tailed, unpaired Student's t-test, $P=0.597$); however, grip force values of C57BL/6J WT mice were decreased immediately following the swim

when compared to their pre-swim values (Figure 1), in agreement with the results in the Swiss Webster mice. In contrast, grip force responses of C57BL/6J UCP1-KO mice following a forced swim were not significantly different than those of their own pre-swim baseline values, consistent with the attenuated response of brown adipose tissue-ablated Swiss Webster mice.

3.2 Musculoskeletal hyperalgesia induced by BRL37344 and its reversal by either ablation of interscapular brown adipose or by elimination of the UCP1 gene

To test the hypothesis that β_3 adrenergic activation of brown adipose tissue would mimic the hyperalgesic effect of stress on musculoskeletal nociception, the β_3 adrenergic receptor agonist BRL37344 was injected s.c. at 1 mg/kg. When measured 24 h after injection, the average grip force response of the group injected with BRL37344 was decreased compared to that of vehicle-injected control mice. To determine if it is the activation of β_3 adrenergic receptors located specifically in brown adipose tissue that is necessary for the development of musculoskeletal hyperalgesia in response to BRL37344 (as opposed to β_3 adrenergic receptors elsewhere in the body), grip force responses to BRL37344 in mice whose interscapular brown adipose depots were ablated were compared to mice that were sham-operated and served as controls. Baseline grip force values were unaffected by prior ablation when compared with sham-ablated mice (data not shown). However, mice with chemically ablated brown adipose depots exhibited no decrease in their mean grip force values 60 min after injection of BRL36344 while sham-operated mice were hyperalgesic when compared to their corresponding baseline control values (Figure 1) as well as 24 h later (data not shown).

Injection of BRL37344 (10 mg/kg) into WT C57BL/6J mice increased their mean rectal temperature, a response indicative of thermogenesis. However, the effect of BRL37344 on grip force values in C57BL/6J mice, like the hyperalgesic effect of a forced swim, was less than that in Swiss Webster mice. As a result, neither WT nor UCP1-KO mean grip force values after injection were significantly less than their respective baselines taken prior to injection, in spite of the fact that a higher dose of BRL37344 was used in C57BL/6J mice than in Swiss mice. However, there was none-the-less a significant difference between the effects of BRL37344 on grip force in WT and UCP1-KO mice suggesting that the absence of UCP1 differentially influences their grip force response to β_3 adrenergic activity.

3.3 Relationship of brown adipose tissue and repeated daily swim-induced hyperalgesia

Thermogenesis reflects the acute activation of brown adipose tissue by sympathetic stimulation whereas adaptive thermogenesis reflects the increase in brown adipose tissue mass as a result of chronic cold or stress [23]. To determine whether musculoskeletal responses to thermogenesis (induced by acute stress) differ from those that lead to adaptive thermogenesis (chronic stress), Swiss Webster mice were subjected to the forced swim at 26°C every day for an additional 14 days. Grip force responses and rectal temperatures were measured immediately after each daily forced swim. Grip force responses of mice subjected to the swim were lower than in the unstressed control group with the greatest decreases in grip force observed on day three and persisting until day 15 (Figure 2A).

Rectal temperatures of mice taken immediately following the grip force assay indicate decreased core body temperatures due to exposure to the 26°C swim (Figure 2B). The lowest value was on day one in response to the first daily swim, after which the ability to defend against the cold improved during the remaining daily swims.

Experimental and control groups were sacrificed on day 15, 24 h after the last swim, and their interscapular brown adipose extracted and weighed. This depot of tissue was heavier in mice exposed to a daily swim than in unstressed control mice (Figure 2C) indicating that daily swims for two weeks was a sufficiently chronic time-interval to increase the mass of brown adipose.

3.4 Effect of daily injection of β_3 adrenergic agonist on mechanical hyperalgesia

Grip force and rectal temperatures were measured after daily s.c. injections of 1 mg/kg of BRL37344 for 15 days. This dose was found to be sufficient to increase rectal temperatures 15 min after its injection (Figure 3C). Grip force responses were measured before as well as 60 min (Figure 3A) and 24 h (Figure 3B) after each of the daily injections. Sixty min after each injection, BRL37344 and saline each decreased the average grip force response compared to their respective baseline values. There was also no difference between responses in the BRL37344 group and vehicle-injected controls (Figure 3A), suggesting that the stress of the injection alone was sufficient to influence the immediate grip force measures. When measured 24 h after each daily injection, grip force responses of the group injected with BRL37344 were still decreased on days 1-5 compared to vehicle-injected control mice (Figure 3B). After the first week of testing, habituation to the effect of BRL37344 developed as there was no longer a difference between grip force values of BRL37344-injected mice compared to vehicle-injected controls when tested 24 h after their injection. No such tolerance developed to the ability of BRL37344 to increase rectal temperatures 15 min after injection when compared to vehicle over the same 2-week interval (Figure 3C).

In all mice, we removed and weighed the interscapular brown adipose depot 24 h after the final injection of BRL37344 or vehicle. This depot was heavier in mice injected daily with BRL37344 than in vehicle-injected controls (Figure 3D), consistent with hypertrophy secondary to adaptive thermogenesis.

3.5 Confirmation that decreases in grip force reflect hyperalgesia

Swim stress-induced hyperalgesia in the grip force assay was previously shown to be inhibited by 10 mg/kg of morphine [2]. To confirm that decreases in grip force values produced by an adrenergic agonist were also due to mechanical hyperalgesia and not musculoskeletal weakness, mice were injected with 1 mg/kg of BRL37344 for 3 days, 3 mg/kg for 3 days and 10 mg/kg for 2 days. Four h after the last injection of BRL37344, mice had grip force responses that were significantly lower than after saline (Figure 4). Mice were then injected i.p. with either saline or 10 mg/kg of morphine and retested 30 min later, the same morphine-challenge used to test swim stress-induced hyperalgesia [2]. The decrease in grip force 4 h after the last injection of BRL37344 was reversed by injection of morphine but not saline. Control mice that were not injected with BRL37344 were unaffected by

morphine, consistent with the inability of morphine to enhance grip force responses above those of normal healthy mice. Together these data confirm that, like stress-induced hyperalgesia [2], BRL37344-induced decreases in grip force responses reflect musculoskeletal hyperalgesia rather than weakness.

3.6 Effect of Rose Bengal ablation of interscapular brown adipose on daily swim-induced hyperalgesia

To determine whether activation of brown adipose tissue is necessary for the persistent musculoskeletal hyperalgesia observed after a forced swim, the brown adipose of 12 mice was partially ablated using Rose Bengal, as described in methods [22; 55]. Two weeks later, baseline grip force values were unaffected by prior ablation when compared with sham-ablated mice (Figure 5A). However, when exposed to the forced swim, mice whose interscapular brown adipose was not ablated not only exhibited greater decreases in grip force responses immediately after the swim than previously ablated mice (Figure 1), when tested daily thereafter, these differences persisted for the first 5 days of the two-week study (Figure 5A).

To determine if it is the activation of β_3 adrenergic receptors located in brown adipose tissue that is necessary for the persistent mechanical hyperalgesia in response to BRL37344, interscapular brown adipose depots were ablated in one group of mice while those in the other group were sham-operated and served as controls. Sham and brown adipose tissue-ablated mice were injected s.c. daily for an additional 14 days with 1 mg/kg of BRL37344 and grip force measured before as well as 60 min (Figure 5B). Once again, baseline grip force values were unaffected by prior ablation when compared with sham-ablated mice. However, mice with chemically ablated brown adipose depots exhibited no decrease in their mean grip force values 60 min after injection of BRL36344 while sham-operated mice were hyperalgesic when compared to their corresponding baseline control values (Figure 1). These differences in response to BRL37344 at 60 min persisted and were even greater throughout the remaining two weeks of daily injections and testing (Figure 5B), however, tolerance developed to the effect of BRL37344 measured 24 h after injection (data not shown)

We then questioned whether the BRL37344-injected mice were still hyperalgesic in response to a forced swim. We also wished to determine whether the hyperalgesic effect of a swim is mediated by β_3 adrenergic receptor activity. To address these issues, after sham-operated mice were tested for their grip force on day 16 (Figure 5B), they were randomized into two groups, one injected s.c. with 2 mg/kg of SR59230A, a β_3 receptor antagonist, and the other with vehicle. Fifteen min later, mice were subjected to a forced swim and the grip force tested immediately thereafter. Mice injected with vehicle were more hyperalgesic after the swim than prior to the swim (baseline values indicated by the dotted line), while mice injected with SR59230A prior to the swim had significantly higher grip force values (Figure 5C). These data suggest that mice did not become desensitized to swim-induced hyperalgesia following two weeks of daily injections of BRL37344, and that forced swim-induced hyperalgesia requires β_3 adrenergic receptor activity.

Success of chemical ablation on intrascapular brown adipose tissue was confirmed by measuring the weight of this depot at the end of the study. Although daily swim and daily injection of mice with BRL37344 were each found to increase the weight of interscapular brown adipose (Figures 5D and 5E) when compared to those treatments in unablated sham mice (Figures 2C and 3D), the interscapular brown adipose depot of mice whose tissue was chemically ablated weighed less than that of mice whose interscapular brown adipose was not, whether the mice were exposed to daily swims (Figure 5D) or to daily injection of BRL37344 (Figure 5E).

3.7 Comparison of body weight and temperature of UCP1-KO and wild type (WT) mice

Deletion of a gene does not always impact the system in which the protein functions due to compensatory mechanisms during development. To assess the consequences of UCP1 deletion on relevant aspects of general health, we monitored weight and body temperature. UCP1-KO mice did not differ from WT controls in their initial body weight (Figure 6A) and both WT and UCP1-KO mice became heavier, increasing their weight to a similar degree over the next 21 days of normal feed and housing. To confirm the contribution of brown adipose tissue UCP1 activity to the regulation of body temperature following a cold stress, we compared rectal temperatures in UCP1-KO mice to those of UCP1-replete WT controls. The average resting rectal temperature of UCP1-KO mice did not differ from WT controls (Figure 6B), consistent with the inactivity of brown adipose activity during thermoregulation at rest. However, temperatures of UCP1-KO mice taken immediately after the forced swim were lower than WT controls, indicating a greater ability of mice with UCP1 to defend against the cold than those whose brown adipose function is deficient due to the deletion of the UCP1 gene (Figure 6B). This confirms that the stress-induced activation of brown adipose in WT mice is incompletely compensated for in UCP1-KO mice. Differences in temperature regulation did not appear to influence thermal nociception as the tail flick latency in UCP1-KO mice did not differ from that of WT mice. (KO: mean=3.96±0.42 sec, n=6; WT mean=4.08±0.40 sec, n=6, 2-tailed, unpaired Student's t-test, P=0.840)

4. Discussion

Body temperature and pain are both sensitive to stress, perhaps due to overlapping patterns of innervation along thermoregulatory and nociceptive pathways [12; 17]. We theorized that a relationship exists between brown adipose and stress-induced musculoskeletal pain [23; 28; 33] based on the fact that stress activates brown adipose via sympathetic nerves [29] that have collaterals projecting to surrounding muscle [47]. The present study supports this hypothesis as musculoskeletal hyperalgesia is produced by a forced swim stress, as previously reported [2], is replicated in Swiss-Webster mice by β_3 adrenergic receptor activity, and it is attenuated by ablation of a large depot of brown adipose tissue, by β_3 adrenergic receptor antagonism and by the elimination of the UCP1 gene expression in UCP1-KO mice. These findings suggest that development of stress-induced musculoskeletal hyperalgesia is dependent, in part, on activation of brown adipose or its associated neuronal pathways.

Brown adipose is found in many sites throughout the body, however, the interscapular depot is a major contributor to thermogenesis in rodents [7]. To assess the importance of brown adipose on muscle pain, we eliminated interscapular brown adipose tissue depot in mice using light-activated Rose Bengal, a model of tissue ablation successfully used to destroy the olfactory bulb in mice [22]. Ablation was deemed successful based on the lower weight of interscapular brown adipose compared to sham-operated controls after two weeks of daily swims. Chemical ablation had no effect on baseline grip force responses, confirming that brown adipose does not impact musculoskeletal nociceptive sensitivity in the absence of stress to initiate thermogenesis. It also establishes that possible damage to surrounding tissues during ablation does not impair behavioral responses associated with the nociceptive assay. However, ablation of this depot was sufficient to inhibit the hyperalgesia produced by a forced swim. The UCP1-gene is paramount to brown adipose tissue's thermogenic abilities [7]. Similar to ablation of a brown adipose depot, molecular deletion of the UCP1 gene necessary for thermogenesis had no effect on baseline grip force values in the absence of stress, however, knock out of this gene attenuated decreases in grip force caused by the forced swim. Together these two lines of evidence, produced using different strategies to interfere with brown adipose activity, converge to support the conclusion that swim stress-induced musculoskeletal hyperalgesia is dependent, in part, on intact and activated brown adipose pathways.

Injection of the β_3 adrenergic agonist BRL37443 mimicked the musculoskeletal hyperalgesic effect of forced swim and SR59230A, the β_3 adrenergic antagonist, blocked the hyperalgesic effect of the swim. This indicates that activation of β_3 adrenergic receptors is not only sufficient but also necessary to induce swim-induced musculoskeletal hyperalgesia. In addition to the dense localization of β_3 adrenergic receptors in brown adipose, these receptors are also located in gall bladder, urinary bladder, and brain, but absent in skeletal muscle [11; 27], eliminating an effect directly on muscles. The attenuation of BRL3744-induced hyperalgesia by prior ablation of the interscapular brown adipose depot strongly suggests that it is adrenergic activation specifically in brown adipose that is necessary for musculoskeletal hyperalgesia induced by β_3 adrenergic activity.

Decreases in grip force produced by BRL37443 and by swim stress are both reversed by morphine [1]. As morphine is incapable of enhancing strength but has potent antinociceptive activity, this indicates that decreases in grip force observed after either injection of BRL37443 or a swim stress were each due to musculoskeletal hyperalgesia and not muscular weakness.

If hyperalgesia were dependent on the magnitude of stress, the UCP1-KO mice would be predicted to be more hyperalgesic than WT mice as UCP1-deficient mice have an impaired ability to defend against the cold, as reflected in their lower body temperature following the swim. A lower body temperature would cause a greater stress, leading to a greater hyperalgesic responses. The lower body temperature observed in UCP1-KO mice following the swim cannot be attributed to differences in body sizes as their average weights did not differ from those of WT mice. Their resting body temperatures also did not differ from WT controls, consistent with previous studies [18]. However, WT mice were hyperalgesic following a swim despite their ability to maintain a higher body temperature during the

swim. This indicates that it is the presence of UCP1, which is localized primarily in brown adipose tissue, rather than the degree of stress that affects the magnitude of the hyperalgesic response to stress.

Many types of stress are capable of activating brown adipose tissue [4; 23; 28]. For example, repeated exposure of mice to cold increases blood flow, UCP1 content, and brown adipose tissue mass, changes that reflect adaptive thermogenesis [15]. Even exposure of rats to cold for just two days causes brown adipose tissue to hypertrophy [6; 9]. This is similar to the increased weight of interscapular brown adipose we observed after two weeks of daily swims. Although direct measurement of UCP1 or thermogenic activity would be necessary to reflect a change specifically in thermogenic function, the gradual increase in daily rectal temperature following the swim together with the increased weight of interscapular brown adipose suggest that our stress protocol is sufficient to activate brown adipose and may even initiate a degree of adaptive thermogenesis. Similar increases in interscapular brown adipose tissue weight after daily injections of BRL37443 likely reflect protracted activation of this tissue, similar to increases in brown adipose brought about by elevated circulating catecholamines associated with pheochromocytoma [23; 28].

The production of musculoskeletal hyperalgesia solely by injection of a β_3 adrenergic agonist in Swiss Webster mice and its elimination by a β_3 adrenergic antagonist provides evidence consistent with the concept that sympathetic activity plays a vital role in the modulation of chronic pain states [35; 48]. Although these compounds also act at other adrenergic receptors at high doses (BRL37344 at β_1 and β_2 ; SR59230A at α_1), the greater affinity of each for β_3 sites makes this their probable target. Rats exposed to sound stress develop mechanical hyperalgesia that is counteracted by prior sympathectomy [26]. Increases in sympathetic tone also aggravate chronic pain conditions like fibromyalgia and complex regional pain syndrome [5; 14; 33]. Although stress also activates the hypothalamic-pituitary-adrenocortical axis, administration of glucocorticoids fails to alleviate fibromyalgia pain [10]. Manipulation of glucocorticoids also fails to alter musculoskeletal hyperalgesia in mice produced by lipopolysaccharides, a chemical form of stress [24].

Although our findings implicate brown adipose in the generation of stress-induced musculoskeletal hyperalgesia in normal healthy mice, the exact mechanism by which this occurs is unclear. One possibility involves referred pain carried along axon collaterals that project to brown adipose as well as surrounding skeletal muscle [48]. These nerves may be either sympathetic or primary afferent C-fibers, as both innervate muscle as well as brown adipose [52] and both are associated with hyperalgesic conditions. Primary afferent C-fibers innervating brown adipose normally convey information regarding temperature. As a result, these primary afferent fibers decrease brown adipose activity by feeding back and inhibiting thermogenesis [54]. Desensitization of primary afferent fibers expressing TRPV1 sites, by injection of resiniferatoxin, causes a protracted (30 days) musculoskeletal hyperalgesia in mice at the same time that it causes a protracted thermal antinociception [1]. It's implausible that this hyperalgesia results from an action on primary afferent C-fibers projecting to muscle as these fibers are not dense and their desensitization would more likely result in antinociception. However, desensitization of primary afferent C-fibers projecting to brown

adipose would be expected to interfere with feedback inhibition and increase sympathetic activity in response to stress in brown adipose as well as surrounding muscle. Indeed, impaired small diameter primary afferent fibers have been reported in skin biopsies from patients with fibromyalgia syndrome [51]. Further studies are required to determine whether primary afferent C-fibers innervating brown adipose is similarly impaired.

Summary

Our data confirm that musculoskeletal hyperalgesia, as measured using the grip force assay in mice, can be transiently induced by either a forced swim stress or the activation of β_3 adrenergic receptors. Swim stress-induced hyperalgesia is attenuated by either prior ablation of brown adipose tissue or by elimination of UCP1 enzymatic activity. While brown adipose is typically associated with thermogenesis, our data provide evidence that brown adipose or its associated nerves support musculoskeletal hyperalgesia induced by stress. We speculate that this system may be linked to stress-induced increases in musculoskeletal pain in a variety of painful human conditions. For example, nerves projecting to brown adipose also project collaterals to regions surrounding tender points of fibromyalgia, primarily in supraclavicular regions, but also in supra-axial, peri-renal and subcutaneous areas. This anatomical overlap may provide collateral innervation of tissue adjacent to brown adipose. Consistent with this, the temperature is lower in skin above tender points [21], suggesting vasoconstriction. Future studies designed to identify the relevant fibers as either sympathetic nerves or primary afferent neurons from brown adipose and surrounding tissues may provide us with a novel target to treat muscle pain like that in patients with fibromyalgia.

Acknowledgments

This work was supported by a grant from NIH from the National Institutes on Arthritis and Musculoskeletal and Skin Diseases [AR056092] and support from the College of Pharmacy and College of Veterinary Medicine at the University of Minnesota.

References

1. Abdelhamid RE, Kovacs KJ, Honda CN, Nunez MG, Larson AA. Resiniferatoxin (RTX) causes a uniquely protracted musculoskeletal hyperalgesia in mice by activation of TRPV1 receptors. *J Pain*. 2013; 14(12):1629–1641. [PubMed: 24188863]
2. Abdelhamid RE, Kovacs KJ, Pasley JD, Nunez MG, Larson AA. Forced swim-induced musculoskeletal hyperalgesia is mediated by CRF2 receptors but not by TRPV1 receptors. *Neuropharmacology*. 2013; 72:29–37. [PubMed: 23624287]
3. Bexis S, Docherty JR. Role of alpha 1- and beta 3-adrenoceptors in the modulation by SR59230A of the effects of MDMA on body temperature in the mouse. *Br J Pharmacol*. 2009; 158(1):259–266. [PubMed: 19422394]
4. Brooks SL, Neville AM, Rothwell NJ, Stock MJ, Wilson S. Sympathetic activation of brown-adipose-tissue thermogenesis in cachexia. *Biosci Rep*. 1981; 1(6):509–517. [PubMed: 7295902]
5. Bruhl S. An update on the pathophysiology of complex regional pain syndrome. *Anesthesiology*. 2010; 113(3):713–725. [PubMed: 20693883]
6. Bukowiecki L, Collet AJ, Follea N, Guay G, Jahjah L. Brown adipose tissue hyperplasia: a fundamental mechanism of adaptation to cold and hyperphagia. *Am J Physiol*. 1982; 242(6):E353–359. [PubMed: 6953766]
7. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev*. 2004; 84(1):277–359. [PubMed: 14715917]

8. Cawthorne MA, Sennitt MV, Arch JR, Smith SA. BRL 35135, a potent and selective atypical beta-adrenoceptor agonist. *Am J Clin Nutr.* 1992; 55(1 Suppl):252S–257S. [PubMed: 1345889]
9. Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, Wang S, Fortier M, Greenberg AS, Obin MS. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *J Lipid Res.* 2005; 46(11):2347–2355. [PubMed: 16150820]
10. Clark S, Tindall E, Bennett RM. A double blind crossover trial of prednisone versus placebo in the treatment of fibrositis. *J Rheumatol.* 1985; 12(5):980–983. [PubMed: 3910836]
11. Coman OA, Paunescu H, Ghita I, Coman L, Badararu A, Fulga I. Beta 3 adrenergic receptors: molecular, histological, functional and pharmacological approaches. *Rom J Morphol Embryol.* 2009; 50(2):169–179. [PubMed: 19434307]
12. Craig AD, Andrew D. Responses of spinothalamic lamina I neurons to repeated brief contact heat stimulation in the cat. *J Neurophysiol.* 2002; 87(4):1902–1914. [PubMed: 11929910]
13. da Silva Torres IL, Cucco SN, Bassani M, Duarte MS, Silveira PP, Vasconcellos AP, Tabajara AS, Dantas G, Fontella FU, Dalmaz C, Ferreira MB. Long-lasting delayed hyperalgesia after chronic restraint stress in rats-effect of morphine administration. *Neurosci Res.* 2003; 45(3):277–283. [PubMed: 12631463]
14. Drummond PD, Finch PM. Persistence of pain induced by startle and forehead cooling after sympathetic blockade in patients with complex regional pain syndrome. *J Neurol Neurosurg Psychiatry.* 2004; 75(1):98–102. [PubMed: 14707316]
15. Fellenz M, Triandafillou J, Gwilliam C, Himms-Hagen J. Growth of interscapular brown adipose tissue in cold-acclimated hypophysectomized rats maintained on thyroxine and corticosterone. *Can J Biochem.* 1982; 60(8):838–842. [PubMed: 7127189]
16. Halter JB, Pflug AE, Porte D Jr. Mechanism of plasma catecholamine increases during surgical stress in man. *J Clin Endocrinol Metab.* 1977; 45(5):936–944. [PubMed: 925142]
17. Hayes NL, Rustioni A. Spinothalamic and spinomedullary neurons in macaques: a single and double retrograde tracer study. *Neuroscience.* 1980; 5(5):861–874. [PubMed: 7413086]
18. Hofmann WE, Liu X, Bearden CM, Harper ME, Kozak LP. Effects of genetic background on thermoregulation and fatty acid-induced uncoupling of mitochondria in UCP1-deficient mice. *J Biol Chem.* 2001; 276(15):12460–12465. [PubMed: 11279075]
19. Imbe H, Iwai-Liao Y, Senba E. Stress-induced hyperalgesia: animal models and putative mechanisms. *Front Biosci.* 2006; 11:2179–2192. [PubMed: 16720304]
20. Janig W, Baron R. Complex regional pain syndrome is a disease of the central nervous system. *Clin Auton Res.* 2002; 12(3):150–164. [PubMed: 12269546]
21. Jeschonnek M, Grohmann G, Hein G, Sprott H. Abnormal microcirculation and temperature in skin above tender points in patients with fibromyalgia. *Rheumatology (Oxford).* 2000; 39(8):917–921. [PubMed: 10952750]
22. Kang HM, Jin J, Lee S, Ryu J, Park C. A novel method for olfactory bulbectomy using photochemically induced lesion. *Neuroreport.* 2010; 21(3):179–184. [PubMed: 20042896]
23. Kataoka N, Hioki H, Kaneko T, Nakamura K. Psychological stress activates a dorsomedial hypothalamus-medullary raphe circuit driving brown adipose tissue thermogenesis and hyperthermia. *Cell Metab.* 2014; 20(2):346–358. [PubMed: 24981837]
24. Kehl LJ, Kovacs KJ, Larson AA. Tolerance develops to the effect of lipopolysaccharides on movement-evoked hyperalgesia when administered chronically by a systemic but not an intrathecal route. *Pain.* 2004; 111(1-2):104–115. [PubMed: 15327814]
25. Kehl LJ, Trempe TM, Hargreaves KM. A new animal model for assessing mechanisms and management of muscle hyperalgesia. *Pain.* 2000; 85(3):333–343. [PubMed: 10781907]
26. Khasar SG, Green PG, Levine JD. Repeated sound stress enhances inflammatory pain in the rat. *Pain.* 2005; 116(1-2):79–86. [PubMed: 15936144]
27. Krief S, Lonqvist F, Raimbault S, Baude B, Van Spronsen A, Arner P, Strosberg AD, Ricquier D, Emorine LJ. Tissue distribution of beta 3-adrenergic receptor mRNA in man. *J Clin Invest.* 1993; 91(1):344–349. [PubMed: 8380813]
28. Kuji I, Imabayashi E, Minagawa A, Matsuda H, Miyauchi T. Brown adipose tissue demonstrating intense FDG uptake in a patient with mediastinal pheochromocytoma. *Ann Nucl Med.* 2008; 22(3):231–235. [PubMed: 18498040]

29. Larson AA, Pardo JV, Pasley JD. Review of overlap between thermoregulation and pain modulation in fibromyalgia. *Clin J Pain*. 2014; 30(6):544–555. [PubMed: 23887348]
30. Leblais V, Pourageaud F, Ivorra MD, Guibert C, Marthan R, Muller B. Role of alpha-adrenergic receptors in the effect of the beta-adrenergic receptor ligands, CGP 12177, bupranolol, and SR 59230A, on the contraction of rat intrapulmonary artery. *J Pharmacol Exp Ther*. 2004; 309(1):137–145. [PubMed: 14718590]
31. Lofgren M, Norrbrink C. Pain relief in women with fibromyalgia: a cross-over study of superficial warmth stimulation and transcutaneous electrical nerve stimulation. *J Rehabil Med*. 2009; 41(7): 557–562. [PubMed: 19543667]
32. Lopez M, Varela L, Vazquez MJ, Rodriguez-Cuenca S, Gonzalez CR, Velagapudi VR, Morgan DA, Schoenmakers E, Agassandian K, Lage R, Martinez de Morentin PB, Tovar S, Nogueiras R, Carling D, Lelliott C, Gallego R, Oresic M, Chatterjee K, Saha AK, Rahmouni K, Dieguez C, Vidal-Puig A. Hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance. *Nat Med*. 2010; 16(9):1001–1008. [PubMed: 20802499]
33. Martinez-Lavin M, Vidal M, Barbosa RE, Pineda C, Casanova JM, Nava A. Norepinephrine-evoked pain in fibromyalgia. A randomized pilot study [ISRCTN70707830]. *BMC Musculoskelet Disord*. 2002; 3:2. [PubMed: 11860612]
34. Matthias A, Ohlson KB, Fredriksson JM, Jacobsson A, Nedergaard J, Cannon B. Thermogenic responses in brown fat cells are fully UCP1-dependent. UCP2 or UCP3 do not substitute for UCP1 in adrenergically or fatty acid-induced thermogenesis. *J Biol Chem*. 2000; 275(33):25073–25081. [PubMed: 10825155]
35. McMahon SB. Mechanisms of sympathetic pain. *Br Med Bull*. 1991; 47(3):584–600. [PubMed: 1794073]
36. Nedergaard J, Golozoubova V, Matthias A, Asadi A, Jacobsson A, Cannon B. UCP1: the only protein able to mediate adaptive non-shivering thermogenesis and metabolic inefficiency. *Biochim Biophys Acta*. 2001; 1504(1):82–106. [PubMed: 11239487]
37. Nishiyori M, Ueda H. Prolonged gabapentin analgesia in an experimental mouse model of fibromyalgia. *Mol Pain*. 2008; 4:52. [PubMed: 18990235]
38. Nisoli E, Tonello C, Landi M, Carruba MO. Functional studies of the first selective beta-3-adrenergic receptor antagonist SR 59230A in rat brown adipocytes. *Mol Pharmacol*. 1996; 49(1): 7–14. [PubMed: 8569714]
39. Oriowo MA, Chapman H, Kirkham DM, Sennitt MV, Ruffolo RR Jr, Cawthorne MA. The selectivity in vitro of the stereoisomers of the beta-3 adrenoceptor agonist BRL 37344. *J Pharmacol Exp Ther*. 1996; 277(1):22–27. [PubMed: 8613923]
40. Pertovaara A. Noradrenergic pain modulation. *Prog Neurobiol*. 2006; 80(2):53–83. [PubMed: 17030082]
41. Porsolt RD. Animal model of depression. *Biomedicine*. 1979; 30(3):139–140. [PubMed: 573643]
42. Porsolt RD, Bertin A, Blavet N, Deniel M, Jalfre M. Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity. *Eur J Pharmacol*. 1979; 57(2-3):201–210. [PubMed: 488159]
43. Porsolt RD, Deniel M, Jalfre M. Forced swimming in rats: hypothermia, immobility and the effects of imipramine. *Eur J Pharmacol*. 1979; 57(4):431–436. [PubMed: 258612]
44. Quintero L, Moreno M, Avila C, Arcaya J, Maixner W, Suarez-Roca H. Long-lasting delayed hyperalgesia after subchronic swim stress. *Pharmacol Biochem Behav*. 2000; 67(3):449–458. [PubMed: 11164071]
45. Ryu V, Garretson JT, Liu Y, Vaughan CH, Bartness TJ. Brown adipose tissue has sympathetic-sensory feedback circuits. *J Neurosci*. 2015; 35(5):2181–2190. [PubMed: 25653373]
46. Sarzi-Putini P, Atzeni F, Diana A, Doria A, Furlan R. Increased neural sympathetic activation in fibromyalgia syndrome. *Ann N Y Acad Sci*. 2006; 1069:109–117. [PubMed: 16855138]
47. Sbarbati A, Cavallini I, Marzola P, Nicolato E, Osculati F. Contrast-enhanced MRI of brown adipose tissue after pharmacological stimulation. *Magn Reson Med*. 2006; 55(4):715–718. [PubMed: 16506160]
48. Sinclair DC, Weddell G, Feindel WH. Referred pain and associated phenomena. *Brain*. 1948; 71(2):184–211. [PubMed: 18890914]

49. Suarez-Roca H, Quintero L, Arcaya JL, Maixner W, Rao SG. Stress-induced muscle and cutaneous hyperalgesia: differential effect of milnacipran. *Physiol Behav.* 2006; 88(1-2):82–87. [PubMed: 16624346]
50. Suarez-Roca H, Silva JA, Arcaya JL, Quintero L, Maixner W, Pinerua-Shuhaibar L. Role of mu-opioid and NMDA receptors in the development and maintenance of repeated swim stress-induced thermal hyperalgesia. *Behav Brain Res.* 2006; 167(2):205–211. [PubMed: 16214233]
51. Uceyler N, Zeller D, Kahn AK, Kewenig S, Kittel-Schneider S, Schmid A, Casanova-Molla J, Reiners K, Sommer C. Small fibre pathology in patients with fibromyalgia syndrome. *Brain.* 2013; 136(Pt 6):1857–1867. [PubMed: 23474848]
52. Vallbo AB, Hagbarth KE, Torebjork HE, Wallin BG. Somatosensory, proprioceptive, and sympathetic activity in human peripheral nerves. *Physiol Rev.* 1979; 59(4):919–957. [PubMed: 227005]
53. Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL, Tugwell P, Campbell SM, Abeles M, Clark P, et al. The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. *Arthritis Rheum.* 1990; 33(2):160–172. [PubMed: 2306288]
54. Yoneshiro T, Aita S, Kawai Y, Iwanaga T, Saito M. Nonpungent capsaicin analogs (capsinoids) increase energy expenditure through the activation of brown adipose tissue in humans. *Am J Clin Nutr.* 2012; 95(4):845–850. [PubMed: 22378725]
55. Zhang S, Kluge B, Huang F, Nordstrom T, Doolen S, Gross M, Sarmiere P, Holmberg EG. Photochemical scar ablation in chronically contused spinal cord of rat. *J Neurotrauma.* 2007; 24(2):411–420. [PubMed: 17376003]

Figure 1.

Summary of the effect of swim stress (Swim) or injection of BRL37344 (BRL), a β_3 adrenergic receptor agonist, on the average change in grip force responses ($\pm$ SEM) compared to mice that did not swim (C) or were injected with vehicle (Veh). Mice were also forced to swim (panel A) or injected with BRL (panel B) following ablation of interscapular brown adipose tissue (Abl) compared to mice that were sham-operated (Sham). All mice were Swiss Webster except those used to determine the effect of a forced swim (panel A) or injection of BRL37344 (B) in C57BL/6J mice not expressing the UCP1 gene (KO) or UCP1 replete controls (WT). Grip force values were taken before (designated a zero) and immediately (within 5 min) after forced swims that lasted for 15 min in water maintained at 26°C. BRL37344 was injected s.c. at a dose of 1 mg/kg in Swiss Webster mice and 10 mg/kg in UCP1-KO and WT C57BL/6J mice. Brown adipose tissue was chemically ablated by injection of Rose Bengal combined with high intensity illumination of the interscapular brown adipose depot two weeks prior to testing. Control mice were also injected with Rose Bengal but not illuminated. Grip force values were taken 24 h after the injection of BRL37344 in ablated/sham mice and 60 min after the injection BRL67344 in the WT and UCP1-KO mice. In all figures, asterisks (*) depict statistically significant differences between the groups indicated ($P < 0.05$) using a two-tailed unpaired Student's t-test to compare grip force responses. The hashtag (#) designates a group that only differed

significantly ($P < 0.05$) from its own baseline control value taken on the same day, when evaluated using a two-tailed, paired Student's t-test. The number of mice/group is indicated at the base of each column.

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Figure 2.

Effect of a daily forced swim on mean ($\pm$ SEM) grip force responses, rectal temperature and interscapular brown adipose tissue (BAT) weight. The forced swim lasted for 15 min in water maintained at 26°C. Grip force values (A) and rectal temperatures (B) of Swiss Webster mice were taken immediately (within 5 min) after each daily swim and compared to mice not subjected to a swim stress. The weight of interscapular BAT (C) was measured in both groups 24 h after the final swim. An unpaired, two-tailed Student's t-test was used to compare the effect of a daily forced swim on grip force responses and rectal temperatures to mice not exposed to the daily swim on the same day of testing. In all figures, asterisks indicate statistically significant differences between mice exposed to a swim and control mice when $P < 0.05$. Throughout all figures, SEM is calculated for all points but not shown graphically where it is smaller than the size of the symbol depicting the mean. The number of female mice/group is indicated in the key and at the bottom of each column in panel C.

Figure 3.

Effect of a daily injection of BRL37344, a β_3 adrenergic receptor agonist, on mean ($\pm$ SEM) grip force responses, rectal temperature and interscapular brown adipose tissue (BAT) weight. Grip force values (A and B) and rectal temperatures (C) of Swiss Webster mice were taken 60 min (A) or 24 h (B) after each daily injection of BRL37344 (1 mg/kg daily s.c. for 15 days) and compared to mice injected with vehicle. Rectal temperature was taken 15 min and 24 h after each injection (C). The weight of interscapular BAT (D) was measured in both groups 24 h after the final daily injection. An unpaired, two-tailed Student's t-test was used to compare the effect of BRL37344 to that of vehicle on daily grip force responses, rectal temperatures 15 min after injection, rectal temperatures 24 h after injection, and BAT weights. Asterisks indicate statistically significant differences between groups when $P < 0.05$. SEM is calculated for all points but not shown graphically where it is smaller than the size of the symbol depicting the mean. The number of female mice/group for all panels is indicated at the bottom of each column in panel D.

Figure 4.

Effect of morphine on the mean ($\pm$ SEM) decrease in grip force responses induced by BRL37344. Swiss Webster mice were injected s.c. with either vehicle or 1 mg/kg daily for 3 days, 3 mg/kg daily for 3 days, and 10 mg/kg of BRL37344 daily for 2 days followed 4 h later by an additional injection i.p. of 10 mg/kg of morphine or saline. Values represent the mean ($\pm$ SEM) grip force responses before (Vehicle or BRL37344 only) and 30 min after injection of morphine or saline, as indicated in the legend. Asterisks indicates a significant difference ($P < 0.05$) between the treatments indicated as determined using one-way ANOVA followed by the Newman-Keuls post hoc analysis. The number of female mice/group is indicated at the bottom of each column.

Figure 5.

Comparison of the effect of chemical ablation of the interscapular brown adipose depot on the mean ($\pm$ SEM) grip force responses and brown adipose tissue weights after 14 daily forced swims or 14 daily injections of BRL37344. Two weeks prior to swims or injections, brown adipose tissue was chemically ablated by injection of Rose Bengal combined with high intensity illumination of the BAT depot, as described in detail in methods. Control mice were also injected with Rose Bengal but not illuminated (Sham). Panels A and D reflect data from mice that were subjected to a 15-min forced swim in water maintained at 26°C. Panels B, C and E reflect data from mice who were injected s.c. daily with 1 mg/kg of BRL37344. Grip force measurements were taken before and 0 min after a forced swim (A) or 60 min after each injection of BRL37344 (B). Brown adipose tissue was weighed in mice 24 h after the final swim (D) or final injection of BRL37344 (E). Panel C depicts the response of mice (used in panel B) to a forced swim 1 day after the last daily injection of BRL37344 and 15 min after an additional s.c. injection of SR59230A, a β_3 adrenergic receptor antagonist, or vehicle. The dotted line indicates the average baseline grip force prior to injection of SR59230A. An unpaired, two-tailed Student's t-test was used to compare the effect of a daily swim or a daily injection in BAT-ablated mice to sham-operated mice tested on the same day. Asterisks indicate statistically significant differences between the two groups of mice when $P < 0.05$. The number of male mice/group is indicated at the bottom of each column in panels D and E. The number of mice/group for panel A is the same as that for panel D, and the number for panel B is the same as that for panel E.

Figure 6.

Effect of a daily forced swim on the rectal temperatures of UCP1-KO and wild type (WT) C57BL/6J mice before (Baseline) and immediately after the daily swim and on their body weights throughout the study. Values in panel A reflect mean ($\pm$ SEM) rectal temperatures at baseline compared to that immediately after a 15-min forced swim (at 26°C). The values in panel B represent the mean ($\pm$ SEM) body weights when mice arrived (day 1) and at the end of the study (day 33). An unpaired, two-tailed Student's t-test was used to compare temperatures between the two groups. A paired two-tailed Student's t-test was used to compare changes in these values due to swim within each group. Asterisks indicate statistically significant differences between the two groups indicated when $P < 0.05$. The number of female mice/group is indicated at the bottom of each column.