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After a cold conditioning swim, UCP2-deficient mice are more able to defend against the cold than wild type mice

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Abstract

Uncoupling protein 2 (UCP2) is widely distributed throughout the body including the brain, adipose tissue and skeletal muscles. In contrast to UCP1, UCP2 does not influence resting body temperature and UCP2-deficient (-/-) mice have normal thermoregulatory responses to a single exposure to cold ambient temperatures. Instead, UCP2-deficient mice are more anxious, exhibit anhedonia and have higher circulating corticosterone than wild type mice. To test the possible role of UCP2 in depressive behavior we exposed UCP2-deficient and wild type mice to a cold (26°C) forced swim and simultaneously measured rectal temperatures during and after the swim. The time that UCP2-deficient mice spent immobile did not differ from wild type mice and all mice floated more on day 2. However, UCP2-deficient mice were more able to defend against the decrease in body temperature during a second daily swim at 26°C than wild type mice (area under the curve for wild type mice: 247.0 ± 6.4 ; for UCP2-deficient mice: 284.4 ± 3.8 , $P < 0.0001$, Student's t test). The improved thermoregulation of wild type mice during a second swim at 26°C correlated with their greater immobility whereas defense against the warmth during a swim at 41°C correlated better with greater immobility of UCP2-deficient mice. Together these data indicate that while the lack of UCP2 has no acute effect on body temperature, UCP2 may inhibit rapid improvements in defense against cold, in contrast to UCP1, whose main function is to promote thermogenesis.

Keywords

Uncoupling protein 2; Thermoregulation; Acclimation; Forced swim test; UCP2-deficient; Corticosterone

1. Introduction

Uncoupling protein 2 (UCP2), a member of the mitochondrial uncoupling protein family, protects cells from excess reactive oxygen species (ROS) produced by the electron transport

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Competing interests

The authors declare that they have no conflict of interest.

chain [1]. In contrast to UCP1 that is found almost exclusively in brown adipose tissue [2], UCP2 is widely distributed throughout the body including the pancreas [3], spleen, kidney [4], immune cells [5], brain [6], and the CNS [7]. This distribution has implicated UCP2 in multiple pathologies including diabetes [8], Parkinson's disease [9], cancer [10, 11], atherosclerosis [12], anxiety [13] and depression [14].

UCP2 is found in brain regions that control stress and neuroendocrine function, including the paraventricular hypothalamic nucleus. UCP2-deficient mice are more anxious than wild type mice in the elevated plus maze [13] in response to defeat stress [13]. UCP2-deficient mice exhibit more anhedonia, measured using the glucose preference test, and have higher concentrations of circulating corticosterone than wild type mice when subjected to chronic mild stress [14]. UCP2-deficient mice have an increased turnover of brain dopamine and serotonin [13], two neurotransmitters involved in depression [15, 16] as well as anxiety [17, 18]. To our knowledge no studies have examined the effect of the UCP2 deficiency on depressive behavior in the forced swim test, one of the most widely used assays of depressive behavior. Therefore we measured immobility during the forced swim test by comparing the responses of UCP2-deficient mice to those of wild type controls.

UCP2 is not thought to have a role in thermogenesis as the body temperature of UCP2-deficient mice is unchanged during a single cold exposure [4, 19-21]. Yet stressed mice have an increased concentration of UCP2 in the hypothalamus [14], a region of the brain important in thermogenesis and temperature regulation [22]. In many animal models of depression, including chronic mild stress: [5,6]; olfactory bulbectomy: [7]; after high doses of lipopolysaccharides: [8-11]; tail suspension test: [12], depressive-like behavior occurs coincident with changes in body temperature. We therefore also measured rectal temperature as well as immobility during and after 2 daily forced swims to determine whether stress-induced increases in UCP2 contribute to longer-term changes in thermoregulation or depressive behavior brought about by a conditioning swim.

2. Methods & materials

2.1. Animals

Adult male and female wild type C57BL/6J (WT) and UCP2-deficient mice (3 breeding pairs, previously cryopreserved), also known as UCP2-knockout (KO) mice (Jackson Laboratories; Bar Harbor, MA) weighing 20-25 g were housed four per cage (males) or five per cage (females) and allowed to acclimate for at least one week prior to use. The homozygote UCP2-deficient mice were on a pure C57BL6/J background, similar to the WT mice. All UCP2-deficient mice were bred in our animal facility and all offspring were used in the experiments.

Mice were allowed free access to food and water, and housed in a room with a constant temperature of 23°C on a 12-h light–dark cycle. Every group of mice was used only once. All experimental procedures and measurements were done blinded to the treatment. This study was performed according to the guidelines of the International Association for the Study of Pain, the University of Minnesota Animal Care and Use Committee, and the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal

Resources, National Research Council (DHEW Publication NIH 78-23, revised 1995). All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available.

2.2. Body temperature measurement

Rectal temperatures were measured as a reflection of body temperature, and for ease in writing, significant decreases in rectal temperature during a cold swim are expressed as hypothermia. Rectal temperatures were routinely taken in a room with an ambient temperature of 25°C. The animal was placed under a clean towel to create a barrier on three sides, and its colonic temperature was measured using a rectal thermometer (ETI Microtherma 2K Thermometer connected to a RET-3 rectal probe), as previously described [23-25].

2.3. Forced swim test

The forced swim test is one of the most commonly used assays of depressant-like activity in rodents, originally described by Porsolt [26-28]. Each mouse was placed individually into a large, 3 L beaker (diameter: 15 cm, height: 20 cm) containing water maintained at 26°C or 41°C. Mice were forced to swim for 15 min for two consecutive days. The water level was deep enough (18 cm) so the tail of the mouse never touched the bottom. The first 5 min of each 15-min swim was recorded using a Flip (Cisco) or Handycam (Sony) video camera. After the swim, mice were removed from the water and their rectal temperature measured, after which time mice were towed dry and returned to their home cage. The amount of time spent immobile, including times during which small paw movements helped the mouse stay afloat rather than propel it forward, was subsequently evaluated by observers unaware of the treatment of each mouse.

Immobility was counted during the first 5 min as immobility did not increase significantly beyond that time. However, swimming the full 15 min on day 1 was important for its conditioning effect on immobility measured the next day. When the mice are tested again 24 h later, floating times are immediately increased, consistent with the previously reported potentiative effect of conditioning swim on immobility [29]. Fifteen-min conditioning swims on day one are frequently used [26-29] and we previously reported depressive-like behavioral data using that experimental design [24].

2.4. Corticosterone assay

Twenty-four h after the first daily swim, mice were decapitated and trunk blood collected for analysis of corticosterone. Another group of mice was similarly killed for blood collection 24 hr after their second daily swim. Blood was collected in plastic tubes containing EDTA on ice until samples were centrifuged (2500 g) for 30 min at 4°C, and the plasma was stored at -20°C until assayed [30]. Corticosterone was assayed in duplicate samples of mouse plasma using the ImmuChem™ Double Antibody Corticosterone 125I RIA kit from ICN Biochemicals Inc. (Costa Mesa, CA).

2.5. Statistical analysis

Data were expressed as mean $\pm$ SEM. Paired Student's t-test was used to compare values on day 1 to those on day 2 within the same group of mice, while unpaired Student's t-test was used to compare values of different treatment groups on the same day. When correlations between changes in rectal temperature and changes in immobility times were analyzed linear regression analysis was used to determine P values and construct the line of best-fit. Statistical analysis was performed using a two-way analysis of variance (ANOVA) followed by Bonferroni's post hoc analysis when 2 groups were compared at the same time point and one-way ANOVA followed by Bonferroni's post hoc analysis was used to compare different time points in the same treatment group. In all cases, a cutoff of $P < 0.05$ was used to establish statistical significance. The number of mice/experimental group is given on the figures on the bottom of the columns in bar graphs or following the group name on the line graphs.

3. Results

3.1. The effect of UCP2 deficiency on forced swim-induced immobility

There was no difference in the time that UCP2-deficient mice spent immobile compared to wild type controls, during either the first or the second daily swim at 26°C. In addition, all mice floated longer during the second daily swim than they did during the first swim (Fig. 1).

3.2. The effect of UCP2 deficiency on thermogenesis during cold and warm swims

When exposed to a swim in a 26°C bath, the rectal temperature of mice decreased during the entire time they were submerged and was lowest at the end of the swim (Fig. 2A,C). After mice were removed from the water, their rectal temperatures recovered gradually and were completely restored to values no different than pre-swim temperatures by 30 min after the swim (Fig. 2A). Rectal temperatures of wild type and UCP2-deficient mice did not differ from each other during the first swim in a 26°C bath (Fig. 2A), consistent with previous studies [4, 19-21]. On the second day, rectal temperatures of UCP2-deficient mice ($33.75 \pm 0.16^\circ\text{C}$, $n=14$) did not decrease during the swim as much as those of wild type mice ($32.6 \pm 0.19^\circ\text{C}$, $n=15$) and recovery, to values that did not differ from those prior to the swim, was faster in UCP2-deficient mice (15 min) than in the wild type controls (30 min) (Fig. 2C). On day 2, the whole 'hypothermic' area under the curve was calculated and confirmed that the UCP2-deficient mice have an improved defense against the cold (wild type: 247.0 ± 6.4 ; UCP2-deficient: 284.4 ± 3.8 , $P < 0.0001$, Student's t test). Together these data indicate that UCP2-deficient mice adapted more readily to the decrease of the body temperature than wild type mice, but only when tested 24 h after a conditioning swim.

To determine whether (the lack of UCP2 protein) UCP2 is involved in the adaptation to any other changes in rectal temperature, we also tested additional mice during a warm (41°C) swim. The mean rectal temperature of mice increased significantly during the warm swim (Fig. 2B,D), but after mice were removed from the water their rectal temperatures dropped below baseline and reached their lowest point 5 min after the swim. On both days mice gradually recovered from this hypothermia but even 30 min after the swim, they did not

reach their pre-swim temperatures (Fig. 2B,D). In contrast to the swim at 26°C, there was no difference in rectal temperatures between wild type and UCP2-deficient mice at any point during or after the swim at 41°C on either day (Fig. 2B,D).

3.3. Immobility of mice during warm swims

Similar to the swim at 26°C, there was no difference in the duration of immobility of UCP2-deficient mice and wild type controls during either the first or the second daily swim at 41°C. All mice spent more time floating during the second daily swim than they did on the first day of swim (Fig. 3).

3.4. Relationship between immobility and rectal temperature

In many animal models of depression the depressive-like behavior is coincident with changes in body temperature or thermoregulation [31-33]. To examine the relationship between rectal temperature at the end of the swim and immobility during the swim we examined the correlation coefficients between rectal temperature and immobility of mice during each daily swim, but found no significant relationship between these data (data not shown). We then compared the changes in rectal temperatures after each swim (from day 1 to day 2) to the corresponding changes in immobility (between day 1 and day 2). In wild type mice, there was a positive correlation between their increased ability to defend against cold (26°C) and the increase in immobility (Fig. 4A). In contrast, the same correlation was not statistically significant in UCP2-deficient mice (Fig. 4C). After 2 daily warm swims (41°C), an increased ability to defend against heat (less increase in rectal temperatures during the second day warm swim) resulted in a negative correlation between immobility and rectal temperature in UCP2-deficient mice (Fig. 4D), but not in wild type mice (Fig. 4B).

3.5. Blood concentrations of corticosterone in wild type and UCP2-deficient mice

To determine whether the improved ability of UCP2-deficient mice to defend against cold during the second swim at 26°C is associated with different degrees of stress to the mice, we compared circulating concentrations of corticosterone, a stress hormone, in UCP2-deficient mice to that in wild type mice 24 h after each daily swims. Wild type and UCP2-deficient mice are known to have similar resting concentrations of circulating corticosterone [14, 34], however, we found that UCP2-deficient mice had a higher concentration of circulating corticosterone than wild type mice when measured 24 h after a swim at 26°C (Fig. 5B), coinciding temporally with a greater defense against cold in UCP2-deficient mice than in wild type mice (Fig. 5A).

4. Discussion

UCP2 is the only uncoupling mitochondrial protein expressed in the brain [6, 35, 36] where it is implicated in anxiety and depression. Because anxiety is a condition that is frequently, but not always comorbid with depression, it is useful to differentiate their mediation from each other. Relevant to a potential role in depression, UCP2-deficient mice exhibit anhedonia and have higher circulating corticosterone after 3 weeks of unpredictable chronic mild stress than wild type mice [13, 14]. To determine the role of UCP2 in a commonly used

measure of depressive behavior in rodents, we tested mice in the forced swim test but observed no difference between the duration of immobility of UCP2-deficient and wild type mice and all mice floated longer on day 2 than on day 1. Together these data indicate that UCP2 is not involved in the regulation of this acute depressive behavior and the potentiative effect of the conditioning swim was also unaffected by UCP2. Thus, UCP2 appears to be involved in depression only after chronic stress, as described previously [13, 14].

UCP2 is expressed in regions of the brain that are responsible for thermoregulation and thermogenesis [6, 35, 37, 38] where UCP2 produces heat by uncoupling brain mitochondria [38]. In support of this, the hypothalamus, a brain region rich in UCP2, has a higher temperature than surrounding brain regions and core body temperature [38]. In addition, mice designed to over express UCP2 in hypocretin-expressing neurons of the hypothalamus have elevated hypothalamic temperatures when compared to sham mice [37]. Yet in spite of this, the body temperatures of UCP2-deficient mice respond identically to a single ambient cold exposure (24 h at 5°C) as those of wild type mice [19], as indicated by a lack of difference in their rectal temperatures after a single swim. While this eliminates the possibility of a tonic influence on body temperature regulation, UCP2 might have a role in adaptive changes that influence challenges to thermoregulation. Specifically, hypothalamic neurons that are sensitive to a cold environment (16 h at 4°C) in the rat become even more densely innervated by UCP2-containing nerves when activated by a single cold exposure [38]. To determine whether exposure to conditioning swims at lower than normal body temperature alters subsequent responses to this challenge, we compared rectal temperature changes in response to two daily swims at 26°C in UCP2-deficient and wild type mice. We found that after a conditioning swim at 26°C, UCP2-deficient mice were less hypothermic (i.e. less of a decrease in rectal temperature) than wild type mice and the duration of their hypothermia after the swim was shorter. Together these data indicate that a single swim at 26°C primed UCP2-deficient mice to defend against the decrease in body temperature 24 h later more than it did the wild type mice. Rather than contributing to thermogenesis in a fashion similar to UCP1, it appears that the presence of UCP2 inhibits subsequent increases in thermogenesis.

Based on our results, lack of UCP2 protein promotes the ability to maintain body temperature in the face of cold, as changes in the ability to defend against the cold from day 1 to day 2 are much faster than in UCP2-expressing wild type mice. Thus, one might speculate that the role of UCP2 in temperature regulation may be to avoid radical changes in body temperature. When mice are exposed to a cold swim, their brain and body temperatures drop by 6-7 °C [39]. This decrease is detected by thermo-sensitive hypothalamic neurons that are activated to increase brain and body temperatures [40]. These activated neurons become innervated with numerous UCP2-containing axonal terminals that lead to an increase in local hypothalamic temperature [38]. This hyperthermia caused by UCP2 then attenuates whole body thermogenesis [37] during or in response to a second cold exposure. During this effort to maintain normal body temperatures, UCP2 appears to contribute more to restoration of normal body temperatures after cold than after warm swims. One caveat is that UCP2-deficient mice may develop adaptive changes to compensate for the deficiency of that enzyme.

Despite the similarity in immobility between UCP2-deficient and wild type mice, there was a positive correlation between enhanced depressive behavior from day 1 to day 2, and the enhanced ability to defend against the cold but not warmth over the same time interval in wild type mice. This correlation suggests that depressive behavior and defense against the cold are each regulated by mechanisms that involves UCP2 as similar correlations were not apparent in UCP2-deficient mice during cold swims. In contrast to the adaptation to decreases in body temperature during a swim at 26°C, there was a better correlation between the adaptation to heat during a warm (41°C) swim in UCP2- deficient mice than in wild type controls, as evidenced by the negative correlation between changes in immobility and changes in rectal temperature over this time interval. The significance of these relationships is not clear, one might speculate that they relate to the increased immobility and changes in thermoregulation observed in models of depression.

We found that the decreased hypothermia in UCP2-deficient mice after a second swim was coincident with an elevated concentration of blood corticosterone 24 h after the initial swim. Mice usually have only a transient increase in plasma corticosterone immediately following a cold swim [23], however, UCP2-deficient mice maintained an elevated concentration of corticosterone for 24 h after the conditioning swim. This suggests that corticosterone, released in response to the stress, is normally inhibited by increasing concentrations of UCP2, perhaps in a feedback inhibitory fashion. Corticosterone is known to inhibit stress-induced hyperthermia in rats [41, 42] by activating type II glucocorticoid receptors located on neuronal circuits between the hippocampus and hypothalamus [42], but the relationship between these two with respect to UCP2 is unclear.

5. Conclusions

In summary, lack of UCP2 protein does not affect immobility in the forced swim test, a test that is frequently used to quantify depressive behavior [26-28]. This suggests that although UCP2 may be important in anxiety-like behavior, it is not likely involved in depressive behavior in mice used for the screening of antidepressant compounds. More importantly, UCP2 might be involved in changes in thermogenesis that are triggered by at least one single conditioning cold exposure. The role of UCP2 may be to prevent excessive changes in defense against the cold, perhaps by feeding back and inhibiting changes that support adaptive thermogenesis.

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HIGHLIGHTS

- UCP2 deficiency has no effect on depressive behavior measured in the forced swim test
- UCP2-deficient mice defend their body temperature against cold better than wild type
- UCP2 may antagonize adaptive thermogenesis

Fig. 1. Mean ($\pm$ SEM) duration of immobility during two daily forced swims at 26°C

The mean time that wild type (WT) and UCP2-deficient (UCP2 KO) mice spent immobile during the first 5 min of the swim was measured on day 1 (D1) and day 2 (D2). The number of mice/group is indicated at the bottom of the columns. Statistically significant differences ($P < 0.05$) between testing days is marked using an asterisk.

Fig. 2. Mean ($\pm$ SEM) changes in rectal temperatures during 2 daily forced swims at 26°C (A,C) and 41°C (B,D)

Rectal temperature (°C) of wild type (WT) and UCP2-deficient (UCP2 KO) mice was measured during and after a 15-min forced swim using a rectal probe. The number of mice/group is indicated in the legend. Statistically significant differences ($P < 0.05$) between treatment groups at the same testing time is marked using an asterisk.

Fig. 3. Mean ($\pm$ SEM) duration of immobility during 2 daily forced swims at 41°C
 The mean time that wild type (WT) and UCP2-deficient (UCP2 KO) mice spent immobile during the first 5 min of the swim was measured on day 1 (D1) and day 2 (D2). The number of mice/group is indicated at the bottom of the columns. Statistically significant differences ($P < 0.05$) between testing days are marked using an asterisk.

Fig. 4. Correlation between changes in rectal temperature and changes in immobility between day 1 and day 2

The data represent the correlations between individual changes in rectal temperatures after each of two daily swims compared to the individual change in time spent immobile during each of two daily swims. Lines represent the linear regression of data derived from the experiments depicted in figures 1-3. Linear regression analysis was used to determine P values and construct the lines of best-fit. P values less than 0.05 (as in panels A and D) are taken as statistically significant, as indicated by an asterisk and by solid lines of best fit.

Fig. 5. Mean ($\pm$ SEM) rectal temperature (A) and resting concentration of circulating corticosterone (B) after 2 daily forced swims at 26°C

The rectal temperature (A) and resting concentration of corticosterone (B) of wild type (WT) and UCP2-deficient (UCP2 KO) mice was measured on day 1 (D1) and day 2 (D2). Rectal temperatures were taken immediately after each swim (A) and corticosterone was measured in trunk blood sampled at death 24 h after the swims (B) in water maintained at 26°C. A cutoff of $P < 0.05$ was used to establish statistical significance, as indicated by an asterisk. The number of mice/group is indicated at the bottom of each column.