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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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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.

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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.

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 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

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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], and 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 2 K 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 toweled 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-minute 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 hours 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 h 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 ImmuChemTM Double Antibody Corticosterone 125I RIA kit from ICN Biochemicals Inc. (Costa Mesa, CA).

2.5. Statistical analysis

Data were expressed as mean ± 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).

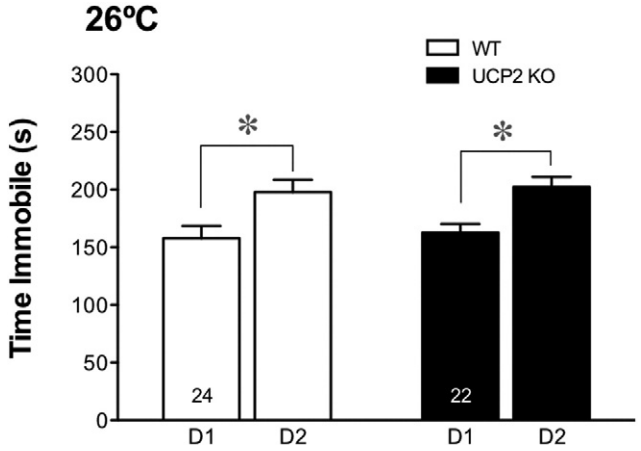

Fig. 1. Mean (±SEM) duration of immobility during two daily forced swims at 26 °C. The mean time that wild type (WT) and UCP2-deficient (UCP2 KO) mice spent immobile during the first 5 min of the swim was measured on day 1 (D1) and day 2 (D2). The number of mice/group is indicated at the bottom of the columns. Statistically significant differences ($P < 0.05$) between testing days are marked using an asterisk.