UCP1 is essential for adaptive adrenergic nonshivering thermogenesis

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Golozoubova, Valeria, Barbara Cannon, and Jan Nedergaard. UCP1 is essential for adaptive adrenergic nonshivering thermogenesis. Am J Physiol Endocrinol Metab 291: E350–E357, 2006. First published April 4, 2006; doi:10.1152/ajpendo.00387.2005.—Participation of brown adipose tissue [through the action of the uncoupling protein-1 (UCP1)] in adaptive adrenergic nonshivering thermogenesis is recognized, but the existence of a response to adrenergic stimulation in UCP1-ablated mice implies that a mechanism for an alternative adaptive adrenergic thermogenesis may exist. Here, we have used UCP1-ablated mice to examine the existence of an alternative adaptive adrenergic nonshivering thermogenesis, examined as the oxygen consumption response to systemically injected norepinephrine into anesthetized or conscious mice acclimated to different temperatures. We confirm that UCP1-dependent adrenergic nonshivering thermogenesis is adaptive, but we demonstrate that the adrenergic UCP1-independent thermogenesis is not recruitable by cold acclimation. Thus, at least in the mouse, no other proteins or enzymatic pathways exist that can participate in or with time take over the UCP1 mediation of adaptive adrenergic nonshivering thermogenesis, even in the total absence of UCP1. UCP1 is thus the only protein capable of mediating cold acclimation-recruited adaptive adrenergic nonshivering thermogenesis.

uncoupling protein-1; brown adipose tissue; norepinephrine; oxygen consumption; muscle thermogenesis; basal metabolic rate

MAMMALS ACUTELY EXPOSED TO COLD increase their metabolism acutely by shivering, resulting in heat production (thermogenesis). This thermogenesis is facultative, i.e., it occurs only when the mammal is in the cold. Mammals chronically exposed to cold acquire an alternative means of facultative heat production; they substitute with time the process of shivering heat production with nonshivering thermogenesis (16). The acquisition of nonshivering thermogenesis temporally coincides with the acquirement of a much enhanced thermogenic response to an injection of norepinephrine (19). The enhanced response thus represents an adaptive adrenergic thermogenesis. An increased response to injected or infused norepinephrine is therefore generally equated with an increased capacity for nonshivering thermogenesis (20). This adaptive adrenergic thermogenesis is thus the one resulting from the replacement of shivering with nonshivering thermogenesis as an effect of acclimation to cold. We will therefore refer to it here as adaptive adrenergic nonshivering thermogenesis (even though clearly all heat production resulting from norepinephrine injection can formally be said to be nonshivering).

The ability to exchange shivering with nonshivering thermogenesis is fully dependent upon the presence of uncoupling protein-1 (UCP1) in the animals. This is clearly demonstrated (10) in studies with the UCP1-ablated mice developed in the laboratory of L. P. Kozak (7). UCP1-dependent thermogenesis is located to brown adipose tissue (2). Brown adipocytes from UCP1-ablated mice are fully devoid of adrennergically induced thermogenesis (25). However, several studies in UCP1-ablated mice have demonstrated that, in these animals there is still a response to adrenergic stimulation (7, 10, 11, 13). Considering the total inability to recruit adaptive nonshivering thermogenesis in UCP1-ablated mice (10), the presence of this response to adrenergic stimulation in the intact animal raises questions concerning the nature of adaptive adrenergic nonshivering thermogenesis. Particularly, the question may be raised whether a UCP1-independent, alternative form of adaptive adrenergic nonshivering thermogenesis could exist. The existence of such an alternative adaptive adrenergic thermogenesis has been suggested in relation to metabolic control in general (23, 24, 35). The localization of this alternative thermogenesis has been discussed to be muscle (1, 12, 21, 23, 24). This alternative possibility has been discussed particularly concerning larger mammals (such as adult man) that are generally supposed to lack significant amounts of brown adipose tissue and thus of UCP1.

To examine whether an alternative (i.e., UCP1-independent) adaptive adrenergic nonshivering thermogenesis exists, we acclimated wild-type and UCP1-ablated mice to a warm (thermonutral) environment and to a relatively cold environment. We then examined whether any adaptive adrenergic nonshivering thermogenesis could be induced. It is the conclusion of our investigations that UCP1 has a unique role as the mediator of adaptive adrenergic nonshivering thermogenesis; no other protein or metabolic pathway can mediate this process. This conclusion has been stated in earlier reviews (2, 28, 29).

MATERIALS AND METHODS

Animals. The UCP1-ablated mice were the progeny of those described by Enerbäck et al. (7). The mice were bred in the Institute. Ethical permission was obtained from the Animal Ethics Committee of the North Stockholm Region. For most experiments, two independent experimental series were performed. In the main series, controls used were of the C57BL/6 strain (B & K Universal, Stockholm, Sweden), the blastocyst donor strain for the UCP1-ablated mice, and the original line of UCP1-ablated mice were the experimental animals. For the second, confirmatory series, the results of which are shown in the supplementary information (the online version of this article contains supplemental data), the UCP1-ablated mice had been backcrossed into the C57BL/6 strain for six to seven generations and then outcrossed, and two lines were established from the outcrosses, the UCP1+/− and the UCP1−/− lines. All animals were fed ad libitum (Rat and Mouse Standard Diet No. 1, BeeKay Feeds; B & K Universal), had free access to water, and were kept on a 12:12-h light-dark cycle in single cages. Wild-type and UCP1-ablated female mice were divided into two age- and weight-matched groups. One group was acclimated to 30°C [referred to as warm-acclimated (WA) mice];
control experiments confirmed that this temperature was thermoneutral for both wild-type and UCP1-ablated mice, as no decrease in metabolic rate was observed at 32 vs. 30°C, whereas an increase was observed at 28 and 26 vs. 30°C. The second group was acclimated to 18°C [referred to as cold-acclimated (CA) mice]. Both groups were acclimated for at least 1 mo before the start of the experiments.

Body temperature. The body temperature of the mice was measured at their respective ambient temperature with a rectal probe (RET-3; Physitemp Instruments, Clifton, NJ) after 1 mo of acclimation, in the middle of the light period.

Oxygen consumption/thermogenesis. Oxygen consumption measurements were performed in a closed-circuit系统, principally as in Dicker et al. (4), with a flow rate of 0.42 l/min and a chamber volume of 1.8 liters. The system was calibrated daily. Oxygen consumption and ambient temperature data were collected every 6 s via a MacLab/2e (AD Instruments, Castle Hill, Australia). Oxygen consumption was recalculated to milliliters of O2 per minute per kilogram of body weight0.75 for each animal.

Metabolic rate (lowest observed and average) of conscious animals was determined at 30 and at 18°C. The lowest observed metabolic rate was defined as the lowest value of oxygen consumption stable for at least 4 min within a period of 2.5–3 h in the metabolic chamber. The lowest observed metabolic rate determined in this way at an ambient temperature of 30°C (thermoneutrality) is referred to as resting metabolic rate (RMR). The average metabolic rate (AvMR), which includes activity, was defined as the mean oxygen consumption over a period of 1 h (600 data points) starting 1.5 h after the animal was placed in the metabolic chamber.

In studies on anesthetized animals, mice were first injected with pentobarbital sodium (pentobarbitalum natricum, 95 mg/kg body wt ip; Apoteksbolaget, Stockholm, Sweden) dissolved in physiological saline (Pharmacia, Stockholm, Sweden). When sensation had been lost (5–10 min after the injection), the animals were placed in the metabolic chamber at 33°C; pilot experiments have shown that this environmental temperature suffices to allow the anesthetized mice to maintain a stable body temperature during the experiment. The metabolic rate under anesthesia (MRan) was defined as the mean oxygen consumption over a 10-min period after a stable level had been reached. About 20 min after the injection of barbiturate, norepinephrine [1 mg/kg body wt (i.e., 0.53 mg free base (3.13 μmol per kg)], final volume 0.1 ml, (−)-arterenol bitartrate; Sigma] was injected subcutaneously in a final volume of 0.1 ml, or physiological saline was injected. Only one substance was tested on each experimental occasion. Data from animals that arose from anesthesia during the course of the experiments were excluded. After the experiment, all the animals were allowed to recover for 24 h at 30°C. After the recovery, the animals that had been acclimated earlier to 18°C were moved back to 18°C.

In studies on nonanesthetized animals, the mice were placed in the metabolic chamber (at 30°C) and allowed to adapt to the surroundings for 2.5–3 h. They were then injected with either norepinephrine as above but intraperitoneally, or with physiological saline, in alternating order, with a 1.5-h interval between injections.

For estimation of the effect of substances, RMR or, in the case of anesthetized animals, MRan, was calculated individually for each trace and subtracted from the running data list. The curves presented are the means of these individual curves, and the standard error shown is the standard error of these means. When a curve represents the difference between independent values (different sets of animals), it is calculated as the difference between the mean curves; the uncertainty of the mean values in these cases is estimated as the sum of the standard errors of the original mean curves used in the calculation.

RESULTS

To examine whether UCP1-independent adrenergic nonshivering thermogenesis is adaptive, we acclimated wild-type and UCP1-ablated mice to thermoneutral temperature (WA) and to a relatively cold environment (CA, 18°C; see below). We examined the response in two independent experimental series, with the main series using C57BL/6 mice as wild-type mice and the original line of UCP1-ablated mice (7) and the confirmatory series using UCP1+/− and UCP1−/− lines obtained from backcrossing the UCP1-ablated mice into C57BL/6 for six to seven generations. The results of the two series were qualitatively identical and quantitatively very similar. Therefore, the results of the confirmatory backcrossed animals are displayed in the supplementary material in parallel to Figs. 2 and 3, and will not be further discussed in the text.

In Fig. 1A, we demonstrate that, within the time frame of the experimental series, the body weights of the mice were not significantly affected by the two acclimation conditions or by genotype. Because there was no difference in body weight, the outcome of the study is not influenced by the way the metabolic rates are presented; we present data expressed as milliliters of O2 per minute per kilogram of body weight0.75. The data from thermoneutrally acclimated mice are presented first, followed by the effect of acclimation to relative cold.

Significance of UCP1 for the response to norepinephrine in WA mice. After acclimation to a thermoneutral temperature (WA), the RMR (i.e., the lowest metabolic rate observed at 30°C; Fig. 1B) and the AvMR (i.e., the mean metabolism at 30°C; including activity, etc.; Fig. 1C) were the same in UCP1-possessing and UCP1-ablated mice.

Animals such as these, acclimated to temperatures within their thermoneutral zone, do not require extra heat to maintain euthermy and are therefore not expected to demonstrate any adaptive adrenergic nonshivering thermogenesis. Thus, to examine the lowest level of adrenergic thermogenesis that can be expected to occur, we examined the response of these animals to norepinephrine injection. In conscious mice, analysis of thermogenic responses to injections is complicated by the so-called “stress” response to saline, which can be eliminated by anesthesia. However, volatile anaesthetics cannot be used because they directly inhibit brown adipose tissue function (5, 31, 32), but barbiturates are without this effect (30). Therefore, we used barbital anesthesia. Barbital anesthesia in itself, as expected, led to a decrease in RMR, but in this respect there was no difference between wild-type and UCP1-ablated mice (Fig. 1D). The rates (MRan) in both cases were ~7 ml O2/min 1·kg−0.75, i.e., 78% of the rates of nonanesthetized mice. In the barbital-treated mice, there was no metabolic effect of saline injection (Fig. 2A).

When norepinephrine was injected into the anesthetized WA wild-type mice, it elicited a clear increase in the metabolic rate (Fig. 2A), corresponding to nearly a doubling of the MRan of these mice. In the thermoneutrally acclimated UCP1-ablated mice, norepinephrine was also able to induce a metabolic response (Fig. 2B), which amounted to an increase of about 50% of the MRan. This response could not originate from an adrenergic, but UCP1-independent thermogenic process specifically in brown fat cells, because the brown fat cells of these animals are unable to respond thermogenically to norepinephrine (25). The UCP1-independent thermogenic response to norepinephrine must therefore occur in tissues other than brown adipose tissue.

Because the response to norepinephrine in UCP1-ablated mice (Fig. 2B) was smaller than that in wild-type mice (Fig. 2A), it was possible to estimate the contribution of UCP1 to the
response to norepinephrine by calculating the running difference between these responses. This difference, which thus represents the UCP1-dependent thermogenic response, is shown in Fig. 2C. As is evident, even in wild-type mice acclimated to thermoneutral temperature a sufficient amount of UCP1 was present to allow for a small, but clearly significant, response to norepinephrine, amounting to about 3 ml O₂·min⁻¹·kg⁻⁰·₇₅, i.e., about a 40% increase over MRan.

**Effect of acclimation to cold on adrenergically induced thermogenesis.** When mice are transferred from a thermoneutral temperature to a “cold” environment (i.e., any temperature below thermoneutrality), they activate thermogenic mechanisms to defend their body temperature. Initially they shiver, but with time nonshivering thermogenesis is recruited; this thus represents an adaptive nonshivering thermogenesis. The standard cold acclimation temperature is 4°C. To examine whether adaptive adrenergic nonshivering thermogenesis exists in UCP1-ablated mice, it would be preferable to utilize these standard conditions. However, UCP1-ablated mice cannot tolerate acute exposure to 4°C (7, 10). Although the UCP1-ablated mice can survive at 4°C if they are successively acclimated to lower temperatures (10), they have a much-reduced survival time (mean time ~100 days and an increased mortality seen much earlier). Therefore, acclimation to 4°C may not be optimal. We showed earlier in 4°C-acclimated mice that a response to norepinephrine injection was seen (10); it was smaller than that of wild-type mice, but it was not investigated whether it had been increased due to cold acclimation. In addition, the adverse conditions for the UCP1-ablated mice living at 4°C may have negatively affected the response.

Therefore, for the present studies, a milder cold stress (18°C) was chosen. Both wild-type and UCP1-ablated mice tolerated acclimation to 18°C well (Fig. 1A), and the body temperature of the two types of mice acclimated to 18°C was well defended and equal in the UCP1-consuming and UCP1-ablated mice (37.1 ± 0.2 vs. 37.3 ± 0.2°C) and slightly higher than that observed in the WA mice (36.8 ± 0.2 vs. 36.9 ± 0.2°C; n = 5–6, no significant effect of genotype). That chronic activation of thermogenic mechanism(s) was necessary for acclimation to 18°C was evident from the increase in AvMR at that temperature. In wild-type mice, the metabolic rate increased from ~13 ml O₂·min⁻¹·kg⁻⁰·₇₅ at 30°C to ~26 ml O₂·min⁻¹·kg⁻⁰·₇₅ at 18°C, i.e., about a doubling (Fig. 1E).
After ~1 mo at 18°C, the RMRs (i.e., the lowest rates measured in conscious mice temporally transferred to 30°C) of the 18°C-acclimated wild-type mice and UCP1-ablated mice had become higher than those of the mice acclimated to 30°C (Fig. 1B). The average metabolic rates (also at 30°C but including activity) also tended to be increased (Fig. 1C). Furthermore, the metabolic rate of anesthetized CA mice was also higher than those of the WA mice (9.7 and 10.6 ml O$_2$·min$^{-1}$·kg$^{-0.75}$ for the wild-type and UCP1-ablated mice, respectively; Fig. 1D).

The responses of WA and CA wild-type mice to norepinephrine were then compared (Fig. 3; the data on WA mice are identical to those in Fig. 2 but are shown with small symbols to facilitate comparison). As seen in Fig. 3A, the response to norepinephrine in the CA wild-type mice was at least double that in the WA mice. Thus acclimation to 18°C had led to the expected recruitment of adaptive adrenergic nonshivering thermogenesis. The difference between the response to norepinephrine in WA and CA wild-type mice is calculated in Fig. 3C (top curve) and amounted to ~10 ml O$_2$·min$^{-1}$·kg$^{-0.75}$. This value thus represents the cold-acclimation recruited, norepinephrine-induced thermogenesis in UCP1-possessing mice.

The ability of the UCP1-ablated mice to recruit adrenergic nonshivering thermogenesis as an effect of cold acclimation was then examined. As seen in Fig. 3B, the response to norepinephrine was virtually indistinguishable in mice acclimated to the two temperatures. Even a 10-fold higher dose of norepinephrine did not elicit a higher response in the 18°C-acclimated UCP1-ablated mice (not shown). This absence of effect of cold acclimation is even more evident in Fig. 3C (bottom curve), where the difference between the responses in CA and WA mice is depicted. In the UCP1-ablated mice, the difference curve did not deviate significantly from zero. Thus no UCP1-independent adrenergic nonshivering thermogenesis could be recruited by cold acclimation in the UCP1-ablated mice.

Verification of the absence of UCP1-independent adaptive nonshivering thermogenesis also in conscious animals. Despite the problems in interpretation of experiments in conscious animals, we felt it necessary to examine whether any adaptive, UCP1-independent adrenergic process exists in conscious mice, since it may be hypothesized that such a process could require participation of central control regions that could have become inhibited during anesthesia.

In wild-type conscious mice, the response to norepinephrine was higher in both WA and CA animals (Fig. 4A) than in anesthetized mice (Fig. 3A), and it was clearly recruited by cold acclimation (Fig. 4A); the recruitable part of the response is depicted in Fig. 4C (top curve). This response was very high, ~15 ml O$_2$·min$^{-1}$·kg$^{-0.75}$ i.e., some 50% higher than that seen in anesthetized mice. In contrast, the response to norepinephrine in the UCP1-ablated mice (Fig. 4B) was not recruited by cold acclimation. The adaptive part of the response is depicted in Fig. 4C (bottom curve); it does not differ from zero. Thus, also in conscious animals, no UCP1-independent adrenergic nonshivering thermogenesis could be identified.

**DISCUSSION**

In the present investigation, we demonstrate that adrenergic UCP1-independent thermogenesis is not recruitable by accl-
formation to cold. Thus, at least in the mouse, no other mechanism exists for adaptive adrenergic nonshivering thermogenesis than that mediated by UCP1. No other proteins or enzymatic pathways can participate in or with time partly take over the mediation of adaptive adrenergic nonshivering thermogenesis, even in the total absence of UCP1. A direct comparison between the recruitability of the UCP1-dependent and lack of recruitability of the UCP1-independent adrenergic nonshivering thermogenesis is made in Fig. 5 in anesthetized (Fig. 5A) and conscious (Fig. 5B) mice. Qualitatively, the outcome was independent of the state of consciousness.

Cold acclimation-recruited increase in RMR is UCP1 independent. In both anesthetized and conscious animals, and in both wild-type and UCP1-ablated mice, we observed an increase in RMR due to cold acclimation (Fig. 1). Because the increase is observed at thermoneutral temperature, where extra heat production is not necessary, this increase in RMR is not facultative (i.e., it is not initiated by the animal as a compensation for acute heat loss), and therefore, a thermoregulatory role cannot be ascribed to it. It does contribute to the total heat production, but its extent is far from the increase needed for thermal balance at 18°C. Such a minor increase in RMR (i.e., the rate observed at 30°C) in CA animals could have been suggested earlier to be due to the increased amount of UCP1 found in the CA animals under the provision that UCP1 would display some “leakiness”, i.e., that UCP1, even in unstimulated tissue, would be slightly uncoupling. However, the fact that the increase was quantitatively identical in UCP1-possessing and UCP1-ablated mice demonstrate that the increase is UCP1 independent. The presence of UCP1 thus does not affect RMR. Bioenergetically, this means that UCP1 is neither constitutively uncoupling nor even slightly “leaky.” It requires an external stimulus to become active. An increase in the amount of endogenously expressed UCP1 thus does not in itself alter basal metabolism. This conclusion is principally in agreement with observations in animals where UCP1 levels have been increased pharmacologically but where additional sympathomimetic stimulation was necessary to observe the increased thermogenic capacity (34, 36).

The UCP1-independent increase in basal metabolism may be related to the fact that the mice defended a slightly higher body temperature when acclimated to 18 than to 30°C (+0.3°C), to the increased food intake in the colder environment, or to the increase in serum T3 levels observed in CA animals (6).

UCP1-independent adrenergic thermogenesis is not recruitable. Even in the UCP1-ablated mice, a thermogenic response to norepinephrine is observed (Figs. 2–5) (7, 10, 11, 13). The existence of a UCP1-independent adrenergically induced increase in thermogenesis is not unexpected, because a response to norepinephrine injection has also been observed in animal groups, such as reptiles and amphibians, that fully lack UCP1 and brown adipose tissue (14, 15), i.e., in ectothermic animals devoid of adaptive metabolic thermogenesis.

Fig. 3. Cold acclimation-recruited NE-induced thermogenesis in anesthetized mice. WT (A) and UCP1-ablated (B) mice were acclimated to 18°C for ≥ 1 mo. Curves were constructed as in Fig. 2, from which the corresponding data from 30°C-acclimated mice are also shown here for reference, but with small circles. Data in A, B, and C are means ± SE of 6 animals in each group. C: cold acclimation-recruited response to NE of the wild-type and UCP1-ablated mice (−/−); curves were calculated from the data in A and B.
Although a thermogenic response to norepinephrine was observed in UCP1-ablated mice, the magnitude of this response was identical in CA and in WA mice (Figs. 3–5). Thus this UCP1-independent adrenergic thermogenesis should not be considered as a form of thermoregulatory thermogenesis. The localization and molecular mediation of this nonadaptive thermogenesis cannot be elucidated from the present experiments, except that it is not brown fat derived [because no adrenergically induced thermogenesis can be elicited in brown fat cells from UCP1-ablated mice (25)]. An injection of norepinephrine mimics an unregulated general sympathetic activation. The response may thus be said to represent the pharmacological summation of the stimulation of all adrenoceptors in the body and thus include contributions from most tissues, including, e.g., muscle. It is likely that it represents the metabolic cost for the synthesis of ATP utilized in cellular processes that are stimulated by norepinephrine in different tissues.

Only UCP1-dependent adrenergic thermogenesis is adaptive. For an animal in a thermoneutral environment, the recruitment level of brown adipose tissue would be expected to be minimal. It is therefore noteworthy that a significant UCP1-dependent adrenergic nonshivering thermogenesis exists even under these conditions (Fig. 2C). Because no extra heat is needed under thermoneutral conditions, the purpose of this adrenergic UCP1-dependent thermogenesis cannot be thermoregulatory; it may instead be related to the phenomenon of metaboloregulatory thermogenesis (2).

It is evident from the data in Fig. 3 that it is only in the UCP1 possessing wild-type mice that acclimation to cold leads to an increase in the response to norepinephrine injection. Thus, as is evident in Fig. 3C, the entire increase in the capacity for adrenergic thermogenesis acquired as an effect of

Fig. 4. Cold acclimation-recruited NE-induced thermogenesis in conscious mice. Responses to NE of WT (A) or UCP1-ablated mice (B) acclimated to 30°C (small circles) or to 18°C (large circles) were compared. Data are means ± SE of 4 animals in each group. Curves were constructed as in Fig. 2. C: cold acclimation-recruited response of WT and UCP1-ablated mice was calculated from the data in A and B.

Fig. 5. Schematic summary of thermogenic components. Different thermogenic components in anesthetized (A) and conscious (B) UCP1-ablated (U−) and WT (U+) mice without any stimulation (−) or upon NE injection. Graph is based on data presented in Figs. 1–4.
acclimation to cold is dependent on the presence of UCP1; only the UCP1-dependent processes are adaptive.

Brown adipose tissue does not have an additional, indirect role in thermogenesis. The possible phenomenon of an additional, indirect thermogenic function of brown adipose tissue in adaptive adrenergic nonshivering thermogenesis has classically been discussed [the so-called “mediatory role” (17)]. This role would result from brown adipose tissue producing some of its heat in an indirect way, e.g., through the release of fatty acids to the circulation (26). In their turn, these fatty acids may activate thermogenesis in other tissues. Because, e.g., lipolysis in the brown-fat cells from the UCP1-ablated mice is fully functional (25), such a mediatory role of brown adipose tissue should be evident even in the UCP1-ablated mice. However, because no adaptive adrenergic nonshivering thermogenesis could be evoked in the UCP1-ablated mice, it is clear that brown adipose tissue does not mediate nonshivering thermogenesis in other tissues.

Auxiliary heat production in heart and respiratory muscle. In the seminal paper of Foster and Frydman (8) that points to brown adipose tissue as at least the “major” site for adrenergic adaptive thermogenesis, the authors stated that “at least” 60% of the adaptive adrenergic nonshivering thermogenesis occurs in brown adipose tissue. This would apparently leave a significant amount for other tissues. Indeed, the authors report a fourfold increase in blood flow to heart and respiratory muscles in norepinephrine-injected animals. However, because no adaptive adrenergic nonshivering thermogenesis can be observed in the UCP1-ablated mice, the increased oxygen consumption in the heart and the respiratory muscles is induced only when there is a demand from brown adipose tissue for extra oxygen. The oxygen consumption (heat production) in the heart and respiratory muscles can thus be seen as auxiliary, i.e., as heat derived from the muscular work necessary to provide oxygen for the brown fat-derived heat. Of course, physiologically, this auxiliary heat also contributes to the thermal balance of the animal, but the heart and the respiratory muscles possess no independent ability to perform adaptive adrenergic nonshivering thermogenesis in themselves.

No adaptive adrenergic nonshivering thermogenesis in muscle. Adaptive adrenergic nonshivering thermogenesis was initially expected to be localized in muscles, mainly because of their large mass and high potential metabolic capacity. However, it is now accepted that in rodents, adaptive adrenergic nonshivering thermogenesis is localized at least to a significant extent to brown adipose tissue (18). Nonetheless, it has been maintained that “alternative” adaptive adrenergic nonshivering thermogenesis exists, deriving from organs other than brown adipose tissue, especially muscle. In this connection, it may be pointed out that Foster and Frydman (8) demonstrated that the blood flow to skeletal muscles in rats was increased somewhat (by ~50%) during norepinephrine-induced thermogenesis (but, to be noted, equally so in WA and CA animals). Since cold acclimation could not increase the amount of norepinephrine-induced thermogenesis in the UCP1-ablated mice, the present results indicate that such an increased blood flow to muscle is not associated with an adaptive thermogenesis in rodents.

Especially in larger mammals [including adult man, which has generally been considered to be principally devoid of functional brown adipose tissue (3, 22)], it has been doubted that brown adipose tissue should have a significant role in adaptive adrenergic nonshivering thermogenesis, and this role has instead been ascribed to muscle (1, 12, 21, 24). The evidence presented here demonstrates that, even in the absence of UCP1, a mouse still has no ability to recruit any adaptive adrenergic nonshivering thermogenesis from skeletal muscles despite the fact that the lack of UCP1 should have provoked the recruitment of any other potential mechanism in this model organism. Although we clearly cannot present experimental evidence concerning the situation in larger mammals, including man, we would find it unlikely that a mere increase in animal size would alter muscle cellular metabolism in such a way that an alternative to UCP1-dependent thermogenesis would appear, especially because this property should have then developed independently in the muscles of species of different mammalian orders. Therefore, we suggest that adaptive adrenergic nonshivering thermogenesis, as defined here, is not located to muscle in any mammalian species.

No metabolic pathways other than that through UCP1 contribute to adaptive adrenergic nonshivering thermogenesis. All metabolism is basically thermogenic, but a series of metabolic processes in various tissues have been considered to have thermogenesis as their function, e.g., substrate cycles, Ca^{2+} cycling, increases in cell membrane permeability (ion leaks) leading to compensatory increased Na^{+}-K^{+}-ATPase activity, etc. Many of these processes have been suggested to be the molecular background for adaptive adrenergic nonshivering thermogenesis. Although these processes are probably thermogenic in a bioenergetic sense, and although they may be stimulated by norepinephrine, they cannot be the molecular background for cold acclimation-recruited adaptive adrenergic nonshivering thermogenesis because such a thermogenesis does not exist in the absence of UCP1, at least not in the mouse. Similarly, it must be concluded that the members of the mitochondrial carrier protein family referred to as UCP2, UCP3, etc. (as reviewed in Ref. 27) cannot contribute to adaptive adrenergic nonshivering thermogenesis. The present data, however, cannot exclude that these carriers may contribute to other types of thermogenesis.

In conclusion, the present investigation demonstrates that, although a thermogenic response to adrenergic agents does exist in the absence of UCP1, this response is not recruitable by cold acclimation, i.e., it is nonadaptive, and UCP1 is the only protein that is able to mediate adaptive adrenergic nonshivering thermogenesis.

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