Activation of brown adipose tissue thermogenesis by electrical stimulation to the dorsal surface of the tissue in rats

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ABSTRACT

Brown adipose tissue (BAT) is a heat-producing organ that plays an important role in maintenance of energy homeostasis. The purpose of this study was to test a novel method for stimulating BAT thermogenesis in rats. Application of electrical field stimulation to the dorsal surface of interscapular BAT caused a substantial rise in tissue temperature without affecting rectal temperature. The electrical stimulation failed to raise BAT temperature on the 2nd day after surgical sympathetic denervation. This is unlikely to be due to loss of thermogenic capacity, since neither UCP1 contents nor norepinephrine-induced thermogenesis were diminished 2 days after the denervation. A pharmacological experiment revealed that the BAT thermogenesis induced after electrical stimulation is mediated through β-adrenoceptors. The present study demonstrates that electrical stimulation applied to the dorsal surface of BAT is able to activate thermogenesis of BAT through mediation of norepinephrine released from sympathetic nerves. Our findings may provide a basis for developing a novel therapeutic procedure for obesity and related disorders.

Brown adipose tissue (BAT) is a heat-producing organ that plays an important role in maintenance of energy homeostasis in newborns and cold-exposed mammals (3, 9, 15). BAT is also important for maintaining energy balance during spontaneous hyperphagia, as it is activated in response to diet (11). It has been widely believed that BAT contributes little, if anything, to the maintenance of energy homeostasis in adult humans. However, recent studies have revealed that BAT is active not only in newborns but also in adults (4, 13, 19, 20). The fact that BAT thermogenesis in adult humans can be acutely activated by cold stimulus (7, 13) offered a novel therapeutic strategy to reduce body weight and to prevent weight gain.

BAT thermogenesis is activated by sympathetic nerves that richly innervate the tissue (2, 3, 9). Cellular events associated with heat production involve binding of norepinephrine (NE) released from sympathetic nerve endings to β-adrenergic receptors, increased breakdown of triglycerides into fatty acids, and increase in mitochondrial oxidation. The presence of uncoupling protein 1 (UCP1) in BAT allows a short-circuit of the proton gradient across the inner mitochondrial membrane, thus uncoupling fuel oxidation with ATP synthesis (9). It is reasonable to expect that increases in BAT mass and UCP1 expression are effective as a therapeutic approach for obesity. However, the increased thermogenic capacity of BAT is not directly linked with enhancement of basal metabolic rate. Indeed, hyperplastic BAT that has developed in rodents adapted to the cold becomes functionally inactive immediately after
transferring to warmer conditions (6, 8). Thus, effective methods for stimulating BAT thermogenesis are required to utilize the tissue function for body weight control.

The purpose of this study was to explore a novel method for stimulating BAT thermogenesis in rats. For this purpose, we examined whether application of electrical field stimulation to the dorsal surface of interscapular BAT activates thermogenesis. This trial was based on the expectation that electrical field stimulation would activate sympathetic nerve endings and thereby cause NE release.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (SLC, Japan) aged 11–13 weeks and weighing 340–380 g were used in this study. The rats were kept in plastic cages at 22 ± 1°C with a 12-h : 12-h light : dark cycle (lights on at 0600–1800) and supplied both laboratory chow and water ad libitum. All experimental procedures were approved by the Animal Care and Use Committee of Gifu University.

Measurement of BAT temperature. Rats were anesthetized with urethane (500 mg/kg ip). Each animal was placed on a DC heating pad (Homeothermic Blanket System; Harvard Apparatus, Massachusetts, USA) and rectal temperature was maintained at 37.0 ± 0.2°C throughout the experiment. The ventral surface of BAT was partially separated from underlying muscle, and a small thermistor with a diameter of 1 mm was placed under the pad of BAT (12). A similar thermistor was also inserted 5 cm into the rectum. The thermistors were connected to a Power Lab system (AD Instruments, Colorado, USA; model IT-18) and BAT and rectal temperatures were continuously recorded.

Electrical field stimulation of interscapular BAT. Electrical field stimulation was applied to interscapular BAT by using a custom-made bipolar electrode composed of a pair of metal pads (each 30 mm²). Skin of the scapular region was removed and the electrodes were set as shown in Fig. 1. Single square-wave pulses (500 μs in duration and 20 V in intensity) of various frequencies (5–100 Hz) were applied for 20 s by using an electronic stimulator (model SEN-3301; Nihon Kohden, Tokyo, Japan). Propranolol ((±)-propranolol hydrochloride, Wako, Osaka, Japan), when used, was given intraperitoneally at a dose of 20 mg/kg body weight (16) 10 min before the application of electrical stimulation.

Surgical denervation of sympathetic nerves. Sympathetic denervation of interscapular BAT was performed as described previously (17). Briefly, under pentobarbital anesthesia (50 mg/kg ip), a transverse incision was made just anterior to the right scapula, and the ventral surface of the interscapular BAT was partially separated from underlying muscles. The scapula was raised to expose the five intercostal nerve bundles entering right BAT pad. A section of each nerve bundle was removed. Experiments were begun 2 days after unilateral denervation. This period was chosen on the basis of observation that degeneration of nerves occurred on the 2nd day after injury (5, 10).

Western blot analysis. Tissue samples were obtained separately from intact and denervated pads of BAT. The tissues were homogenized in a lysis/extraction reagent (CellLytic™-MT; Sigma, St. Louis, MO, USA) and centrifuged at 10,000 x g for 5 min at 4°C. The fat cakes were discarded, and the infranatants were used for Western blot analysis with a rabbit anti-rat UCP1 antibody (MERCK, Darmstadt, Germany). Procedures for Western blot analysis were the same as those described previously (18). Immunostained proteins were revealed with an ECL system (GE Healthcare, Buckinghamshire, UK). Chemiluminescent signals were detected and visualized by using a LAS-1000 Lumino image analyzer (Fuji Film). Densitometry analyses were carried out by using ImageJ software.
the electrical stimulation of BAT. The rise in BAT temperature was frequency-dependent (Fig. 2B).

Effect of sympathetic denervation on rise in BAT temperature
Surgical denervation of the sympathetic nerves diminished the rise in BAT temperature (Fig. 3). In the denervated BAT, electrical stimulation at a frequency of 20 Hz caused a slight increment of tissue temperature (0.09 ± 0.04°C, n = 6), which was significantly lower than that observed in intact innervated BAT (0.32 ± 0.05°C, n = 6).

Thermogenic activity of BAT at 2 days after sympathetic denervation
To determine whether thermogenic capacity of BAT was retained at 2 days after sympathetic denervation, UCP1 levels were examined by Western blot analysis. As shown in Fig. 4, UCP1 contents in denervated BAT were not significantly different from those in intact BAT.

In addition, the effect of intravenous infusion of NE was examined. In contrast to electrical stimulation, infusion of NE at a dose of 5 μg/kg body weight increased tissue temperature not only in intact BAT but also in denervated BAT (Fig. 5). The net increment in denervated BAT was 0.29 ± 0.10°C (n = 6), which was comparable to that in intact BAT (0.33 ± 0.09°C, n = 6).
play a pivotal role in activating thermogenesis in BAT. Accordingly, stimulation of the ventromedial hypothalamus, which activates the sympathetic nerves entering BAT, or direct electrical stimulation of the sympathetic nerve bundles increases interscapular BAT temperature in rats (12). Action potentials, which propagate along the axon to the nerve terminal, activate voltage-gated Ca\(^{2+}\) channels (VGCCs). Ca\(^{2+}\) entry through presynaptic VGCCs initiates release of neurotransmitters. Accordingly, we expected that electrical field stimulation applied to the surface of BAT would activate presynaptic VGCCs at the sympathetic nerve terminals, leading to thermogenesis as a consequence of NE release. The stimulation method used in this study causes a substantial rise in BAT temperature. As discussed below in detail, NE released from the sympathetic nerves would be essential for the stimulation-induced rise in BAT temperature. Therefore, the present study provides evidence that BAT thermogenesis can be directly activated by local application of electrical stimulation.

The increase in BAT temperature after electrical stimulation is unlikely to reflect systemic rise in body temperature, since rectal temperature was not increased in response to the stimulation. Furthermore, the non-stimulated BAT pad on the left side in the same rat did not show a rise in temperature, even when temperature of the stimulated BAT pad was increasing. These findings suggest that the electrical stimulation protocol exerts effects exclusively on the local area where field stimulation was applied. In accordance with this, heart rate was unchanged and no abnormal electrocardiograph (ECG)

Effect of \(\beta\)-adrenergic blocker on rise in BAT temperature

Electrical stimulation was applied before and 10 min after an intraperitoneal injection of a \(\beta\)-adrenergic blocker, propranolol (Fig. 6). The net increments of temperature before injection of the blocker were 0.34 \(\pm\) 0.08°C and 0.52 \(\pm\) 0.09°C in response to electrical stimulation at 20 and 50 Hz, respectively. The levels were significantly diminished (0.12 \(\pm\) 0.04°C and 0.18 \(\pm\) 0.08°C, respectively) after injection of the blocker.
was recorded in rats to which the stimulation was applied (unpublished observation). This is important since it proves that the novel method for activating BAT thermogenesis has no serious side effects.

Surgical denervation of the sympathetic nerves prevented the rise in BAT temperature in response to electrical stimulation. One possible explanation for this observation is that the thermogenic capacity of BAT is strongly suppressed by the denervation. We tested this hypothesis by examining UCP1 contents and NE-induced thermogenesis in intact and denervated BAT pads. The UCP1 contents were not significantly changed at 2 days after the denervation. This is consistent with the results of a previous study showing that mRNA levels of UCP1 on the 4th day after sympathetic denervation are not significantly reduced in rats (14). Moreover, intravenously infused NE, which directly acts on brown adipocytes, increased tissue temperature of denervated BAT in a manner similar to that of intact BAT. These results suggest that loss of sympathetic innervation for 2 days is not sufficient to diminish the thermogenic capacity. It is thus reasonable to conclude that intact innervation of the sympathetic nerves is essential for activating BAT thermogenesis by the electrical stimulation method.

A pharmacological experiment revealed that the BAT thermogenesis induced after electrical stimulation is mediated through β-adrenoceptors. This indicates that the indispensable role of sympathetic nerves in the stimulation-dependent thermogenesis appears to be largely due to release of NE from the nerve endings. Although the precise mechanism remains to be elucidated, it is most probable that voltage-gated channels located at the nerve endings are activated, resulting in the release of NE.

In the present study, we described a novel method to activate the BAT thermogenesis. Because recent studies have revealed that BAT is active not only in newborns but also in adult humans (4, 13, 19, 20), it is hoped to apply the method used in this study to therapeutic approach for humans. Evidently, the method is still not sufficient for clinical application. It would be worth noting, however, that an application of a relatively high-intensity electrical stimulation from surface of the skin slightly but detectably increased BAT temperature (unpublished data). Thus, the electrical stimulation may be accessible to activation of human BAT if stimulus protocol is well optimized. Chronic exposure to a cold environment promotes BAT hyperplasia and consequently increases the capacity of non-shivering thermogenesis (3, 9). Since the adaptive changes are totally dependent on activation of sympathetic nerves, it is also expected that consecutive electrical stimulation can induce hyperplasia of BAT. Taken together, our findings may provide a basis for developing a novel therapeutic procedure for obesity and related disorders including hyperlipidemia (1).

In summary, the present study demonstrates that an electrical stimulation applied to the dorsal surface of BAT is able to activate thermogenesis in the tissue. This effect is likely to be achieved via release of NE from the endings of sympathetic nerves.

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