J. Biochem. **143,** 385–393 (2008)
doi:10.1093/jb/mvm233

Impaired Basal Thermal Homeostasis in Rats Lacking Capsaicin-sensitive Peripheral Small Sensory Neurons

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Received October 3, 2007; accepted November 19, 2007; published online December 13, 2007

We studied the effects of selective loss of capsaicin-sensitive primary sensory neurons on thermosensation and thermoregulation in rats. Neonatal capsaicin treatment in rats caused a remarkable decrease in the number of small-diameter neurons in the dorsal root ganglion (DRG) compared with their number in the control rats. Gene expression analysis for various thermo-sensitive transient receptor potential (TRP) channels indicated marked reductions in the mRNA levels of TRPV1 (70%), TRPM8 (46%) and TRPA1 (64%), but not of TRPV2, in the DRG of capsaicin-treated rats compared with those in the control rats. In addition to the heat and cold insensitivity, capsaicin-treated rats showed lower rectal core temperature, higher skin temperature and decreased sensitivity to ambient temperature alteration under normal housing at room temperature, suggesting impaired thermosensation and change in thermoregulation in the rats. Uncoupling protein 1 (UCP1) expression and the thermogenic ability in brown adipose tissues were attenuated in the capsaicin-treated rats. These results indicate a critical role of capsaicin-sensitive sensory neurons in both heat and cool sensation and hence in basal thermal homeostasis, which is balanced by heat release and production including UCP1 thermogenesis, following sensation of the ambient temperature.

Key words: dorsal root ganglion, sensory neuron, thermoregulation, transient receptor potential channel, uncoupling protein 1.

Abbreviations: BAT, brown adipose tissue; DRG, dorsal root ganglia; LE, living environment; TE, test environment; Trectal, rectal temperature; TRP, transient receptor potential; Tskin, tail skin temperature; UCP, uncoupling protein.

Body temperature in homeothermic animals is controlled at a constant level by the balance between heat release and production. Animals sense the ambient temperature at all times, presumably through thermoreceptors belonging to the transient receptor potential (TRP) family of ion channels (temperature-sensitive channels) in primary sensory neurons of the periphery; and, based on the thermosensation, the central nervous system (CNS) regulates the responses to maintain body temperature (*1–3*). Several physiological mechanisms such as cutaneous vasoreaction or insulation are involved in the control of heat loss in animals. In particular, cutaneous vasodilation is the major mechanism for heat loss from the skin surface (*4–6*). Effective heat loss is achieved through various body parts, *e.g.* the hand in humans and the tail in rats, both of which have a high surface-to-volume ratio, absence of hair or fur and a high density of arteriovenous anastomoses. In rats, ~25% of the basic metabolic heat can be dissipated at the tail (*7*).

Likewise, heat production by thermogenic organs, such as muscle and brown adipose tissue (BAT), controlled *via* CNS, is indispensable (*8*). To date, BAT-specific uncoupling protein (UCP1) is known to be the most potent thermogenic protein (*9*, *10*); and the critical role of UCP1 in adaptive non-shivering thermogenesis in a cold environment has been verified by studies using UCP1-deficient mice (*11*, *12*). UCP2 and UCP3, homologues of UCP1, were discovered in 1997, but their roles in thermogenesis seem to be low (*8–10*). With respect to the connection between thermosensation and thermoregulation, Jancso-Gabor *et al.* (*13*) were the first to show that capsaicin treatment of adult rodents causes desensitization of assumed warmth detectors and impaired regulation of rectal core temperature, suggesting the involvement of capsaicin-sensitive neurons in thermoregulation. However, the roles of these sensory neurons

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and their thermoreceptors in the mechanism of thermal homeostasis still remain to be understood.

Recent advances towards understanding the molecular mechanism of thermosensation including that in the noxious range have been made since the cloning of the capsaicin receptor (vanillloid receptor 1, VR1/TRPV1) by Julius' group in 1997 (*14*). TRPV1 is located in small-diameter neurons with unmyelinated C-fibres and thinly myelinated Aδ-fibres in sensory ganglia such as the dorsal root and trigeminal ganglia. After the discovery of TRPV1, a series of other thermoreceptors sensing different ranges of ambient temperature were identified by using the techniques of expression cloning and electrophysiology *in vitro* (*15–23*). Thermoreceptors are gated by noxious heat (>43°C, TRPV1; >52°C, VRL1/TRPV2), warmth to hot (about 25–40°C, TRPV3 and TRPV4), cold to cool (about 15–25°C, TRPM8) and cold (<18°C, ANKTM1/TRPA1). In addition, recent studies using gene knockout mice have clarified the physiological and pathological roles of these receptors. For example, mice lacking TRPV1 show a deficiency in response to noxious heat and in the development of heat hyperalgesia (*24*), but the mutant mice maintain a normal resting body temperature (*25*). TRPV4-knockout mice exhibit an impaired pressure sensation (*26*), whereas circadian body temperature and thermoregulation in the mutant mice were normal at warm environmental temperatures of 25–35°C (*27*). Two studies using TRPA1-deficient mice demonstrated that TRPA1 is essential for transduction of chemical stimuli in nociceptor sensory neurons, although the role of TRPA1 in sensing noxious cold is controversial (*28*, *29*). Most recently, three groups have reported a predominant role of TRPM8 in cold sensation by using mice lacking this receptor (*30–32*). Nevertheless, the contribution of these receptors to the regulation of thermal homeostasis *in vivo* remains to be fully understood. To extend our understanding about the roles of primary sensory neurons in thermosensation and basal thermal homeostasis and to assess the involvement of thermoreceptors in the mechanisms, we investigated the thermal responses and heat production in conscious animals with selective loss of capsaicin-sensitive primary afferent neurons in the present study.

MATERIALS AND METHODS

Animals—F344/N pregnant rats were obtained from Japan SLC. On Day 1 after birth, the neonatal rats were injected or not intraperitoneally with capsaicin (Cap, 50 mg/kg of BW; Wako, Japan) dissolved in a solvent of 10% ethanol, 10% Tween-80, and 80% saline, as described earlier (*33*). The newborn rats in both groups were weaned at the age of 4 weeks and reared until they were 9–10 weeks old, during which time they were given a regular chow diet (Labo MR stock, Nihon Nousan Co., Japan) and tap water *ad libitum* under a 12-h:12-h light–dark cycle at 23 ± 1°C. In the present study, five independent experiments were conducted; and a total of 35 (19 females and 16 males) and 33 (13 females and 20 males) animals were used in control and Cap groups, respectively. The physiological analyses were conducted after maturation of the rats (after 8 weeks of age) under the conscious condition, because anaesthetization greatly affects the regulation of body temperature (*34*). After a series of analyses, the rats were killed by decapitation; and tissues including BAT and skeletal muscles were then dissected for RNA and/or protein analyses. The lumbar dorsal root ganglia (DRG, level 1–5) were removed and frozen on dry ice. The average body weights of 9-week-old rats in the control and capsaicin groups were 124.4 ± 2.0 and 119.1 ± 3.3 g, respectively, for females and 187.4 ± 3.9 and 186.1 ± 4.1 g, respectively for males. All experiments were carried out according to the institutional guidelines for animal care and the principles in the Helsinki Declaration.

Physiological Analysis—The hot-plate test was performed in an aluminium cage (D165 mm × W250 mm × H100 mm), the floor temperature of which was controlled at 52 ± 1°C by a hot plate (MODEL PC-420, Corning) beneath it. The floor temperature of the test cage was monitored with a thermal sensor (TD-300, Shibaura Electronics, Tokyo). The latency until rats showed the first signs of discomfort (paw-lifting, -licking or -shaking) was recorded with a cutoff time of 60 s. The cold-plate test was designated to assess any difference in thermal sensation to cold temperature. For this purpose, we used an experimental setup similar to that for the hot-plate test, in which the aluminium cage, which had been placed in a polystyrene box, was surrounded by small ice cubes. The floor temperature of the test cage, monitored as in the hot-plate test, was ~1°C. Rats showed similar signs of discomfort to those in the hot-plate test, although the responses to the cold stress took a longer time compared with those to the hot stress. The shortest and longest latency times in the cold-plate test were 34.6 s in the control group and 216.7 s in the capsaicin group, respectively. In the cold tolerance test, rats were maintained in a cold room (5°C) individually for 5 h. Rectal temperature (Trectal) of the animals was measured every hour with an electronic thermistor equipped with a rectal probe (Mon-a-therm 4070TM, Mallinckrodt Medical Inc., St. Louis, MO). Changes in the skin-surface temperature (Tskin) of conscious rats were recorded at 10-s intervals by use of an infrared thermographic device, Thermotracer (TH5100, NEC San-ei, Tokyo Ltd., Tokyo) or Thermo-Viewer (JTG-5200, JEOL Ltd., Tokyo), as described (*6*), which is a good tool to evaluate heat release in conscious, unrestrained animals non-invasively and successively. We determined the Tskin in the area of the tail, because it has no fur, thus facilitating accurate analysis, and is a crucial site for regulation of heat release in rodents (*4*, *35*). After the Tskin of rats in their regular cage (living environment, LE: 23.5–24.3°C) had been recorded for several minutes, the rats were transferred singly to a new cage without wooden chips (test environment, TE: 21.5–22.4°C). This transfer gave the rats a drop of ~1–2°C in the ambient temperature around them. Two cages were set in the field of the device, and the recording for two rats from each group was done at the same time for 5 min after transferring the rats to the TE. The highest skin temperature in a fixed area of the tail was measured by using image analysing software (TH51-701, NEC San-ei, or TG-5000CNTA, JEOL Datum., Tokyo).

Histological Analysis—Horizontal sections (15-μm thickness) of the lumbar DRGs were cut on a cryostat and thaw-mounted on Superfrost slides. The slides were stained with cresyl violet and thionine and observed with OptiPhoto2 (Nikon). The microscopic images were scanned by a digital camera (HC-2000, Fujix). The contours of thionine-stained neurons in the images were traced, and the cell diameter was measured by using a digital caliper (Mitutoyo, Japan).

RNA Analysis—For northern blot analysis, total RNA (10 or 20 μg), isolated from the DRG, BAT or gastrocnemius muscles by use of TRIzol reagent (GIBCO BRL), was electrophoresed on 1.25% formaldehyde–agarose gels. The separated RNA was transferred onto a GeneScreen membrane (NEN^{TM} Life Science Products Inc.; Boston, MA) in 10 × saline sodium citrate (SSC) by capillary blotting and was immobilized by exposure to ultraviolet light (0.35 J). Blots were hybridized with probes (labelled with [^{32}P] dCTP) for the mRNAs of TRPV1, TRPV2, TRPM8, UCP1 and 18S rRNA, as previously described (*36*). The cDNA probes for TRPV1, TRPV2 and TRPM8 mRNAs were produced from positions 81 to 580 of the rat VR1 sequence (GenBank accession No. AF029310), from positions 208 to 676 of the rat VRL1 sequence (GenBank accession No. AF129113), and from positions 446 to 3765 of the rat TRPM8 sequence (GenBank accession No. AY072788), respectively, by using rat DRG total RNA and the reverse transcription PCR technique. The PCR products were sequenced after subcloning into the pCRII or pCR2.1 vector (Invitogen, CA, USA). The blots were hybridized sequentially with the probes after having stripped away the previous probe. Each probe was confirmed to react with the specific mRNA. Hybridization signals were quantified with a Fuji Bioimage Analyzer. Gene expression of TRPA1 was evaluated by the technique of real-time quantitative PCR. After first-strand cDNAs for TRPA1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs had been synthesized with 1 μg total RNA and SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA), the cDNAs were analysed by using Light Cycler FastStrat DNA Master^{PLUS} SYBR Green I and Light Cycler 2.0 system (Roche Diagnostics, Mannheim, Germany). The gene expression of TRPA1 was normalized to the level of that of GAPDH. The sequences of primers used were the following: TRPA1, GAAAACTAAGCAAGTACGAG (forward) and CTCCCAC TGAAATTAGGTAG (reverse); GAPDH, forward primer, ACCACAGTCCATGCCATCAC (forward) and TCCACCA CCCTGTTGCTGTA (reverse).

Biochemical Analysis—Immunodetection of UCP1 and cytochrome oxidase subunit IV (COX IV) was performed by using the mitochondrial fraction isolated from BAT, as described previously (*11*). The protein concentration of the fraction was measured with a BCA protein assay kit (Pierce, Rockford, IL, USA). Equal amounts of mitochondrial protein (2 μg) were separated on 12.5% gels (Daiichi Pure Chemicals; Tokyo, Japan) and transferred onto Immobilon polyvinylidene difluoride membranes (Millipore Corporation; Bedford, MA, USA). The membranes were incubated with affinity-purified rabbit polyclonal antibodies specific for UCP1 (STRATA GENE, USA) or monoclonal antibody specific for COX IV (Molecular Probes, Inc., USA). After the secondary antibody reaction for 1 h at room temperature, the specific signals were detected by using an ECL kit (Amersham Pharmacia Biotech). The resulting images were quantified with NIH Image (version 1.63). Thermogenic activity was evaluated by using the mitochondrial fraction isolated from BAT, as previously described (*37*). Briefly, [^{3}H] GDP-binding was measured by incubation of 1 mg/ml mitochondria with 1 μM [8, 5′-^{3}H] GDP (specific radioactivity 32.5 Ci/mmol, PerkinElmer Life Sciences, Boston, MA, USA) in the presence or absence of unlabelled 1 mM GDP at 37°C for 15 min in an assay buffer of 100 mM sucrose, 0.1 mg/ml fatty acid-free bovine serum albumin, 2 μM rotenone and 10 mM sodium Na–Tris (hydroxymethyl) methyl-2-aminoethane sulphonic acid (pH 7.2). After the reaction mixture had been centrifuged at 8,500*g* for 5 min, the pellets were recovered, dissolved in 20 μl of 5% SDS, and then transferred into vials for scintillation counting. The mitochondrial samples pooled from six rats were used for Scatchard plot analysis.

Statistical Analysis—Data were expressed as means ± SE. The statistical significance of the data was assessed by using the unpaired Student's *t*-test or repeated measure analysis of variance (ANOVA).

RESULTS

Reduction in Small-diameter Neurons and Expression of TRPV1, TRPM8 and TRPA1 in DRG of Capsaicin-treated Rats—Capsaicin treatment of neonatal rats resulted in a marked reduction in the small-diameter DRG neurons (Fig. 1). The number of neurons with the diameter <20 μm in Cap rats reduced to 23% of the control rats. The number of small-diameter neurons (20–<30 μm) in Cap rats was about a half compared to that in the control rats. These results indicated a marked loss of small-diameter neurons in the DRG of Cap rats. On the other hand, there was no significant difference in the number of medium-diameter neurons (30–<40 μm) between the control and Cap groups. The numbers of medium- to large-diameter neurons (40–<50 and 50–<60 μm) were rather greater in Cap rats than in the control rats. When the effect of the capsaicin treatment on the expression of TRP channels in the DRG was examined by northern blot analysis (Fig. 2A), an ~70% decrease in the TRPV1 mRNA level in the DRG was found in Cap rats. The results also indicated a significant reduction (about 46%) in the TRPM8 mRNA level in the DRG of Cap rats, compared with that of the control rats; however, there was no difference in the TRPV2 mRNA level between the two groups. Moreover, a marked reduction (about 64%) in the TRPA1 mRNA level was detected in Cap rats (Fig. 2B).

Distinct Thermosensation and Thermoregulation in Rats Lacking Capsaicin-sensitive Neurons—To ascertain the functional defect expected for the rats lacking capsaicin-sensitive small sensory neurons in their DRGs, we first examined the response of rats to noxious heat. In the hot-plate test, Cap rats exhibited a significantly longer response latency than the control

rats (Fig. 3A). We then performed the cold-plate test to examine whether the reduction in small sensory neurons affected the sensation of cold temperature as well (Fig. 3B). When the rats were put on a cold plate (1°C), they showed several signs of discomfort such as paw-lifting, -licking and -shaking, which were quite similar to those in the hot-plate test. There was a tendency for a longer latency in the Cap group than in the control group ($P = 0.0895$), although the latency time was not significantly different between the two groups.

We also performed a cold tolerance test to examine whether the reduction in small sensory neurons affected the regulation of body temperature (Trectal) in the cold (Fig. 3C). The core temperature was slightly but significantly lower in Cap rats (37.3 ± 0.1°C) than in the control rats (37.6 ± 0.1°C) at the room temperature of 23°C (Time 0). When the rats were exposed to the cold at 5°C, Cap rats retained tolerance against the cold just like the control rats did; whereas the decrease in the Trectal of rats in the first 1 h seemed to be slow in Cap group (Δ 0.2°C) compared with that in the control group (Δ 0.6°C). The Trectal of rats was reduced 0.8 and 0.6°C by 5 h of cold exposure in the control and Cap group, respectively. We then measured the Tskin, an index of heat release, by using an infrared thermographic device. In contrast to the Trectal (Fig. 3C), the Tskin in the regular cage was slightly higher in Cap group than in the control group (27.1 ± 0.2 and 26.7 ± 0.1°C, respectively, $P < 0.01$; Fig. 3D). To further test the sensitivity to a small change in ambient temperature, the rats were transferred from their LE (LE, 23.5–24.3°C) to the TE (TE, 21.5–22.4°C), a drop of ~1–2°C. The Tskin of rats immediately decreased just after the change in ambient temperature in both groups (at 0 time), though the initial fall in Tskin was considerably smaller (~1.7°C) in Cap rats than in the control rats (~2.7°C). After that, the Tskin in the control rats was kept at a constant level, while the Tskin in Cap rats further decreased gradually and became close to the level in the control rats by 5 min (Fig. 3D).

Decrease in UCP1 Thermogenic Ability in the BAT of Capsaicin-treated Rats—To determine whether the lack of capsaicin-sensitive neurons affected the thermogenic ability, we examined the expression of UCPs in major thermogenic tissues; *i.e.* BAT and skeletal muscles. As shown in Fig. 4A, we detected a decrease in the steady-state level of UCP1 mRNA in the BAT of Cap group (about 80% of the control). There were no

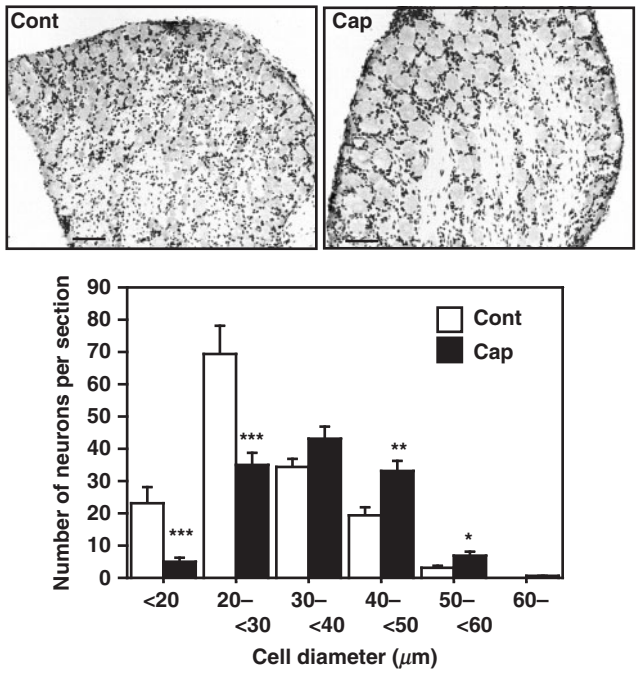

Fig. 1. **Histological analysis of the DRG in rats with neonatal treatment of capsaicin.** Tissue sections of the lumbar DRGs in the control (Cont) and capsaicin-treated (Cap) rats were stained with cresyl violet and thionine. Representative images are shown (scale bar, 100 μm). Cell soma diameter of the DRG neurons was measured and the data are expressed as mean ± SE. The numbers of sections analysed were 14 for the control group and 18 for capsaicin group. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus control group.

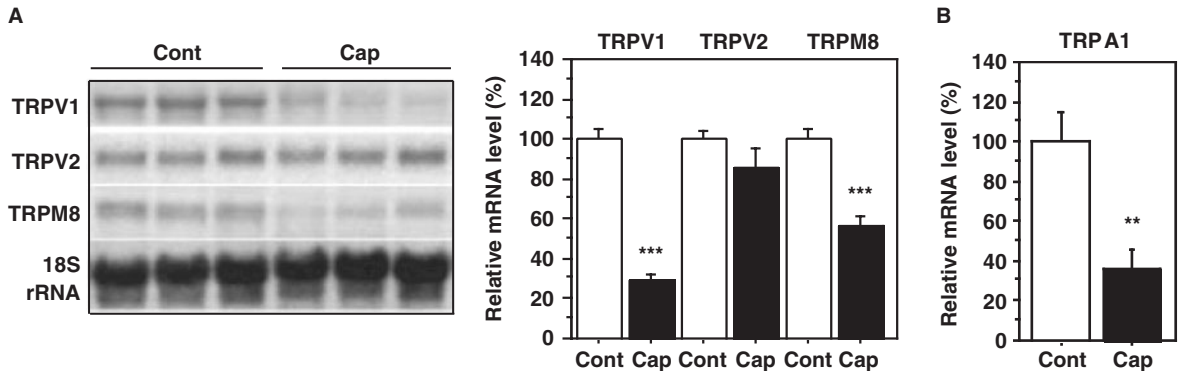

Fig. 2. **mRNA levels of TRPs in the DRG of rats.** (A) mRNA of TRPV1, TRPV2 and TRPM8 was analysed by northern blots using total RNA (10 μg) from DRGs of the control (Cont) and capsaicin-treated (Cap) rats, as described in MATERIALS AND METHODS section. The relative levels of mRNA for TRPs are expressed as means ± SE after normalization by 18S rRNA levels. The numbers of rats were 28 and 32 (for TRPV1 and TRPM8), and 12 and 11 (for TRPV2), in the control and capsaicin groups, respectively. $^{***}P < 0.001$ versus control group. (B) mRNA of TRPA1 was measured by real-time PCR and expressed as relative to GAPDH mRNA. There was no significant difference in the GAPDH mRNA levels between the groups. Data are expressed as means ± SE ($n = 8$ for control group, $n = 9$ for capsaicin group). $^{**}P < 0.01$ versus control group.

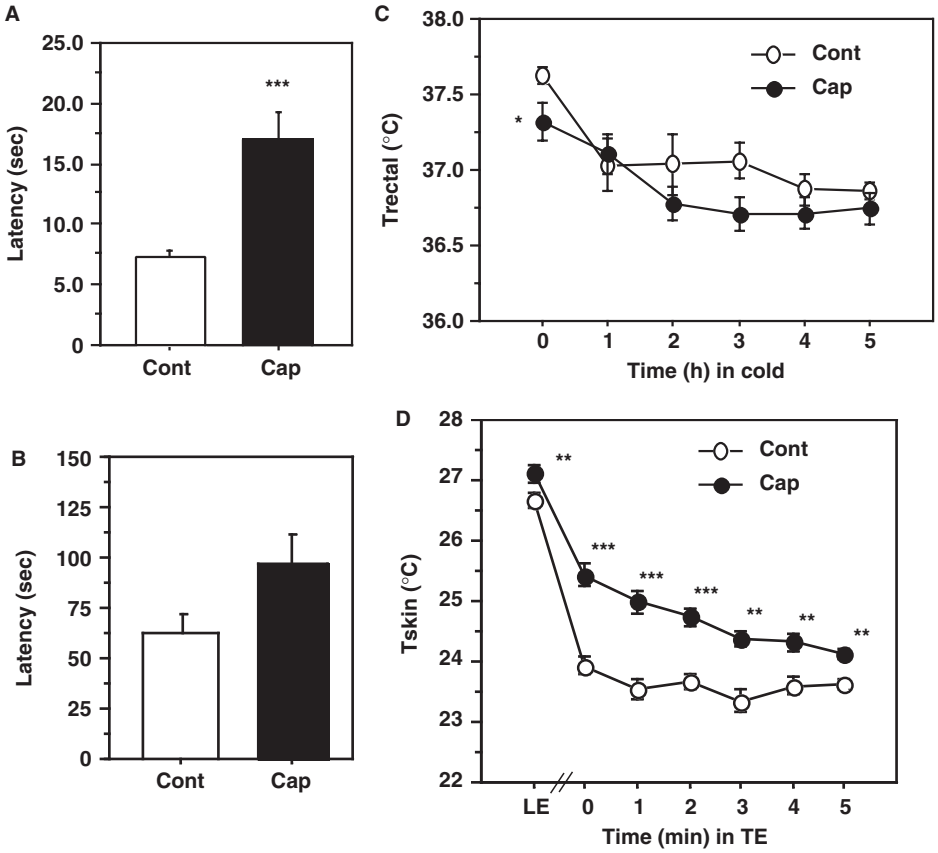

Fig. 3. **Thermal responses to various stimuli in the control (Cont) and capsaicin-treated (Cap) rats.** (A) Hot-plate test at $52 \pm 1^{\circ}C$ ($n = 24$ for the control group, $n = 22$ for capsaicin group). (B) Cold-plate test at $1^{\circ}C$ ($n = 8$ for the control group, $n = 10$ for capsaicin group). (C) Cold tolerance test. Rectal temperature (Trectal) of the rats exposed to a cold temperature of $5^{\circ}C$ was measured as described in MATERIALS AND METHODS section. The data for time 0 are those obtained at $23^{\circ}C$ ($n = 13$ for the control group, $n = 16$ for capsaicin group). (D) Regulation of heat release in response to a small change in ambient temperature. Tail skin temperature (Tskin, an index of heat release) of the unrestrained rat ($n = 6$ for each groups) was recorded for 5 min by an infrared thermographic device before and after the rats had been transferred from their living environment (LE: 23.5–24.3°C) to the test environment (TE: 21.5–22.4°C). Data are expressed as means ± SE. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ versus control group.

significant differences in UCP2 and UCP3 levels in the BAT or skeletal muscles between the two groups (data not shown). The decreased expression of UCP1 in the BAT of Cap rats was confirmed by the decrease in protein level, which was 67% of the control (Fig. 4B). In contrast, the COX IV level in Cap group was 30% higher than that in the control group. The GDP-binding activity, a marker of thermogenic activity, was significantly lower in the BAT of Cap rats than in that of the control ones (Fig. 4C). In addition, Scatchard plot analysis showed a considerable decrease in total GDP-binding sites (Bmax), but not in binding affinity (Kd; Fig. 4D), in Cap rats compared with those in the control rats.

DISCUSSION

To understand the mechanism of thermal homeostasis, many studies have been performed previously. Jancso-Gabor *et al*. (*13*) for the first time reported the effect of capsaicin treatment of adult rodents on thermoregulation. After this, capsaicin has been used as a tool to study the mechanism of thermoregulation as well as that of nociception, leading to the discovery of the capsaicin receptor, TRPV1 (*14*). Later Osaka *et al*. (*38*, *39*) demonstrated the role of capsaicin-sensitive nerve fibres in the regulation of BAT thermogenesis and thermal homeostasis by using anaesthetized rats. However, the role of capsaicin-sensitive neurons in the sensation of ambient temperature in conscious animals remained unknown. Likewise, the degree of desensitization by capsaicin treatment of mature rodents in previous studies is unclear. In the present study, therefore, we used neonatal capsaicin treatment, which is different from the desensitized rat model, as it is well established that a single capsaicin administration selectively destroys small sensory neurons in the peripheral ganglia in newborn rats, but not in mature rats (*33*, *40*).

Indeed, Mezey *et al*. (*41*) showed that the capsaicin treatment of neonatal rats significantly decreased the number of TRPV1-positive cells in their DRG, whereas the treatment did not affect the expression and/or distribution of the mRNA in the CNS except in the spinal trigeminal nucleus. Our results of the histological and RNA analyses of DRG also suggested a marked

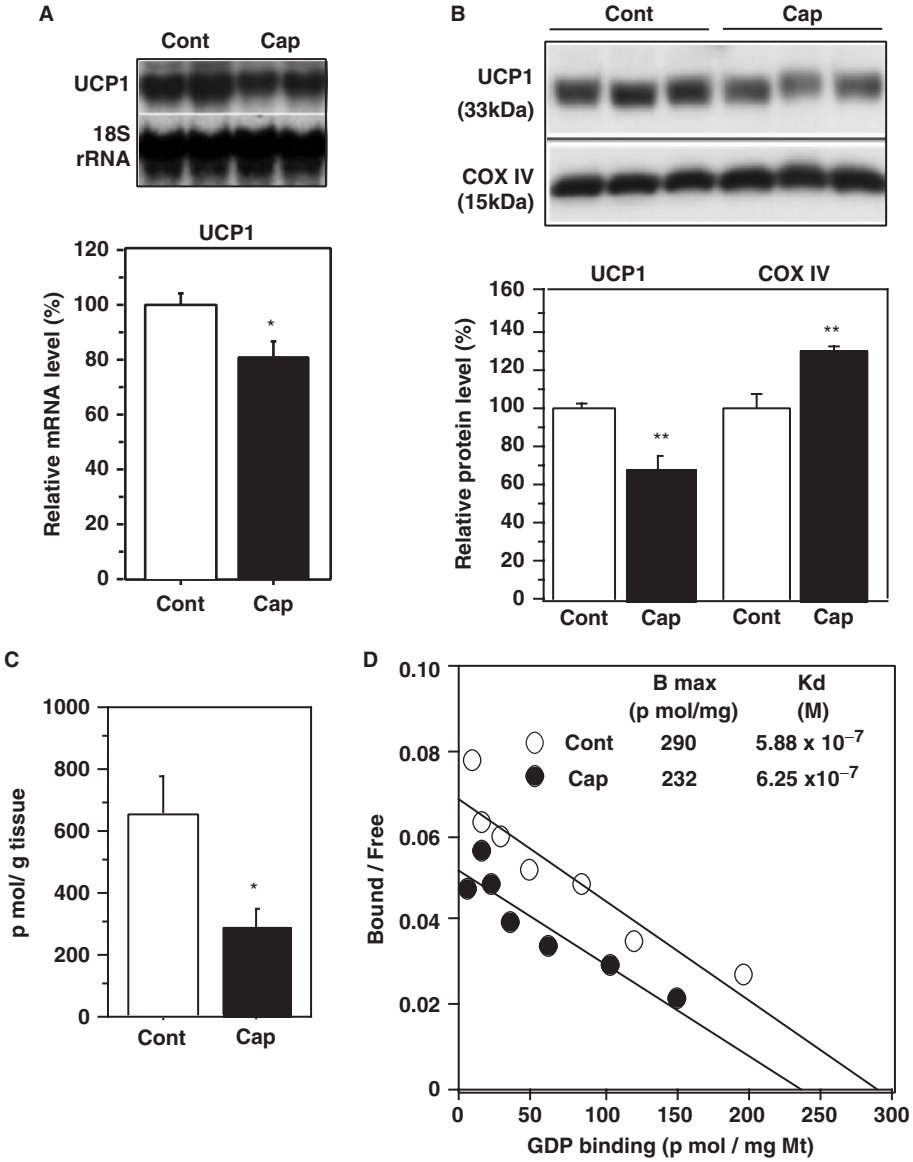

Fig. 4. **Reduction in UCP1 expression and thermogenic ability in the BAT of capsaicin-treated rats.** (A) Northern blots using total RNA (20 μg) of BAT from the control (Cont) and capsaicin-treated (Cap) rats were hybridized with the probes for UCP1 or 18S rRNA as described in MATERIALS AND METHODS section. The numbers of rats were 23 and 26 in the control and capsaicin groups, respectively. (B) Immunodetection of UCP1 and COX IV. (C) GDP-binding activity. (D) Scatchard plot analysis. Western blot analysis and GDP-binding assay using mitochondrial proteins isolated from the BAT of rats were performed as described in MATERIALS AND METHODS section. Data are expressed as means ± SE ($n = 6$ for each group). $^{*}P < 0.05$ and $^{**}P < 0.01$ versus control group.

reduction in the number of small sensory neurons expressing TRPV1 in the DRG of Cap rats. On the other hand, an increase in the numbers of medium- to large-diameter neurons was detected in the DRG of Cap rats. We presently do not know the reason; however, there may be a compensatory mechanism for the loss of small-diameter neurons in Cap rats. In addition, we found that the loss of capsaicin-sensitive small-diameter neurons was associated with a marked decrease in the mRNA level of TRPA1. This result may be reasonable, because TRPA1 is co-expressed with TRPV1 in a subset of nociceptive sensory neurons (*42*). We also found a significant reduction in the mRNA level of TRPM8 in the DRG of Cap rats. The present data suggest that TRPM8 was expressed in a subpopulation of TRPV1-expressing neurons in the rat DRG, which is consistent with the report of McKemy *et al*. (*18*), although the co-expression of these receptors in same subpopulation was controversial (*19*, *42*). Story *et al*. (*22*) have demonstrated that NGF treatment of DRG neurons isolated from adult rats elicited co-expression of TRPM8 and TRPV1 in culture. One may consider another possibility that the death of capsaicin-sensitive neurons caused a decrease in TRPM8-expressing neurons by some

unknown mechanism. Although the expression of TRPV3 in DRGs of monkeys and humans was reported previously (*21*, *23*), we could not detect a clear signal for TRPV3 in the rat DRG, in agreement with Peier *et al*. (*20*). These observations led us to the experiments to examine the effects of loss of the small sensory neurons expressing TRPV1, TRPA1 and/or TRPM8 on thermoregulation in rats.

To assess the responsiveness of rats to the ambient temperature, we used four different methods, *i.e*. the hot-plate test for the sensation of high temperature (~52°C), cold-plate test for the sensation of low temperature (~1°C), cold tolerance test for the regulation of body temperature in the cold (~5°C) and infrared thermography for the sensation of mild temperature (~23°C). As a result of these methods, rats lacking capsaicin-sensitive small sensory neurons displayed not only the heat-insensitivity but also a tendency to be insensitive to noxious cold, suggesting that heat and cool sensors are in the same subset of small sensory neurons. A reduction in the neurons expressing TRPM8 and/or TRPA1 might cause this insensitivity of cold sensation in rats, as suggested by knockout studies (*29–32*). Moreover, we found novel phenotypes indicating changes in thermal homeostasis in Cap rats. Particularly, the thermographic analysis revealed greater heat release in Cap rats than in the control ones. This finding may indicate an adaptive response to dissipate excess heat for the control of body temperature, which was significantly lower in Cap rats than in the control rats; because heat release from the body surface to the environment is huge and profoundly affects the regulation of body temperature (*6*, *39*, *43*). In addition, Cap rats were significantly insensitive to a small change in ambient temperature around 23°C as well as to noxious temperature, suggesting that capsaicin-sensitive small sensory neurons in DRG is responsible to sense the mild temperature. This may be related to a considerable decrease in the mRNA level of TRPM8, which functional range covers the ambient temperature in a normal animal facility (*18*, *19*). In the cold tolerance test, the change in Trectal after 1 h of cold exposure was smaller in Cap rats than in the control rats. Since TRPA1 senses the lowest temperature (<18°C) among the members of the thermosensitive TRP channel family (*22*), the blunt response of Trectal to acute cold exposure in Cap rats could be associated with the decrease in the number of TRPA1 and an impaired sensation of cold temperature.

Adaptive thermogenesis is an important response in mammals to environmental alterations such as cold and excessive food intake in order to maintain body temperature or energy homeostasis (*8–10*). BAT and skeletal muscle are the principal thermogenic organs and dominantly express UCP1 and UCP3, respectively. The critical role of UCP1 in adaptive thermogenesis in the cold was verified by studies using UCP1-knockout mice (*11*, *12*). With respect to the effect of capsaicin on the regulation of body fat, Cui and Himms-Hagen (*44*) reported an atrophy of and decrease in the UCP1 level in the BAT of the capsaicin-desensitized rats; however, the functional change in thermosensation was not determined. Our results showed a significant decrease in BAT theromogenic ability in Cap rats, which displayed a lowered core temperature and an increased heat release under normal housing conditions. Interestingly, these phenotypes were contrast to those in UCP1-knockout mice, in which thermosensation seems to be normal but the core temperature tended to be higher than that in wild-type mice (*45*). Several groups including our group have demonstrated that induction of UCP1-independent thermogenesis and strong vasoconstrictor response contributed to the regulation of body temperature in mice lacking UCP1 under the thermal conditions of ~20–25°C in the normal animal facilities (*6*, *12*, *45*). The difference in thermoregulation between Cap rats and UCP1-knockout mice may be related to that of the target tissue in these animal models. Namely, capsaicin-sensitive afferent neurons were destroyed and hence several types of thermo-sensitive channels were markedly reduced in Cap rats, which caused abnormal integration of thermosensation and affected the mechanisms to maintain body temperature such as thermogenesis in BAT (an effector of thermoregulation). In UCP1-knockout mice, however, BAT thermogenesis was eliminated by the target manipulation of *Ucp1* gene but thermosensation was normal, in which UCP1-independent thermogenesis was induced and heat conservation was strengthened for homeothermic regulation. Taken together, the results in the two animal models suggest that normal recognition of ambient temperature *via* thermosensory neurons is a crucial factor to elicit proper thermal responses in homeothermic regulation. Furthermore, the changes in thermoregulation in Cap rats could be related to a decline in sympathetic nervous activity, because norepinephrine stimulates UCP1 expression and BAT thermogenesis and simultaneously has a vasoconstriction effect, which decreases peripheral blood flow and suppresses heat loss from the body (*12*, *43*). Scatchard plot analysis indicated a decrease in BAT thermogenic capacity and a reduced demand for UCP1 thermogenesis in Cap rats. The present data also suggest a role of UCP1 in the regulation of basal thermal homeostasis. If there is an intimate interplay between thermosensation and thermoregulation, how is the thermoregulation in mice lacking TRP channels such as TRPM8 and TRPA1? Unfortunately, the studies using knockout mice did not look into the consequences of loss of cold sensitivity on thermoregulation and core temperature, as pointed out by Nilius and Voets (*46*). Because body temperature is controlled by the balance between heat loss and production, the changes in thermosensation by gene knockout of TRP channels may affect thermal responses such as thermogenesis through regulation of UCP1 expression.

Thus, the present study indicated a critical role of capsaicin-sensitive small sensory neurons in thermosensation not only of noxious heat but also of mild room temperature. The change in thermosensation by loss of the capsaicin-sensitive neurons profoundly affected basal thermal homeostasis, which is balanced by heat release and production. It is plausible that the decreased sensitivity of Cap rats to a change in the ambient temperature in the LE was originated, at least in part, from the marked reduction in the numbers of TRPM8- and/or TRPA1-expressing neurons, coincident with the reduction in TRPV1-expressing small-diameter neurons

relating to another phenotype of heat insensitivity. In addition, UCP1 thermogenesis appears to be involved in basal thermal homeostasis in rats. However, further studies such as those using double-knockout animals are required to understand the precise mechanisms of the cross-talk between thermosensation and thermoregulation and their contribution to basal thermal homeostasis *in vivo*.

We are grateful to Ms T. Suzuki, I. Nakano and Z. Wang for their skillful technical assistance. We also thank for Dr M. Tominaga for critical reading of the manuscript. This work was supported by a grant from the program Health Sciences Research Grants, Comprehensive Research on Aging and Health, of the Ministry of Health, Labor and Welfare (to H.Y.) and in part by research funding from CREST (Core Research for Evolutional Science and Technology (to N.M.). Most of this study was performed at Department of Molecular Genetics in National Institutes for Longevity Sciences.

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