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Neonatal capsaicin treatment in rats affects TRPV1-related noxious heat sensation and circadian body temperature rhythm

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ABSTRACT

The transient receptor potential vanilloid 1 (TRPV1) is a cation channel that serves as a polymodal detector of noxious stimuli such as capsaicin. Therefore, capsaicin treatment has been used to investigate the physiological function of TRPV1. Here, we report physiological changes induced by treating neonatal rats with capsaicin. Capsaicin (50 mg/kg) (cap-treated) or vehicle (vehicle-treated) was systemically administered to newborn SD rat pups within 48 h after birth. TRPV1 expression, intake volume of capsaicin water, and noxious heat sensation were measured 6 weeks after capsaicin treatment. Circadian body temperature and locomotion were recorded by biotelemetry. Expression of Per1, Per2, Bmal1 and Hsf1 (clock genes) was also investigated. Neonatal capsaicin treatment not only decreased TRPV1 expression but also induced desensitization to noxious heat stimuli. Circadian body temperature of cap-treated rats increased significantly compared with that of vehicle-treated rats. Additionally, the amplitude of the circadian body temperature was reversed in cap-treated rats. Expression of the hypothalamic Hsf1 and liver Per2 clock genes followed a similar trend. Therefore, we suggest that these findings will be useful in studying various physiological mechanisms related to TRPV1.

1. Introduction

Capsaicin is the main component of hot chili peppers and it is responsible for their spicy flavor and pungent taste. Capsaicin that has been subcutaneously injected into newborn rat pups acts as a neurotoxin that specifically destroys a subset of small-diameter primary afferents [1].

The transient receptor potential vanilloid 1 (TRPV1) channel is a member of a larger family of transient receptor potential ionic channels [2]. The TRPV1 channel is a ligand-gated non-selective cation channel that is permeable to Ca^{2+}. *In vitro*, it is activated by noxious heat, as well as by protons, capsaicin and some endogenous factors [3]. For this reason, capsaicin is considered to be a tool to investigate sensory fiber functions mediated by TRPV1, such as touch, pain, and taste including thermosensation [4,5].

Studies of TRPV1 have provided various sensory mechanisms. For example, blocking TRPV1 with various antagonists can elicit hyperthermia in rodents [6]. However, this effect has not been shown in TRPV1 knockout mice [7,8]. These results suggest that tonical TRPV1 activity *in vivo* regulates body temperature [9]. Additionally, repeated injection of capsaicin at a high dose could induce desensitization of noxious heat responses in rodents [8,10].

Many studies on various sensory mechanisms mediated by TRPV1 have been conducted *via* chronic treatment of capsaicin in adult rodents. Moreover, only one study has reported on the physiological effects of reducing the expression of TRPV1 in neonates [11].

In this study, we examined the changes in TRPV1 expression induced by neonatal capsaicin treatment in rats and investigated the physiological responses to noxious heat stimuli related to the thermal range of TRPV1. In particular, core temperature was recorded using biotelemetry in unrestrained rats that were treated with capsaicin as neonates. Similar data were collected from rats that were treated with vehicle for comparison.

2. Materials and methods

2.1. Animals

Animal facilities were approved by the Association of Assessment and Accreditation of Laboratory Animal Care (AAALAC), and all experiments were performed under the institutional guidelines established by the Institutional Animal Care and Use Committee at Yonsei University (IACUC-2012-0177). Pregnant Sprague–Dawley (SD) rats were obtained 1 week before parturition, housed individually in plastic cages with soft bedding, and allowed to deliver. Pups in each litter were randomly

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assigned to the experimental groups and weaned at postnatal day 21, at which time they were separated based on sex and housed in groups of three to five until the end of the experiment. Only males were used in the present study. All animals were maintained in a 12-hour light/dark cycle (light on, 08:00) at 22–25 °C with free access to food and water.

2.2. Neonatal capsaicin treatment

As described previously [12], SD rat pups were systemically administered 10 μl/g (body weight) capsaicin (cap-treated) (50 mg/kg, Sigma, MO, USA) or vehicle (vehicle-treated) (saline containing 10% Tween 80 and 10% ethanol) within 48 h of birth.

2.3. TRPV1 immunofluorescence

Six weeks after capsaicin treatment, rats were deeply anesthetized with pentobarbital (50 mg/kg, i.p., Sigma, MO, USA) and perfused with heparinized saline followed by 4% paraformaldehyde and 0.1% picric acid in 0.1 M phosphate buffer (PB, pH 7.4). The S1 dorsal root ganglia (DRG) were harvested by laminectomy and then cryoprotected within 30% sucrose in 0.1 M PB at 4 °C overnight. The DRG were sectioned at 14 μm on the cryostat. Sections were blocked with 1% bovine serum albumin and 10% normal goat serum in 0.05 M phosphate buffered saline for 1 h at room temperature. They were then incubated with guinea pig polyclonal anti-TRPV1 (1:1000, Chemicon, CA, USA). The sections were incubated with a mixture of FITC-conjugated secondary antibodies at RT for 1 h. Fluorescent signals were detected with a fluorescence microscope (BX51, Olympus, Tokyo, Japan). Six to ten sections were randomly selected for each rat and the number of TRPV1-immunofluorescent cells was counted.

2.4. Intake volume of capsaicin water

To assess the ability to taste capsaicin, intake volume of capsaicin water was assessed using a paired preference test [13]. Bottles of capsaicin-dissolved water (1.65 μM in 0.05% ethanol) and vehicle water (0.05% ethanol) were placed in each cage. Rats were given free access to capsaicin-dissolved and vehicle water. The intake volume of each bottle was measured daily for 10 days.

2.5. Evaluation of noxious heat sensation

As described previously [14], paw-withdrawal latency to radiant infrared (IR) heat stimulation was measured using a plantar test apparatus (IITC Plantar Analgesia Meter, IITC Inc., Woodland Hills, CA, USA). The cutoff time was 20 s to prevent potential tissue damage. Tails were dipped in hot water (43 °C) to measure the tail-withdrawal latency. The cutoff time was 15 s. All experiments were performed three times at 15-minute intervals to avoid adaptation to the hot temperature. Results were calculated as average withdrawal behavior.

2.6. Measurement of circadian rhythm

Physiological changes were measured by using small implantable transponders (HR E-Mitter, PDT-4000, Bend, OR, USA). The transponders were implanted into the abdominal cavity of rats under deep gas anesthesia using isoflurane (0.5–2%, Hana Pharm. Co., Ltd., Seoul, Korea). All circadian temperature and locomotion data were received by an ER4000 receiver (56 × 29 × 7 cm, RS 232 serial, Bend, OR, USA) and automatically recorded on a main computer. After measuring for 10 consecutive days, data were separated according to the 12-hour light (day)/dark (night) cycle to analyze daily temperature and locomotion.

2.7. Real-time PCR analysis

Rat hypothalamus and liver tissues were harvested 3 h after light on (zeitgeber time (ZT) 3) or off (ZT15) under euthanasia. Each tissue was chopped to 2-mm^2 samples and biopsies were collected to isolate total RNA using an RNeasy® Micro kit (Qiagen®, Venlo, Limburg, Netherlands) according to the manufacturer's instructions. The total RNA was reverse transcribed to cDNA using a Reverse Transcription System (Promega, Madison, WI, USA). Real-time PCR was performed in a total reaction volume of 20 μl containing a SYBR Green PCR Master Mix (10 μl, Applied Biosystems, CA, USA), a primer pair (1 μl each of 10 pmol/μl primers) and diluted cDNA (8 μl, 500 ng/μl) using a real-time PCR system (#7000, Applied Biosystems, CA, USA). The relative expression of clock genes was determined in comparison to a housekeeping gene (GAPDH), using the $2^{-\Delta\Delta C_T}$ method [15]. Primer pairs for rat Per1, Per2, Bmal1, Hsf1 and GAPDH are listed in Table 1.

2.8. Statistical analysis

All data are presented as mean ± SEM. Statistical significance was analyzed using Student's t-test or the Mann–Whitney rank sum test, depending on normality. A $P < 0.05$ was considered to be statistically significant. All statistical analyses were carried out using SigmaStat (ver. 3.5, Systat Software Inc., IL, USA).

3. Results

3.1. Neonatal capsaicin treatment reduces the number of TRPV1-expressing DRG neurons

The percent of TRPV1-immunofluorescent DRG neurons in rats treated neonatally with capsaicin decreased to 5.12%. This result represented a significant 15.8% decrease compared to that of the vehicle-treated group (Fig. 1A and B). By real-time PCR, TRPV1 mRNA decreased approximately 40% in the L5 DRG of the cap-treated group compared with the vehicle-treated group (Fig. 1C).

3.2. Neonatal capsaicin treatment impaired sensing of capsaicin and noxious heat stimuli

The vehicle-treated rats clearly preferred vehicle water to capsaicin-containing water. The cap-treated rats did not show a preference between capsaicin water and vehicle water. The cap-treated rats took in significantly more capsaicin water than did the vehicle-treated rats (Fig. 2A). In the plantar test (Hargreaves' method), the cap-treated rats showed significantly longer paw-withdrawal latency in response to noxious infrared radiant heat (Fig. 2B). Tail-withdrawal latency after immersion in a 43 °C water bath was also remarkably increased in the cap-treated rats (Fig. 2B).

3.3. Neonatal capsaicin treatment induces abnormal rhythm of circadian body temperature while the rhythm of locomotor activity remains normal

Circadian rhythm was separated by a 12-hour light and dark cycle. The abdominal temperature of rats belonging to either the cap- or vehicle-treated rats changed clearly between night and day (Fig. 3A).

Table 1
The oligonucleotide pairs for real-time PCR.

Gene	Forward	Reverse
Per1	*GGCTCCGGTACTTCTCTTTC*	*AATAGGGGAGTGGTCAAAGG*
Per2	*CATGGCCAGTGTGCAGAGAG*	*GAGGCCACCAAACAGACAGG*
Bmal-1	*AACCCGTGGACCAAGGAAGT*	*GTGAGCTGTGGGAAGGTTGG*
Hsf1	*CTGGTGCACTACACGGCTCA*	*GTTGTGCTGGCTTGACCTAG*
GAPDH	*ACAACCACAACGATTCTTCTGTAA*	*CCTTTACAAGTTTTGCTGTGCTAA*