ORIGINAL ARTICLE

Effects of Moxibustion on Body Core Temperature Responses in Rats

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Synopsis
We examined the neurological and pathological effects of moxibustion on body temperature in the rat by using in vivo physiological and pathological approaches. To mimic direct and indirect moxibustion, the forelimb skin of rats was stimulated by heat at 80 or 40°C. Heat at 40°C evoked rapid increases in rectal temperature and heart rate. Surgical and chemical sympathectomy antagonized these effects. In contrast, heat at 80°C evoked slow increases in rectal temperature and heart rate, as well as in blood tumor necrosis factor-α and interleukin-6. Although the concentrations of these pyrogens increased significantly, surgical and chemical sympathectomy antagonized these effects. Collectively these data demonstrate that moxibustion may increase body core temperature mainly through sympathetic nerve stimulation, and that low-temperature stimulation is more effective than high-temperature treatment. Therefore, indirect moxibustion or moxibustion at low temperature is safer than direct moxibustion.

Key words: thermoreceptor, pyrogen, autonomic system

Introduction
Moxibustion is a traditional Chinese medical therapy widely used in East Asian countries [1]. In this therapy, skin is heated directly or indirectly by burning moxa. In direct moxibustion, burning moxa in a stick is applied onto or above an acupuncture point. In contrast, indirect moxibustion uses salt, ginger, or garlic under the burning moxa to avoid skin damage [2–5]. The needle-warming technique is also used to avoid skin damage [6]. In this technique, the skin is stimulated by needle penetration at the acupuncture point, and burning moxa applied over the needle warms the skin around the acupuncture point. With all of these techniques, moxibustion evokes a warm sensation. However, the exact mechanism by which moxibustion induces this warm sensation is unknown.

Information on skin temperature is received by heat receptors [7] and then conducted through
afferent neuronal pathways to the thermoregulatory center in the brain [8–10]. When the skin temperature exceeds 45 °C, heat nociceptors are activated and the tissue is damaged [11]. Pyrogens released from degenerating tissue are sensed by the thermoregulatory center. Information from the skin thermoreceptors is integrated with immune signals in the thermoregulatory center. Command signals are then provided to peripheral effectors through efferent neuronal and endocrine pathways. Sympathetic nerves control nonshivering thermogenesis in brown adipose tissue, as well as cutaneous vasomotion and tachycardia; somatic nerves mediate shivering thermogenesis, which drives skeletal muscles [12, 13].

Pyrogens released from degenerating tissue can cause the set point of the thermoregulatory center to increase. Efferent thermogenetic pathways are then activated to increase the body temperature. If moxibustion were to activate pyrogen release, then the warm sensation that patients experienced would be caused by skin inflammation. However, moxibustion therapists are very careful not to burn the skin, and the treatment evokes a warm sensation in the absence of skin tissue damage. The mechanisms by which moxibustion causes the warm sensation are therefore unknown. Our aim was to examine the neurological and pathological effects of moxibustion on body temperature by using physiological and pathological approaches in a rat model. We examined [1] whether moxibustion changes body core temperature, [2] whether moxibustion induces the production of pyrogens, especially tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), levels of which increase after skin burn, and [3] if moxibustion does change body core temperature, whether sympathetic and somatic efferent nerves are involved in thermogenesis.

Materials and Methods
A total of 50 male Sprague–Dawley rats (Japan SLC, Inc., Hamamatsu) in the weight range 230 to 260 g were used. Animals were maintained in a standardized environment under a 12-h light-and-dark cycle (lights on at 7:00 a.m.) with ad libitum access to food and water. All procedures were approved by the Osaka University Intramural Animal Care and Use Committee and the Animal Ethics Committee of Morinomiya University of Medical Sciences in accordance with the guidelines of the National Institutes of Health, USA, and all efforts were made to minimize the number of animals used.

Moxibustion evokes multisensory sensations, including touch, pressure, warmth, pain, and chemical senses [6]. We therefore used a thermostimulator to eliminate the influence of sensations other than warmth. To mimic direct and indirect moxibustion, the forelimb skin of the rats, corresponding to the acupuncture point Arm Five Li, was stimulated by heat at 80 or 40°C.

Experiment 1: Body temperature responses to indirect and direct moxibustion
Twenty rats were allocated to two groups: an indirect moxibustion group (n = 10) and a direct moxibustion group (n = 10). Anesthesia was induced and maintained by pentobarbital sodium (50 mg/kg, i.p.), and the level of responsiveness to anesthesia was monitored by toe pinch. Rectal temperature and heart rate were monitored continuously throughout the experiment with a thermometer (PTC-201, Unique Medical Co., Ltd., Tokyo) and polygraph (DEN751S, Unique Medical Co., Ltd., Tokyo), respectively. The dorsal surface of the upper forelimb in an area corresponding to the acupuncture point Arm Five Li (LI13) was shaved and rinsed preoperatively. A Pain Meter Thermo (UDH-105, Unique Medical Co., Ltd., Tokyo) was used for thermal stimulation (Fig. 1). In the indirect moxibustion

![Fig. 1](image-url)
group, 10 sets of stimulation for 30 s at 40°C, with an interval of 10 s between sets, were applied to LI13. In the direct moxibustion group the same treatment was given but at 80°C. At 2 and 6 h after the last stimulation, blood was collected from the external carotid vein into sterile tubes containing pyrogen-free heparin (10 U/mL) and then centrifuged (5000 g, 4°C, 10 min). Plasma was stored at –70°C until further processing. IL-6 and TNF-α in the plasma samples were measured by specific ELISA kits (Quantikine ELISA Kits, R&D Systems Inc., MN, USA). The minimum detectable concentration was less than 5 pg/mL for TNF-α and less than 20 pg/mL for IL-6.

**Experiment 2: Effect of surgical sympathectomy on body temperature responses**

Rats were subjected to surgical removal of the superior and cervical ganglia and stellate ganglia (n = 10) or to sham surgery (n = 10). Under halothane anesthesia (2.0%), the animal was placed in a supine position on an electric heating pad and its body temperature was monitored with a rectal thermistor probe throughout the entire surgical procedure. The level of anesthesia was occasionally monitored by toe pinch. All wound margins were anesthetized with small injections of lidocaine hydrochloride. The ventral surface of the neck was shaved and rinsed preoperatively. The salivary glands were exposed through a 2 cm vertical incision and then retracted to expose the strap muscles to permit the identification of each superior cervical ganglion and stellate ganglion. The ganglia were carefully and totally removed from both sides by using microsurgical forceps; the sham surgery employed the same surgical approach but without the removal of ganglia. Following completion of the surgical procedures, the wound was cleansed and then closed with nylon sutures. After recovering from anesthesia, the animals received a single dose of ampicillin (30 mg/kg) and were replaced in their plastic cages on receiver boards. Body temperature was continuously recorded. The same thermal stimulation, followed by physiological recordings and pyrogen assays, was conducted as described in Experiment 1.

**Experiment 3: Effect of chemical sympathectomy on body temperature responses**

Rats (n = 5) were given an injection (100 mg/kg, i.p.) of 6-hydroxydopamine hydrobromide (6-OHDA; Sigma, St. Louis, MO, USA) dissolved in phosphate-buffered saline (PBS, pH 7.2) containing 0.1% ascorbic acid for 3 days. Sham-injected animals (n = 5) given PBS alone served as controls. After 6 days, thermal stimulation, followed by physiological recordings, was conducted on the 6-OHDA-treated and sham-injected animals.

![Figure 2](image)

**Figure 2** Effects of thermal stimulation on core temperature and heart rate.

Rectal temperature (A) and heart rate (B) were monitored continuously. Stimulation at 40°C (white columns) and 80°C (black columns) induced increases in rectal temperature and heart rate. These peaked 30 min after stimulation at 40°C and 3 h after stimulation at 80°C. Graphs show peak values after stimulation. Data are means ± SD; ANOVA analysis with Bonferroni-Holm correction, *P < 0.05 vs. corresponding values in the control groups.
**Statistical analysis**

All data were expressed as means ± SD. Statistical differences between experimental groups were determined by analysis of variance (ANOVA). Bonferroni-Holm correction was applied to obtain corrected $P$ values for multiple comparisons. Levels of $P < 0.05$ were considered significant.

**Results**

**Body temperature and heart rate are elevated in response to indirect and direct moxibustion**

Rectal temperature and heart rate were monitored continuously to evaluate the effects of moxibustion. Although these values dropped quickly and then became unstable after initiation of anesthesia, they were recorded as non-heated values when they recovered and stabilized. Stimulation of LI13 at 40°C induced increases in rectal temperature and heart rate. Rectal temperature and heart rate increased rapidly and peaked 30 min after stimulation (36.1 ± 0.3°C compared with 33.1 ± 0.3°C unstimulated; 400.1 ± 8.0 bps compared with 330.1 ± 8.2 bps unstimulated; $P < 0.05$). No inflammatory signs were seen on the surface of the skin at LI13. Stimulation at 80°C also induced increases in rectal temperature and heart rate. However, these peaked 3 h after stimulation (36.0 ± 0.3°C compared with 33.0 ± 0.4°C unstimulated; 405.3 ± 5.2 bps compared with 332.2 ± 5.1 bps unstimulated; $P < 0.05$) (Fig. 2). Severe burns were seen on the surface of the skin at LI13. Stimulation at 80°C also induced significant increases in circulating TNF-α levels, which peaked 2 h after stimulation (520.0 ± 20.3 pg/mL compared with 95.2 ± 20.1 pg/mL with 40°C stimulation, $P < 0.05$). Circulating interleukin-6 levels also increased but peaked 6 h after stimulation (340.0 ± 25.2 pg/mL compared with 100.2 ± 25.1 pg/mL with 40°C stimulation, $P < 0.05$) (Fig. 3).

**Effect of surgical sympathectomy on body temperature responses**

Body weight before surgery did not differ between the animals that underwent surgical cervical ganglionectomy and the sham-operated animals.

In the sham-operated group, 40°C stimulation of LI13 induced increases in rectal temp-
temperature and heart rate. Rectal temperature and heart rate increased significantly and peaked at 30 min (36.1 ± 0.3°C compared with 33.1 ± 0.2°C unstimulated; 400.2 ± 9.1 bps compared with 332.1 ± 5.3 bps unstimulated; P < 0.05) (Fig. 4A, 4C).

No inflammatory signs were seen on the surface of the skin at LI13. Stimulation at 80°C also induced increases in rectal temperature and heart rate. However, they peaked 3 h after stimulation (36.0 ± 0.3°C compared with 33.0 ± 0.4°C unstimulated; 409.5 ± 4.9 bps compared with 332.1 ± 5.3 bps unstimulated; P < 0.05) (Fig. 4B, 4D). Clear reddening was seen on the surface of the skin at LI13.

In contrast, in the surgical cervical ganglionection group, no significant changes were seen in rectal temperature or heart rate after thermal stimulation. Stimulation at 40°C had no effects on rectal temperature or heart rate (33.1 ± 0.2°C compared with 33.1 ± 0.2°C unstimulated; 332.6 ± 5.0 bps compared with 332.4 ± 5.0 bps unstimulated; P > 0.05) (Fig. 4A, 4C). Stimulation at 80°C also had no effect on rectal temperature or heart rate (33.1 ± 0.1°C compared with 33.0 ± 0.4°C unstimulated; 331.4 ± 7.4 bps compared with 330.0 ± 8.4 bps unstimulated; P > 0.05) (Fig. 4B, 4D), although, it did cause clear reddening on the surface of the skin at LI13 (Fig. 5).

Figure 4   Effects of surgical sympathectomy on body temperature responses. Rectal temperature (A, B) and heart rate (C, D) were measured after stimulation at 40°C (A, C) or 80°C (B, D) with or without surgical sympathectomy. Graphs show peak values after stimulation. Data are means ± SD; ANOVA analysis with Bonferroni-Holm correction, *P < 0.05 vs. corresponding values in control groups.
Effect of chemical sympathectomy on body temperature responses

Body weight before surgery did not differ between the animals that underwent chemical sympathectomy and the sham-operated animals. With chemical sympathectomy, no significant changes were seen in rectal temperature or heart rate after thermal stimulation. Stimulation at 40°C had no effect on rectal temperature (33.1 ± 0.2°C compared with 33.0 ± 0.2°C unstimulated) or heart rate (330.2 ± 5.0 bps compared with 329.6 ± 5.6 bps unstimulated; \( P > 0.05 \)). Stimulation at 40°C also had no effects on rectal temperature (33.0 ± 0.3°C compared with 32.9 ± 0.2°C unstimulated) or heart rate (331.4 ± 7.4 bps compared with 330.0 ± 8.4 bps unstimulated; \( P > 0.05 \)) (Fig. 6), although stimulation at 80°C caused severe burns on the surface of the skin at LI13.

Discussion

Our results provide experimental evidence to support the involvement of moxibustion in body core temperature increase and thermogenesis. Stimulation of LI13 at both 40°C and 80°C significantly increased rectal temperature and heart

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**Figure 5** Effects of surgical sympathectomy on blood pyrogen levels. TNF-\( \alpha \) (A) and IL-6 (B) were measured after stimulation at 40 and 80°C with or without surgical sympathectomy. Graph shows peak values after stimulation. Data are means ± SD; ANOVA analysis with Bonferroni-Holm correction, * \( P < 0.05 \) vs. corresponding values in control groups.

**Figure 6** Effects of chemical sympathectomy on body temperature responses. Rectal temperature (A) and heart rate (C) were measured after thermal stimulation at 40°C (white columns) and 80°C (black columns) with or without 6-OHDA injection. Graphs show peak values after stimulation. Data are means ± SD; ANOVA analysis with Bonferroni-Holm correction.
rate. Stimulation at 80°C increased the concentrations of circulating TNF-α and IL-6; however, stimulation at 40°C had no effects. Surgical cervical ganglionectomy antagonized the effects of thermal stimulation on rectal temperature and heart rate. However, surgical cervical ganglionectomy did not change the effect of stimulation at 80°C on circulating TNF-α and IL-6. Chemical sympathectomy also antagonized the effects of thermal stimulation on rectal temperature and heart rate.

Body temperature is regulated through a variety of involuntary thermoregulatory responses. Thermoreceptors in the skin [7], abdominal cavity [14], brain [15], and spinal cord [16] send information through sensory afferent neuronal pathways to the thermoregulatory center, which is located in the hypothalamus. In inflammation, immune signals are delivered to the brain and trigger the production of prostaglandin E2 (PGE2), which is sensed by the thermoregulatory center. The thermoregulatory center integrates this information and then provides command signals to peripheral effectors via sympathetic and somatic nerves [12, 13].

**Effect of moxibustion on the cardiovascular system**

In hot environments, evaporative cooling and cutaneous vasodilation occur in animals. Tail blood flow is crucial for dissipating body heat in rats and dissipates 25% of heat production during periods of heat stress, because the rat’s thick, hairy skin prevents evaporative cooling or sweating [17]. When skin heat receptors detect an increase in temperature, vasodilation occurs in the tail artery. The rat tail has one large artery, the medial caudal artery, which stems from the abdominal aorta and median sacral artery [18, 19]. Therefore, increases in abdominal and sacral blood flow and poor evaporative cooling are possible causes of rectal temperature rise. Moreover, heat receptors in the skin are thought to be involved in the feed-forward neural mechanism [20, 21]. We found here that stimulation at 40°C caused a relatively rapid rectal temperature increase. This change in rectal temperature is likely involved in the feed-forward mechanism.

Skin warming also increases heart rate [20]. Warming-induced tachycardia increases cardiac output and arterial pressure, which then increase tail blood flow, resulting in a rapid increase in rectal temperature.

Indirect moxibustion is likely involved in these neural mechanisms and thus evokes a sensation of warmth in human patients, although rats are far smaller than humans.

**Effect of moxibustion on the immune system and on body core temperature**

TNF-α, a macrophage product, is involved in systemic inflammation and stimulates the acute-phase reaction of fever [22]. TNF-α shares many biologic properties with interleukin-1 (IL-1), which acts directly as an endogenous pyrogen on the thermoregulatory center in the hypothalamus. Unlike TNF-α levels, IL-1 levels are not increased systemically in response to thermal injury [23]. TNF-α levels rapidly increase in the plasma, peaking 2 h after thermal injury [24]. We did not investigate the kinetics of pyrogen concentrations; however, the concentration of circulating TNF-α 2 h after stimulation at 80°C was significantly higher than that after stimulation at 40°C.

IL-6 blood levels increase significantly within the first 6 h after burn injury [24]. IL-6 levels rise when IL-1 and TNF-α have been synthesized. Therefore, there is a delay between the increase in circulating TNF-α levels and the increase in IL-6 levels [25]. These pyrogens are capable of crossing the blood-brain barrier and initiating synthesis of PGE2 in the thermoregulatory center, thereby changing the body’s temperature set point [26]. Inflammatory cytokines that are released from immune cells act on the endothelial cells of blood vessels in the brain and thereby induce the expression of enzymes for biosynthesis of PGE2 [27, 28]. These enzymes include cyclooxygenase-2 [27, 28], suggesting that IL-6 prolongs PGE2 synthesis and thermogenesis. Our stimulation at 80°C was far more severe than that in the direct moxibustion usually performed in humans. However, moxibustion at 38°C and 46°C causes inflammatory reactions in the rat. Therefore, prolonged thermal sensation is partially involved in cytokine-mediated thermogenesis.
Effect of moxibustion on sympathetic control of thermogenesis

In rats, interscapular brown adipose tissue (BAT) is a major organ of heat production through nonshivering thermogenesis. Sympathetic nerves regulate BAT by activating $\beta_3$-adrenoceptors [29, 30]. During fever, pyrogen-induced PGE$_2$ raises the set point in the hypothalamus and then sympathetic nerves activate BAT thermogenesis. Sympathetic neurons arising largely from the third cervical ganglia innervate BAT [30]. In the rat, the cervical sympathetic chains have two prominent ganglia, the superior cervical ganglion and the inferior cervical ganglion. The superior cervical ganglion sends axons to cranial nerves 9 to 12 and cervical spinal nerves 1 to 4. The inferior cervical ganglion and the first two or three thoracic ganglia are fused to make the stellate ganglion, which innervates the lower cranial and uppermost thoracic cranial nerves [31]. Therefore, removal of these ganglia prevents BAT thermogenesis and tail vasoconstriction. Our surgical sympathectomy abolished moxibustion-induced hyperthermia and tachycardia, whereas stimulation at 80°C increased plasma pyrogen levels. These results suggest that the increase in body core temperature upon stimulation at 80°C is caused mainly by pyrogen-induced PGE$_2$.

6-OHDA destroys the adrenergic nerve terminals in adult and newborn rats [32, 33]. In adult rats, intravenous administration of 6-OHDA almost completely abolishes vasoconstrictor responses in the renal artery [34]. Moreover, surgical lumbar sympathectomy almost abolishes the rapid increase in response to whole body heating [35]. Our results showed that chemical sympathectomy as well as surgical sympathectomy abolished moxibustion-induced hyperthermia and tachycardia. However, the responses to stimulation at 40 and 80°C were mediated by different neural response mechanisms.

Conclusion

Our results suggest that direct and indirect moxibustion increases body core temperature. Even though the rat is a small animal and its metabolic system is different from that of humans, moxibustion at low temperature, or indirect moxibustion, should be the first choice for moxibustion therapists.

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