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Application of camphor to the skin has been empirically thought to improve blood circulation. However, camphor's effects on blood circulation to the skin and on thermal sensation have not been well elucidated. In this study, we examined its effects on the quality of sensation as well as on skin and muscle blood flow in human. Nine adults (average age 37 ± 9.4 years) participated in the study. Petroleum jelly containing 5%, 10%, 20% camphor, or 2% menthol was separately applied to the skin on the medial side of one forearm of each subject. Just after the application, camphor at each concentration induced a cold sensation in a dose-dependent manner. Within 10 min, each subject reported that the cold sensation had faded, after which it was replaced by a warm sensation. As reported previously, a cold sensation was induced by application of 2% menthol, but the subjects did not adapt to that sensation. In addition, menthol did not induce a warm sensation at all. Application of menthol has been shown to increase blood flow in the skin. Finally, we measured blood flow in skin and muscle after the application of camphor or menthol. Application of camphor or menthol separately induced increases in local blood flow in the skin and muscle. The present results indicate that camphor induces both cold and warm sensations and improves blood circulation.

Key words camphor; cold sensation; warm sensation

Camphor, which is isolated from the wood of the camphor laurel tree (*Cinnamomum camphora*), has long been applied as a traditional medicine.¹⁾ Camphor has been pharmaceutically applied as an analgesic, antipruritic, antispasmodic, anti-inflammatory rubefacient.^{2,3)} In addition, camphor has been used as an antiseptic, contraceptive, aphrodisiac, and lactation suppressant.⁴⁾ Because camphor has mild expectant, nasal decongestant, and cough-suppressant effects, camphor in ointments, oils, and inhalants has been widely used as a home treatment for colds even recently.⁴⁾

Although the warm or burning sensation properties and improvement in blood circulation by camphor are widely known, there remains the question of whether camphor produces a cold or warm sensation in human skin. Camphor has been shown to activate heat-sensitive transient receptor potential (TRP) vanilloid subtypes 1 (TRPV1) and 3 (TRPV3).^{5,6)} Camphor also activates human and rat cold-sensitive channel transient receptor potential melastatin 8 (TRPM8) expressed in HEK293 cells.⁷⁾ Herein, we focused on the quality of sensation that camphor evoked when it was applied to human skin. The warm sensation induced by local heating of the skin increases skin blood flow, while the cold sensation induced by local cooling decreases skin blood flow.^{8–11)} However, application of menthol, which induces a cold sensation, increases skin blood flow.¹²⁾ In the present study, we also examined blood flows in the skin and muscle to explore the effects of camphor on blood flow.

MATERIALS AND METHODS

Ethical Considerations The content of this study was approved by the Ethics Committee of Asahikawa Medical University (approval ID: 560).

Subjects There were 9 subjects (2 males and 7 females).

Their age, height, body weight, and BMI were 37 ± 9.4 years old, 161.0 ± 7.3 cm, 57.3 ± 8.8 kg, and 21.3 ± 3.4 , respectively. These subjects had no clinical symptoms or other signs of skin and circulatory disorders. Each was informed of the aims, methods, and procedures involved and consented to participate in the present study.

Chemical Stimulation Each concentration of *dl*-camphor (Junsei Chemical Co., Ltd., Tokyo, Japan) and *l*-menthol (Nacalai Tesque, Kyoto, Japan) was suspended in petroleum jelly (white Vaseline; KENEI Pharmaceutical, Osaka, Japan). One gram of ointment was applied to the skin of the right forearm into a circular ring with a 4 cm diameter. The ring was placed on the right forearm, a skin blood-flow-meter probe was loaded inside the ring and a muscle blood-flow-meter probe was loaded next to the ring. The subjects did not smell camphor odor from ointment containing 5%, 10%, and 20% camphor during experiments. There were no significant changes in heart rates, systolic and diastolic blood pressures, and ear-drum temperatures.

Measurement of Perceived Sensation The subjects were asked to report on the intensity of the warmth or coldness on their skin once every minute after the ointment was applied, and those intensities were recorded in the range of 0 (no sensation) to 4 (maximum sensation). The latency for induction of cold and warm sensations by camphor was defined as the earliest time when a subject felt a cold sensation. The duration was defined as a period between the beginning and the end of the sensation.

Measurement of Skin and Muscle Blood Flow Skin blood flow was recorded from the surface of the right forearm using a laser Doppler blood flow-meter (ALF21RD, ADVANCE Co., Ltd., Tokyo, Japan), and blood flow rate was transmitted to a PC through PowerLab (ADInstrument, Sydney, Australia) to be recorded. Muscle blood flow rate was measured with a noninvasive tissue oxygen monitor (NIRO-120, Hamamatsu Photonics, Hamamatsu, Japan) for

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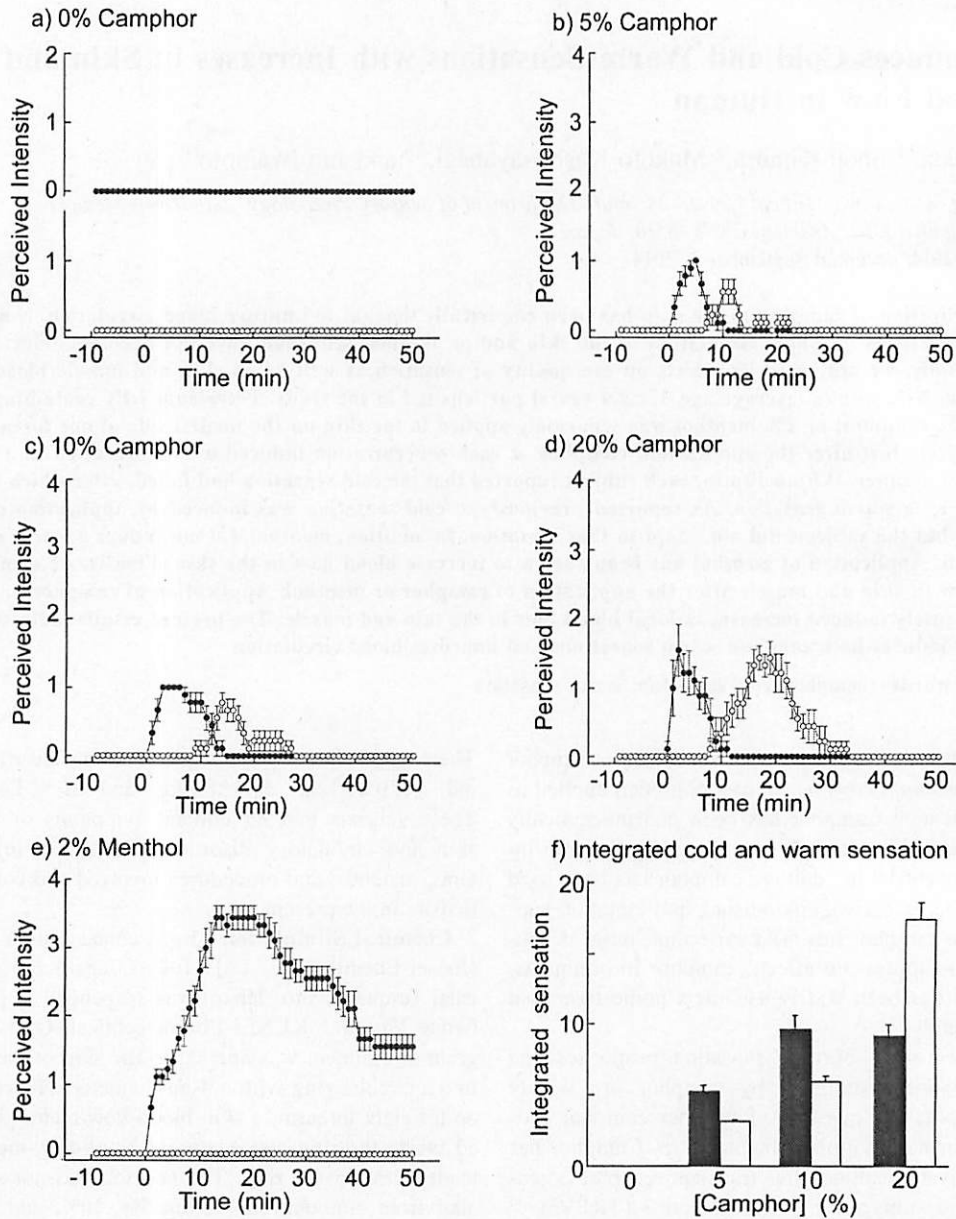


Fig. 1. Mean Ratings of the Perceived Intensity of the Cold (Closed Circle) and Warm (Open Circle) Sensations after Application of 0% (a), 5% (b), 10% (c), or 20% (d) Camphor and 2% Menthol (e); Cold (Closed Column) and Warm (Open Column) Sensations Integrated during 50min after the Stimulation (f)

Data indicate the mean±S.E.M. (n=9).

which near-infrared spectroscopy was used, and muscle blood flow data were recorded to a computer every 0.5s. For the determination of the latency of changes in skin and muscle blood flow, we drew a straight line passing points before changes induced by camphor by eye. We also drew a straight line passed points where a steepest change in blood flow occurred by eye. The latencies for changing in blood flow was defined as the location where these two lines intersect.

Experimental Procedure All experiments were performed at 25–26°C with 31–32% relative humidity. The subjects sat upright in a comfortable and supportive chair with their right arm supported at heart level. A ring was fitted to the medial side of the forearm (right flexor carpi radialis muscle), a skin blood flow-meter probe was fitted in the ring, and a muscle blood flow-meter was placed close to the ring.

The subjects were kept at rest for 10min (control), 1g of ointment was applied uniformly to the skin within the ring, and the variation of each measurement was recorded for 50min.

Statistical Analysis The data were compared by ANOVA with Fisher's protected least significant difference (PLSD) *post-hoc* testing. Statistical analyses were performed with StatView version 5.0 (SAS Institute, Cary, NC, U.S.A.). Data are expressed as the mean±S.E.M. (n=9).

RESULTS

Application of 5% camphor to the forearm induced a cold sensation in all subjects with a time latency of 2.3 ± 0.5 min and a duration of 5.0 ± 0.3 min, while that of Vaseline did not induce a cold sensation (Fig. 1). Applications of 10% and 20%

camphor also induced a cold sensation with time latencies of 2.0 ± 0.3 and 2.0 ± 0.7 min and durations of 9.4 ± 1.0 and 7.3 ± 1.2 min, respectively. After the end of the cold sensation, all subjects then reported a warm sensation. Application of 5%, 10%, and 20% camphor induced a warm sensation with latencies of 12.6 ± 2.3 , 13.9 ± 1.1 , and 12.4 ± 1.0 min and durations of 3.1 ± 0.2 , 6.9 ± 1.4 , and 13.2 ± 0.8 min, respectively. Application of 2% menthol induced only a cold sensation, which lasted throughout the measurement period in all subjects. The perceived intensity of the cold sensation was cast into a two-factor ANOVA as follows: concentration of camphor (0, 5, 10, and 20%) and time. This analysis revealed the main effect of the camphor concentration ($F(3, 1920) = 64.303$, $p < 0.0001$) and a significant interaction between the concentration and time ($F(177, 1920) = 6.706$, $p < 0.0001$). Perceived intensity of the warm sensation was also cast into a two-factor ANOVA

as follows: camphor concentration (0, 5, 10, and 20%) and time. This analysis revealed the main effect of the camphor concentration ($F(3, 1920) = 125.74$, $p < 0.0001$) and a significant interaction between the concentration and time ($F(177, 1920) = 6.426$, $p < 0.0001$). Figure 1f shows the integrated magnitudes of cold sensation induced by various concentrations of camphor. The magnitude of cold sensation increased with the increase in camphor concentration to 10% and was unchanged despite further increases. The magnitude of warm sensation increased with an increase in camphor concentration.

Then, we measured the effects of camphor on skin and muscle blood flow. Relative values of skin blood flow increased with a time latency of 4.1 ± 3.0 min by application of 5% camphor. Similarly, application of 10% or 20% camphor resulted in a gradual increase in skin blood flow in all subjects, with time latencies of 1.2 ± 0.4 and 2.7 ± 2.0 min,

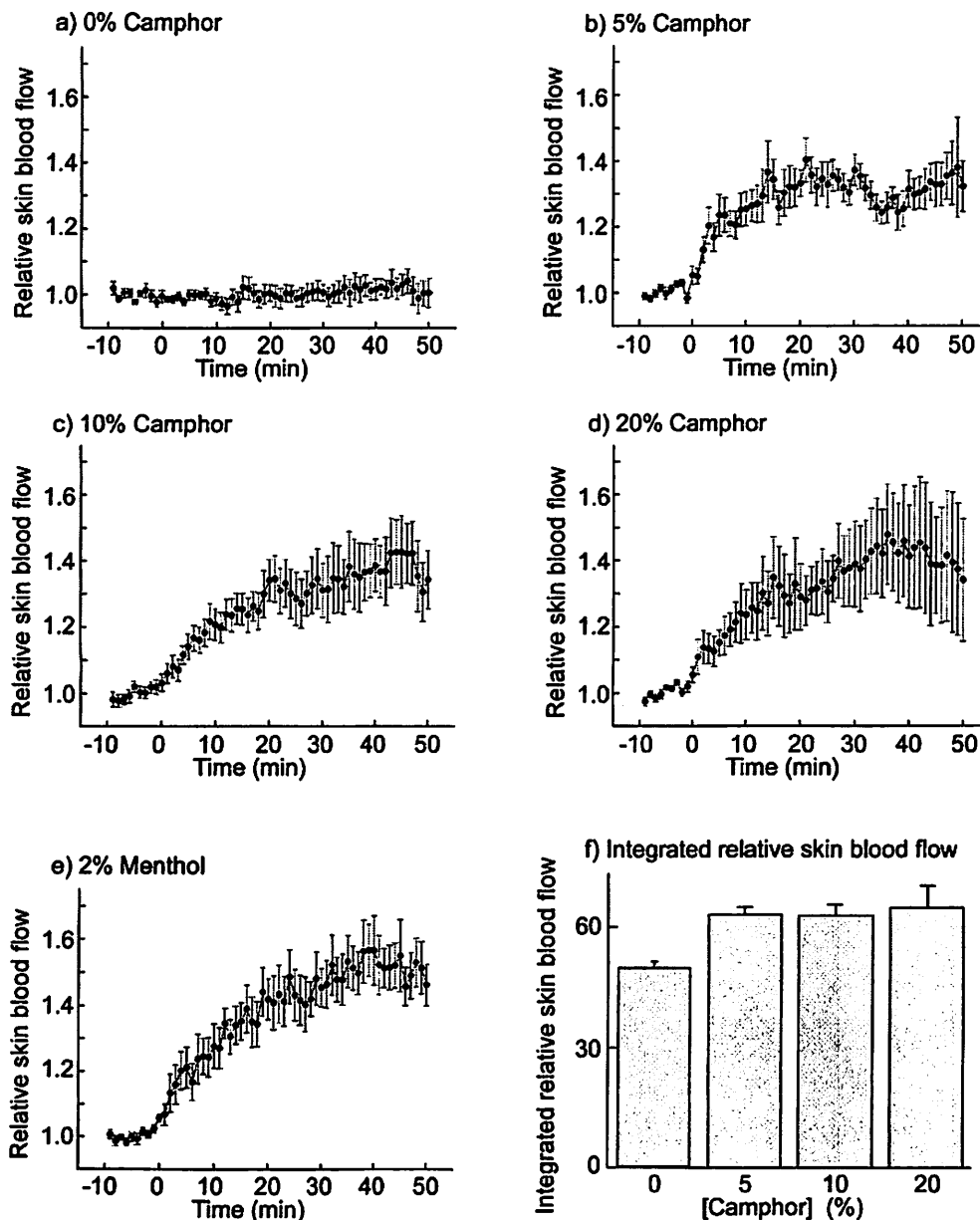


Fig. 2. Magnitude of Relative Skin Blood Flow after Application of 0% (a), 5% (b), 10% (c), or 20% (d) Camphor and 2% Menthol (e) Integrated relative skin blood flow after application of 0%, 5%, 10%, and 20% during 50 min after the stimulation. Data indicate the mean \pm S.E.M. ($n=9$).

respectively. Relative values of skin blood flow were cast into a two-factor ANOVA as follows: concentration of camphor (0, 5, 10, 20%) and time. This analysis revealed the main effect of camphor concentration ($F(3, 1920)=1173.674, p<0.0001$). Figure 2f shows the integrated magnitude of cold sensation induced by various concentrations of camphor. Relative skin blood flow after application of 5%, 10%, or 20% camphor was higher than that without camphor ($p<0.0001$).

The muscle blood flow significantly increased, similar to the case with skin blood flow, with time latencies of $1.5\pm 0.7, 2.8\pm 1.0, \text{ and } 3.0\pm 1.2\text{ min}$, respectively. Values of muscle blood flow were cast into a two-factor ANOVA as follows: camphor concentrations (0, 5, 10, and 20%) and time. This analysis revealed the main effect of camphor concentration

($F(3, 1920)=311.67, p<0.0001$) and a significant interaction between concentration and time ($F(177, 1920)=1.378, p<0.005$). Figure 3f shows the integrated magnitude of cold sensation induced by various concentrations of camphor. Relative skin blood flow after application of 5%, 10%, or 20% camphor was higher than that without camphor ($p<0.0001$). Finally, values of time latencies of induction of a cold sensation, and increases in skin and muscle blood flow were cast into a two-factor ANOVA as follows: measurements and camphor concentrations (5, 10, and 20%). This analysis did not reveal the main effect of measurements ($F(2, 72)=0.103, p=0.9024$) and camphor concentration ($F(2, 72)=0.223, p=0.8009$). A *post-hoc* test indicated that there were no significant differences between values in latencies of induction of cold sensation, and

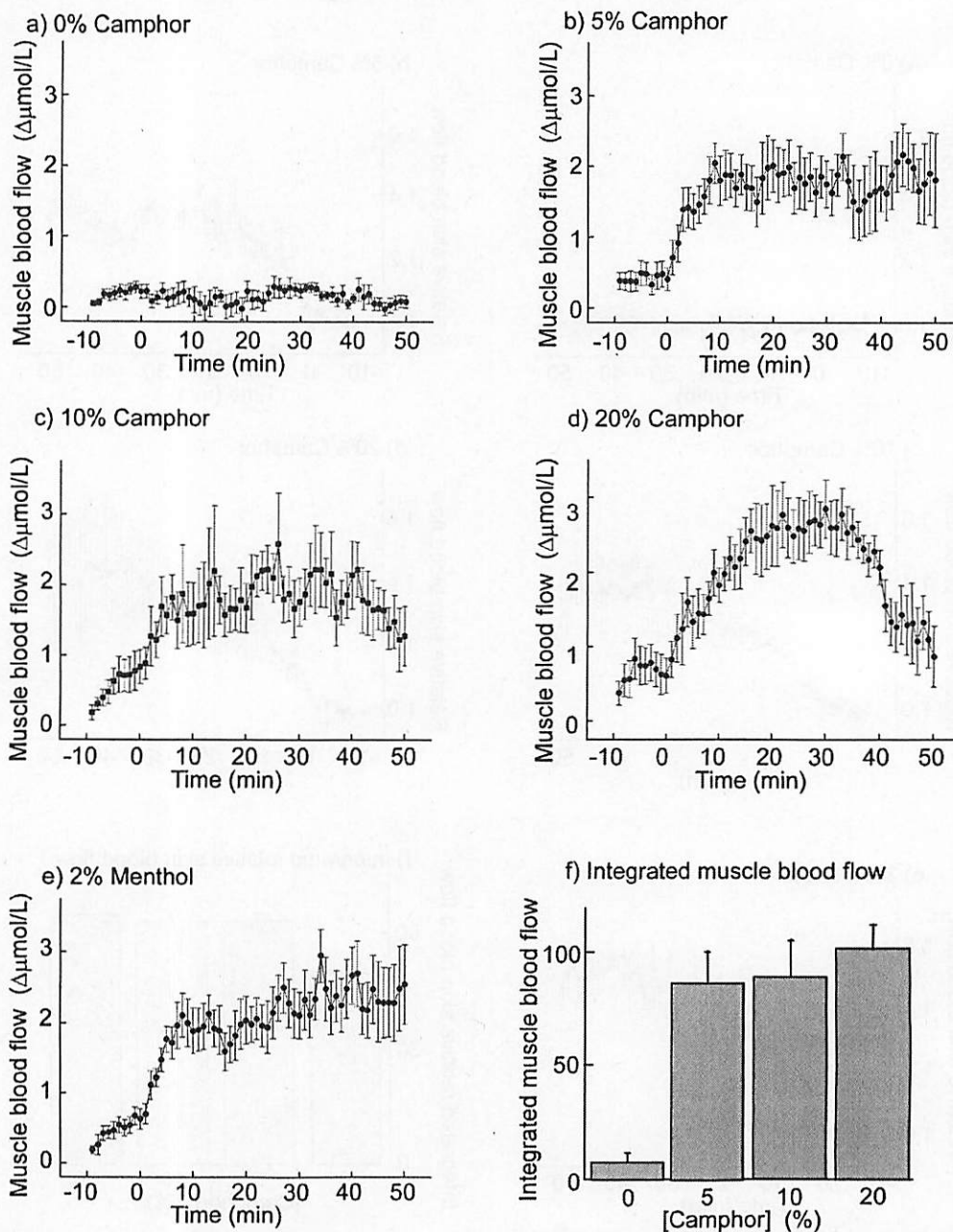


Fig. 3. Magnitude of Muscle Blood Flow after Application of 0% (a), 5% (b), 10% (c), or 20% (d) Camphor and 2% Menthol (e) Integrated muscle blood flow after application of 0%, 5%, 10%, and 20% during 50 min after the stimulation. Data indicate the mean \pm S.E.M. ($n=9$).

increases in skin and muscle blood flow.

DISCUSSION

In this study, we have demonstrated that in human, topical application of camphor to the forearm skin elicits cold and warm sensations. We also demonstrated that camphor produces a significant increase in local skin and muscle blood flow.

Application of camphor on the human skin enhances cold and warm sensations.¹² Camphor applied in the amount of 20 mM to the volar forearm increased the perceived intensity of the cutaneous sensations produced during heating from 33 to 43°C and cooling from 33 to 18°C.¹³ This indicates that camphor affects activities of both the cold and warm receptors. As camphor activates heat-sensitive TRPV1 and TRPV3,^{4,5} it is possible that it induced a warm sensation by activating these TRPs at the free nerve endings in the forearm skin. Inhalation of camphor, eucalyptus, or menthol induced a cold sensation in the nose along with the sensation of improved airflow.¹⁴ Our results were consistent with this observation. A recent study showed that camphor also activates human and rat TRPM8 expressed in HEK293 cells.⁷ As shown in Fig. 1e, the subjects did not adapt to the menthol-induced cold sensation during the measurement, while they did adapt to the camphor-induced cold sensation about 10 min after the stimulation. Application of 1 mM camphor to *Xenopus* oocytes expressing rat TRPM8 did not induce any responses.¹⁵ In addition, Chinese hamster ovary cells expressing mouse TRPM8 also did not respond to the application of 0.25–2 mM of camphor.¹⁶ Therefore, the magnitude of TRPM8 activation induced by camphor may be weaker than that induced by menthol.

Skin blood flow rose to an initial peak within 1–3 min by local heat applied to the human arm, followed by a sustained secondary rise to a plateau from about 10 min after stimulation.^{9–11} Minson *et al.* explained that a rapid increase in skin blood flow is induced by local heating mediated by the axon reflexes and that a slower increase is induced by nitric oxide (NO).⁹ The warm sensation induced by camphor faded within 30 min, while skin and muscle blood flow sustained during stimulation. Similarly, sustained increases in skin blood flow induced by local heating remain after the end of stimulation.⁹ A slowly increases in skin blood flow that relies on local production of NO.⁹ Therefore, it is possible that sustained increases in skin blood flow induced by camphor during and after the warm sensation are mediated by NO. The mechanism of regulation of NO production during heat stimulation has not been made clear. Local heating normally activates warm-sensitive TRPV1 and TRPV3.^{5,6} As described above, camphor activates TRPV1 and TRPV3.^{5,6} Therefore, the initial increases in blood flow by camphor may be induced by the axon reflex. In the present study, increases in skin blood flow induced by camphor did not show an initial transient peak, while there was a remarkable delay in the initiation of increases in blood flow. We applied camphor suspended in Vaseline to the forearm. Therefore, camphor took more time to reach the free nerve endings that induce the axon reflex. After the subjects adapted to the cold sensation, camphor induced a warm sensation. It is possible that camphor, similar to local heating, induces sustained increases in NO-induced blood flow.

Local cooling of human skin induces decreases in skin blood flow.¹⁰ However, topical application of 3% menthol,

which induces a cold sensation, to human skin has been reported to induce increases in blood flow.¹² In the present study, application of 2% menthol to the forearm skin also increased local blood flow in skin and muscle. These results suggest that the cold sensation induced by menthol does not directly regulate skin blood flow. Wasner *et al.* showed that menthol activates C nociceptors,¹⁷ suggesting that menthol increases cutaneous blood flow *via* the axon reflex. TRPM8 mRNA and protein are present in smooth muscle of rat tail, femoral and mesenteric arteries, and thoracic aorta.¹² Therefore, menthol may also directly induce vasodilatation *via* TRPM8 channels in the artery.¹² Similarly, it is possible that the cold sensation induced by camphor also does not directly regulate skin blood flow.

In the present study, we showed that camphor induced a cold sensation followed by a warm sensation. One possible explanation for this phenomena is as follows. First, we hypothesize that low concentration of camphor activates human TRPM8 but not human TRPV1 and TRPV3. In the initial period of stimulation, the concentration of camphor around free nerve endings is low. Therefore, camphor induces the cold sensation. Concentration of camphor around free nerve endings becomes higher over time. Then, camphor induces the warm sensation. Obviously, further experimental studies are needed to verify this explanation.

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