PAIN

Pain 84 (2000) 133–139

www.elsevier.nl/locate/pain

Research Papers

Computer-assisted infrared thermographic study of axon reflex induced by intradermal melittin

Natsu Koyama[a,*], Kazuhiko Hirata[b], Koichiro Hori[b], Kenjiro Dan[b], Toshikatsu Yokota[a]

[a]*Department of Physiology, Shiga University of Medical Science, Seta, Otsu 520-2192, Japan*
[b]*Department of Anesthesiology, School of Medicine, Fukuoka University, Nanakuma, Jyonan-ku Fukuoka 814-0180, Japan*

Received 28 September 1998; received in revised form 13 July 1999; accepted 22 July 1999

Abstract

The aim of the present study was to investigate whether melittin, the principal toxin of the honeybee (*Apis mellifera*) venom, can be used as an algogenic agent in the study of pain in humans. Five micrograms of melittin in 0.5 ml of saline was intradermally injected into the volar aspect of the forearm. Resultant pain was scored by a visual analogue scale (VAS), and skin temperature change was analyzed by means of a computer-assisted infrared thermography. Intradermal melittin temporarily produced severe pain, followed by a sustained increase in skin temperature. The skin temperature increase peaked in about 10 min and outlasted 1 h. Topical application of 10% lidocaine gel did not significantly suppress the melittin-induced pain, but markedly suppressed both the increase in the peak temperature and the area of temperature increase. In conclusion, 5 μg of melittin is sufficient to produce pain in humans and 10% lidocaine gel differentially decreases the melittin-induced axon reflex without any significant analgesic effect. © 2000 International Association for the Study of Pain. Published by Elsevier Science B.V.

Keywords: Melittin-induced pain; Honeybee venom; Axon reflex; Lidocaine; Thermography

1. Introduction

Honeybee venom produces pain and accompanying inflammation. Lariviere and Melzack (1996) have introduced the bee venom test as an animal model of persistent pain in place of the formalin test. The optimal dose of bee venom for behavioral experiments in rats appears to be between 0.1 and 0.2 mg. We tried the same bee venom test ourselves in a pilot study. Bee venom of this dose brought forth an immediate pain peaking within 3 min and an axon reflex flare lasting about 2 h. Besides the immediate pain, delayed erythema and edema larger than the primary flare appeared with heavy itch sensation. These delayed reactions continued for 4 days in the longest case. These results indicate that the bee venom test is unsuitable for experiments in human subjects. Bee venom contains many physiologically active substances; a biogenic amine (histamine), peptides or small proteins (apamin, melittin and mast cell degranulating peptide), enzymes (phospholipase A_2 and hyaluronidase) and so on (Habermann, 1972; Chahl and Kirk, 1975; Lariviere and Melzack, 1996). Although many ingredients might synergistically produce strong pain, it is desirable to determine which ingredients of bee venom are responsible for the algogenic action of bee venom. Histamine is a well-known prurigenic and algogenic substance. Apamin has not been directly implicated in pain production: it characteristically produces a long-lasting excitation of the central nervous system (Habermann, 1972). Melittin is a 26 amino acid amphipathic peptide with a highly positive charge at the C-terminal (Dempsey, 1990). Melittin has been assumed to produce pain by directly acting on nerve fibers and/or by releasing potassium ions through cell lysis (Habermann, 1972). It has also been shown that melittin is a phospholipase A_2 activator in vitro (Hassid and Levine, 1977; Shier, 1979; Fletcher and Jiang, 1993). Thus melittin stimulates arachidonic acid release and subsequent formation of both eicosanoids and leukotrienes in a variety of cells (Hassid and Levine, 1977; Shier, 1979; Salari et al., 1985; Fletcher and Jiang, 1993). In addition, melittin has been found to accelerate kallikrein release from cellular membrane (Nishimura et al., 1980), and to increase interleukin-1 (IL-1) and tumor necrosis factor (TNF) production in a time- and dose-dependent manner (Bomalaski et al., 1995). All of these mechanisms may contribute to melittin-induced pain.

The aim of the present study was to examine whether

* Corresponding author. Tel.: +81-77-548-2147; fax: +81-77-548-2146.
E-mail address: natsu@belle.shiga-med.ac.jp (N. Koyama)

0304-3959/00/$20.00
PII: S0304-3959(99)00192-X

melittin can be used as an algogenic agent in the study of pain in humans. In addition, effects of topical application of 10% lidocaine gel on melittin-evoked responses were studied using a computer-assisted infrared thermography.

2. Methods

Seven healthy adults, including the authors, (2 women and 5 men) from our departments were subjected to the present study (age range 34–68 years, mean age 42.3 ± 10.9 years). All of them gave their informed consents and the study was approved by the local ethics committee. Five micrograms of melittin (Sigma), dissolved in 0.05 ml normal saline was intradermally injected into the volar aspect of the forearm with a sterile tuberculin syringe with or without 10% lidocaine gel pretreatment 1 h before melittin. Ten percent lidocaine gel (10 g lidocaine; 9.8 ml propylene alcohol; 1.3 g hyviswako 104; 1.1 g di-2-propanolamine; 30 ml ethanol; aqua distillata) was prepared by the pharmacy of the Fukuoka University Hospital. Experiments were conducted in a thermoneutral environment of a temperature-controlled laboratory (25 ± 0.5°C). Experiments commenced when thermally steady state and thermal balance of the skin were reached after the injection site of the skin had been cleaned by an alcohol cotton swab. During recordings, subjects quietly lay in a supine position without moving. Lidocaine gel was applied within a 20-mm diameter circle. After removing lidocaine gel, the skin was cleaned, and melittin was intradermally injected into the center of the circle where lidocaine gel had been applied. Melittin-induced pain was scored by a visual analogue scale (VAS) ranging from 0 (no pain) to 10 (most intense pain imaginable). Melittin-induced skin temperature change was monitored by a computer-assisted infrared thermograph (Thermotracer TH3100ME, NEC-SANEI, Japan). The area of thermographic recording covered the proximal four fifths of the forearm. The operation range of the sensor was 3–5.3 μm wavelength, i.e. in the so-called 'thermal infrared range'. The sensitivity of the sensor was 0.1°C at 30°C object temperature. The thermal noise was reduced through on-lined averaging of data almost simultaneously obtained by eight sensory elements to construct a single frame. Averaged thermograms of eight single frames recorded every 0.8 s were successively displayed on the monitor by a frame shift average method. The averaged thermograms were stored on a hard disk (12-bit resolution) every 1 min during the last 3 min prior to, and the first 10 min after intradermal melittin injection and thereafter every 5 min.

2.1. Data analysis

Following each experiment, 11 successive thermograms recorded prior to and during the first 60 min period after the melittin injection were subjected to analysis by a thermo-image processing program (TH31-701, NEC-SANEI, Japan). Original thermograms were enlarged 2-fold, placing the injection site at the center of the monitor screen. The area thus displayed on the monitor screen was analyzed. In all experiments, this area covered the whole area where temperature increase occurred centering around the injection site. In each enlarged thermogram, peak temperature was automatically measured using TH31-701. The mean of peak temperatures in three thermograms recorded prior to the injection was defined as basal peak temperature. Changes in temperature (T) were calculated by subtracting the basal peak temperature. Moreover, 0.2°C-interval isothermal lines were mapped and the area of each interval was measured using TH31-701. In time course analyses of melittin-induced pain and of temperature changes, Wilcoxon analysis and two factor repeated ANOVA were used. P-values < 0.05 were considered statistically significant. Data are presented by mean and standard error (SE).

3. Results

Intradermal melittin (5 μg) immediately caused painful sensation after the injection in all subjects. The pain disappeared within 3 min and severe pain disappeared within 1 min. Pretreatment with lidocaine gel did not significantly affect the time course of melittin-induced painful sensation. Fig. 1a shows time courses of mean VAS scores of pain following melittin injection in two separate series of seven experiments with and without lidocaine gel pretreatment. In both cases, the mean VAS score was maximum 10 s after melittin. It was 7.57 ± 0.22 and 8.57 ± 0.40 with and without lidocaine gel pretreatment, respectively. ANOVA revealed no significant difference in melittin-induced VAS score changes between with and without lidocaine gel pretreatment ($F_{1,12} = 0.559$, $P < 0.1$). Melittin caused neither itch nor warm sensation. Melittin produced a flare surrounding a wheal near the injection site. The flare disappeared within 2 h and no delayed reaction was produced except in two cases. In these two cases there was a faint reddening less than 10 mm in diameter without any sensation, next day.

Fig. 1b shows mean time courses of peak temperature changes following melittin injection in two separate series of seven experiments with and without lidocaine gel. Without lidocaine pretreatment the basal peak temperature was 34.37 ± 0.26°C and the peak temperature became 34.60 ± 0.34°C 1 min after melittin injection ($\Delta T = 0.20 \pm 0.12$). Thereafter it increased: it was 35.26 ± 0.23°C ($\Delta T = 0.86 \pm 0.19$) 3 min after melittin injection, reached a maximum temperature of 35.77 ± 0.26°C, ($\Delta T = 1.40 \pm 0.23$) after 10 min and then gradually decayed. Changes in the peak temperature were statistically significant 3 and 10 min after melittin injection. Any skin temperature decrease did not occur in the visible flare except in the swollen injection site during recordings. The maximum value of the peak temperature was 34.60±0.31°C ($\Delta T = 0.63 \pm 0.14$) follow-

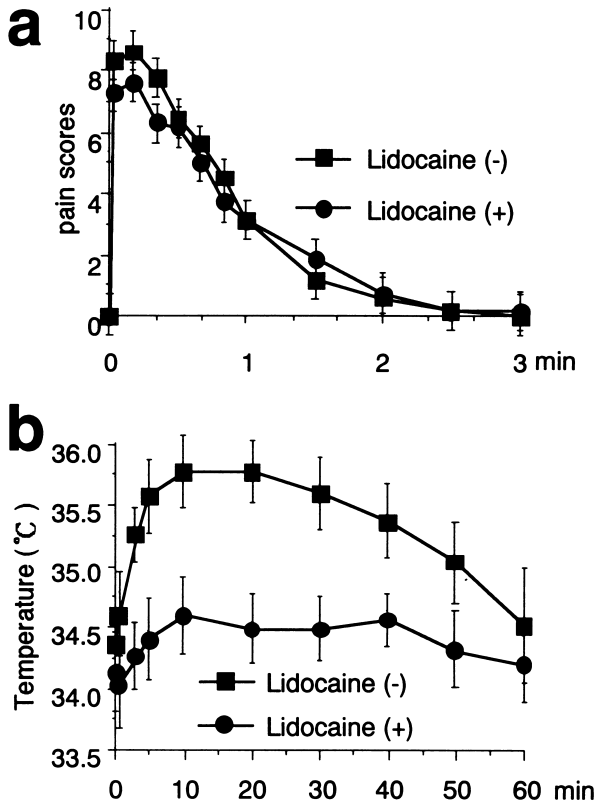

Fig. 1. Mean time courses of changes in pain scored by a visual analog scale (VAS) ranging from 0 (no pain) to 10 (most intense pain imaginable) (a), and in the skin temperature (b) after intradermal melittin (5 μg) ($n = 7$). Melittin caused painful sensation immediately after the injection, and the pain disappeared within 3 min. The skin temperature started to increase about 3 min after melittin, peaked in 10 min and then decayed gradually. Pretreatment with 10% lidocaine gel did not significantly suppress the melittin-induced pain but markedly reduced the temperature increase.

ing melittin injection with lidocaine pretreatment. ANOVA revealed a significant difference in the peak temperature change between with and without lidocaine gel pretreatment ($F_{1,12} = 6.408$, $P < 0.05$). Although lidocaine pretreatment significantly curtailed the temperature increase following intradermal melittin injection, the peak time was unaffected by lidocaine pretreatment.

Figs. 2 and 3 illustrate examples of thermograms. Without lidocaine the skin temperature remarkably increased following the intradermal melittin injection (Fig. 2), and topical lidocaine gel markedly inhibited the skin temperature increase (Fig. 3). Fig. 4 illustrates examples of skin temperature distribution in histograms of 0.2°C-interval isothermal map before and 10 min after melittin. To produce these histograms, areas of every 0.2°C interval in the isothermal map were measured using TH31-701. In Fig. 4a, the total area of temperature increase above the basal peak temperature was 409.5 mm^2, 10 min after the injection without lidocaine. In Fig. 4b, it was 177.5 mm^2, 10 min after the injection with lidocaine. Table 1 summarizes the mean areas of temperature increase 10 min after melittin injection. The time courses of changes in the mean total area of temperature increase above the basal peak temperature are shown in Fig. 5a. It was 443.6 ± 88.2mm^2 and 141.2 ± 68.7 mm^2, 10 min after the injection without and with lidocaine, respectively (Fig. 5a). The difference between with and without lidocaine was statistically significant for all ΔT values in the table. The time courses of changes in the mean area of temperature increase are shown in Fig. 5b.

4. Discussion

The present study demonstrated that 5 μg of melittin is sufficient to produce pain without any dangerous side effect. Since melittin constitutes about 50% of the total dry weight of bee venom, this dose is equivalent to one twentieth of the melittin content of the optimal dose of the bee venom test. The bee venom test is a potentially useful animal model of experimental tonic pain to elucidate persistent clinical pain (Lariviere and Melzack, 1996). On the other hand, the melittin test may be a useful experimental phasic pain test in humans because subjects need not endure any long-lasting pain.

The axon reflex is a phenomenon that an activation of one branch of a nociceptive fiber by a noxious stimulus results in antidromic invasions of action potentials into adjacent branches of the fiber which, in turn, cause the release of vasodilatory substances from the terminals of the fiber. Lewis (1942) proposed that the axon reflex is a nocifensor response. The major vasodilatory substances released from unmyelinated C fibers is calcitonin gene-related peptide with some contribution from substance P (Lembeck, 1983; Hua et al., 1995). The vasodilatory neuropeptides may reach arterioles by diffusion and they may also act via release of other mediators from mast cells and blood vessels in the neighborhood of the initially stimulated terminals. The increased blood flow thus induced would facilitate the removal of endogenous or exogenous irritants or toxic substances. Increased plasma extravasation could, in addition, allow a faster elimination of larger molecules by the lymph flow (Lewis, 1942; Lembeck, 1983). The axon reflex vasodilatation may also play an important role in healing of injury. Clinically, loss of both nociception and axon reflex vasodilatation has been implicated in diabetic foot ulceration, and it was proposed that early and prolonged nerve growth factor treatment at an appropriate dose may provide rational prophylaxis for their condition (Anand et al., 1996). It was suggested that the loss of nociceptive function predisposes to patients to diabetic foot ulcerations in two ways: by impairment of protective pain sensation and by impairment of neurogenic inflammatory response to injury (Parkhouse and Le Quesne, 1988).

Previously, the axon reflex has been studied by measuring the area of visible flare and/or by measuring the cutaneous blood flow using a laser Doppler flowmeter following application of histamine (Lembeck, 1983; Forster et al., 1995), capsaicin (Fitzgerald, 1983; Maggi and Meli, 1988; Holzer,

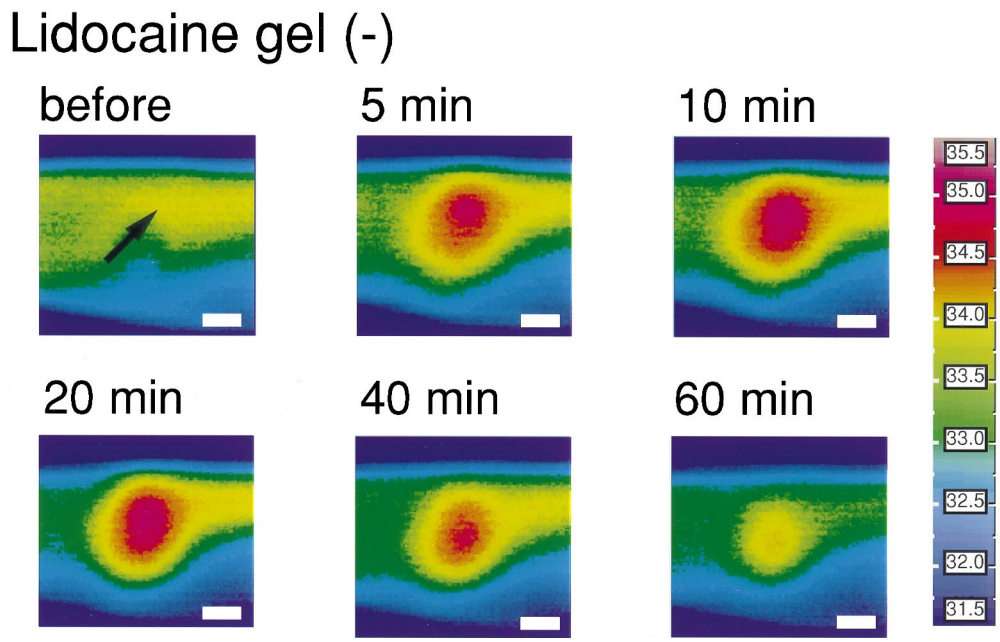

Fig. 2. A series of thermograms showing changes in the temperature distribution due to intradermal injection of melittin into the volar aspect of the forearm without lidocaine gel pretreatment. Five micrograms of melittin was injected at the site indicated by an arrow. The skin temperature around the injection site increased. The skin temperature calibration is shown in a color scale code. White bar: 10 mm.

1991; Morris et al., 1995) or mustard oil (Jancso et al., 1983). The visible flare is related to the number of erythrocytes in superficial capillaries, and usually the flare size is used as an indicator of the axon reflex. The laser Doppler flowmetry measures cutaneous blood flow in a circumscribed area, and usually the magnitude of increase in blood flow is used as an indicator of the axon reflex. The thermography can detect the temperature of superficial skin, but the warm area detected by the thermography reflects increased blood flow in subcutaneous tissues due to their larger volume and mass (Francis et al., 1979). One obvious advantage of the thermography over the laser Doppler flow-

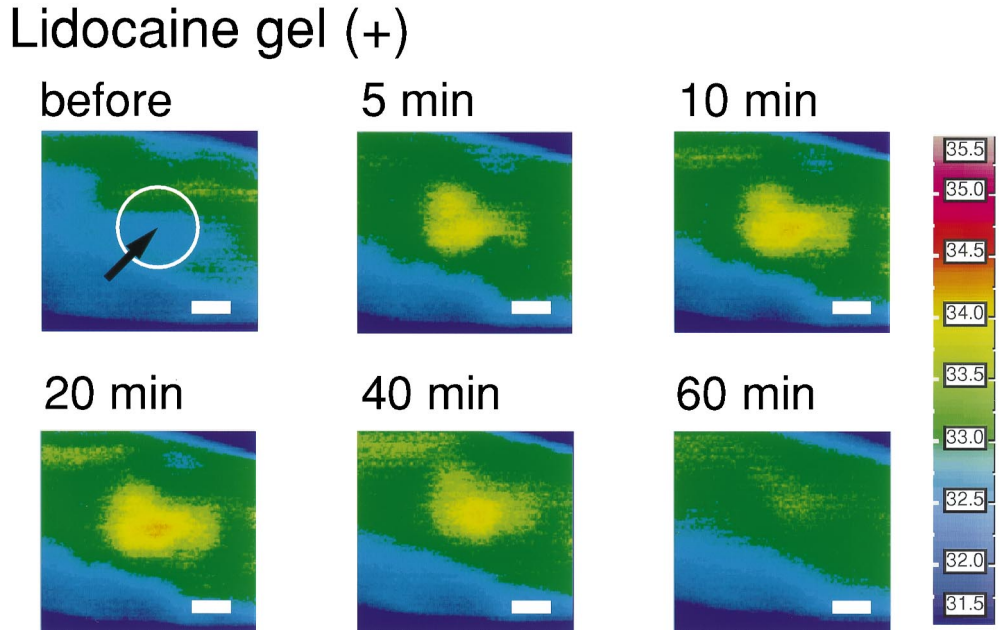

Fig. 3. A series of thermograms showing changes in the temperature distribution due to intradermal injection of melittin into the volar aspect of the forearm with lidocaine gel pretreatment. Five micrograms of melittin was injected at the site indicated by an arrow after removing lidocaine gel which had been applied within a white circle (diameter is 20 mm). The skin temperature increase was less marked compared with Fig. 2. The skin temperature calibration is shown in a color scale code. White bar: 10 mm.

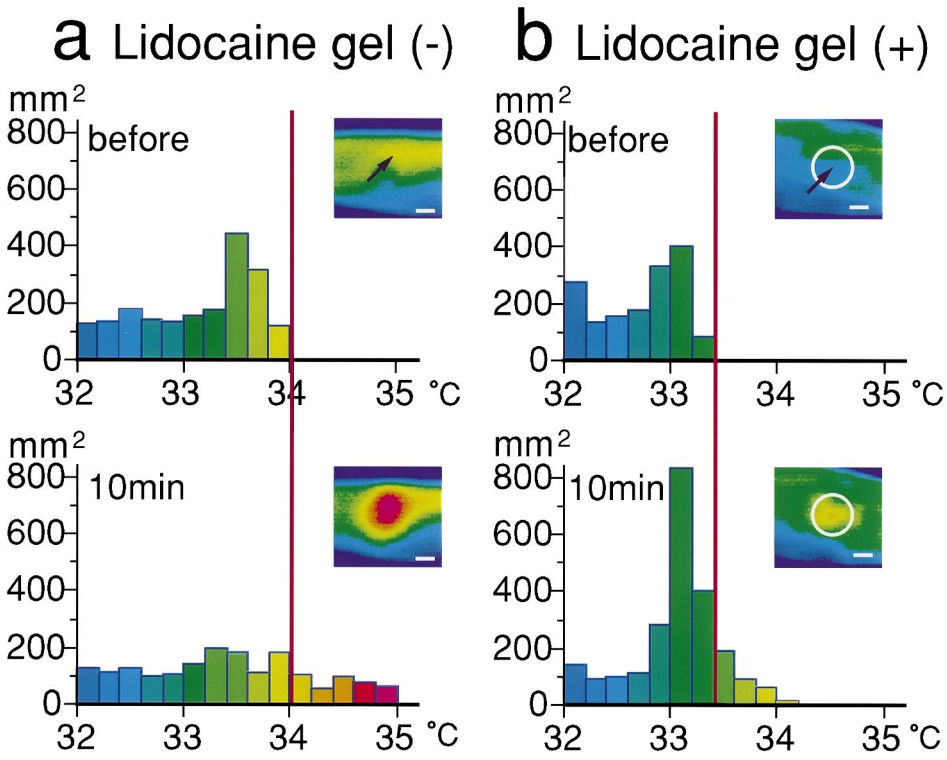

Fig. 4. Examples of skin temperature distribution histograms of 0.2°C-interval-isothermal map before and 10 min after melittin. (a) Without lidocaine pretreatment; (b) with lidocaine pretreatment. The total area of temperature increase above the basal peak temperature was 409.5 and 177.5 mm^2, 10 min after the injection without and with lidocaine, respectively.

metry is that thermography can monitor a larger area compared with the laser Doppler flowmetry. Furthermore, it is possible to reconstruct an isothermal map with the computer-assisted thermography. It appears that the flare induced by chemicals is stronger and bigger than mechanically induced flare. According to a recent microneurographic study, innervation territories of mechanically activated C nociceptor in human skin are of similar size as axon reflex flares evoked by noxious mechanical stimuli, about 25 mm in diameter (Schmidt et al., 1997). This size is comparable to the area of melittin-induced temperature increase as measured by the thermography in the present study.

Forster et al. (1995) compared the visible flare and warming reaction recorded by infrared thermograph following intracutaneous histamine application. The visible flare but not the warming reaction was suppressed by topically applied local anesthetic cream. The visible flare did not closely correspond to the thermal reaction. They concluded that the vascular axon reflex is differently organized in different layers of the skin.

Table 1
Mean areas (mm^2) of temperature increase above the basal peak temperature + ΔT, 10min after the injection

ΔT (°C)	Lidocaine (−) (mean ± SE)	Lidocaine (+) (mean ± SE)
0	443.6 ± 88.2	141.2 ± 68.7
0.2	308.1 ± 83.6	76.1 ± 47.2
0.4	212.3 ± 75.8	60.3 ± 29.9
0.6	137.5 ± 62.5	35.9 ± 24.4
0.8	85.1 ± 45.1	4.5 ± 3.9
1.0	43.7 ± 27.7	0.0 ± 0.0
1.2	31.1 ± 22.8	0.0 ± 0.0

It is known that histamine produces a sensation of itch due to excitation of C fibers with polymodal or chemospecific nociceptors and that histamine is a potent vasodilator by itself. In sharp contrast, melittin-induced temperature increase was markedly suppressed by topically applied lidocaine gel, in the present study. The difference between our results and those of Forster et al. (1995) suggests that melittin-induced temperature increase detected by infrared thermography is primarily due to axon reflex rather than direct action of melittin on blood vessels.

Another chemical which has been used for the study of axon reflex, capsaicin, is the main pungent chemical in 'hot' chilli peppers, and elicits a sensation of burning pain. Morris et al. (1995) showed a clinical significance of the flare and vasodilatation response to topical capsaicin in discriminating postherpetic neuralgia (PHN) patients with allodynia and burning pain from patients with burning pain alone: when tested at the site of clinical pain, patients with allodynia and burning pain exhibited a significantly greater reduction in axon reflex activity compared with patients with

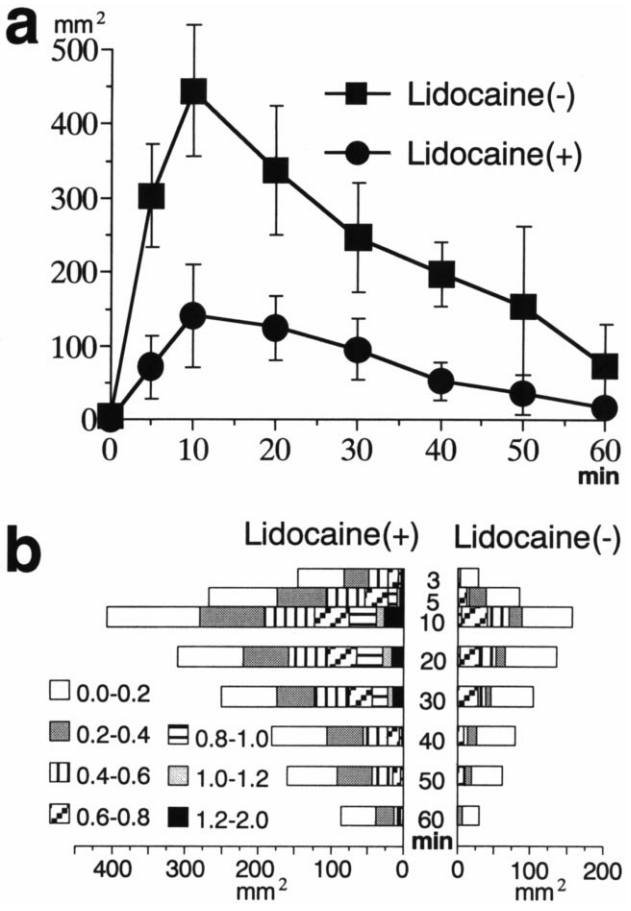

Fig. 5. Time course of changes in the mean total area of temperature increased after intradermal melittin ($n = 7$). (a) Mean time courses of changes in the total area of temperature increase above the basal peak temperature. (b) Mean time courses of changes in the area of temperature increase every 0.2°C interval. The mean total area of temperature increase peaked in 10 min irrespective of lidocaine. Lidocaine gel inhibited the temperature increase.

burning pain alone. They concluded that the laser Doppler recorded blood flow is more appropriate than the flare size. The present data suggest that the thermographic recording of the spatial distribution of melittin-induced temperature increase can be used for the same purpose, i.e. to test the impairment of C fibers in neuropathies such as PHN.

A problem with the present melittin test was that subjects must lie in a supine position without moving more than 1 h. This was almost intolerable toward the end. Since it became clear that the maximum change occurs in about 10 min, the test can be terminated within 30 min for clinical purposes.

Magerl et al. (1996) reported that topical application of mustard oil on the forearm induced skin temperature decrease of the ipsilateral palm in human volunteers. In contrast, the melittin-induced skin temperature change of the forearm observed in the present study was always an increase. A possible reason why we could not record any vasoconstrictor response in the forearm may be as follows: the palm does have a numerous arteriovenous anastomoses (AVA), but the forearm skin does scarcely. The regulation of blood flow through an AVA is governed principally by the sympathetic nervous innervation in response to reflex activation (Sherman, 1963).

In the present experiments, topical application of lidocaine gel had no significant effect on the melittin-induced pain, but differentially reduced the melittin-induced skin temperature increase. The skin temperature increase reflects increased blood flow in subcutaneous tissues. These results are consistent with the previous report that dilute mepivacaine, given as an intravenous regional block, had no effect on the VAS rating of capsaicin-induced pain sensation but differentially decreased the spread of capsaicin-induced flare (Kalman et al., 1998). Pretreatment with local anesthetics abolishes the flare induced by capsaicin, but it does not suppress the blood flow increase due to intracutaneously injected CGRP. Thus local anesthetics have little direct influence on vasodilatatory action of neuropeptides released from sensory nerve ending (Larkin and Williams, 1993). It was suggested that local anesthetics block conduction in the peripheral arborizations of thin afferent fiber most potentially. Similarly, a topical application of 10% lidocaine gel may suppress retrograde conduction in peripheral arborizations of C-nociceptors without blocking orthodromic conduction in the same afferent fibers. Alternatively, lidocaine gel may have no effect on pain sensation due to excitation of A-δ fiber nociceptors. Ten percent lidocaine gel has been used to relieve post-herpetic neuralgia. Although 10% lidocaine gel could not suppress the melittin-induced pain, it may relieve neuropathic pain by suppressing ectopic discharges generated by just suprathreshold depolarization (Rowbotham et al., 1995; Koyama et al., 1997).

Acknowledgements

We wish to thank all staff members for their participation in this study and Drs Nishio and Sakuragi for their valuable advice.

References

Anand P, Terenghi G, Warner G, Kopelman P, Williams-Chestnut RE, Sinicropi DV. The role of endogenous nerve growth factor in human diabetic neuropathy. Nat Med 1996;2:703–707.

Bomalaski JS, Ford T, Hudson AP, Clark MA. Phospholipase A2-activating protein induces the synthesis of IL-1 and TNF in human monocytes. J Immunol 1995;155:4027–4031.

Chahl LA, Kirk EJ. Toxins which produce pain. Pain 1975;1:3–49.

Dempsey CE. The actions of melittin on membranes. Biochim Biophys Acta 1990;1031:143–161.

Fitzgerald M. Capsaicin and sensory neurons – a review. Pain 1983;15:109–130.

Fletcher JE, Jiang MS. Possible mechanisms of action of cobra snake venom cardiotoxins and bee venom melittin. Toxicon 1993;31:669–695.

Forster C, Greiner T, Nischik M, Schmelz M, Handwerker HO. Neurogenic flare responses are heterogeneous in superficial and deep layers of human skin. Neurosci Lett 1995;185:33–36.

Francis JE, Roggli R, Love TJ, Robinson CP. Thermography as a means of blood perfusion measurement. J Biomech Eng 1979;101:246–249.

Habermann E. Bee and wasp venoms. Science 1972;177:314–323.

Hassid A, Levine L. Stimulation of phospholipase activity and prostaglandin biosynthesis by melittin in cell culture and in vivo. Res Commun Chem Pathol Pharmacol 1977;18:507.

Holzer P. Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons. Pharmacol Rev 1991;43:143–201.

Hua XX, Wong S, Jinno S, Yaksh TL. Pharmacology of calcitonin gene related peptide release from sensory terminals in the rat trachea. Can J Physiol Pharmacol 1995;73:999–1006.

Jancso G, Husz S, Simon N. Impairment of axon reflex vasodilatation after herpes zoster. Clin Exp Dermatol 1983;8:27–31.

Kalman S, Linderfalk C, Wardell K, Eintrei C, Lisander B. Differential effect on vasodilatation and pain after intradermal capsaicin in humans during decay of intravenous regional anesthesia with mepivacaine. Reg Anesth Pain Med 1998;23:402–408.

Koyama N, Morikochi Y, Yokota T. Inhibition of high frequency firing of peripheral nerve fiber following intravenous lidocaine administration (Preliminary report). Pain Res 1997;12:115–120.

Lariviere WR, Melzack R. The bee venom test: a new tonic-pain test. Pain 1996;66:271–277.

Larkin SW, Williams TJ. Evidence for sensory nerve involvement in cutaneous reactive hyperemia in humans. Circ Res 1993;73:147–154.

Lembeck F. Sir Thomas Lewis's nocifensor system, histamine and substance-P-containing primary afferent nerves. Trends Neurosci 1983;6:106–108.

Lewis T. Pain, New York: Macmillan Company, 1942. pp. 192.

Magerl W, Koltzenburg M, Schmitz JM, Handwerker HO. Asymmetry and time-course of cutaneous sympathetic reflex responses following sustained excitation of chemosensitive nociceptors in humans. J Aut Nerv Sys 1996;57:63–72.

Maggi CA, Meli A. The sensory-efferent function of capsaicin-sensitive sensory neurons. Gen Pharmacol 1988;19:1–43.

Morris GC, Gibson SJ, Helme RD. Capsaicin-induced flare and vasodilatation in patients with post-herpetic neuralgia. Pain 1995;63:93–101.

Nishimura K, Alhenc-Gelas F, White A, Erdos EG. Activation of membrane-bound kallikrein and renin in the kidney. Proc Natl Acad Sci USA 1980;77:4975–4978.

Parkhouse N, Le Quesne PM. Impaired neurogenic vascular response in patients with diabetes and neuropathic foot lesions. New Engl J Med 1988;318:1306–1309.

Rowbotham MC, Davies PS, Field HL. Topical lidocaine gel relieves postherpetic neuralgia. Ann Neurol 1995;37:246–253.

Salari H, Braquet P, Borgeat P. Stimulation of lipoxygenase product synthesis in human leukocytes and platelets by melittin. Mol Pharmacol 1985;28:546–548.

Schmidt R, Schmelz M, Ringkamp M, Handwerker HO, Torebjörk HE. Innervation territories of mechanically activated C nociceptor units in human skin. J Neurophysiol 1997;78:2641–2648.

Sherman JL. Normal arteriovenous anastomoses. Medicine 1963;42:247–267.

Shier WT. Activation of high levels of endogenous phospholipase A2 in cultured cells. Proc Natl Acad Sci USA 1979;76:195–199.