Effects of a menthol-based analgesic balm on pressor responses evoked from muscle afferents in cats

Brian G. Ragan, MS; Amanda J. Nelson, MS; Jonathan H. Foreman, DVM, MS; Gerald W. Bell, EdD; Gary A. Iwamoto, PhD

Objective—To evaluate changes in heart rate (HR) and mean arterial pressure (MAP) as indicators of changes in pressor response for muscle afferents after topical application of menthol (MEN)-based analgesic balm.

Animals—11 decerebrate cats.

Procedure—Pressor responses were reflexively evoked by static contraction of hind limb muscles, which are caused by group III and IV afferents. Responses were monitored without interference from anesthesia or effects of higher brain function by the use of decerebrate cats. After obtaining baseline data, MEN analgesic balm (1.9%) was applied to the skin over contracting muscles of 1 hind limb in 6 cats; petrolatum was applied to 5 control cats. Muscle contractions were evoked every 10 minutes, alternating between hind limbs, for 120 minutes. Peak MAP and HR were analyzed.

Results—Peak MAP responses evoked by static muscle contraction for the ipsilateral hind limb were significantly attenuated 20 minutes after application, but approached baseline values 40 minutes after application. The pressor response was significantly decreased 20 minutes after application during the last 12 seconds of the stimulus, which was attributed to group IV afferents. There were no significant differences in HR responses.

Conclusions and Clinical Relevance—Application of MEN analgesic balm to the skin over contracting muscles significantly decreased the pressor response to static muscle contractions. This suggests that topical application of MEN has effects on responses evoked from receptors located in muscles. The MEN analgesic balm appeared to attenuate the pressor response 20 minutes after application, but it was a short-term effect. (*Am J Vet Res* 2004;65:1204–1210)

Veterinarians and trainers routinely use analgesic balms for the treatment of animals with minor muscle conditions.[1] Physically active people engaged in sports and exercise also have a long history of the use of analgesic balms for relief of minor pain. Topical analgesics have grown into an annual industry of $150 million, with almost 60 million adults in America using an analgesic balm at some time.[2] However, the efficacy and mechanism of action of analgesic balms have been poorly studied in veterinary and human patients. Analgesic balms are a principal focus of research by our laboratory group. In 1 study,[3] we documented that topical use of capsaicin ointment over the distal palmar digital nerves causes significant reductions in **heart rate (HR)** and lameness score for up to 4 hours after application in horses with experimentally induced foot pain. In another series of experiments,[4] the effects of a combination of capsaicin and methyl salicylate in decerebrate cats documented an attenuation of the pressor response evoked by muscular contraction 20 minutes after application.

A wide range of possible explanations exists for the actions of analgesic balms, and these possible mechanisms are dependent on the composition of each specific product. **Menthol (MEN)**, methyl salicylate or trolamine salicylate, and capsaicin (capsicum) are active ingredients commonly used in analgesic balms. Capsaicin is believed to cause depletion of substance P from nociceptive fibers, resulting in a temporary blockade of the transmission of pain impulses to higher centers.[3] Menthol is also widely used as the sole active ingredient in many analgesic balms; however, its mechanism is not well understood, except as a counterirritant.

Pressor responses that result from muscle contractions are believed to be an important mechanism for increasing blood pressure during exercise. Although part of the blood pressure response during exercise is a result of a volitional control mechanism (ie, central command), muscle contractions stimulate small-diameter sensory nerve fibers in skeletal muscles, which evokes a reflex pressor response. These muscle afferents are usually referred to as group III (fine myelinated) and IV (nonmyelinated) afferents.[5] Sensory fibers from skin are named such that A-delta and C fibers correspond to group III and IV afferents, respectively. The sensation of pain in muscle is caused by the action of group III and IV afferents. Group III afferents generally discharge during muscle contraction in a pattern consistent with mechanical events in the muscle, whereas group IV afferents appear to be primarily sensitive to chemical changes during muscle contraction. Both afferent groups respond to bradykinin and arachidonic acid (the prostaglandin precursor), both of which are potential algesic substances.[5] Furthermore, cyclooxyge-

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From the Departments of Veterinary Biosciences (Ragan, Nelson, Iwamoto) and Veterinary Clinical Medicine (Foreman), College of Veterinary Medicine, and the Athletic Training Education Program (Ragan, Nelson, Bell, Iwamoto), Neuroscience Program (Iwamoto), and Department of Kinesiology (Ragan, Nelson, Bell, Iwamoto), College of Applied Life Studies, University of Illinois, Urbana, IL 61801.
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Address correspondence to Dr. Iwamoto.
†Deceased.

nase inhibitors blunt the putative prostaglandin part of the pressor response to muscle contraction.[6]

For decades, acute experiments in animals have provided important insight into the pressor response to muscle contraction.[5] This method involves stimulating the ventral roots or peripheral nerves at a low threshold to induce muscle contractions. Although animals cannot actually report on sensory events, the cardiovascular effects resulting from muscle contractions can be used as a measurement of the extent to which the effects of high-threshold activity of group III and IV afferents are modified by MEN. In another study[4] conducted by our laboratory group in which we used the pressor response to muscle contractions, we documented that topical application of a commercially available analgesic balm containing methyl salicylate and capsaicin reduced the pressor response after application to skin overlying the contracting muscles, which corresponds to the dermatomes of the same level as those supplying the contracting muscles. The purpose of the study reported here was to use the same methods to investigate the effects of topical application of MEN analgesic balm alone on muscle contraction-evoked changes in **mean arterial pressure (MAP)** and HR. It was necessary to use decerebrate cats in the study to eliminate possible effects from higher brain centers on MAP and HR because consciousness or anesthesia could have affected the results for intact (ie, nondecerebrate) cats. Our hypothesis was that topical application of MEN would attenuate MAP and HR responses evoked by muscle contractions.

Materials and Methods

Animals—Eleven healthy adult male and female cats were used in the study. All procedures were approved by the University of Illinois Institutional Animal Care and Use Committee (protocol S9B 250/8349).

Animal preparation—All methods used have been described elsewhere.[7] Cats were anesthetized with halothane (1% to 5%) in a mixture of 67% nitrous oxide and 33% oxygen. An endotracheal tube was inserted to ensure airway patency and enable use of a ventilator. End-tidal carbon dioxide concentration was maintained between 3.5% and 4.5%[a] while the cats remained on the ventilator. Body temperature was maintained at 37°C by use of heating pads and a heating lamp. A catheter was inserted in a common carotid artery of each cat for measurement of blood pressure.[b] Another catheter was inserted in an external jugular vein of each cat for infusion of fluids. The HR was derived from the blood pressure signal.[c] Cats were positioned in sternal recumbency (**Figure 1**). Hair was clipped over the head, back, and hind limbs by use of a No. 40 blade.[d] The skin was not shaved or the hair cut too short because of the risk of skin damage. Dorsal laminectomy was performed from L4 to L7 to provide access to the ventral roots of L7 and S1. The calcaneal tendon was isolated along with a small portion of the calcaneus and used to monitor tension of the triceps surae (gastrocnemius and soleus muscles).[8] Care was taken to minimize the size of the skin incision to the area immediately over the tibiotarsal joint (hock) region to prevent the possibility of applying analgesic balm on an open wound. Each cat was then rigidly mounted in a stereotaxic frame[e] and a Goteborg-style spinal fixation device. The calcaneal tendons were attached to force transducers.

Midcollicular decerebration was then performed.[7] Briefly, this involved use of a blunt spatula to remove all neural tissue rostral to the plane formed by the bony tentorium cerebelli. This procedure allowed the inhalant anesthesia to be discontinued and thus provided a background activity free from the confounding effects of anesthetics or centers of consciousness. This also ensured that the cats did not feel any pain. This procedure is recognized by the Committee on Guidelines for the Use of Animals in Neuroscience and Behavioral Research as one that eliminates all possibility of pain and the need for general anesthesia.[9] It is generally recognized in all cases of midcollicular decerebration (including those reported here) that some decerebrate rigidity or increase in extensor muscle tone is evident.

The cats were eventually weaned from the ventilator and allowed to breathe spontaneously. The ventral roots of L7 and S1 were isolated, and a pool of mineral oil was maintained over the exposed neural tissue to prevent desiccation of the tissues. At the conclusion of the experiments, the cats were each administered an overdose of sodium pentobarbital (300 mg/kg, IV).

Muscular contraction and application of MEN—After the surgical procedures, a period of approximately 120 minutes was allowed for stabilization of each cat. Resting tension of the triceps surae was initially 200 to 400 g (ie, less than the optimum for tension generation) so that this mechanism could be engaged at a later time to increase evoked tension as the muscles became slightly fatigued during the recording of the responses. The ventral roots were then stimulated to evoke the pressor response attributable to muscle contractions. The ventral roots of L7 and S1 were stimulated[f] for 30 seconds by use of 0.1-millisecond pulses with a stimulus frequency of 30 Hz at an intensity of 3 times the motor threshold while monitoring the tension generated by static contraction of the triceps surae. Although the triceps surae was monitored, this stimulation activated the entire L7 to S1

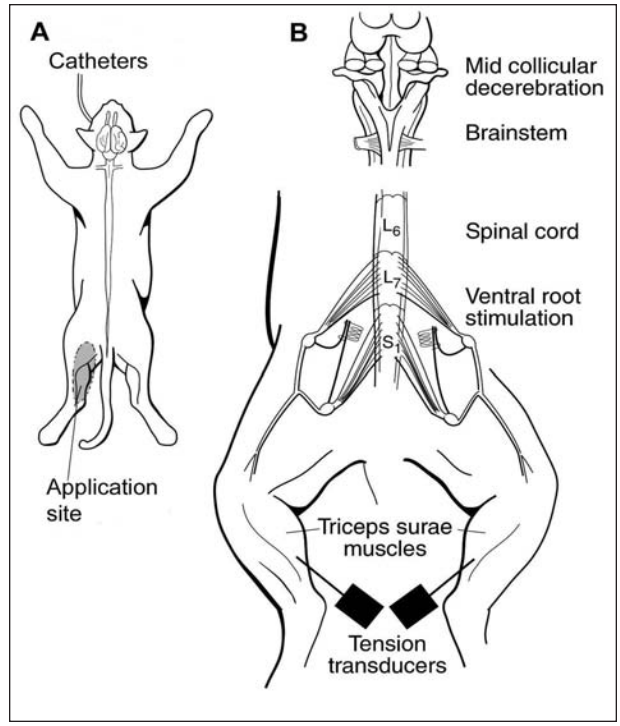

Figure 1—Diagrams depicting a cat in sternal recumbency and the site of application of a menthol (MEN)-based analgesic balm or petrolatum (control treatment) to a hind limb (A) and the experimental arrangement for the decerebrate cats (B). Catheters were used to record mean arterial pressure (MAP) and heart rate (HR). Tension transducers were attached to a small portion of the calcaneus and used to monitor tension of the triceps surae (gastrocnemius and soleus muscles).

distribution. We used a submaximal stimulus frequency to minimize muscle fatigue during the course of the experiment.

After 3 consecutive stimulations in which similar muscle tension, MAP, and HR were obtained, cats were assigned to receive 1.9% MEN ointment or petrolatum. Six cats received the MEN analgesic balm, and 5 cats served as control animals and received the petrolatum. One cat in the MEN group was excluded from analyses because of technical or equipment malfunction; thus, there were 5 cats in each group.

The MEN analgesic balm or petrolatum was applied (thickness of approx 2 mm) to cotton cast padding, which was then placed on the area of the caudal thigh on 1 hind limb (Figure 1). We lightly massaged the padding for a few seconds to ensure that the compound was past the hair stubble and in good contact with the skin. We also were careful to ensure that the MEN balm was not applied to open wounds. On the basis of precise knowledge of homologous structures in dogs,[10] the area of application included the area of L7 and S1 dermatomes and included the fields of the caudal cutaneous femoral nerve and distal cutaneous sural nerves.

Collection of data—Nerve roots on the contralateral limb (ie, hind limb that did not receive application of MEN analgesic balm or petrolatum) were stimulated, and baseline data recorded. Ten minutes later (time 0), nerve roots on the ipsilateral limb (ie, hind limb to which MEN analgesic balm or petrolatum was topically applied) were stimulated and data recorded. This was followed immediately by the application of the MEN analgesic balm or petrolatum. Subsequent stimulation of nerve roots alternated from the contralateral to the ipsilateral hind limb at intervals of 10 minutes for 40 minutes after topical application. Thus, nerve roots on the contralateral hind limb were stimulated 10 minutes before and 10 and 30 minutes after topical application, whereas nerve roots on the ipsilateral hind limb were stimulated immediately before and 20 and 40 minutes after topical application. This technique provided a 20-minute recovery period for the muscles on each hind limb in an attempt to minimize fatigue. Data were recorded at each time point.

Statistical analysis—Data were analyzed with two 2 × 3 mixed ANOVAs by use of a between-group factor of treatment (MEN and control) and within-subjects factor of time (at baseline, 20 minutes after application, and 40 minutes after application) for the dependent variables (MAP and HR responses). Repeated-measure ANOVA of the contralateral limb response was performed for 10 minutes before application, 10 minutes after application, and 30 minutes after application to act as another control value. Since the type of afferent input is dependent to some extent on the time during the stimulus bout that the effects are measured, with the response for group III (mechanical) afferents preceding that for group IV (chemical) afferents,[5] 2 repeated-measures ANOVAs were performed on the MEN group for the peaks during the first 12 seconds and last 12 seconds of the 30-second stimulus at baseline, 20 minutes after application, and 40 minutes after application. Follow-up contrasts of mean differences were performed. Values of $P \leq 0.05$ were considered significant.

Results

Ipsilateral stimulation—An ipsilateral effect was detected with the pressor response evoked by muscle contraction greatly attenuated at 20 minutes after application (**Figure 2**). Values had almost completely returned to baseline at 40 minutes after the application of MEN analgesic balm. Application of MEN on the ipsilateral side affected peak of the response evoked without regard to when the peak was detected during the contraction (**Figure 3**). Mean ± SEM values of MAP for stimulation of the ipsilateral hind limb at 0, 20, and 40 minutes after application were 18.00 ± 4.37, 7.39 ± 2.54, and 12.47 ± 3.44 mm Hg, respectively. There was a significant interaction (ie, a decrease) in the peak MAP but no effect on peak HR response. Follow-up contrasts of mean differences revealed a significant decrease in the MAP response for the MEN group at 20 minutes after application.

To ensure that attenuation of the pressor response was not attributable to skin vasodilation, the MAP immediately before stimulation (ie, muscle contraction) at baseline and 20 minutes after application were examined. Mean MAP for baseline was 117.2 ± 6.0 mm Hg, whereas it was 116.7 ± 6.0 mm Hg 20 minutes after application. A dependent *t* test did not indicate a significant difference between MAP at baseline and 20 minutes.

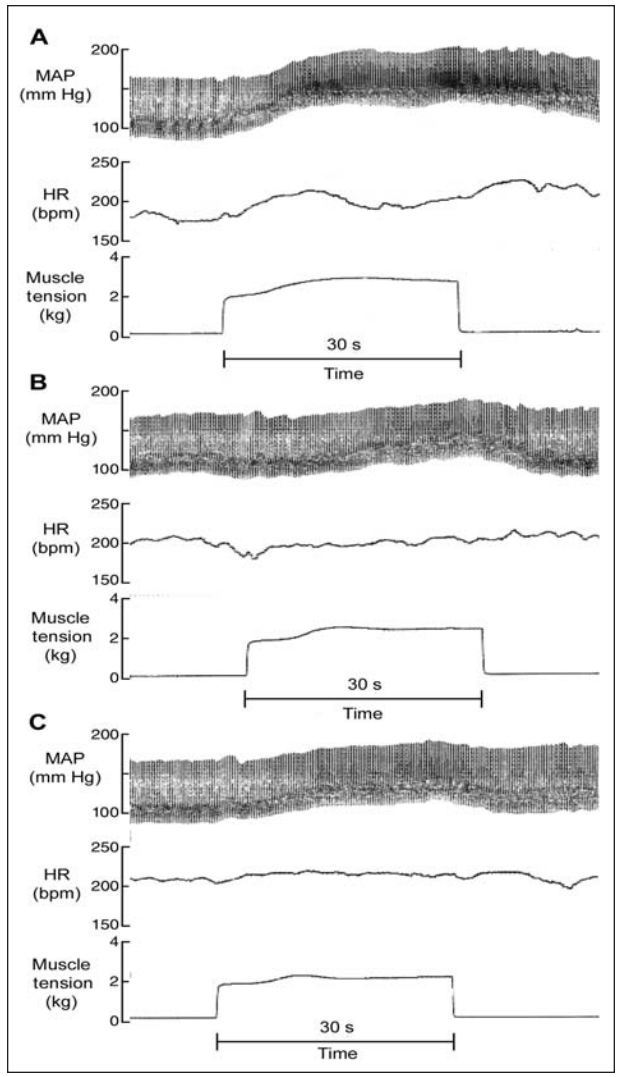

Figure 2—Representative recordings of MAP and HR responses to static muscle contraction during a 30-second stimulus before (A) and 20 minutes (B) and 40 minutes (C) after application of MEN analgesic balm. Stimulus was applied to the hind limb that received the topical application of MEN. Time of application was designated as time 0. bpm = Beats per minute.

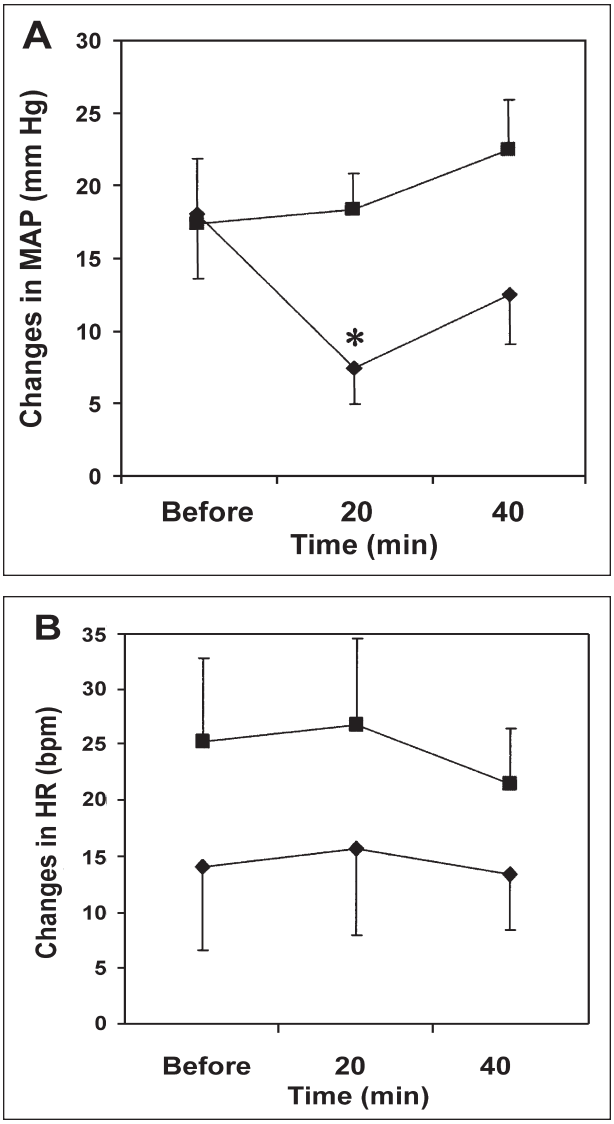

Figure 3—Mean ± SEM peak responses for MAP (A) and HR (B) to static muscle contraction during a 30-second stimulus in 5 cats that received topical application of petrolatum (control cats; squares) and 5 cats that received topical application of MEN analgesic balm (diamonds). Data were recorded before application, 20 minutes after application, and 40 minutes after application. Time of topical application was designated as time 0. Stimulus was applied to the hind limb that received the topical application of petrolatum or MEN analgesic balm. Some peaks were evident early during the stimulus (during the first 12 seconds of the 30-second stimulus) and others near the end of the stimulus (during the last 12 seconds of the 30-second stimulus). *Value differs significantly ($P \leq 0.05$) from the value obtained before topical application.

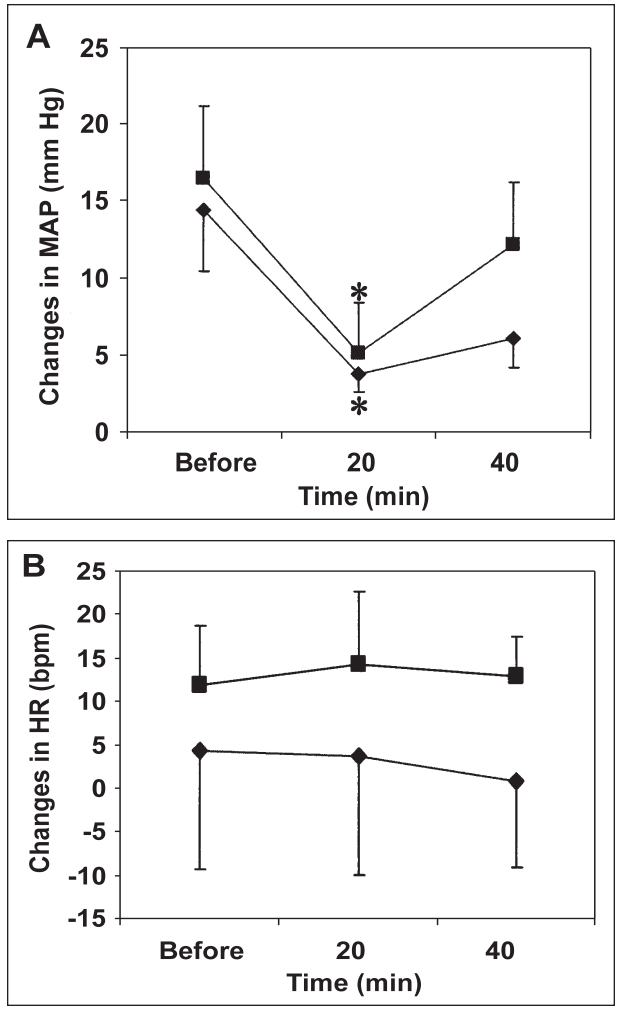

Figure 4—Mean ± SEM peak responses for MAP (A) and HR (B) to static muscle contraction during the first 12 seconds (diamonds) and last 12 seconds (squares) of a 30-second stimulus in 5 cats that received topical application of MEN analgesic balm. Data were recorded immediately before application, 20 minutes after application, and 40 minutes after application. Time of topical application was designated as time 0. Stimulus was applied to the hind limb that received the topical application of the MEN analgesic balm. Mean peak responses for the first 12 seconds and last 12 seconds of each 30-second stimulus were analyzed separately. *Value differs significantly ($P \leq 0.05$) from the value obtained before topical application of MEN analgesic balm.

Contralateral stimulation—We did not detect significant effects on peak MAP and HR responses within the time course of the study after stimulation of the contralateral hind limb. The MAP responses for 10 minutes before application, 10 minutes after application, and 30 minutes after application were 9.13 ± 3.74, 8.66 ± 1.38, and 8.13 ± 4.48 mm Hg, respectively.

Timing of putative afferent input—A significant peak MAP response was detected during the first 12 seconds (attributed mainly to group III afferents) and the last 12 seconds (attributed mainly to group IV afferents) of the 30-second stimulus. Analysis indicated that there was a significant decrease in both of these parts of the pressor response 20 minutes after MEN application (**Figure 4**). Mean peak MAP during the first 12 seconds of the stimulus of the ipsilateral hind limb at 0, 20, and 40 minutes after application was 14.40 ± 4.03, 3.79 ± 1.25 , and 6.13 ± 1.95 mm Hg, respectively. Mean peak MAP during the last 12 seconds of the stimulus of the ipsilateral hind limb at 0, 20, and 40 minutes after application was 16.46 ± 4.73, 5.20 ± 3.21, and 12.2 ± 4.13 mm Hg, respectively. There was not a significant effect on HR observed for the first 12 seconds or the last 12 seconds of the 30-second stimulus.

Discussion

Results of the study reported here provide additional evidence that the pressor response to muscle contraction mediated by small myelinated and unmyelinated muscle afferents may be significantly attenuated by the application of an analgesic balm to the skin overlying the contracting muscles. Although another study[4] conducted by our laboratory group documented the effects of an analgesic balm containing capsaicin and methyl salicylates on the pressor response, we did not have information on MEN. Menthol is the sole active ingredient of many analgesic balms. Menthol also differs distinctly in the sensation experienced by humans, in contrast to the sensation provided by other counterirritants. Menthol is an enigma that gives rise to paradoxic sensations. In humans, it generates a feeling of coolness but nonetheless acts as a counterirritant that has some of the qualities of capsaicin (ie, a feeling of heat or warmth), which indicates that thermoreceptors may be involved. Menthol is a stimulant of cold receptors.[11-13] Cooling of the skin is detected by cutaneous cold receptors, which include A-delta and C fibers.[12] However, another study[12] revealed that approximately half of cold- and MEN-sensitive neurons in the dorsal root ganglion are also sensitive to capsaicin.

On the basis of analysis of our data, MEN appears to have an effective time course that differs from the effective time course for a mixture of capsaicin and methyl salicylates.[4] Menthol had a dramatic effect on the evoked MAP response 20 minutes after application, almost completely eliminating it. However, MEN does not appear to have a long-term effect on evoked MAP responses, which is in contrast to the long-term effect for a mixture of capsaicin and methyl salicylates.[4] Responses 40 minutes after application had nearly returned to baseline values. This finding is consistent with those of another study[11] in mice in which investigators reported that the antinociceptive effect of MEN peaked 30 minutes after application and quickly returned to baseline values within an hour. Thus, although our study[4] on capsaicin and methyl salicylate revealed an additional attenuation effect 40 minutes after application, MEN appeared to act during a much shorter time course in the study reported here. In another study[14] that involved only capsaicin, we found that the time course for the effects of capsaicin is similar to that for MEN.[14] It has been suggested that methyl salicylate may act as a counterirritant and a nonsteroidal anti-inflammatory agent. The action of the salicylate may account for differences in effects detected 40 minutes after application in our other study.[4] It seems likely that the salicylate portion of the analgesic balm penetrated the tissues beneath the skin to have some effects on sensory nerve endings through local penetration and blood-borne routes, presumably on formation of prostaglandin metabolites (detected by group IV afferents).[15,16] The IV administration of indomethacin can also reduce the pressor response to muscle contraction.[6] There is a report[17] of salicylates penetrating and having an effect in the subcutaneous tissues over time; thus, this could be a possible explanation for the difference in MEN responses in the study reported here, compared with results for capsaicin and methyl salicylate analgesic balms in other studies[4,14] conducted by our laboratory group.

It should be emphasized that although the results reported here document the effects of MEN, they do not provide an indication of exactly where in the nervous system the effects are exerted. There are some possible mechanisms for these effects. Menthol could act in a manner similar to that as capsaicin in the sense that MEN engages the direct effects on sensory fibers or terminals.[11] However, integrative effects on CNS circuitry may also account for analgesic properties. Capsaicin, MEN, and, perhaps, methyl salicylate are believed to hyperstimulate or cause a counterirritant effect that is poorly understood.[2] Some authors have also speculated that the rubbing that accompanies application of analgesic balms may be responsible for increasing the activity of large-diameter afferents and therefore initiation of the gate-control mechanism.[18,19] Although often not explicitly stated, still others have speculated that the sensation of warmth that accompanies application of an analgesic balm is related to the analgesia of superficial heating modalities.[20] However, application of analgesic balms does not lead to temperature changes.[g] Finally, some consider that a placebo effect is the most likely source of the analgesic effect and that these substances have no mechanism of action other than by the power of suggestion.[21]

Assuming that MEN acts as a counterirritant at least somewhat similar to capsaicin, 1 possibility for the attenuation is that MEN-stimulated, cutaneous, small-diameter afferents inhibit group III and IV afferent traffic from the muscles as a result of synaptic action within the CNS. Capsaicin is an agonist for small-diameter, high-threshold cutaneous afferents and causes secondary hyperalgesia.[22] In addition, capsaicin may act directly to block nerve fibers[23] or deplete transmitter substances.[24-26,h] When applied directly to the common peroneal nerve, capsaicin can attenuate the pressor response to muscle contraction[27] in much the same manner as for the study reported here. However, effects for the contralateral limb were also observed in that study.[27] Several possible explanations exist for this discrepancy in effects for the contralateral limb. The difference may be attributable to the activation of cutaneous afferents in a manner consistent with clinical practices and the receptive fields represented by the entire peroneal nerve, which would also include afferents to the cutaneous tissues, muscles, and joints. Systemic absorption of capsaicin can result in direct central cardiac effects, such as bradycardia.[1,28] Another possibility may be in the relative strength of the counterirritant. Differences in concentrations of the active ingredient may be responsible for differences in analgesia. Although the concentration of 1.9% MEN is commonly used, it is a relatively low concentration by clinical standards. There are also numerous examples from the literature on motor neurophysiologic mechanisms that indicate that motor reflex pathways are enhanced or inhibited by the stimulation of cutaneous fields.[29] There are also many examples of inhibitory skin fields having an effect on signal transmission of high-threshold afferents. Because capsaicin activates

about half of the MEN-sensitive neurons,[12] MEN may be well suited for the role of a counterirritant.

Nociceptive stimuli are defined as stimuli that are frankly damaging to tissues or have the potential to cause tissue damage. Ergoreception refers to muscle contraction, the fundamental event underlying exercise. However, pain is not as clearly identified. Pain is greatly dependent on the context in which nociceptive or nonnociceptive stimuli are encountered.[19] A nociceptive stimulus may not be perceived as painful, which is often the case during athletic contests and for battlefield conditions (ie, during the heat of battle).[19] However, bitter complaints of pain may be encountered later for even comparatively mild stimuli. One interpretation of the stimulus is that it may contain nociceptive and ergoreceptive components because the stimulus is a high-intensity tetanic contraction that closely resembles that seen during a muscle cramp.

Other possible ways exist in which a skin-stimulation mechanism could engage a central integrative mechanism. The original gate theory should not be completely dismissed, but it seems unlikely that MEN operates through this mechanism. Menthol activates small-diameter thermoreceptor afferents[12] and not large fibers that are needed for blocking nociceptive input in the gate control theory.[19] In addition, had skin contact caused activation of large fibers, we should have detected some effect as a result of application of the petrolatum. Perhaps the strongest possibility is that this phenomenon is linked to the **diffuse noxious inhibitory control (DNIC)** system.[30,31] The DNIC system is believed to be a major mechanism for endogenous suppression of pain. Activation of the DNIC system involves a supraspinal relay, which exerts a diffuse inhibitory effect on noxious sensory input.[32] The effects of a DNIC system are likely to include presynaptic and postsynaptic inhibition. Of the several transmitter systems implicated as possible mediators of the DNIC system, 1 intriguing candidate identified in this process would be the supraspinally mediated endogenous release of opiates. These endogenous opiates are also centrally released at a strictly local spinal level in response to peripheral stimulation.[33] This possibility is supported by investigators[11] who reported that the antinociceptive effects of MEN administered orally (hot-plate latency and abdominal constriction) or intracerebroventricularly (abdominal constriction) were acting centrally and dependently on the opioid system. They reported that naloxone, an unspecific opioid antagonist, and a κ-opioid antagonist prevented the antinociceptive effects of MEN. It would appear from the results of one of the other studies[4] conducted by our laboratory group that the effects of analgesic balms may sometimes influence the response from contralateral skin fields; however, because we did not detect any contralateral effects in the study reported here, this tends to leave unresolved the participation of a diffuse mechanism.

We did not detect evidence of an effect of MEN on HR in the study reported here. There were no significant differences attributable to MEN application for HR responses. The HR responses to muscular contraction evoked by the stimulus of ventral nerve roots are typically small[7]; therefore, it is difficult to determine changes in HR. Because the change in HR responses attributable to MEN was nonsignificant, the most likely explanation is that the reduction in blood pressure responses caused by MEN was attributable to a reduction in the peripheral resistance evoked by muscle contraction. We did not detect changes in the response to muscle contraction of the contralateral hind limb. There were no significant changes in the MAP and HR responses after stimulation of the contralateral hind limb; thus, it is likely that the ipsilateral effects were not simply a result of deterioration of the response over time

It also remains possible that the analgesic changes may have been related to changes in blood flow to the skin. In 1 study,[i] blood flow to the skin was increased 3- to 4-fold after application of an analgesic balm. Certainly this would appear to be an effect of analgesic balms that is commonly observed.

We conclude that cardiovascular effects attributable to activation of group III and IV muscle afferents can be significantly attenuated by the application of an analgesic balm containing MEN. The time course for action of analgesic balms containing only MEN as an active ingredient differs from the time course of action for a mixture of capsaicin and methyl salicylate[4] but is similar to the time course for capsaicin alone.[14] This also documents that analgesic balm applied to the skin in a manner consistent with common clinical practice has effects on signals that originate beneath the skin surface. From this initial determination of a pharmacologic effect, there are many possible avenues for investigation, including the extent of effects on afferent nerve fibers and single units within the CNS. Much is known about the physiologic mechanisms of the pressor response attributable to muscle contraction. For example, substance P plays an integral role in the response, and the pathways to and from the brainstem underlying the response have been identified.[5] It is likely that future studies will incorporate experiments to investigate the role of these factors in the response of animals to the application of an analgesic balm.

[a]LB-2 Medical Gas Analyzer, Sensormedics, Yorba Linda, Calif.
[b]Statham P23D transducer, Gould Inc, Cleveland, Ohio.
[c]Gould Biotach, Gould Instrument Systems Inc, Valley View, Ohio.
[d]A5 Clippers, Oster, McMinnville, Tenn.
[e]Kopf 1700 sterotaxic frame, David Kopf Instruments, Tujunga, Calif.
[f]AstroMed Grass S48, Astro-Med Inc, West Warrick, RI.
[g]Draper DO, Trowbridge CA, Wells AM, et al. The Thermacare heat wrap increases paraspinal muscle temperature greater than the icy-hot patch and the mentholatum patch (abstr). *J Athl Train* 2003;38:S45.
[h]Ghersetich I, Bianchi B, Lotti T. Capsaicin: therapeutic activities on itch by depleting sensory neuropeptides fibers in the skin(abstr). *J Invest Dermatol* 1999;112:608.
[i]Shellock FG. Effect of a topically applied counterirritant/analgesic on skin blood flow (abstr). *Med Sci Sports Exerc* 1987;19:S49.

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