Heterotopic CO₂ Laser Stimulation Inhibits Tooth-Related Somatosensory Evoked Potentials

Keiko Fujii-Abe, DDS, PhD,* Yuka Oono, DDS, PhD,† Katsunori Motohashi, DDS, PhD,‡ Haruhisa Fukayama, DDS, PhD,§ and Masahiro Umino, DDS, PhD†

*Department of Dental Anesthesiology, School of Dental Medicine, Tsurumi University, Yokohama; †Anesthesiology and Clinical Physiology, Department of Oral Restitution, Division of Oral Health Sciences, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan

Reprint requests to: Keiko Fujii-Abe, DDS, PhD, Department of Dental Anesthesiology, School of Dental Medicine, Tsurumi University, Tsurumi 2-1-3, Tsurumi-ku, Yokohama 230-8501, Japan. Tel: 81-45-580-8342; Fax: 81-45-573-9599; E-mail: fujii-keiko@tsurumi-u.ac.jp.

Abstract

Background. The diffuse noxious inhibitory control (DNIC) effect is the neurophysiological basis for the phenomenon that heterotopic “pain inhibits pain” in remote areas of the body. The effect of DNIC is mediated by spino–bulbo–spinal loops and a final postsynaptic inhibitory mechanism. The DNIC effect depends on intensity, duration, quality, and application site of conditioning stimulation and stimulated nerve fiber-type. DNIC induced by CO₂ laser conditioning stimulation has, however, not yet been investigated, and the present study was designed to examine this.

Methods. As the indicator of test stimulation, the late component of somatosensory evoked potentials (SEPs) induced by electrical tooth stimulation and pain intensity were examined under CO₂ laser conditioning stimulation. As the conditioning stimulus, CO₂ laser energy (λ = 10.6 μm, spot size Ø = 2 mm) was applied to the dorsum of the left hand.

Results. The maximum reductions in SEP amplitude and pain intensity evaluated using a visual analog scale were 34.7% and 28.7%, respectively during CO₂ laser conditioning stimulation. No aftereffect was observed.

Conclusion. The present study revealed that CO₂ laser radiation attenuated the late component of SEPs induced by electrical tooth stimulation, triggering the DNIC effect but with no aftereffect.

Key Words. DNIC; CO₂ Laser; Somatosensory Evoked Potentials

Introduction

The activity of spinal dorsal horn nociceptive (NS) neurons in anesthetized rats can be depressed by applying heterotopic noxious stimuli [1,2,3]. Such phenomenon has been termed diffuse noxious inhibitory control (DNIC). DNIC has been observed not only in animals [4,5] but also in humans [6–8]. In the trigeminal nerve territory, the occurrence of DNIC has also been reported in animals [2,3,9] and humans [7,10,11]. Hu [12] and Dallel et al. [13] reported that the trigeminal subnucleus caudalis and oralis in rats are also involved in pain perception and modulation by DNIC. In addition, the results of both animal [2,3,9] and human studies [7,10,11,14–17] revealed that an aftereffect of an inhibitory nature lasted for several minutes following the DNIC effect. This aftereffect is a conceivably major feature in DNIC and could persist for several minutes as long-lasting poststimulus effects [2,3,9,18]. DNIC was effective on both unmyelinated and myelinated fibers, but it was much more effective on the former [19,20].

Various forms of stimulation, including capsaicin [7], thermal [14,21], ischemic [11], and electrical stimulation [15,19] of the skin or other tissues have been used in human subjects as conditioning stimuli in order to study DNIC. However, most of these stimuli activate not only nociceptors but also tactile mechanoreceptors.

Radiant heat stimulation, such as from a pulse of CO₂ laser energy, excites free nerve endings in the superficial skin layers, and the afferent information is conducted by small-myelinated Aδ and unmyelinated C fibers without provoking tactile mechanoreceptors. Therefore, we tested DNIC on trigeminal-evoked somatosensory evoked potentials (SEPs) in humans by CO₂ laser stimulation, which allows activation of Aδ and C nociceptors of the skin as conditioning stimuli. In this study, the electrical tooth stimulation was used as test stimulation because tooth pulp is mostly innervated by not only Aδ fibers but also C fibers, although Aβ fibers are included [22–25]. The purpose of this study is to determine whether the
amplitude of the late component of SEPs and pain intensity induced by electrical tooth stimulation would be modified by pain-mediated noxious stimuli elicited with the CO2 laser and whether an aftereffect would occur with the noxious conditioning stimuli.

Materials and Methods

DNIC Study

Subjects

After the experimental protocol was approved by the ethics committee of Tokyo Medical and Dental University, nine healthy volunteers, aged 24 to 28 years (25.9 ± 0.9 years, mean ± SD; three men and six women) participated in the DNIC study. The inclusion criteria for the subjects were as follows: is over the age of 20 years; is healthy and pain-free; and has the ability to give informed consent. The exclusion criteria were as follows: has any neurological, psychiatric, neuromuscular, endocrine, cardiorespiratory, and metabolic disorders; unable to give informed consent; and currently uses analgesic drugs, antidepressants, sedatives, or cough suppressants. The subjects were fully informed about the procedure and aims of the study, and their consent was obtained in accordance with the Declaration of Helsinki. The subjects were informed prior to their participation that they were free to withdraw from the experiments at any time, that the study concerned the perception of pain, and that there was no chance of tissue injury.

Experimental System

The system was composed of an electrical tooth stimulation unit for application of the test stimulation, an SEP recording unit to objectively estimate pain, and the CO2 laser system to deliver the conditioning stimulus. The electrical tooth stimulation unit and the dental-evoked SEP recording unit were previously described [10,11].

Test Stimulation Procedure

Electrical Tooth Stimulation

Electrical tooth stimulation was selected as the test stimulation for this study. Because the electrical tooth stimulation method has been described in detail, the procedure is only briefly described here [10,19,26,27]. After a healthy, unfilled upper right incisor was isolated by a rubber dam, a plastic tube (4 mm in diameter, 13.8 mm2 in area) was fixed on the labial surface of the tooth to prevent electrical leakage to gingival and periodontal tissues. A copper wire was inserted into the paste and was connected to an isolated constant-current electrical stimulator (SEN-3301, Nihon Kohden, Tokyo, Japan). For each subject, electrical tooth stimuli with single rectangular wave pulses of 1 ms duration were generated using an isolated constant-current electrical stimulator (SEN-3301; Nihon Kohden). The stimuli were manually presented at intervals of 1 s through an isolator (SEN-3301; Nihon Kohden). To determine the intensity level of the test stimulation for each subject, the electrical stimuli to the tooth were gradually increased from 0 μA to an intensity that caused a faint pain sensation and then to the strongest bearable pain. The average values for faint pain sensation and strongest bearable pain were calculated for each individual. The values generating the faint and strongest pain bearable sensations were, respectively, designated as “A” and “E.” The electrical values between A and E were divided equally into four parts for each subject. The five different electrical current values obtained were termed A to E (A < B < C < D < E). To determine the intensity level of the test stimulation, this electrical tooth stimulus intensity was approximately 1.4 times the pain threshold, and the visual analog scale (VAS) value of the D stimuli was about 48.6 mm.

To avoid the effects of attention caused by the noxious conditioning stimulation on hand, we asked the subjects to concentrate their full attention on the tooth where the electrical stimuli was applied and required them to count the number of stimuli and declare it after each session.

Recording of SEPs Induced by Electrical Tooth Stimulation

The SEP recordings were performed according to a previously described method [10,19,22]. Evoked potentials were recorded with Ag/Ag–Cl electrodes according to the international 10–20 system at Cz (vertex) in reference to the earlobe contralateral to the site of stimulation. The electrodes had impedances below 5 KΩ. Sixty-four single pulses were delivered to the tooth for averaging evoked potentials. The evoked potentials generated by electrical tooth stimuli were recorded and fed to an averaging system (Neuro-pack-four mini, MEB-5304; Nihon Kohden). All the signals were fed into an EEG amplifier, bandpass-filtered at 0.1 to 50 Hz. Artifacts of more than 100 μV during the SEP recording were automatically excluded from summation. A waveform with latencies between ~50 and 450 ms was described. An upward deflection of the SEP waveform was defined as N (negative) and a downward deflection as P (positive), and the second major reflections were N2 and P2, respectively.

The amplitudes of the late component with latencies between 150 and 250 to 300 ms (N2–P2) were analyzed. The amplitudes of the late component were calculated between –50 and 450 ms was described. An upward deflection of the SEP waveform was defined as N (negative) and a downward deflection as P (positive), and the second major reflections were N2 and P2, respectively.

The amplitudes of the late component with latencies between 150 and 250 to 300 ms (N2–P2) were analyzed. The amplitudes of the late component were calculated between –50 and 450 ms was described. An upward deflection of the SEP waveform was defined as N (negative) and a downward deflection as P (positive), and the second major reflections were N2 and P2, respectively.
analogue scale, which ran from zero pain on the left to maximum pain on the right. The lengths from the left end to the points marked were scored in millimeters as the pain intensity of perception. The subjective pain responses were evaluated immediately after the SEP recording.

Conditioning Stimulation Procedure

The subjects were asked to sit in a reclining chair to ensure a state of good muscular relaxation and rest, and they wore protective goggles in accordance with the guidelines for the control of laser hazards. CO₂ laser energy (λ = 10.6 mm, spot size Ø = 2 mm) was delivered to the dorsum of the right hand (Lasery 15Z, Nippon Infrared Industries Co. Ltd., Kawasaki, Japan). CO₂ laser with 200 ms pulse width was radiated at a 1-second interstimulus interval. In the present study, we employed a 1-second interval, although CO₂ laser stimulation was delivered at 3 to 10 seconds [16,29,30]. The latencies of laser-evoked potentials LEPs induced by a CO₂ laser radiated onto the hand were distributed between 340 and 480 ms (interval: every 3 seconds, randomized between 3 and 10 seconds, or randomized between 20 and 40 seconds) in Aδ fiber-mediated stimuli [7,31,32] and 922.2 ± 87.6 ms (interval: randomized between 9 and 11 seconds) in C fiber-mediated stimuli [7].

The incident fluence was set at 17 to 20 mJ/mm² to elicit a sharp pinprick-like pain. In order to avoid habituation, sensitization, receptor fatigue, and skin burning, irradiated points were moved slightly for each stimulus.

The CO₂ laser was radiated asynchronously with tooth stimulation with a 500-ms delay between the CO₂ laser and electrical tooth stimulation because electrical tooth stimulation produced the SEP late component between 150 and 250 to 300 ms. The laser beam was applied to the dorsum of the left hand within innervations of the radial nerve. For the control session, the laser hand piece was placed over the dorsum of the hand in exactly the same manner as for the noxious stimulus, but laser energy was not emitted.

Experimental Design

The study was performed under uniform conditions (23 to 25°C temperature, minimum noise environment, with measurements performed between 3 and 7 PM). Two experimental sessions (i.e., a noxious session and a control session) were performed for each subject on separate days (Figure 1). The noxious CO₂ laser stimulation was applied during the noxious session. Conditioning stimulation was performed for 3 minutes from 8.5 to 11.5 minutes after the start of the study. In each session, the SEP recording was performed and the VAS values were determined four times: at 0 (5 minutes before the conditioning stimulation or sham operation: control baseline), at 10 (1.5 minutes after the start of the conditioning stimulation or sham operation), at 20 (8.5 minutes after the removal of the conditioning stimulation), and at 30 minutes.

Figure 1 SEP recording and VAS evaluation. Two experimental sessions were performed in each subject. In the noxious session, the noxious CO₂ laser stimulation was applied to the dorsum of the right hand for 3 minutes, but the laser handpiece was set up over the dorsum of the hand in exactly the same manner as for the noxious stimulus, but laser energy was not emitted in the control session. The SEP recording was performed and VAS values were determined at four times for 30 minutes in both sessions.

(18.5 minutes after the removal of the conditioning stimulation or sham operation).

Statistics

A repeated-measure analysis of variance was performed for intersession comparisons of the amplitude of the late component, the N2 and P2 latencies, and the VAS values between the noxious and control sessions. Findings were considered significant at P < 0.05.

Results

SEP Waveforms

Figure 2 shows typical SEP recordings generated by electrical tooth stimulation during the noxious and control sessions. The major downward deflection of the SEP waveform generated by electrical tooth stimulation was observed at a latency of between 150 and 300 ms in both the noxious and control sessions. The amplitudes of the late component (N2–P2) significantly decreased during CO₂ laser conditioning stimulation in the noxious session. However, no differences in either the SEP waveform or the latency of the late component were observed during the study.

Latency of the Late Component

Neither the latency of N2 nor P2 in the late component exhibited significant intersession or intrasession changes (Tukey–Kramer: P > 0.1) (Table 1).
Amplitude of the SEP Late Component

In the noxious session, the amplitude of the late component (N2–P2) significantly decreased only at 10 minutes (during conditioning stimulation) \((P < 0.05)\) compared with the control session (Figure 3, Table 2). The magnitude of the amplitude reductions was 34.7\% (2.57 \(\mu V\)) at 10 minutes. The attenuated amplitude recovered to its control value at 20 and 30 minutes. The detailed data are described in Table 2.

VAS Values

In the noxious session, the VAS values also decreased only at 10 minutes (during conditioning stimulation) \((P < 0.01)\) compared with the control session (Figure 4, Table 2). The magnitude of the reductions in the VAS values was 28.7\% (14.2 mm) at 10 minutes. The attenuated VAS value recovered to its control value at 20 and 30 minutes. The detailed data are described in Table 2.

Discussion

Noxious Stimulation Procedures

Of the components of the SEP obtained by delivery of painful electrical tooth stimulation, the amplitude of the late component with latencies between 150 and 300 ms is more closely associated with stimulus intensity \([8,26]\) and

Table 1  Latency of the somatosensory evoked potential late component (\(N = 9; \text{mean} \pm \text{standard deviation}\))

<table>
<thead>
<tr>
<th></th>
<th>0 minutes (ms)</th>
<th>10 minutes (ms)</th>
<th>20 minutes (ms)</th>
<th>30 minutes (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control session</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency of N2</td>
<td>145.1 ± 22.4</td>
<td>155.7 ± 27.6</td>
<td>153.9 ± 23.6</td>
<td>148.4 ± 27.8</td>
</tr>
<tr>
<td>Latency of P2</td>
<td>263.2 ± 24.1</td>
<td>256.3 ± 28.0</td>
<td>271.4 ± 41.0</td>
<td>259.8 ± 16.6</td>
</tr>
<tr>
<td><strong>Noxious session</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency of N2</td>
<td>144.3 ± 17.8</td>
<td>152.1 ± 20.5</td>
<td>155.0 ± 27.9</td>
<td>144.0 ± 20.2</td>
</tr>
<tr>
<td>Latency of P2</td>
<td>263.4 ± 35.8</td>
<td>262.2 ± 27.0</td>
<td>253.9 ± 26.2</td>
<td>262.2 ± 30.4</td>
</tr>
</tbody>
</table>

\(\leftrightarrow \text{CO}_2\text{ laser period}\)
subjective painfulness [28]. Therefore, in the present study, the method of recording SEPs induced by electrical tooth stimulation was selected for the evaluation of objective pain intensity because it is valid and reliable. In electrical tooth stimulation, A<sub>d</sub> and C fibers are mainly activated because the majority of the afferent fibers are the NS afferents conducting in the A<sub>d</sub> and C fiber range in the tooth pulp. However, the tooth pulp also contains some A<sub>b</sub> fibers [22–25]. Thus, the possibility that activated A<sub>b</sub> fibers may contribute to the SEP components cannot be completely excluded in the present study. The SEP components evoked by electrical tooth stimulation for A<sub>b</sub> fibers are earlier than those for A<sub>d</sub> and C fibers because its conduction velocity is faster than those of A<sub>d</sub> and C fibers. Chudler et al. [33] reported that the SEP components evoked by electrical tooth stimulation for A<sub>b</sub> fibers are early, such as between about 10 and 60 ms.

Pain sensation caused by electrical tooth stimulation was subjectively evaluated with a VAS [11,34,35].

In the present study, we selected CO<sub>2</sub> laser stimulation as the conditioning stimulation because it can activate A<sub>d</sub> and C nociceptors [31,36,37] depending on the laser energy, and the NS impulse is conveyed through A<sub>d</sub> or C fibers and reaches the cerebral cortex via the spinothalamic tract [37]. Thus far, there have been numerous studies on the DNIC effect in which CO<sub>2</sub> laser was used in the test stimulation. In these studies, CO<sub>2</sub> LEPs were objective indicators of the DNIC effect [7,8,16,38,39]. However, CO<sub>2</sub> laser has rarely been employed as the conditioning stimulus. CO<sub>2</sub> laser radiation can stimulate both A<sub>d</sub> and C fibers in an intensity-dependent manner [29,30,40,41]. The intensity of 17 to 20 mJ/mm<sup>2</sup> of CO<sub>2</sub> laser energy used as conditioning stimuli in the present study could also activate both A<sub>d</sub> and C fibers.

**DNIC Effect and Its Mechanism**

The results of the present study revealed that the magnitudes of the reduction in the SEP amplitude and VAS values were 34.7% and 28.7%, respectively, during CO<sub>2</sub> laser stimulation on the hand. Although the maximal effects occurred during conditioning stimulation, no after-effect was observed.

**Table 2** The data of somatosensory evoked potential amplitudes and visual analog scale values (N = 9; mean ± standard deviation)

<table>
<thead>
<tr>
<th></th>
<th>Amplitude (µV)</th>
<th>0 minutes</th>
<th>10 minutes</th>
<th>20 minutes</th>
<th>30 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control session</td>
<td>8.4 ± 2.2</td>
<td>8.4 ± 2.5</td>
<td>8.3 ± 2.5</td>
<td>8.9 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>Noxious session</td>
<td>7.4 ± 1.7</td>
<td>4.8 ± 2.1</td>
<td>6.9 ± 2.2</td>
<td>6.7 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>VAS (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control session</td>
<td>50.8 ± 8.91</td>
<td>51.2 ± 11.3</td>
<td>48.4 ± 7.72</td>
<td>48.7 ± 14.9</td>
<td></td>
</tr>
<tr>
<td>Noxious session</td>
<td>49.4 ± 9.57</td>
<td>35.2 ± 14.6</td>
<td>48.9 ± 8.68</td>
<td>47.4 ± 16.1</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 3** Changes of SEP amplitudes. In the noxious session (solid line), the amplitude of the late component (N2–P2) was significantly decreased only during the noxious CO<sub>2</sub> laser stimulation (P < 0.05). The attenuated amplitude recovered to its control value at 20 and 30 minutes.
In our previous studies, the maximum decreases in SEP amplitude and VAS value induced by electrical conditioning stimuli on the hand were, respectively, 49.3% and 42.3% with aftereffect [19]. Ischemic conditioning stimuli on the hand also induced the maximum decreases in SEP amplitude of 26.1% and VAS of 21.2% with aftereffect [11]. The heterotopic CO2 laser conditioning stimulation in the present study induced no aftereffect, different from that in the case of electrical and ischemic conditioning stimulation in our previous studies.

Although the duration of conditioning stimulation was 10 minutes in both our previous studies, in the present study, we employed short duration conditioning stimulation; it was 3 minutes in this study. The absence of an aftereffect suggested that this may be due to the short duration of the conditioning stimulation and the phasic laser pulse being different from the electrical or ischemic stimulation in our previous reports [10,11]. The DNIC effects were directly related to the intensity and type of noxious conditioning stimuli and nerve fiber stimulated [11,19]. Our previous studies [10,11] using electrical or ischemic stimulation as tonic and powerful conditioning stimulation showed DNIC effect with aftereffect. Indeed, with strong stimuli, the inhibitory effects were powerful and were followed by long-lasting poststimulus effects that could persist for several minutes [7,10,11,14–17,35,42–44].

Although DNIC affects both unmyelinated C and myelinated Aδ fibers, the effects are larger on unmyelinated C fibers [9,11,39,41,42,45]. This sensitization of C fiber nociceptors following strong stimulation or administration of inflammatory substances results in the development of spontaneous activity, reduced thresholds, and increases in responses to suprathreshold stimulation in the nociceptors [46,47], leading to a long-lasting and strong DNIC effect by C fiber nociceptor stimulation.

The central mechanism of DNIC, however, has not yet been completely elucidated. Le Bars [18] has suggested the involvement of the descending pain inhibitory system modulating the activity of convergent neurons from the lower brain stem. DNIC is involved in brain structures confined to the caudalmost part of the medulla, including the subnucleus reticulare dorsalis and mediated by descending pathways in the dorsolateral funiculi. DNIC occurrence in the trigeminal nerve territory suggests the involvement of the descending pain inhibitory system to the trigeminal spinal nucleus from the lower brainstem.

Previous studies [24,47] have revealed that there are incidences of afferent input to low threshold mechanoreceptor, wide dynamic range (WDR), and NS neurons from the tooth pulp in cat. Some animal studies on DNIC mechanism in the trigeminal nerve territory have revealed that WDR and NS neurons in the subnucleus caudalis and also oralis in the trigeminal spinal nucleus play an important role in pain perception and modulation by DNIC [12,13,48]. Therefore, not only WDR neurons but also NS neurons may be involved in pain intensity and SEP amplitude in the present study.

NS and non-NS inputs from orofacial regions converge onto WDR neurons located in the subnucleus caudalis [13,21,24]. Bereiter et al. [49] reported that trigeminal subnucleus caudalis are organized differently from spinal stems. Hu [12] reported that WDR and NS neurons had an ipsilateral orofacial mechanoreceptive field and were localized within laminae I to VI, especially within the deeper laminae of caudalis. DNIC acts on WDR and NS neurons of the trigeminal nucleus caudalis receiving NS signals from the tooth pulp through a postsynaptic inhibition. Indeed, it has been indicated that DNIC acts on nucleus caudalis convergent neurons by a final postsynaptic inhibitory mechanism involving hyperpolarization of the neu-
ronal membrane in the rat [50]. In the present study, it is considered that DNIC was induced by the descending pain inhibitory system modulating the activity of WDR and NS neurons in the trigeminal nucleus complex from the lower brain stem.

Clinical Implication

The findings of the present study revealed that electrically induced dental pain could be clearly inhibited by noxious CO2 laser stimulation with a tolerable intensity on the hand in normal human subjects. The pain inhibition was mediated by Aδ and C fibers elicited by application of CO2 laser. In the future, a pain inhibitory study based on the CO2 laser-mediated DNIC effect should be also conducted for pathological pain such as pulpitis, periodontitis, periapicalitis, and postsurgical pain in dentistry. The experimental facts indicate the possibility of development of new analgesic method for the dental, oral, and maxillofacial region using heterotopical CO2 laser beam radiation—delivering a noxious but tolerable stimulus different from transcutaneous electrical nerve stimulation or acupuncture. Additionally, our previous studies have found a DNIC effect with electrical or ischemic conditioning stimuli on electrically induced dental pain in a similar manner as in the present study. The use of the CO2 laser in combination with electrical or ischemic stimulation deserves further consideration.

Acknowledgments

This work was supported by Grant-in-Aid for Scientific Research no. 14207088 from the Ministry of Education, Culture, Sports, Science and Technology. Japan.

References


