

# Effects of cryotherapy, transcutaneous electrical stimulation and their combination on femoral nerve electrical activity in rats

Efeitos da crioterapia, estimulação elétrica transcutânea e da sua associação na atividade elétrica do nervo femoral em ratos

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## Abstract

**Background:** Clinical reports suggest that the therapeutic association between cryotherapy (CRYO) and transcutaneous electrical stimulation (TENS) favors local analgesia. **Objective:** To evaluate the electrical activity of the femoral nerve (FNA), at rest and during single and combined application of TENS and CRYO, in rats. **Methods:** Nine adult Wistar rats weighting  $\pm 300$ g were used in this study. After inducing anesthesia (Urethane, 1mg/g i.p.), the right femoral nerve was isolated in order to record the FNA at baseline and during the therapeutic modalities. After attaching the electrodes to the lower third of the right thigh, TENS (50Hz, 10mA) was applied for five minutes, and CRYO and the combined therapy (CT) for ten minutes. The FNA was recorded continuously by means of an action potential amplifier and the recordings from the first, fifth and tenth minutes were subsequently evaluated using arbitrary units (aU). One-way analysis of variance (ANOVA) was used, with Dunnett's test as post-hoc analysis. The values were expressed as the mean  $\pm$ SEM and differences were established at  $p < 0.05$ . **Results:** The femoral nerve activity increased ( $p < 0.01$ ) after TENS ( $0.358 \pm 0.09$ aU) and CT ( $0.230 \pm 0.07$ aU) and was unchanged after CRYO ( $0.063 \pm 0.003$ aU), in relation to the baseline ( $0.009 \pm 0.0003$ aU). In the fifth minute, we observed significant ( $p < 0.05$ ) attenuation of FNA in the CT ( $0.144 \pm 0.027$ aU) in relation to TENS alone ( $0.324 \pm 0.089$ aU). **Conclusions:** The association between CRYO and TENS noninvasive analgesia significantly attenuates the effects produced by TENS alone on the FNA of anesthetized rats.

**Key words:** TENS; nerve activity; cryotherapy; Physical Therapy; analgesia.

## Resumo

**Contextualização:** Relatos clínicos sugerem que a associação terapêutica entre crioterapia (CRIO) e estimulação elétrica transcutânea (TENS) favorece analgesia local. **Objetivo:** Avaliar a atividade elétrica do nervo femoral (ANF), em repouso e durante a aplicação isolada, e associada de TENS e CRIO em ratos. **Métodos:** Foram utilizados nove ratos (Wistar) adultos com peso de  $\pm 300$ g. Após anestesia (Uretana, 1mg/g i.p.), o nervo femoral direito foi isolado para registro da ANF basal e durante as modalidades analgésicas. Depois da fixação dos eletrodos no terço inferior da coxa direita, foram aplicadas TENS (50Hz, 10mA) por cinco minutos, CRIO isolada e terapia associada (TA) por dez minutos. Os registros contínuos da ANF foram realizados por meio de um amplificador de potenciais de ação, avaliados posteriormente no primeiro, quinto e décimo minuto em unidades arbitrárias (Ua). Utilizaram-se a análise de variância (ANOVA) uma via e o teste de Dunnett como *post-hoc*. Valores expressos como média  $\pm$ EPM e as diferenças fixadas em  $p < 0,05$ . **Resultados:** A atividade do nervo femoral aumentou ( $p < 0,01$ ) na TENS ( $0,358 \pm 0,09$ Ua) e na TA ( $0,230 \pm 0,07$ Ua) e ficou inalterada após CRIO ( $0,063 \pm 0,003$ Ua), em relação ao basal inicial ( $0,009 \pm 0,0003$ Ua). No quinto minuto, observou-se uma significativa ( $p < 0,05$ ) atenuação da ANF na modalidade TA ( $0,144 \pm 0,027$ Ua) versus TENS isolada ( $0,324 \pm 0,089$ Ua). **Conclusões:** A associação entre as modalidades analgésicas não-invasivas CRIO e TENS atenua significativamente os efeitos produzidos pela TENS isoladamente sobre a ANF de ratos anestesiados.

**Palavras-chave:** TENS; atividade nervosa; crioterapia; Fisioterapia; analgesia.

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## Introduction

Transcutaneous Electrical Nerve Stimulation (TENS) is an analgesic technique used in a variety of frequencies, intensities and pulse duration, classified as high frequency (>50Hz), low frequency (<10Hz) and burst (alternate high and low frequencies)<sup>1-3</sup>. Conventional TENS is a continuous high-frequency (50 and 150Hz), low-intensity stimulation of the fast conduction nervous fibers. The intensity of TENS should not cause muscle contractions, but only a not-unpleasant feeling of paresthesia, adjusted according to individual sensibility<sup>4</sup>. Studies show that intensities between ten and 30 milliamperes (mA) are more comfortable and do not cause significant fasciculation in the pulse time which varies from 40 to 75µs. In this type of stimulation, analgesia occurs immediately or ten minutes after the application. This effect can last from 20 or 30 minutes up to two hours, which explains why this method is preferably used to treat acute pain<sup>1-5</sup>.

TENS promotes analgesia predominantly through the mechanism of gate control theory of pain, proposed by Melzack and Wall<sup>5</sup>. According to this theory, analgesia is provoked by the selective activation of the large-diameter tactile fibers (A-beta fibers), without activation of the small-diameter nociceptive fibers (A-delta and C fibers). The activity generated in A-beta fibers inhibits the current activity of the nociceptive neurons located in the dorsal horn of the spinal cord<sup>5</sup>. Additionally, the analgesic mechanism of TENS also seems to be related to the activation of endogenous opioid receptors in the spinal cord<sup>6</sup>. Recent studies demonstrate that low-frequency TENS specifically activates µ-opioid receptors, serotonin receptors and spinal muscarinic receptors. Conversely, the analgesia produced by high-frequency TENS activates delta-opioid and muscarinic receptors in the dorsal horn of the spinal cord and the supraspinal delta-opioid receptors<sup>1,6-9</sup>.

Studies show that therapeutic cryotherapy (CRYO) application gradually reduces impulse transmission in the sensitive nerves because of the decreased nerve conduction velocity<sup>10,11</sup>. However, after a prolonged cold compress application, the duration of the action potentials of sensorial nerves can increase due to longer refractory periods. Clinically, CRYO is commonly applied in the management of acute injuries, muscle spasms and inflammatory processes<sup>11-13</sup>.

Studies prove that CRYO and TENS can reduce pain in patients with several conditions such as in the post-operative period<sup>14</sup>. Clinical reports that promote analgesia currently recommend the combination of the two therapeutic modalities (CT) for a better response in pain control. However, the electrophysiological mechanism that involves the nervous conduction and, therefore, the analgesic effects

of the combination of both techniques have not been sufficiently explained in the literature<sup>15-17</sup>. CT creates a paradox among the physiological mechanisms of the two therapeutic modalities: while one reduces the nerve conduction velocity, the other stimulates the nervous fibers. The aim of this study was to evaluate the effects of TENS and CRYO, isolated or combined, on the frequency of the action potentials of the femoral nerve.

## Methods

The investigation was conducted in accordance with the established norms of Guide care and use of laboratory<sup>18</sup> and approved by the Animal Testing Ethics Committee of Universidade Federal do Espírito Santo (Ufes), protocol number 026/2007.

### Animals and experimental design

Nine Wistar rats with body mass between 300 and 350 grams were used in a single group. The animals were maintained in the Research Vivarium of the Postgraduate Program in Physiological Sciences of Ufes. All the procedures were conducted according to the biomedical research guide for use of laboratory animals, as determined by the Federation of Experimental Biology Societies. Rats were maintained in individual cages, on a 12-hour light/dark cycle at a controlled temperature of 22°C, under artificial lighting and with *ad libitum* food and water. The evaluated parameter in this study was the femoral nerve electrical activity (FNA) of anesthetized rats, without painful stimuli, before and during the application of two modalities (isolated and combined) which originate three types of intervention: TENS, CRYO and CT.

The experimental design and the time of treatment applications were defined after observation of the behavior of action potential frequency in pilot studies, as there are no studies which establish the time for these procedures. Therefore, the experiment consisted of the continuous recording of the animals' FNA measures, using integrated FNA arbitrary units (aU), at different time intervals: before, during and after isolated and combined application techniques. A period of six minutes between interventions was established so that the preparation reached stability, as demonstrated in Figure 1. The first, fifth and tenth minute were used as the study period of the three interventions. The FNA measure unit used was the integrated unit, based on the absolute activity of the isolated nerve, filtered and discriminated from the raw electrical activity.

## Surgical procedures and femoral nerve electrical activity records

Rats were initially anesthetized with a single intraperitoneal (i.p.) dose of Urethane ( $1\text{mg}\cdot\text{g}^{-1}$  of body mass). This anesthetic was chosen because it is widely used in experiments which involve electrical activity, and therefore recommended for small changes in nerve activity<sup>19,20</sup>. The animals were placed in the supine position, and the femoral nerve of the right limb was exposed through a 1.5 to 2cm longitudinal rectilinear skin incision on the inner thigh (pelvic area). The space between the hip abductor and the femoral muscles was dissected, and the femoral vascular nerve plexus was identified. A microscope was used (M900, DF Vasconcelos, São Paulo, Brazil) for the femoral nerve selection and subsequent accommodation in silver electrodes to record nervous activity. Mineral oil and petroleum jelly were placed on the incision to avoid nerve lesion, to lubricate and to maintain the integrity of the femoral nerve. After this procedure, the extracellular action potentials at baseline were recorded in an amplifier (NL 104, Neurolog, Digitimer, Welwyn Garden, UK). The signs were filtered (NL 126, Neurolog, Digitimer, Welwyn Garden, UK) and connected to an audio amplifier (NL 120, Neurolog, Digitimer, Welwyn Garden, UK) and linked to an oscilloscope (Tektronix 2205, General Electric, NJ, USA). After that, the signs were processed in a Spike trigger action potential discriminator (NL 200, Neurolog, Digitimer, Welwyn Garden, UK) and in a pulse integrator (NL 601, Neurolog, Digitimer, Welwyn Garden, UK). Those signs were simultaneously converted by the software Acknowledge for Windows (Biopac System, Santa Barbara, CA, USA) for subsequent analysis.

## Isolated transcutaneous electrical stimulation

TENS (EMPI Eclipse Inc., Minneapolis, MN, USA) was applied for five minutes at 50Hz and final sensorial intensity of  $10\text{mA}$ . The electrodes (0.5cm diameter) were attached with adhesive tape to the medial and the lateral area of the right knee joint, before the beginning of the experiment in order to avoid possible changes in the FNA recording. The sensorial intensity was determined by increasing the parameters up to levels in which muscle contractions were not evident. This therapeutic modality characterizes an application of conventional TENS and those parameters remained constant<sup>1,6-9</sup>. Immediately after the TENS application (at the end of the fifth minute), the equipment was switched off, and six minutes were allowed so that the nerve reached stability at the action potential frequency (Figure 1). Studies conducted in the same

laboratory as the present study demonstrate that TENS stimulates the nerve quickly, and its response is the same after five or ten minutes. Therefore, the shortest stimulation period (five minutes) was chosen to minimize possible lesions during nerve exposure.

## Isolated cryotherapy and combined therapies

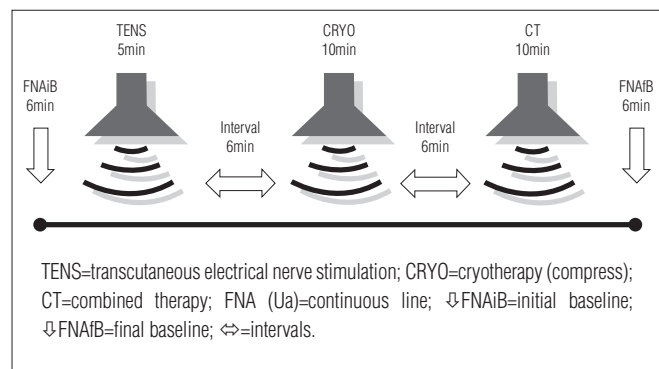
After the frequency was stabilized, CRYO application began with an ice compress over the right knee joint of the back limb for ten minutes. During this period, the femoral nerve action potentials were recorded. At the end of the procedure, the ice was removed and six minutes were allowed once again. After this, CT was applied for ten minutes (Figure 1). The ten-minute period for CRYO application was also based on pilot studies which showed this to be the longest time needed to affect nerve activity. At the end of the study, the animals were euthanized with a lethal dose of anesthetic.

## Statistical analyses

The results were analyzed using statistics software (GraphPad Prism4). One-way analysis of variance (ANOVA), followed by the Dunnett test, was used to compare the variables. Values (aU) were expressed as mean  $\pm$  SEM. The  $\alpha$  level considered for analyses was set at 0.05.

## Results

As demonstrated in Figure 2A, in the first minute after the start of the analgesic modalities, FNA increased significantly ( $p<0.01$ ) in TENS ( $0.358\pm 0.094\text{aU}$ ) and CT ( $0.230\pm 0.074\text{aU}$ ), and it did not change in CRYO ( $0.063\pm 0.037\text{aU}$ ). In the fifth minute, the analgesic modalities TENS ( $0.324\pm 0.089\text{aU}$ ) and CRYO ( $0.035\pm 0.015\text{aU}$ ) maintained the FNA means observed



**Figure 1.** Recording protocol for femoral nerve activity (FNA) in anesthetized rats during application of the noninvasive analgesic modalities.

in the first minute, however CT ( $0.144 \pm 0.027$ aU) attenuated the FNA levels compared to the isolated applications. In the tenth minute, there were no significant differences among the three analgesic modalities, emphasizing that TENS was interrupted in the fifth minute. Figure 2B demonstrates that the mean of FNA at baseline in the anesthetized rats was  $0.009 \pm 0.0003$ aU. This value was similar to those obtained in the intervals of the application of TENS ( $0.004 \pm 0.002$ aU), CRYO ( $0.001 \pm 0.0006$ aU) and CT ( $0.007 \pm 0.001$ aU).

## Discussion

The present study demonstrated the effects of isolated and combined TENS and CRYO on the frequency of femoral nerve

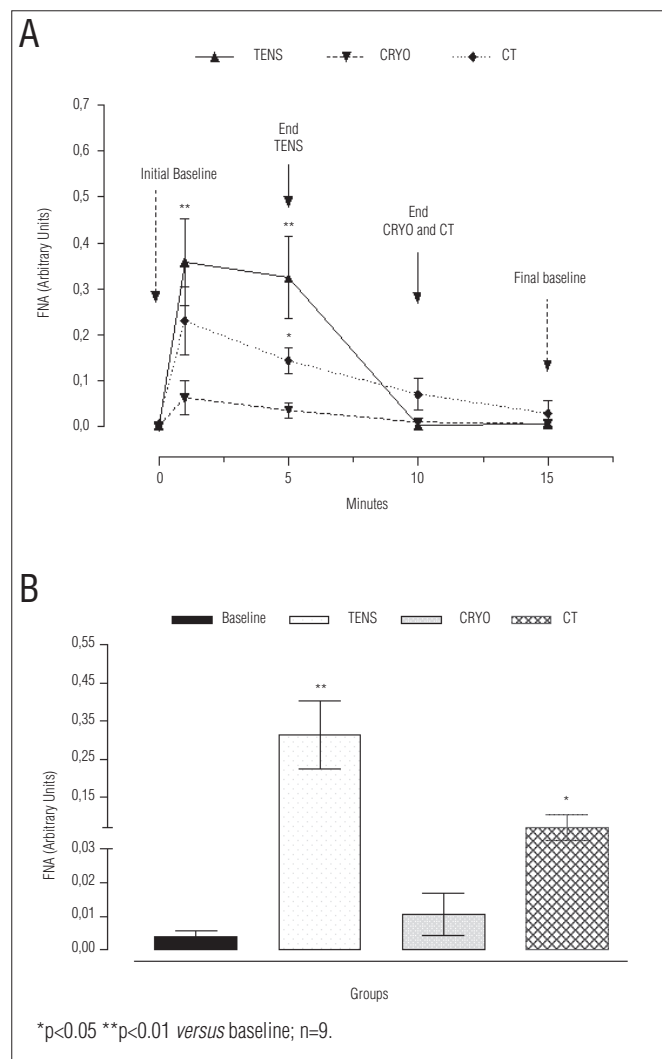
action potentials. The data we obtained showed that isolated TENS in anesthetized rats increases FNA, which was significantly attenuated when CT was used. However, the results also showed that isolated CRYO did not change the FNA of the experimental animals.

These data demonstrated that TENS induces this analgesic effect by promoting peripheral nerve stimulation, specifically in the tactile proprioceptive fibers<sup>1-6</sup>. Conversely, CRYO attenuated FNA possibly because it increases refractory periods and reduces nerve conduction velocity<sup>11-15</sup>. The literature has demonstrated that the use of isolated TENS or CRYO produces significant analgesic effects in acute and chronic inflammatory processes<sup>1,6,10,11,16</sup>. These studies report that CRYO produces analgesia by two main local mechanisms, the neural and the vascular mechanisms. In the neural mechanism, the topical ice application reduces the local temperature which lowers the activation thresholds of tissue nociceptors and, consequently, the transmission signs of pain. With regard to the vascular effects of CRYO, the analgesia is associated with a decrease in blood flow, caused by cold-induced vasoconstriction as well as reduced neural metabolism<sup>11-15</sup>. The means by which these effects occur have yet to be fully explained<sup>11-15</sup>.

Traditionally, TENS promotes analgesia through the selective activation of A-beta tactile fibers, which inhibit the current activity of nociceptive neurons in the dorsal horn of the spinal cord. Moreover, several studies on the presence of pain demonstrate that the analgesic action mechanism of TENS is also related to the activation of opioid receptors in the spinal supraspinal cord<sup>11-5,21-23</sup> and not only through the gate control theory.

In fact, CT has been routinely observed in clinical practice with the purpose of increasing the analgesic effects of these therapies and possibly producing hyperalgesia. In spite of the common (and conventional) simultaneous use of these techniques in physical therapy practice, the literature has little evidence of the benefits of CT.

This study demonstrated that the combined therapy promotes attenuation in FNA which was previously elevated by isolated TENS. This is explained by the reduction in nerve conduction velocity promoted by the ice<sup>10</sup>; cold decelerates the axoplasmic transport, i.e. substance flow along the axon; ATP and creatine phosphate concentrations do not change, therefore, it appears that the blockade is caused by a metabolic reduction in the ATP use by nerves due to reduced enzymatic activity<sup>10-15</sup>. Thus, it is possible to speculate that the use of CT not only opposes the stimulating effect and reduces the therapeutic actions of isolated TENS, but also reduces tactile sensibility<sup>10,11</sup> making it more difficult to identify the ideal intensity for TENS application.



**Figure 2:** A. Temporal evaluation of the effects of the isolated (TENS or CRYO) and combined (CT) analgesic modalities on femoral nerve activity (FNA) in anesthetized rats; B. Definition of baseline FNA before and after application of the noninvasive analgesic modalities. Values expressed as mean  $\pm$  SEM; one-way ANOVA and Dunnett test for multiple comparisons.

However, the sequential use of TENS and CRYO is justified in the presence of pain and other factors, such as osteoarthritis with edema, and because of the arthrogenic muscle disinhibiting effect promoted by CRYO. This occurs because the edemas that accompany the pain of these joint injuries sensitize the capsular mechanoreceptors. These receptors inhibit the spinal alpha motoneurons and, consequently, the signs transmitted to the muscle<sup>10,11</sup>. Although the present study was not conducted during a painful or inflammatory process, the electroneurographic data obtained indicate that the sequential use of TENS and CRYO (in that order) can improve the analgesic pattern<sup>6-8</sup> and allow better joint manipulation, without lowering the pain threshold, respectively. In clinical practice, this fact is incorrectly interpreted as hyperalgesia<sup>10,11</sup>.

New studies involving painful processes are necessary for a better understanding of the action mechanisms involved in neural activity during the use of isolated and combined analgesic techniques. Finally, caution is recommended when applying CRYO and TENS simultaneously in physical therapy clinical practice as this combination produced a new pattern of FNA response in laboratory animals, i.e. FNA was increased by TENS and attenuated by the CT in anesthetized rats.

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