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Increasing intensity of TENS prevents analgesic tolerance in rats

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Abstract

Transcutaneous electrical nerve stimulation (TENS) reduces hyperalgesia and pain. Both low frequency (LF) and high frequency (HF) TENS, delivered at the same intensity (90% motor threshold (MT)) daily, result in analgesic tolerance with repeated use by the 5th day of treatment. The current study tested if 1) increasing intensity by 10% per day prevents the development of tolerance to repeated TENS, and 2) if lower intensity TENS (50 % MT) produces an equivalent reduction in hyperalgesia when compared to 90% MT TENS. Sprague-Dawley rats with unilateral knee joint inflammation (3% carrageenan) were separated according to the intensity of TENS used: Sham, 50% LF, 50% HF, 90% LF, 90% HF, and increased intensity by 10% per day (LF and HF). The reduced mechanical withdrawal threshold following the induction of inflammation was reversed by application of TENS applied at 90% MT and increasing intensity for the first 4 days. On the 5th day, the groups that received 90% MT intensity showed tolerance. Nevertheless, the group that received an increased intensity on each day still showed a reversal of the mechanical withdrawal threshold with TENS. These results show that the development of tolerance can be delayed by increasing intensity of TENS.

Keywords

TENS; intensity; hyperalgesia; pain; opioid; frequency

Introduction

Transcutaneous electrical nerve stimulation (TENS) is used for pain control and is the application of electrical current through electrodes attached to the skin. TENS is a neurostimulation modality that was initially proposed based on the “gate-control” theory of pain.³² However, recent studies show that TENS produces its analgesic effects through release of endogenous opioids in the central nervous system.^{21, 44} Clinically, TENS can be delivered at different frequencies: low frequency (2-10Hz) or high-frequency (50-100Hz). High- and low-frequency TENS analgesia is mediated by different opioid receptors, with

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low-frequency TENS producing analgesia through μ -opioid receptors and high-frequency TENS producing analgesia through δ -opioid receptors.^{21, 44} It is well-known that repeated opioid administration produces analgesic tolerance.^{7, 9, 25, 49} Similarly, repeated TENS administration, high or low frequency, also produces analgesic tolerance.³ This analgesic tolerance occurs at spinal μ - and δ -opioid receptors for low and high frequency TENS, respectively.³

Prevention or delaying tolerance to repeated TENS is clinically useful. Prior work from our laboratory shows pharmacological blockade of spinal receptors involved in opioid tolerance also block tolerance to TENS. Specifically, intrathecal blockade of N-methyl-D-aspartate (NMDA) receptors¹⁹ or of cholecystikinin (CCK) receptors¹³ prevents the development of opioid tolerance. However, non-pharmacological strategies may be more clinically useful for prevention of opioid tolerance. For example, we previously show that alternating frequencies between low and high during treatment delays the onset of tolerance by one week.¹⁰

Clinically, intensity of stimulation has recently been shown to play a critical role in TENS effectiveness with lower intensities of stimulation producing minimal analgesia and higher intensities producing greater analgesia.^{1, 5, 6, 33, 35, 39, 51} Our prior animal studies show TENS given at 90% motor threshold or just above motor threshold produces a strong analgesic effect in animals with joint inflammation.^{3, 43} However, it is not clear if lower intensities would also produce this effect. Further subjects will continuously increase intensity throughout the duration of the stimulation. We propose that continuously increasing intensity of stimulation daily will prevent the development of analgesic tolerance and that lower intensity TENS will be ineffective. The current study tested if: 1) increasing intensity by 10% per day prevents the development of tolerance to repeated TENS, and 2) if lower intensity TENS produces an equivalent reduction in hyperalgesia when compared to 90% mechanical threshold TENS.

Material and methods

Animals

Male Sprague-Dawley rats (N=35), weighing 225–300 g, kept at 12 h dark—light cycle with free access to standard rat chow and water, were used for the experiments. All experiments were approved by University of Iowa Animal Care and Use Committee and were carried out according to the guidelines of the National Institutes of Health.

Behavioral Testing

Before the behavioral tests were applied, animals were acclimated to the testing room and procedures 2x per day for two days. Animals were acclimated to the testing room for thirty minutes after transport to the laboratory from the animal care facilities. They were then placed in transparent Lucite cubicles ($24.6 \times 7.5 \times 7.5$ cm³) on an elevated mesh platform for twenty minutes to acclimate (paw withdrawal threshold test). Animals were also acclimated in a gardener's glove for five minutes (muscle withdrawal threshold test).

For the paw withdrawal thresholds mechanical stimuli with von Frey filaments were applied to the plantar area of the hind paw, as previously described.^{4, 13, 16, 44} Briefly, different bending forces were progressively applied perpendicularly to the plantar aspect of the hind paw. Each filament had one trial that consisted of two consecutive applications of the filament. The lowest bending force at which the rat withdrew its paw from one of the two applications was recorded as the paw withdrawal threshold for mechanical hyperalgesia. This testing method has shown significant statistical test-retest reliability.⁴⁴

For muscle withdrawal thresholds, the animal was restrained in a gardener's glove, the experimenter extended one hind limb, and the knee joint was compressed by using a pair of calibrated forceps.^{30, 42, 52} The tip of the modified forceps was used for compression. The contact area of the forceps is approximately 30 mm². Compression was stopped when the animal withdrew the limb forcefully or when it vocalized. The maximum compression force applied at withdrawal was recorded as the baseline compression threshold in mN for the knee joint of the corresponding limb. Three trials spaced five minutes apart were averaged to obtain one reading at each time point.

Induction of Inflammation

After baseline behavioral measurements, rats were deeply anesthetized with 3-5% isoflurane for approximately five minutes and a solution of 3% carrageenan (0.1 ml, pH 7.4) in sterile saline was injected into the left knee joint to induce inflammation. Following intra-articular injection of carrageenan, the rats were returned to their cages and allowed to recover for 24 hours. Within 24 hours, the animals exhibited signs of inflammation such as; edematous, warm knee joints, and also behavioral signs such as; guarding and decreased weight bearing on the inflamed limb. We previously show that this model produces an acute inflammatory response that converts to a chronic inflammation by one week and lasts through 9 weeks.³⁶ The mechanical hypersensitivity of the paw becomes maximal within 24h and lasts through 8 weeks.³⁶ We previously used this model to examine tolerance to TENS over 2 weeks and showed stable hypersensitivity measures in the sham TENS group.¹⁰

Application of TENS

Active or sham TENS was administered to the inflamed knee joint under light isoflurane anesthesia (1-2% isoflurane) for twenty minutes, for ten days. Before the application of TENS, every day the animals were shaved and their skin was cleaned with 70% alcohol. Pre-gelled electrodes (3.02 cm in diameter modified down to approximately 1.2 cm in diameter) were placed on the medial and lateral aspects of the inflamed knee joint. Following twenty minutes of administration of TENS, rats were removed from anesthesia, the use of TENS discontinued and the pre-gelled electrodes removed.

There were three TENS treatment groups. 1) The sham TENS group was anesthetized and electrodes were placed on their shaved knee joint, but they did not receive TENS treatment (control group). 2) Low frequency TENS group (frequency 4 Hz), subdivided in three different intensities 50% MT, 90% MT and daily increasing intensity. 3) high frequency TENS group (100 Hz), subdivided in three different intensity 50% MT, 90% MT and daily increasing intensity. For the increasing intensity group, initial intensity started at 90% MT on Day 1 and was increased by 10% each day as follows: Day 1 - '90% of motor threshold'; Day 2 '90% of motor threshold + 10%'; Day 3 '(90% of motor threshold + 10%) + 10%'; Day 4 '((90% of motor threshold + 10%) + 10%) + 10%', and so on. Other treatment parameters were kept constant pulse-width (100 μ s) and modulation (normal).^{3, 43} The intensity was determined by increasing the intensity until a visible motor contraction was elicited, and if the animal was of the subgroup with 50% MT, the intensity was decreased by half.

Experimental Design

Baseline paw and joint withdrawal thresholds bilaterally before and 24h after induction of knee joint inflammation. Rats were then lightly anesthetized with 1% to 2% isoflurane for placement of the TENS electrodes and application of TENS for twenty minutes. Withdrawal thresholds were reassessed immediately after TENS and recovery from the anesthesia (10–15min after removal of TENS). The same procedure (assessment of withdrawal thresholds, TENS application, and reassessment of withdrawal thresholds) was done on each day for ten

days. Importantly, 3 rats always were anesthetized with the same vaporizer; at least 1 rat receiving the sham TENS treatment and 1 rat receiving the active TENS treatment were anesthetized at the same time. This procedure ensured that there always were animals in the sham TENS treatment group that received the same dose of anesthesia. Further the tester was blinded to the experimental group. A separate study participant applied TENS.

Data analysis

Differences between groups (different frequencies) and subgroups (different intensities) of treatment were tested with a two-way ANOVA for dependent samples across time (before inflammation, before TENS, and after TENS, on each day of treatment). Parametric paired T-test was used to analyze changes in mechanical withdrawal threshold of the hind paw and the calibrated forceps (knee joint withdrawal threshold) at each time point (before and after TENS stimulation on the same day). Post-hoc testing between groups was performed with a Tukey's test. P value <0.05 was considered significant.

Results

Twenty-four hours following injection of carrageenan into the knee joint, the paw and muscle withdrawal threshold to mechanical stimuli were reduced ipsilaterally in all groups and subgroups. There were no significant differences between groups after induction of inflammation (Figure 2A, 2B, 3A and 3B).

Daily intensity of TENS for each group is summarized in Figure 1. The intensity of TENS remained constant for all days applied for the 50% MT and 90% MT intensities. Further, there is a daily increase in intensity in the group where intensity was increased by 10% each day. The groups were significantly different from each other with increasing intensity > 90% MT > 50% MT > sham ($p<0.001$).

The sham TENS group and high frequency TENS group with 50% MT intensity showed no change in the reduced paw withdrawal thresholds in all days analyzed. However, the 90% MT ($p=0.003$ and $p=0.002$) and increasing intensity ($p<0.001$ for both) groups were significantly greater than the sham and 50% MT groups (Figure 4A). Similarly, for joint withdrawal thresholds the 90% MT and increasing intensity TENS were significantly higher than sham ($p=0.0001$ and $p=0.001$) and 50% MT intensity TENS ($p=0.0001$ and $p=0.0001$) (Figure 4B).

The decreased paw withdrawal threshold following induction of inflammation was reversed by application of TENS on the first four days in both the high and low frequency group with 90% MT intensity, as well as the group with increasing intensity TENS. Nevertheless, after four days of TENS application, in the groups that received 90% MT intensity, TENS was ineffective and thus there was a development of analgesic tolerance. (Figure 2A, 2B, 3A and 3B) The low and high frequency TENS groups that received TENS with a 10% increase in intensity daily showed a significant increase in mechanical withdrawal threshold of the paw and muscle after TENS session, until ninth day ($p<0.001$). As shown in Figures 2A, 2B, 3A and 3B, on tenth day there was no difference between before and after TENS application ($p<0.001$).

Discussion

The current study shows that we can delay the onset of tolerance to repeated application of TENS by continuously increasing the intensity of stimulation. We further show that low intensities of stimulation, 50% MT, have no analgesic effect. The current study shows the same pattern of analgesia and development of tolerance to repeated application of TENS

delivered at the same intensity each day as we have shown previously.^{3, 10, 12, 19} Our prior studies were able to show prevention of tolerance with both pharmacological and non-pharmacological approaches. Specifically, we show that blockade of NMDA glutamate or cholecystinin receptors during TENS treatment prevents the onset of tolerance after repeated TENS.^{13, 19} We further showed that alternating frequency of stimulation in the same session, or between sessions, also delays the onset of tolerance to repeated application of TENS.¹⁰ It may be that combining alternating or mixed frequencies of TENS with increasing intensity will delay the onset of tolerance even farther. As patients routinely use TENS on a daily basis, and clinical trials generally give just one frequency of stimulation, it will become increasingly important to find effective strategies to alleviate tolerance. Having multiple strategies for prevention of tolerance available to clinicians is critically important for maintaining long-term efficacy in patient populations.

Development of Tolerance

Clinically, understanding if TENS produces tolerance and if there is cross tolerance between TENS and pharmacological treatments is important for effective delivery. For example, in human subjects we previously show that repeated daily application of TENS delivered at the same intensity each day produces analgesic tolerance by the fifth day, thus, confirming and validating animal data.²⁹ Increasing intensity each day could be one way to overcome the development of tolerance to repeated TENS, as we show in the current pre-clinical study. Similar to animal studies, humans subjects with chronic pain that were opioid tolerant showed a reduced effectiveness of low frequency TENS, but not high frequency TENS.²⁶ This data again confirms and validates the prior animal studies that show low frequency TENS, but not high frequency TENS, is ineffective in animals with morphine tolerance.⁴⁵ We suggest understanding the mechanisms of opioid tolerance and how to alleviate opioid tolerance will not only assist in the delivery of opioid pharmaceutical agents, but will also prove useful for the effective delivery of TENS.

The mechanisms of analgesic tolerance are not completely understood, and a number of neurotransmitters and receptors have been described.^{14, 23, 28} Prior studies in our laboratory, show that TENS produces effects by activation of PAG, RVM and spinal cord using opioid receptors in these central sites^{11, 21, 44} and others show peripheral activation of opioid receptors in low frequency TENS analgesia.⁴¹ We also previously show that tolerance to low frequency and high frequency TENS occurs at central opioid receptors in the spinal cord with low-frequency TENS showing cross tolerance to μ -opioid receptor agonists and high frequency TENS showing tolerance to δ -opioid receptor agonists.³ We further show a lack of cross-tolerance between frequencies, between low frequency TENS and δ -opioid agonists, and between high frequency TENS and m -opioid agonists.³ Similarly, there is a lack of cross-tolerance between spinally administered μ - (morphine) and δ -opioid (D-Ala2-D-Leu5 enkephalin) agonists suggesting that the tolerance produced at μ - and δ -opioid receptors occur independent of each other.^{22, 46} Thus, tolerance to low-frequency TENS occurs at spinal μ -opioid receptors and that to high frequency TENS occurs at spinal δ -opioid receptors; preventing tolerance prevents the cross-tolerance in the spinal cord to opioid agonists.

Intensity of stimulation is critical

The current study also shows that a low intensity, 50% MT, has no analgesic effect when compared to a sham TENS while 90% MT intensity produces a full reversal of hyperalgesia. This suggests there is a dose (intensity)-dependent effect for application of TENS, that TENS must be given at an adequate intensity to reduce pain intensity.^{1, 33, 38, 39} Recently, we demonstrated a dose-dependent effect of TENS in healthy human subjects similar to the

current study. Specifically, sub-sensory and sensory threshold TENS have no significant analgesic effect while a strong but comfortable intensity has a significant analgesic effect.³³ We also showed previously that increasing intensity every five minutes within a single session of TENS has a greater analgesic effect than the initial strong but comfortable sensation.²⁹ Similarly, Bjordal and colleagues performed a systematic review of the literature and showed that when TENS is given at adequate intensities to people with postoperative pain or osteoarthritic it is more effective, and when given at inadequate intensities it is ineffective.^{1, 2}

TENS is a sensory modality that acts directly on the nervous system by activating A β primary afferent fibers that subsequently leads to a reduction in central nociceptive cell activity.^{15, 31} Both low and high frequency TENS at sensory intensity clearly activate A β primary afferent fibers. We expect that increasing the intensity increases the number of primary afferent fibers activated. This larger number of afferent fibers activated on each day could result in a greater release of opioids. This is supported by work by Janko and colleagues that show, using microneurography of peripheral nerve activity during TENS, that progressively increasing the intensity increased the amplitude of nerve activity.²⁰ Higher intensities might also activate A δ primary afferent fibers since A δ primary afferent fibers are activated at intensities of at least twice motor threshold. Our increasing intensity group reached this level between days 8 and 10 and thus we could have activated additional analgesic systems that are primarily activated by noxious stimulation.³⁷ In human subjects, intensities up to 20 mA activate large A β afferent fibers and some small nociceptive A δ afferent fibers as determined with microneurography.²⁰ Further increasing intensity to produce a motor contraction activates A δ nociceptors.^{27, 34} Activation of nociceptive afferent fibers could further increase activation of the PAG-RVM pathway as well as activate diffuse noxious inhibitory control (DNIC) pathways.^{17, 18, 50} DNIC is an alternate opioid-mediated analgesia pathway activated in response to noxious stimulation.⁸ Thus, the increasing intensity group likely increased the number of large diameter fibers activated and also activated A δ afferent fibers in sufficient quantities to bring in other analgesic systems.

It is possible that different activation patterns occur after tissue inflammation, and that this changes across the time depending on the severity of inflammation and swelling around the joint, and if the inflammation is acute or chronic. In support, higher electroacupuncture intensities (e.g. 6 V or 20–30 mA) are required to induce effective analgesia in uninjured animals⁴⁰ when compared to electroacupuncture intensities in inflamed animals (i.e. 3 mA).²⁴ Current may be transported to deeper tissues easier with more fluid around the joint, i.e. in the early phases of inflammation, and less well in animals with lower levels of inflammation in the chronic phases or in uninjured animals. Alternatively, enhanced excitability may occur as a result of increased excitability in the pain modulatory circuitry, either spinal or supraspinal.^{12, 47, 48}

In summary, the findings presented in this novel study show that increasing intensity of TENS daily by just 10% can delay the onset of analgesic tolerance to repeated treatment. We further show that lower intensities of stimulation are ineffective. Future studies should confirm strategies to prevent tolerance in human subjects and use adequate intensities to test effectiveness. Similarly, clinicians should be aware of the potential development of analgesic tolerance to repeated use of TENS, and simple non-pharmacological strategies aimed at manipulating TENS parameters that can be employed to prevent this analgesic tolerance could be employed.

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Perspective

Our results showed that increasing intensity in both frequencies of TENS was able to prevent analgesic tolerance. Results from this study suggest that increasing intensities could be a clinical method to prevent analgesic tolerance and contribute to the effective use of TENS in reducing inflammatory pain and future clinical trials.

Figure 1.

Graph representing the daily intensity of TENS from animals receiving low and high frequency at different intensities. Data are represented as means $\pm$ SEM.

Figure 2.

Graph representing mechanical withdrawal threshold of the paw (A) and knee joint (B) in animals that received high frequency TENS. Mechanical withdrawal thresholds are illustrated prior to induction of inflammation (baseline), before (b), and after application of TENS (a). Data are represented as means $\pm$ SEM. *Significantly different between before and after TENS stimulation ($p < 0.005$).

Figure 3.

Graph representing mechanical withdrawal threshold of the paw (A) and knee joint (B) in animals that received low frequency TENS. Mechanical withdrawal thresholds are illustrated prior to induction of inflammation (baseline), before (b), and after application of TENS (a). Data are represented as means $\pm$ SEM. *Significantly different between before and after TENS stimulation ($p < 0.005$).

Figure 4.

Graph representing the summary of effect of TENS in mechanical withdrawal threshold of the paw (A) and knee joint (B) from animals receiving Low and High Frequency of TENS treatment. #Significantly different from Sham and 50% of MT ($p < 0.005$). +Significantly different from 90% of MT ($p < 0.005$).