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Full Length Research Paper

Acupuncture (ST 36) Reduces Jaw Open Reflex in a Rat: A Pilot Study

Greg Goddard. DDS

University of California at San Francisco, Department of Oral and Maxillofacial Surgery San Francisco, California

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Acupuncture has been shown to reduce pain in rats. Morphine is the standard of analgesia and has been shown to reduce the JOR in rats by about 50%. Jaw Opening Reflex is a validated measurement of pain in rats. Other methods of pain response such as tail flick to heat have also been used. Stomach 36 is an important point in reducing pain in both animals and humans. St 36 has been often used in rats because it is easy to locate anatomically on small animals. The purpose of the present study was to test the hypothesis that acupuncture at Stomach 36 would decrease pain as measured by a decrease in the jaw opening reflex of a rat. An electrode is placed in the pulp of the lower incisor of a rat and in the digastric muscle, and after placing an acupuncture needle at St 36, an electrical stimulus was applied to the pulp and the JOR is measured. One rat was tested in a laboratory. After placing an acupuncture needle at St 36, an electrical stimulus was applied to the pulp and the JOR is measured. The change in JOR was measured over time. We had a significant drop in JOR with acupuncture at ST 36 with an N of 1. This pilot study shows promise for more studies using a larger sample size.

Keywords: acupuncture, jaw opening reflex, analgesia, rats, stomach 36.

INTRODUCTION

Acupuncture has been shown to reduce pain in rats. Morphine is the standard of analgesia and has been shown to reduce the JOR in rats by about 50%. Jaw Opening Reflex is a validated measurement of pain in rats. Other methods of pain response such as tail flick to heat have also been used.

Stomach 36 is an important point in reducing pain in both animals and humans. St 36 has been often used in rats because it is easy to locate anatomically on small animals. An electrode is placed in the pulp of the lower incisor and in the digastric muscle. An electrical stimulus is applied to the pulp and the JOR is measured (EMG). There was a significant drop in JOR with acupuncture at ST 36 with an N of 1.

Noxious stimulus-induced antinociception (NSIA) is a recently described form of heterosegmental

antinociception equivalent in magnitude to that of high dose morphine (Gear et al., 1999). NSIA, which would be referred to as pain-induced analgesia in humans, can be induced by paw immersion in hot water (50 °C) or subdermal capsaicin injection (Gear et al., 1999) and lasts up to 2.5 h. NSIA is blocked by antagonists for opioid, dopamine and other receptor subtypes microinjected into nucleus accumbens (Gear et al., 1999; Schmidt et al., 2001; Schmidt et al., 2001; Schmidt et al., 2002), suggesting that noxious peripheral stimuli can activate a novel pain modulation system in the ventral striatum. Paradoxically, other evidence indicates that this supraspinal pain modulation system is also activated by inhibitory treatments at the level of the spinal cord. Spinal intrathecal (i.t.) administration of local anesthetic (Gear and Levine, 2001) or the selective μ -opioid receptor

agonist [D-Ala^2 , N-Me-Phe^4 , Gly^5 -ol]-enkephalin (Gear and Levine, 2001) also induce accumbens-mediated heterosegmental antinociception.

Although it is possible that antinociception dependent on opioid receptors in nucleus accumbens could be activated by two separate pathways originating in the spinal cord—that is, one activated by excitatory spinal input and the other by inhibition of neural activity, it is also possible that one pathway is involved. Such would be the case, for example, if noxious stimulation induces NSIA through a mechanism involving the release of inhibitory neurotransmitters in the spinal cord.

The purpose of the present study was to test the hypothesis that acupuncture at Stomach 36 would decrease pain as measured by a decrease in the jaw opening reflex of a rat.

METHODS

Experiments were performed on a 280–350 g male Sprague-Dawley rat (Bantin and Kingman, Fremont, CA). Experimental protocols were approved by the University of California San Francisco Committee on Animal Research and conformed to IASP guidelines for the study of pain in animals (Zimmermann, 1983). The rat was used for only one experiment.

Nociceptive assay

Changes in nociception were measured as attenuation (i.e. antinociception) or enhancement (i.e. hyperalgesia) of the trigeminal jaw-opening reflex (JOR) electromyographic (EMG) signal (Gear et al., 1999; Gear and Levine, 2001; Mason et al., 1985). This assay was employed because it is segmentally remote from the hind paw where the acupuncture needle is applied, thus allowing separation of heterosegmental effects from any intrasegmental effects that might influence assays such as the paw-withdrawal reflex or the tail flick reflex. Previous studies show examples of the effect of spinal administration of the μ -opioid receptor agonist [D-Ala^2 , N-Me-Phe^4 , Gly^5 -ol]-enkephalin (DAMGO) (Gear and Levine, 2001), systemic morphine treatment (Gear et al., 1999) and intraplantar capsaicin (Schmidt et al., 2002) on the JOR EMG.

Anesthesia

The experiment was performed in a rat anesthetized with an intraperitoneal injection of 0.9-gm/kg urethane and 45 mg/kg α -chloralose (both from Sigma-Aldrich, St Louis, MO). This method provides a state of anesthesia with stable physiological parameters (Buelke-Sam et al.,

1978) and a stable JOR EMG signal over the time period required to complete the surgical preparation and experimental protocols (Gear and Levine, 2001), about 3 h.

Electrode implantation

To evoke the JOR, a bipolar stimulating electrode, consisting of two insulated copper wires (36 AWG), each with 0.2 mm of insulation removed from the tip, one tip extending 2 mm beyond the other, was inserted into the pulp of a mandibular incisor to a depth of 22 mm from the incisal edge of the tooth to the tip of the longest wire and cemented into place with dental composite resin (Citrix, Golden Gate Dental Supply, Inc, South San Francisco, CA). A bipolar recording electrode, consisting of two wires of the same material as the stimulating electrode with 4 mm of insulation removed, was inserted into the anterior belly of the digastric muscle ipsilateral to the implanted tooth to a depth sufficient to completely submerge the uninsulated end of the wire.

JOR electromyogram

In this pilot study to test the effect of acupuncture at St 36 on JOR using 1 rat, the rat was prepared, an electrode was placed in the incisor tooth and hooked up to an electric stimulus, the tooth pulp was stimulated 10 times over 30 seconds and the average JOR was recorded. Tooth pulp stimuli were 0.2 ms square wave pulses delivered at 0.33 Hz. The stimulation current was set at three times threshold⁷ which produced stimulation currents ranging from 1.5 to 2.5 mA. We have found that C-fibers can be activated by a 1 mA current with a pulse duration of 0.2 ms, therefore, our stimulation current was adequate to activate both C- and A-delta fibers, some of which may have been extra-pulpal. Each data point consisted of the average peak-to-peak amplitude of 12 consecutive JOR EMG signals delivered at a frequency of 0.33 Hz. Pre-intervention baseline amplitude was defined as the average of the last three data points, recorded at 5-min intervals, before an experimental intervention. As is customary for JOR studies (Gear et al., 1999; Schmidt et al., 2001; Gear and Levine, 2001; Banks et al., 1992; Zhang et al., 1999), data were normalized for differences in baseline by calculating the percentage change from baseline for each post-intervention data point.

At baseline the JOR was 4.76 and a Seirin 30millimeter .20 guage acupuncture needle was inserted to a depth of 5mm at St 36 and manually stimulated by twirling the needle for 5 seconds. The needle was left in place and JOR measurements were taken every 5 minutes.

Table 1. Percentage decrease in JOR over time

Time	Average EKG(mV)	% decrease
Baseline	4.70 / 4.76 / 4.75	0
1 minute	3.64	23.2
5 minutes	3.65	22.9
10 minutes	3.25	31.4
15 minutes	3.32	30.0
20 minutes	3.34	29.5
25 minutes	3.32	30.0
30 minutes	3.36	29.1

RESULTS

Analgesia measured by a 23.2% decrease in the JOR at 1 minute and 30.0% at 15 minutes, and the JOR decrease held up to the last test at 30 minutes (see Table 1). A 30% JOR decrease is equivalent to a moderate dose of morphine.

DISCUSSION

Stomach 36 acupoint was selected for its analgesic effect, and its ease of finding the point anatomically. The needle was inserted to a depth of 4 to 5mm, which was estimated to be the correct position. St 36 has been used in other acupuncture studies using rats. On 1 rat, acupuncture at St 36 had an effect about equal to a moderate dose of morphine.

This pilot study was only to test the feasibility of using JOR to measure the analgesic effect in rats. Because of the promising results as shown on this rat, further studies using a larger sample size and controls are needed to determine if this method is valid.

CONCLUSION

This pilot study shows promise for more studies using a larger sample size to determine the true effectiveness of acupuncture in reducing analgesia in rats.

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