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Cannabidiol and endogenous opioid peptide-mediated mechanisms modulate antinociception induced by transcutaneous electrostimulation of the peripheral nervous system

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ABSTRACT

Transcutaneous electrical nerve stimulation (TENS) is a non-pharmacological therapy for the treatment of pain. The present work investigated the effect of cannabidiol, naloxone and diazepam in combination with 10 Hz and 150 Hz TENS. Male Wistar rats were submitted to the tail-flick test (baseline), and each rodent received an acute administration (intraperitoneal) of naloxone (3.0 mg/kg), diazepam (1.5 mg/kg) or cannabidiol (0.75 mg/kg, 1.5 mg/kg, 3.0 mg/kg, 4.5 mg/kg, 6.0 mg/kg and 12.0 mg/kg); 10 min after the acute administration, 10 Hz or 150 Hz TENS or a sham procedure was performed for 30 min. Subsequently, tail-flick measures were recorded over a 90-min period, at 5-min intervals. 10 Hz TENS increased the nociceptive threshold during the 90-min period. This antinociceptive effect was reversed by naloxone pre-treatment, was not altered by diazepam pre-treatment and was abolished by cannabidiol pre-treatment (1.5 mg/kg). Moreover, 150 Hz TENS increased tail-flick latencies by 35 min post-treatment, which was partially inhibited by naloxone pre-treatment and totally inhibited by cannabidiol (1.5 mg/kg). These data suggest the involvement of the endogenous opioid system and the cannabinoid-mediated neuromodulation of the antinociception induced by transcutaneous electrostimulation at 10 Hz and 150 Hz TENS.

1. Introduction

Transcutaneous electrical nerve stimulation (TENS) is a non-pharmacological, non-aversive approach without side effects that is widely used in the treatment of pain [1,2]. This technique is used for analgesia induction during post-operative recovery after caesarean surgery, videolaparoscopy, herniorrhaphies and thymectomy and is used in association with opiate drugs in clinical oncology, in which higher opiate doses result in higher tolerance followed by lower analgesic effects [3–8].

Bjordal et al. [8] reported a 26.5% decrease in the analgesic use of 1350 patients submitted to cholecystectomy, haemorrhoidectomy, thoracotomy, total knee arthroplasty or hip prosthesis after the post-operative application of 85 Hz TENS to the surgical incision. Tonella et al. [9] reported the treatment of post-operative pain in patients submitted to abdominal surgery with 150 Hz TENS for 30 min. In addition, 100 Hz TENS elicited effective analgesia during painful interventions in

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the abdomen and venter of 30 patients submitted to caesarean after the effect of anaesthesia [3]. Finally, Pitangui et al. [10] showed the efficacy of 5 Hz and 100 Hz TENS for pain relief in 32 women submitted to episiotomy during normal parturition. Altogether, these findings suggest that both 10 and 150 Hz electrical therapy can have an analgesic effect.

There is evidence that the endogenous opioid system [11–14] and endocannabinoids exert a modulatory influence in the perception of pain [15,16]. The interaction between endogenous opioid peptides and endocannabinoids in nociceptive modulation has also been already demonstrated [17–20].

The neural mechanisms of antinociception induced by TENS and the role played by endogenous opioid peptide receptors and endocannabinoid mediators of transcutaneous electrical nerve stimulation at different frequencies are not entirely clear [20]. In addition, it remains unknown whether analgesia induced by TENS is due to different neural pathways activated by the same electrical stimulation or to distinct currents of electrical stimulation of low (≤10 Hz) and high (≥50 Hz) frequencies. Low-frequency TENS may modulate pain at the supra-spinal level by releasing endogenous opioid peptides and serotonin and at the spinal level by inhibiting the ascending sensory discriminative pathways. However, high-frequency TENS modulates pain only at the spinal level [21].

We cannot rule out that at least part of the TENS effect may be due to anxiety, which modulates the nociceptive thresholds [21–23]. As shown by Melo de Paula et al. [3], 100 Hz TENS decreased post-operative pain after caesarean sections in 14 of 15 women, and one patient had severe anxiety manifested by prolonged crying before and after surgery. Thus, the present work aims to expand the current knowledge about the involvement of endogenous opioid peptide-receptors, endocannabinoid neuromodulation and benzodiazepine/GABA-mediated mechanisms in the antinociceptive processes induced by transcutaneous electrical stimulation at 10 Hz or 150 Hz in Wistar rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (Rattus norvegicus, Rodentia, Muridae), weighing 220–250 g (N = 5, 10 or 12 animals per experimental group), from the animal facility of the Patos de Minas Universitarius Centre (UNIPAM) and Ribeirão Preto Medical School of the University of São Paulo (FMRP-USP) were studied. The rats were kept four to a cage in the experimental room for at least 48 h prior to the experiments, with free access to water and food on a 12/12 h light/dark cycle (lights on at 7:00 am) at 22–23 °C (40–70% humidity). All experiments were performed in accordance with the recommendation of the Commission of Ethics in Animal Experimentation of the UNIPAM (CEUA) (proc. 16/09; 01/11), which is in agreement with the ethical principles in animal research adopted by the Brazilian College of Animal Experimentation (COBEA).

2.2. Antinociceptive procedure

The nociceptive thresholds of each experimental group were compared using the tail-flick test. Each animal was placed in a restraining apparatus (Insight, Ribeirão Preto, São Paulo, Brazil) with acrylic walls, and the tail was placed on a heating sensor (tail-flick Analgesia Instrument; Insight, Brazil). The progressive heat elevation was automatically interrupted at the moment when the animal removed its tail from the apparatus. The current raised the temperature of the coil (Ni/Cr alloy; 26.04 cm in length × 0.02 cm in diameter) 9 °C/s [24], starting at room temperature (approximately 22 °C). Small current intensity adjustments were performed, if necessary, at the beginning of the experiment to obtain three consecutive tail-flick latencies (TFL) of control values (baseline) between 2.5 and 3.5 s at 5-min intervals. If the animal did not remove its tail from the heater within 6 s, the apparatus was turned off to prevent skin damage. Tail-flick latencies were also measured over a 90-min period immediately after electrotherapy procedures.

2.3. Drugs

The following drugs and vehicle were intraperitoneally (ip) administered: naloxone hydrochloride, 3.0 mg/kg (Cristália Laboratories, Ponte Nova, São Paulo, Brazil), whose vehicle was physiological saline solution at 7.5 ml/kg; diazepam at 1.5 mg/kg (Hoffmann-La Roche, Basileia, Switzerland), whose vehicle was 40% propyleneglycol in 60% physiological saline solution at 0.3 ml/kg; cannabidiol at 1.5 mg/kg (approximately 99.9% pure, THC-Pharm, Frankfurt, Germany and STI-Pharmaceuticals, Brentwood, United Kingdom) diluted in 10% dimethyl sulfoxide; and 1% polysorbate 80 in 89% physiological saline solution. Cannabidiol was administered at the following doses: 0.75, 1.5, 3.0, 4.5, 6.0 and 12.0 mg/kg.

2.4. Transcutaneous electrical nerve stimulation

To induce the antinociceptive phenomenon, the TENS unit Vif 993 DUAL (Quark Medical; Piracicaba, São Paulo, Brazil) was used to apply either low- (10 Hz) or high- (150 Hz) frequency stimulation to the skin during 30 min. A sham TENS (0 Hz) procedure was performed for 30 min as a control. To avoid the accommodation of peripheral receptors to the stimulus, 5 mA was added every five min during stimulation, starting with 15 mA. For all applications, the pulse width used was 40 ms. A pair of electrodes that adhered to the skin of the rat's tail was specially made for electrical tail stimulation, and the electrodes covered a 4.0 cm^2 area in the proximal and intermediate third of the tail. The thermal stimulus was applied (during the tail-flick test) between these two electrodes.

2.5. Experimental design

The animals were randomly distributed and maintained in thirty-one groups as follows: A — control pharmacological groups treated with (1) naloxone vehicle + 10 Hz TENS, (2) naloxone vehicle + 150 Hz TENS, (3) diazepam vehicle + 10 Hz TENS, (4) diazepam vehicle + 150 Hz TENS, (5) cannabidiol vehicle + 10 Hz TENS and (6) cannabidiol vehicle + 150 Hz TENS; B — Experimental groups treated with (7) naloxone + 10 Hz TENS, (8) naloxone + 150 Hz TENS, (9) diazepam + 10 Hz TENS, (10) diazepam + 150 Hz TENS, (11) cannabidiol + 10 Hz TENS and (12) cannabidiol + 150 Hz TENS; C — two independent sham TENS groups were performed for each TENS frequency comparison (10 or 150 Hz TENS): (13 and 14) naloxone vehicle + sham TENS, (15 and 16) diazepam vehicle + sham TENS, (17 and 18) cannabidiol vehicle + sham TENS, (19 and 20) naloxone + sham TENS, (21 and 22) diazepam + sham TENS and (23 and 24) cannabidiol + sham TENS; and D — additional groups were performed to generate a dose–response curve for cannabidiol: (25) cannabidiol vehicle + sham TENS, (26) cannabidiol at 0.75 mg/kg + sham TENS, (27) cannabidiol at 1.5 mg/kg + sham TENS, (28) cannabidiol at 3.0 mg/kg + sham TENS, (29) cannabidiol at 4.5 mg/kg + sham TENS, (30) cannabidiol at 6.0 mg/kg + sham TENS and (31) cannabidiol at 12.0 mg/kg + sham TENS.

After the habituation period, on day zero of the experiment, a trichotomy was performed on the tail of each rodent to reduce the impedance (caused by hair and capacitive components of skin tissue) to the passage of electric current. Twenty-four hours after the tail-flick test baseline with 5-min intervals, each rat was administered one of the pre-treatments described above. Ten minutes after drug or vehicle administration, sham, low- or high-frequency TENS was performed for 30 min, followed by tail-flick latency tests for 90 min.