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ORIGINAL RESEARCH

Evaluation of the noradrenergic pathway and alpha-2 and beta-receptors in the modulation of the analgesia induced by transcutaneous electric nerve stimulation of high and low frequencies

Avaliação da via noradrenérgica e dos receptores alfa-2 e beta na modulação da analgesia induzida pela estimulação elétrica nervosa transcutânea de alta e de baixa frequência

Evaluación de la vía noradrenérgica y de los receptores alfa-2 y beta en la modulación de analgesia inducida por la estimulación eléctrica nerviosa transcutánea con alta y baja frecuencia

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ABSTRACT | Transcutaneous electric nerve stimulation is a noninvasive method used in clinical Physiotherapy to control acute or chronic pain. Different theories have been proposed to explain the mechanism of the analgesic action of transcutaneous electric nerve stimulation, as the participation of central and peripheral neurotransmitters. The aim of this study was to evaluate the involvement of noradrenergic pathway and of the receptors alfa-2 and beta in the modulation of analgesia produced by transcutaneous electric nerve stimulation of high and low frequency in Wistar rats after chronic treatment with propranolol or yohimbine intraperitoneally. Animals weighing 200 to 300 g were divided into 9 groups (n=8), which were obtained nociceptive thresholds through the Tail Flick before and after application of TENS for comparing the change of pain. The administration of yohimbine or propranolol at a dose of 3 mg/kg was effective in antagonizing the analgesia induced by high (150 Hz) and low (10 Hz) frequency transcutaneous electric nerve stimulation according to ANOVA test followed by Duncan post hoc test ($p<0.05$). Thus, it is suggested the involvement of alpha-2 and beta noradrenergic receptors in the modulation of transcutaneous electric nerve stimulation-induced analgesia.

Keywords | Pain; Yohimbine; Propranolol/therapeutic use; Transcutaneous Electric Nerve Stimulation; Rats, Wistar.

RESUMO | Estimulação elétrica nervosa transcutânea é um método não invasivo utilizado na clínica de Fisioterapia para controlar dores aguda ou crônica. Diferentes teorias são propostas para explicar o mecanismo de ação analgésica da estimulação elétrica nervosa transcutânea, como a participação de neurotransmissores centrais e periféricos. O objetivo do presente estudo foi avaliar a participação da via noradrenérgica e dos receptores alfa-2 e beta na modulação da analgesia

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induzida pela estimulação elétrica nervosa transcutânea de alta e baixa frequência em ratos Wistar, após tratamento crônico com ioimbina ou propranolol por via intraperitoneal. Animais pesando entre 200 e 300 g foram divididos em 9 grupos (n=8), dos quais se obteve os limiares nociceptivos por meio do *Tail Flick* antes e após a aplicação da estimulação elétrica nervosa transcutânea para comparação de mudança do quadro álgico. A administração de ioimbina ou de propranolol na dose de 3 mg/kg foi efetiva em causar uma antagonização da analgesia induzida pela estimulação elétrica nervosa transcutânea de alta (150 Hz) e baixa frequência (10 Hz) segundo teste ANOVA seguido do teste *post hoc* Duncan (p<0,05). Dessa forma, sugere-se o envolvimento de receptores noradrenérgicos alfa-2 e beta na modulação da analgesia induzida pela estimulação elétrica nervosa transcutânea.

Descritores | Dor; Ioimbina; Propranolol/uso terapêutico; Estimulação Elétrica Nervosa Transcutânea; Ratos Wistar.

RESUMEN | La estimulación eléctrica nerviosa transcutánea es un método no invasivo utilizado en la clínica de Fisioterapia para controlar el dolor agudo y crónico. Diversas teorías son propuestas para explanar el mecanismo de acción analgésico de la estimulación eléctrica nerviosa transcutánea, como la participación de neurotransmisores centrales y periféricos. El objetivo del presente estudio fue evaluar la participación de la vía noradrenérgica y de los receptores alfa-2 y beta en la modulación de analgesia inducida por la estimulación eléctrica nerviosa transcutánea con alta y baja frecuencia en ratos del tipo Wistar, después del tratamiento crónico con yohimbina o propranolol por la vía intraperitoneal. Animales que pesaban 200 y 300 g fueron divididos en nueve grupos (n=8), por los cuales fueron obtenidos los umbrales nociceptivos por medio del *Tail Flick*, antes y después de la aplicación de la estimulación eléctrica nerviosa transcutánea con el intuito de comparar la alteración del cuadro álgico. La administración de yohimbina o propranolol en el dosis de 3 mg/kg fue eficaz en resultar en una antagonización de analgesia inducida por la estimulación eléctrica nerviosa transcutánea con alta (150 Hz) y baja (10 Hz) frecuencia, de acuerdo al test de ANOVA seguido del test post-hoc de Duncan (p>0,05). Por lo tanto, se sugiere el envolvimiento de los receptores noradrenergicos alfa-2 y beta en la modulación de analgesia inducida por la estimulación eléctrica nerviosa transcutánea.

Palabras clave | Dolor; Yohimbina; Propranolol/uso terapéutico; Estimulación Eléctrica Nerviosa Transcutánea; Ratos Wistar.

INTRODUCTION

Pain is present in all the vital cycle of human beings. Lead by great scientific curiosity, it spans psychic and somatic aspects. Although unpleasant and stressful[1], it acts as a protection for the organism[2]. However, when its vital protection functions are surpassed, it may seriously compromise quality of life, and bring damages to daily activities[3].

Transmission of the painful stimulus as well as its inhibition involves multiple mechanisms[4]. Pain sensation may be modified by pain inhibitory endogenous systems, predominantly through the descending pathways of noradrenaline, serotonin, and endogenous opioids[4,5]. The *locus coeruleus*, an important nucleus of the noradrenergic brainstem, is involved in the descending control of nociceptive pathways[6,7]. Catecholamine receptors are classically divided into two main categories: alpha and beta adrenoceptors, and the nociceptive effect of noradrenaline is mainly mediated by alpha-2-adrenoceptors[4,8].

Many techniques have been used, alone or in association with medicine, to provide analgesia to the patient, such as the low-level laser therapy[9], acupuncture[10], and transcutaneous electrical nerve stimulation (TENS)[11]. TENS is a non-pharmacological, noninvasive, of easy use and relatively few counter-indications resource that is already known in the modulation of acute and chronic pain[4,12,13].

Among the countless resources used, TENS and its chemical components of the human body like noradrenaline may be an important new mean to relieve very intense and frequent pains, providing an improvement in patients' quality of life. Studies about the relation between TENS and adrenergic receptors[14-17] are rare and contradictory.

The present paper aimed at assessing the participation of the noradrenergic pathway and its respective receptors — alpha-2 and beta — in the modulation of analgesia induced by TENS in Wistar rats after treatment through the intraperitoneal pathway (IP) with yohimbine (alpha-2-receptor antagonist) and propanol (beta-receptor antagonist).

METHODOLOGY

Animals

Rats from the Wistar line that weighed 200 to 300 g, originated from the vivarium, were used. The animals were grouped in numbers of four inside the polypropylene boxes, exposed to a 12-hour light-dark cycle with a mean temperature of 22 to 24°C; they also had free access to food ("Nuvilab" rat food) and water throughout

the experimental period. After the end of experiments, the animals were sacrificed with lethal injection of xylazine chloride and ketamine (100 and 375 mg/kg respectively). All experiments were performed following the ethical principles of the Ethics Commission in Animal Experimentation and following those adopted by the Brazilian College of Animal Experimentation – COBEA (approval protocol number 08/2009).

Animals were divided into nine groups (n=8). Control Groups (1) and (2) – treated with 0.9% physiological saline solution (NaCl), low and high frequency TENS respectively; Experimental Groups (3) and (4) – treated with alpha-2a antagonist medicine, concomitantly with low and high frequency TENS; Groups (5) and (6) – treated with beta antagonist, concomitantly low and high frequency TENS; Placebo Groups (7), (8) and (9) – treated with 0.9% physiological saline (NaCl) and antagonist drugs, both without TENS for purposes of intrinsic evaluation of the drug effects on the noradrenergic pathway.

Drugs used

Yohimbine (Tocris) and propranolol (Tocris) dissolved in physiological saline solution (NaCl at 0.9%) were used. Selective (yohimbine) and non-selective (propranolol) antagonists of noradrenergic receptors were administered through IP pathway in 3 mg/kg doses[7,18,19].

TENS

For induction of the antinociceptive status, the TENS Vif 993 DUAL (QUARK) equipment was used at low (10 Hz) and high frequencies (150 Hz), with initial amplitude of 15 mA, varying every five minutes (5 mA); and final one of 40 mA, average of 30 minutes of electrical therapy and 1 ms pulse duration. A pair of adhesive electrodes was fixed in the distal and proximal regions of the animal's tail, built specially for stimulation, and sized around 1 cm^2. Animals were calm with the electrodes fixed in their tails during TENS, which could not provide a muscular contraction or stressful stimulation that would provoke the animal's scape.

Nociceptive test - Hyperalgesia Measuring

Animals of each group had their nociceptive thresholds measured with the use of tail removal test. Every animal was put inside a contention cage with acrylic walls and their tails put on the sensor of a heating source (tail-flick – Analgesia Instrument; Stoelting), whose progressive raise was automatically interrupted as soon as the animal removed its tail from the equipment. A small adjustment of amplitude and current was performed whenever necessary, in the beginning of the experiment, with the aim of achieving three consecutive latencies of tail removal (LTR), between 2.5 to 3.5 seconds. These adjustments were done to increase or decrease the level of resistance heat of *Tail-flick* so that the reflex would be between 2.5 and 3.5 seconds.

Experimental protocol

Twenty-four hours after determination of baseline for the tail removal test, independent groups of Wistar rats had been previously treated with only one administration of physiological saline (Groups 1 and 2); alpha-2 noradrenergic antagonist (Groups 3 and 4) or beta (Groups 5 and 6) in the dose of 3 mg/kg through IP pathway for 15 consecutive days.

After 10 minutes of the last administration of noradrenergic antagonist or physiological saline that happened in the 15th day, animals were treated with low or high frequency TENS for 30 minutes, and analgesia test for LTR was performed at 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 minutes.

With the aim of assessing drugs effect (yohimbine and propranolol) on the nociceptive threshold, Groups 7, 8 and 9 took the same amount of physiological saline of noradrenergic antagonists for the same time period; however, they did not take part in the electric therapy, remained for 30 minutes only with the electrodes of TENS on the tail and device off (TENS OFF), right after their thresholds were measured in the same time mentioned.

Statistical analysis

For data analysis, the Statistical Package for the Social Sciences (SPSS) software, version 14.0, was used. Shapiro-Wilk's test was applied to verify normality of data, which followed a normal distribution. Results were statistically analyzed in absolute values of the respective arithmetic means and standard deviation through the analysis of variance (one-way ANOVA) and Duncan post-hoc test, in order to find possible differences between the groups. The significance level adopted in the study was $p<0.05$.

RESULTS

Early treatment with yohimbine or propranolol through the IP pathway provided an antagonization of analgesia induced by TENS, both of high and low frequencies.

ANOVA of repeated measures showed a statistically significant effect between the yohimbine or propranolol treatments in the IP 3 mg/kg dose associated with 150 Hz TENS compared to the group treated with physiological saline at 0.9% 0.2 mL/kg IP associated with 150 Hz TENS [$F_{(2,16)}$= 0.806 to 7.876; p<0.005]. The antinociception seen after applying 150 Hz TENS in the group pre-treated with physiological saline remained from 0 to 60 minutes (Figure 1).

The same findings were seen with low frequency TENS. ANOVA showed statistical difference between the yohimbine or propranolol treatments in the 3 mg/kg IP dose associated with 10 Hz TENS compared to the group treated with physiological saline at 0.9% 0.2 mL/kg IP associated with 10 Hz TENS [$F_{(2,16)}$=0.806 to 7.876; p<0.005]. Antinociception seen after using 10 Hz TENS in the group pre-treated with physiological saline also remained from 0 to 60 minutes (Figure 2).

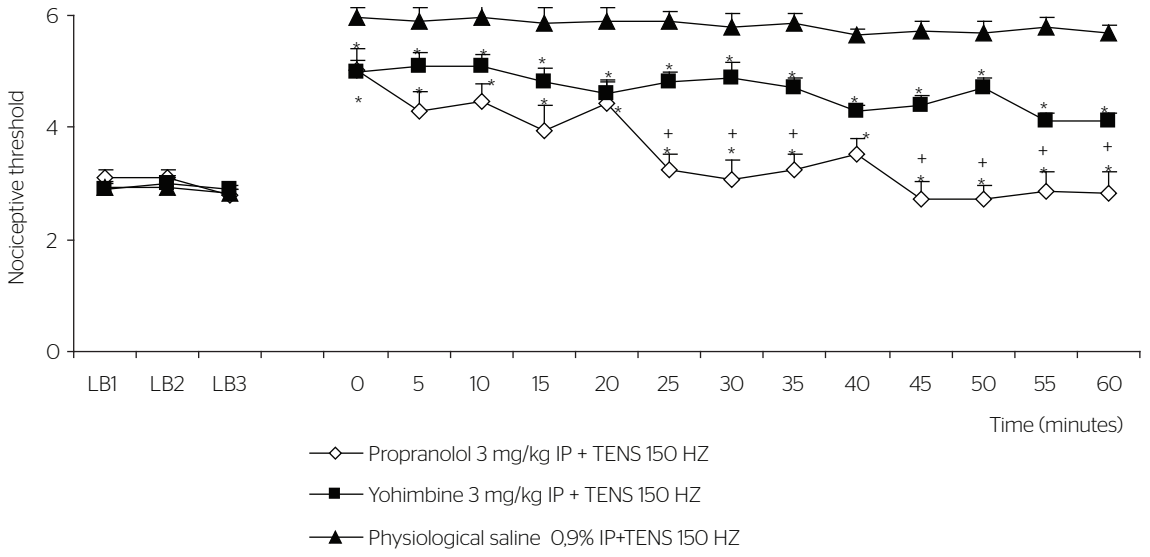
Duncan post-hoc test showed a statistic difference between the group treated with yohimbine and the one treated with propranolol with 150 Hz TENS at 25, 30, 35, 45, 50, 55, and 60 minutes (p<0.05) (Figure 1).

Duncan post-hoc test showed a statistic difference between the group treated with yohimbine and the other treated with propranolol with 10 Hz TENS at 40, 45 and 50 minutes (p<0.05) (Figure 2).

Figure 3 illustrates the absence of effects of the pre-treatment with yohimbine or propranolol in the 3.0 mg/kg dose on the nociceptive threshold. ANOVA of one pathway (one-way ANOVA) did not show a statistically significant effect [$F_{(3,27)}$= 0.38; p<0.05]. Duncan post-hoc test showed absence of significance between the groups (p>0.05) (Figure 3).

DISCUSSION

This paper evaluated the noradrenergic participation in analgesia induced by TENS in laboratory animals. Several and different supraspinal descending inhibitory systems were identified as able to modulate the spinal nociceptive transmission. With the ongoing investigation, places that were limited to structures of the mean line of mesencephalon and medulla increased. Recently, it is known that the solitary nucleus, locus coeruleus — subcoeruleus and the lateral reticular nucleus have a role in the modulation of spinal nociceptive transmission[6]. There are some evidences that injuries of an important origin of such pathways, the locus coeruleus, depress the persistent analgesia that follows tonic-clonic seizures, an effect that also seemed to depend on the participation of noradrenergic alpha- and beta-receptors[7].

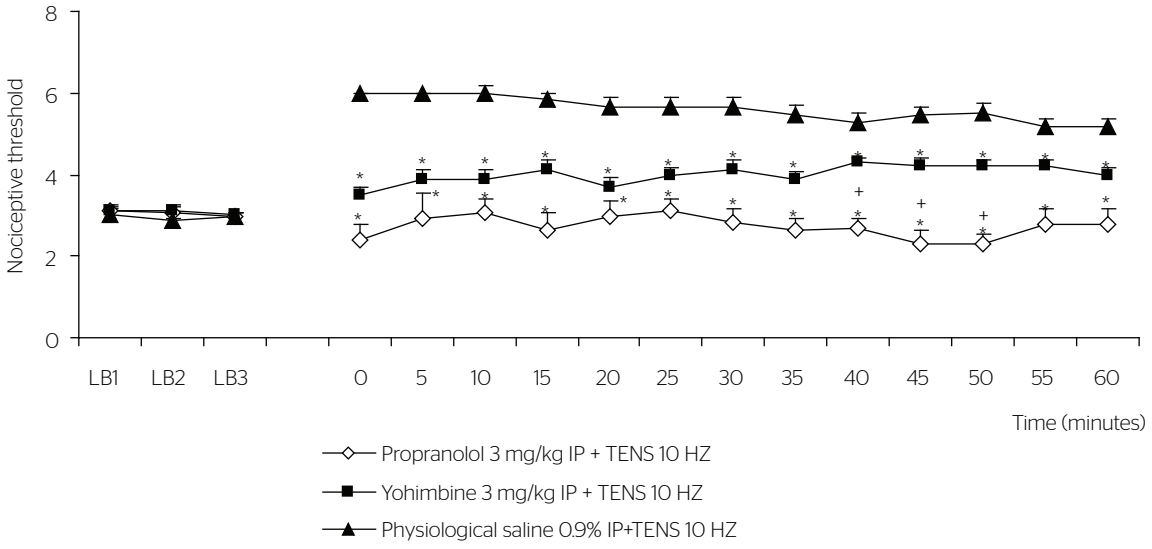


Points represent the means of nociceptive thresholds measured in the different times; bars represent EPM. Nociceptive threshold, y axis, time (s). (*) Statistically significant effect (p<0.05) compared to the group pre-treated with physiological saline at 0.9% 0.2 mL/kg IP + TENS 150 Hz. (+) Statistically significant effect (p<0.05) compared to the groups pre-treated with yohimbine 3 mg/kg IP + TENS 150 Hz according to Duncan post-hoc test

Figure 1. Temporal course of the yohimbine and propranolol injection effect in the 3 mg/kg dose through intraperitoneal pathway (IP) on the nociceptive threshold, here represented by tail removal latency

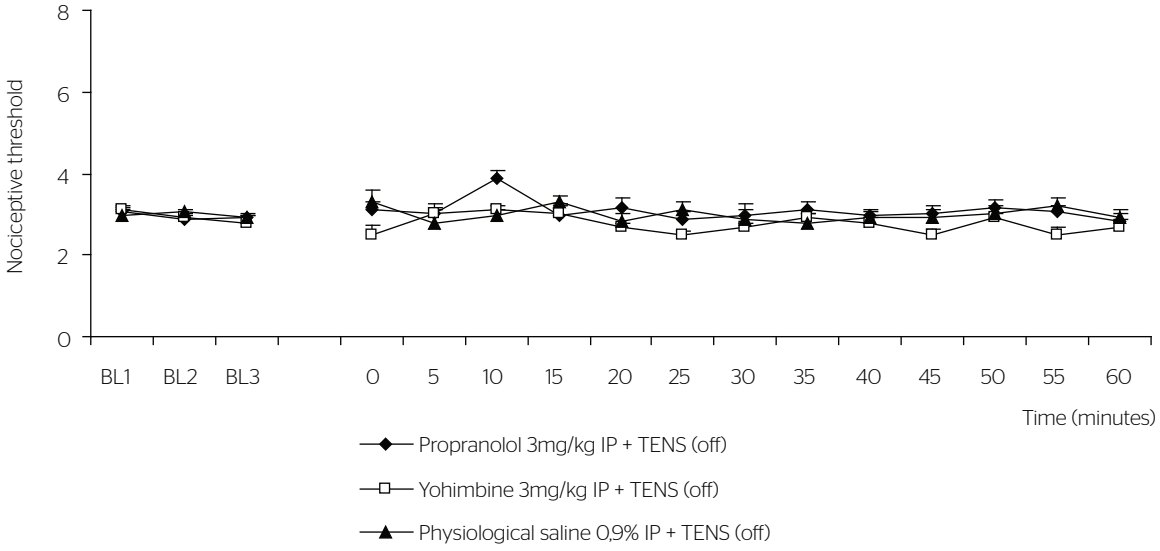
Noradrenergic cells are highly distributed into the mesencephalon and a substantial number of their neurons is in the locus coeruleus and has a significant descending projection to the spinal medulla[5,6,20]. It might be a possibility that the present results about antinociception induced by TENS have as their neural basis the noradrenergic pathway that was originated in the locus coeruleus, an important relay of the endogenous system of pain inhibition and of alpha-2 and beta noradrenergic receptors. Results found support in recent findings, which also suggest that alpha and beta-adrenergic receptors participate of the modulation of analgesia[21-23].

Yohimbine, a drug that is commonly used for the antagonism of alpha-2 noradrenergic receptors[7,8,17,24,25], is a potent and selective alpha-2 adrenergic receptor antagonist[22]. Yohimbine injections in the dorsal horn of the spinal medulla resulted in antagonization of the antinociceptive effect of the stimulation applied in the periaqueductal gray substance[26]. This report suggests that not only noradrenergic receptors of locus coeruleus, but also



Points represent the means of nociceptive thresholds measured in the different times; bars represent EPM. Nociceptive threshold, y axis, time (s). (*) Statistically significant effect (p<0.05) compared to the group pre-treated with physiological saline at 0.9% 0.2 mL/kg IP + TENS 10 Hz. (+) Statistically significant effect (p<0.05) compared to the groups pre-treated with yohimbine 3 mg/kg IP + TENS 10 Hz according to Duncan post-hoc test

Figure 2. Temporal course of the yohimbine and propranolol injection effect in the 3 mg/kg dose through intraperitoneal pathway (IP) on the nociceptive threshold, here represented by tail removal latency



Points represent the means of nociceptive thresholds measured in different times; bars represent EPM. BL1, BL2 BL3 represent the three latency measures of tail removal from baseline (BL). Nociceptive threshold, y axis, time (s). Analysis of variance of a pathway showed absence of a statistically significant difference between the groups (p>0.05)

Figure 3. Temporal course of effect absence of yohimbine and propranolol antagonists administered at 3 mg/kg through intraperitoneal pathway (IP) on the nociceptive thresholds

of the dorsal horn of the spinal medulla may be involved in the antinociceptive processes.

It has been demonstrated that, depending on the dose, some alpha-noradrenergic receptor antagonists present local anesthetic effect[27]. Propranolol is the drug chosen in several studies for the antagonism of beta noradrenergic receptors[7,23,28,29]. It is accepted that, in addition to its classical non-selective antagonist action on beta-noradrenergic receptors, propranolol inhibits ionic currents of high and low tension activated by Ca^{2++} in low concentrations[30] and, in higher concentrations, it moderately suppresses potassium currents[31]. Nevertheless, in the present study, we considered that the propranolol effects happened due to their classical antagonist action on beta-noradrenergic receptors, taking into consideration that propranolol management in the doses used in the present study did not change the basal nociceptive thresholds achieved by means of the tail removal test.

Some theories are proposed to explain TENS action mechanism[4,17]. In 1965, Melzack and Wall suggested the existence of a kind of flood-gate in the medulla dorsal horn. According to this theory, some neurons would have the ability of suppressing the transmission of the painful signal of medulla dorsal horn, therefore closing a hypothetical gate and inhibiting the passage of a painful impulse (Spinal Flood-gate Theory)[4,32]. Spinal and supraspinal mechanisms related to neurotransmitters and their receptors are involved in the mechanism of analgesia induced by TENS[4,17]. Recent data support this theory, both for the low and for the high frequency of TENS[33]. Muscarinic and serotonergic receptors, and opioids are activated by TENS in the spinal medulla and brainstem; peripherally, in the places of TENS application, alpha-2 noradrenergic receptors and opioids are involved in the analgesia induced by TENS[14,34].

TENS effects of high and low frequency were seen in mutant rats that deprived alpha-2 adrenergic receptor. Analgesia induced by TENS both of high and low frequency was reduced in these animals compared to controls. Furthermore, a selective antagonist of the alpha-2 receptor (SK&F 86466) was intra-articularly administered, therefore analgesia induced by TENS was reversed; however, this result was not seen in intrathecal and intracerebroventricular administrations. Data suggest that alpha-2-receptor has some contribution for TENS antihyperalgesia. This result is in agreement with anatomic observations about the localization of alpha-2-receptors in primary afferent neurons and macrophages next to the injured places[14,15]. Non-involvement of spinal alpha-2 noradrenergic receptors in the analgesia of low and high frequency TENS was also verified through intrathecal administration of yohimbine in Radhakrishnan *et al.*[17] study. Spinal serotoninergic receptors participated of the antihyperalgic TENS process of low, but not of the high frequency one[17]. In the present study, involvement of alpha-2 and beta noradrenergic receptors in the analgesia caused by TENS may have happened due to IP administration of antagonists. This method was chosen for allowing an initial and systemic verification of the participation of alpha-2 and beta receptors in the modulation of nociceptive thresholds, since medicine used in this study are able to surpass the hematoencephalic obstruction, thus acting peripherally and centrally, and being used in studies on the antinociceptive process[18,19,35].

Present results demonstrate involvement of alpha-2 and beta noradrenergic receptors after IP administration of specific antagonists in the antinociceptive process induced by TENS. These findings allow us to suggest that electric therapy performed by TENS may be harmed due to the use of noradrenergic antagonists.

CONCLUSION

It might be possible that noradrenergic pathway and alpha-2 and beta-receptors participate of the analgesia modulation induced by TENS, since the administration of yohimbine or propranolol through IP provided a reduction of the nociceptive thresholds with electrical stimulation in high or low frequency.

REFERENCES

1. Guimarães SS. Pelos meandros da dor. Mente e cérebro. 2008;16(188):36-55.
2. Novaes LHM. A dor: presença e papel no psiquismo humano. Rev Dor. 2007;8(2):981-2.
3. Dias S. A versão biológica da dor. Com Ciência [Revista Eletrônica de Jornalismo Científico] 2007 [citado 24 ago. 2012] Disponível em: http://www.comciencia.br/comciencia/?section=8&edicao=24&id=274
4. Santos CMF. Estudo da estimulação elétrica nervosa transcutânea na nocicepção e no edema induzidos pela administração de serotonina intradérmica [dissertação]. Minas Gerais: Escola de Educação Física, Fisioterapia e Terapia Ocupacional, Universidade Federal de Minas Gerais; 2010.

5. Yoshimura M, Furue H. Mechanisms for the anti-nociceptive actions of the descending noradrenergic and serotoninergic systems in the spinal cord. J Pharmacol Sci. 2006;101(2):107-17.
6. Jones SL. Descending noradrenergic influences on pain. Prog Brain Res. 1991;88:381-94.
7. Felippotti TT, dos Reis Ferreira CM, de Freitas RL, de Oliveira RC, de Oliveira R, Paschoalin-Maurin T, *et al*. Paradoxical effect of noradrenaline-mediated neurotransmission in the antinociceptive phenomenon that accompanies tonic-clonic seizures: role of locus coeruleus neurons and α(2)- and β-noradrenergic receptors. Epilepsy Behav. 2011;22(2):165-77.
8. Fairbanks CA, Wilcox GL. Moxonidine, a selective α2-adrenergic and imidazoline receptor agonist, produces spinal antinociception in mice. J Pharmacol Exp Ther. 1999;290(1):403-12.
9. Maher CG. Effective physical treatment for chronic low back pain. Orthop Clin North Am. 2004;35(1):57-64.
10. Kim DH, Cho SH, Song KH, Lee SE, Lee SH, Kwon GO, *et al*. Electroacupuncture analgesia for surgery in cattle. Am J Chin Med. 2004;32(1):131-40.
11. Kararmaz A, Kaya S, Karaman H, Turhanoglu S. Effect of the frequency of transcutaneous electrical nerve stimulation on analgesia during extracorporeal shock wave lithotripsy. Urol Res. 2004;32(6):411-5.
12. Morimoto HC, Yonekura MY, Liebano RE. Estimulação elétrica nervosa transcutânea nas modalidades convencional e acupuntura na dor induzida pelo frio. Fisioter Pesq. 2009;16(2): 148-54.
13. Sluka KA, Walsh D. Transcutaneous electrical nerve stimulation: basic science mechanisms and clinical effectiveness. J Pain. 2003;4(3):109-21.
14. DeSantana JM, Walsh DM, Vance C, Rakel BA, Sluka KA. Effectiveness of transcutaneous electrical nerve stimulation for treatment of hyperalgesia and pain. Curr Rheumatol Rep. 2008;10(6):492-9.
15. King EW, Audette K, Athman GA, Nguyen HO, Sluka KA, Fairbanks CA. Transcutaneous electrical nerve stimulation activates peripherally located alpha-2A adrenergic receptors. Pain 2005;115:364-73.
16. Sluka KA. The Neurobiology of pain and foundations for electrical stimulation. In: Robinson AJ; Snyder-Mackler L (eds). Clinical Electrophysiology. Philadelphia: Lippincott Williams & Wilkins; 2008:107-49.
17. Radhakrishnan R, King EW, Dickman JK, Herold CA, Natalie F. Johnston NF, *et al*. Spinal 5-HT2 and 5-HT3 receptors mediate low, but not high, frequency TENS-induced antihyperalgesia in rats. Pain. 2003;105(1-2):205-13.
18. Freitas RL, Ferreira CM, Ribeiro SJ, Carvalho AD, Elias-Filho DH, Garcia-Cairasco N, *et al*. Intrinsic neural circuits between dorsal midbrain neurons that control fear-induced responses and seizure activity and nuclei of the pain inhibitory system elaborating postictal antinociceptive processes: a functional neuroanatomical and neuropharmacological study. Exp Neurol. 2005;191(2):225-42.
19. Ferreira CMR. Estudo do envolvimento dos receptores noradrenérgicos alfa1, alfa2 e beta do sistema endógeno de inibição de dor nas alterações dos limiares nociceptivos induzidas por crises convulsivas isoladas e durante a fase silenciosa do status epilepticus e no modelo de kindling farmacológico [tese]. Minas Gerais: Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, 2009.
20. Pertovaara A. Noradrenergic pain modulation. Progress in Neurobiology. 2006;80(2):53-83.
21. Kawasaki Y, Kumamoto E, Furue H, Yoshimura M. Alpha 2 adrenoceptor-mediated presynaptic inhibition of primary afferent glutamatergic transmission in rat substantia gelatinosa neurons. Anesthesiology. 2003;98(3):682-9.
22. Binder W, Mousa SA, Sitte N, Kaiser M, Stein C, Schäfer M. Sympathetic activation triggers endogenous opioid release and analgesia within peripheral inflamed tissue. Eur J Neurosci. 2004;20(1):92-100.
23. Saadé NE, Al Amin HA, Barchini J, Tchachaghian S, Shamaa F, Jabbur SJ, *et al*. Brainstem injection of lidocaine releases the descending pain-inhibitory mechanisms in a rat model of mononeuropathy. Exp Neurol. 2012;237(1):180-90.
24. Lalchandani SG, Lei L, Zheng W, Suni MM, Moore BM, Liggett SB, Miller DD, *et al*. Yohimbine dimers exhibiting selectivity for the human alpha 2C-adrenoceptor subtype. J Pharmacol Exp Ther. 2002;303(3):979-84.
25. Sahraei H, Ghazzaghi H, Zarrindast MR, Ghoshooni H, Sepehri H, Haeri-Rohan A. The role of alpha-adrenoceptor mechanism(s) in morphine-induced conditioned place preference in female mice. Pharmacol Biochem Behav. 2004;78(1):135-41.
26. Peng YB, Lin Q, Willis WD. Involvement of alpha-2 adrenoceptors in the periaqueductal gray-induced inhibition of dorsal horn cell activity in rats. J Pharmacol Exp Ther. 1996;278(1):125-35.
27. Morales Aguilera A, Vaughan Williams EM. The effects on cardiac muscle of beta-receptor antagonists in relation to their activity as local anaesthetics. Br J Pharmacol Chemother. 1965;24:332-8.
28. Tchivileva IE, Lim PF, Smith SB, Slade GD, Diatchenko L, McLean SA, *et al*. Effect of catechol-O-methyltransferase polymorphism on response to propranolol therapy in chronic musculoskeletal pain: a randomized, double-blind, placebo-controlled, crossover pilot study. Pharmacogenet Genomics. 2010;20(4):239-48.
29. Park DS, Seo BK, Baek YH. Analgesic effect of electroacupuncture on inflammatory pain in collagen-induced arthritis rats: mediation by alpha2- and beta-adrenoceptors. Rheumatol Int. 2013;33(2):309-14.
30. Akaike N, Ito H, Nishi K, Oyama Y. Further analysis of inhibitory effects of propranolol and local anaesthetics on the calcium current in Helix neurones. Br J Pharmacol. 1982;76(1):37-43.
31. Akaike N, Nishi K, Oyama Y. Inhibitory effects of propranolol on the calcium current of Helix neurones. Br J Pharmacol. 1981;73(2):431-4.
32. Melzack R, Wall PD. Pain mechanisms: a new theory. Science. 1965;19;150(3699):971-9.
33. Sluka KA, Walsh D. Trancutaneous Electrical Nerve Stimulation: Basic Science Mechanisms and Clinical Effectiveness. J Pain. 2003;4(3):109-21.
34. Sluka KA. The Neurobiology of pain and foundations for electrical stimulation. In: Robinson AJ; Snyder-Mackler L, editors. Clinical Electrophysiology. Philadelphia: Lippincott Williams & Wilkins; 2008:107-49.
35. Marcondes Sari MH, Guerra Souza AC, Gonçalves Rosa S, Souza D, Dorneles Rodrigues OE, Wayne Nogueira C. Contribution of dopaminergic and adenosinergic systems in the antinociceptive effect of p-chloro-selenosteroid. Eur J Pharmacol. 2014;725(15):79-86.