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THE ROLE OF THE NORADRENERGIC SYSTEM IN ELECTRICAL STIMULATION-INDUCED ANALGESIA

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ABSTRACT

Transcutaneous electrical nerve stimulation (TENS) is an analgesia inducing method with increasing applications in clinical procedures. In this study we tried to evaluate the role of the adrenergic system and its different adrenoceptor subtypes in TENS-induced analgesia. Two types of low and high frequency electrical stimuli were used in rats through needle electrodes, inserted at the base of their tails, and different alpha and beta adrenergic agonist and antagonists were introduced to these animals. The tail immersion test was used as the analgesiometric test. Our results suggest that the noradrenergic system, through its α_2-receptor, has a significant role in electrical-induced analgesia.

Keywords: TENS, analgesia, adrenoceptors, rat tail immersion.

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INTRODUCTION

There are numerous analgesic drugs which are being used in practice. In addition, in order to alleviate certain types of pain, electrical stimulation methods are being applied. One of these methods is transcutaneous electrical nerve stimulation (TENS).[4,17,27] The effectiveness of this procedure is proven in various disease states such as rheumatoid arthritis,[14] acute facial[8] and chronic oro-facial,[5] ischemic,[21] low back,[2,15] phantom limb,[10] and postoperative[23] pain. Comparing the analgesia producing efficacy of electroacupuncture (EA) and TENS, it has been shown that there is no significant difference in producing antinociception for the two different peripheral conditioning stimulations, when applied at the same sites in rats. It is most likely that common neural mechanisms process the analgesic effects of EA and TENS.[28] Low frequency stimulation (0.5-4 Hz), denoted acupuncture-like TENS (lo TENS), and high frequency stimulation (100 Hz) or conventional TENS (hi TENS) are the most common forms.[1,11]

The 5-hydroxytryptamine[24] and GABA[3] systems have been shown to play a role in electrical stimulation-produced analgesia. Moreover, evidence of an endorphinergic link in the mechanism of acupuncture-like TENS has been provided.[9,25,26] In contrast to acupuncture-like TENS, conventional TENS is not affected by naloxone,[25] even in doses up to 10 mg in adults,[6] indicating that opioids utilizing mu receptors are not involved.

In the present investigation, in order to further study the mechanism which underlies TENS, we tried to evaluate the noradrenergic system's role in TENS. In recent years, there has been considerable interest in the involvement of adrenergic systems in pain and analgesia.[12,20] This has been due, in part, to clinical studies that have demonstrated

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sympathetic nervous system involvement in the pathophysiology of various pain states. Therefore in the present study a series of experiments were designed and carried out to investigate the role of the noradrenergic system in TENS-induced analgesia in rats.

MATERIALS AND METHODS

Subjects and test procedure

50 male and female NMRI rats (219±47g) were used in this study. It has been previously shown that the animal's sex has no effect in TENS analgesia.[13] The rats were housed in plastic boxes (65×40×32 cm) in groups of four with *ad libitum* food and water in the colony room under natural light. All testings were conducted between 09:00 and 17:00 h. For habituation to the test environment, rats were placed in the restrainers 5 minutes prior to testing. The testing room temperature was maintained at 22-24°C. For analgesia induction, a pair of needle electrodes were inserted subcutaneously at the proximal end of the animal's tail, and high frequency stimuli were applied with the following characteristics: frequency, 80 Hz; duration, 2 msec; voltage, 0.25 V. The waveform was sawtooth, which has been proven to be efficient in inducing analgesia.[19] The duration of stimulation was 30 minutes and immediately the analgesic test, i.e., the tail immersion test, was initiated and followed every 5 min. The tail immersion test was performed by immersing the distal one-third of the animal's tail in 52±0.5°C water by a tissue bath regulator.[18] The latency for the rat to curl its tail out of the water was recorded. After establishing a steady nociceptive baseline latency (ranging from 2.5±1.1s), TENS analgesia was induced and thereafter, tail-flick latencies were determined at 5 min intervals following drug administration. A cut-off latency of 12 sec was imposed after which the stimulus was terminated to avoid hurting the tail.

Drug treatments

All drugs were introduced intraperitoneally (ip). The drugs used in this study were propranolol (10 mg/kg; Ciba), a β-adrenergic antagonist, phentolamine (5 mg/kg; Ciba-Geigy), an α-adrenergic antagonist, phenoxybenzamine (5 mg/kg; SKF), an α_1-adrenergic antagonist, yohimbine HCl (0.5 & 1 mg/kg; Merck), an α_2-adrenergic agonist, and reserpine (1 mg/kg; Ciba-Geigy), a catecholaminergic depletor. All drugs were diluted in normal saline.

Statistical analysis

Calculations of statistical significance were performed using the Student's t-test. Critical values that reached a $p<0.05$ level of significance were considered statistically significant. The time-response data are presented for the period of up to 90 minutes following drug injection. All values are presented as mean±S.E.M.

RESULTS

TENS induced a sedative behavior in rats, initiating 5 to 10 mins after starting the stimulation. The pain threshold was 2.5±1.1 sec prior to electrical stimulation and increased to 7.9±1.3 sec after stimulation.

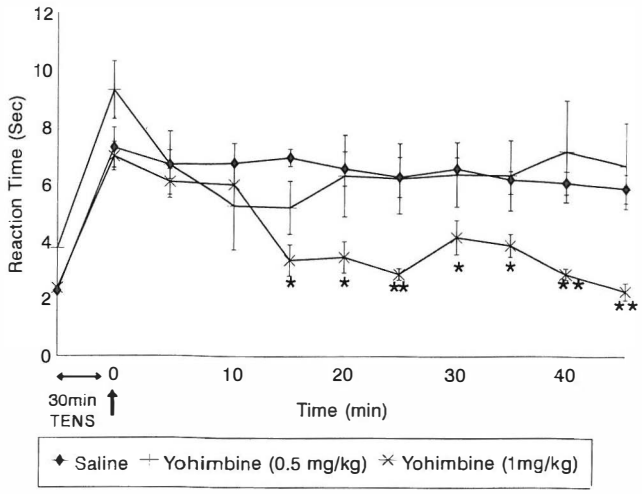

Fig. 1. Comparing the effect of yohimbine (●.5 and 1 mg/kg ip) with saline on hi TENS-induced analgesia. Each point represents mean±S.E.M. ◆= Saline, += Yohimbine (0.5 mg/kg), ★= Yohimbine (1 mg/kg), n= 6.
*($p<0.05$) and **($p<0.005$) significantly different from control levels.
⇑ denotes drug injection.

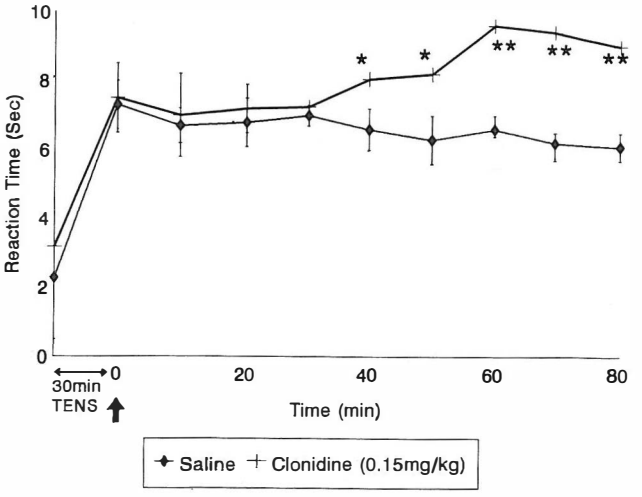

Fig. 2. Comparing the effect of clonidine (0.15 mg/kg ip) with saline on hi TENS-induced analgesia. Each point represents mean±S.E.M. ◆= Saline, += Clonidine (0.15 mg/kg), n= 8.
*($p<0.05$) and **($p<0.005$) significantly different from control levels.
⇑ denotes drug injection.

Table I: Mean±S.E.M. of tail-flick latencies following different drug administration. Propranolol, 10 mg/kg; Phenoxybenzamine, 5 mg/kg; Phentolamine, 5 mg/kg; Yohimbine, 1 mg/kg; Reserpine, 1 mg/kg.

Drug	Tail-Flick Latency Mean±S.E.M. (Seconds)								
	n	0	5	15	25	35	45	55	65
Saline	8	7.4±0.5	7±0.5	6.7±0.6	0.8±0.3	6.9±0.4	6.7±0.6	6.5±0.5	6.6±0.4
Propranolol	7	7.9±0.6	7.5±0.53	7.35±0.56	8.2±0.73	8.1±0.4	6.90±0.66	6.8±1	6.7±0.9
Phenoxybenzamine	5	6.7±0.7	6.4±0.63	5.8±0.53	6.2±0.93	5.8±1.36	6±2.13	6±1.2	6.9±3.6
Phentolamine	5	8.7±0.4	6.8±0.4	7.9±0.9	7.1±0.6	8.6±0.4	7.5±1.16	8.4±1.8	8±1.5
Yohimbine after Reserpine	8	8.4±1.3	8.1±1.8	7.8±1.7	8±1.5	8.7±2.4	7.5±1.4	6.7±2.1	7.8±1.8

Yohimbine (0.5 & 1 mg/kg ip), a selective alpha-2 adrenoceptor antagonist, reversed this analgesia significantly 15 min after drug injection. This effect was dose dependent and persisted after 45 min (Fig. 1). The application of this drug was accompanied with behavioral changes, such as restlessness, and bowel movement. On the other hand clonidine—an alpha-2 receptor agonist—at a dose of 0.15 mg/kg ip, significantly potentiated TENS analgesia (Fig. 2). However, injection of 0.3 mg/kg ip clonidine induced clonic type spasms in the whole body; therefore the test measurement could not proceed. Massive urination was seen with this dose of the drug.

The beta adrenergic antagonist propranolol (10 mg/kg ip), had no statistically significant effect on TENS-induced analgesia. Phentolamine, an alpha adrenergic antagonist (5 mg/kg ip), also showed no significant effect on the animal's nociception. 5 to 10 minutes after injection of phentolamine, the calm behavior of the rats changed into a restless and uneasy manner, accompanied with bowel movement. Administration of phenoxybenzamine (5 mg/kg ip), which has greater alpha-1 adrenergic antagonistic effect, didn't alleviate the produced analgesia, or the behavioral condition of the animals. Table I demonstrates the mean±S.E.M. of the rats' reactions during 70 minutes post-injection of these drugs, as compared with saline injection.

In addition, in 14 rats, following the depletion of the catecholaminergic system by reserpine (1 mg/kg ip) after 24 hrs, the animals underwent TENS. In 8 rats, analgesia was seen after electrical stimulation, and could not be reversed by 1 mg/kg yohimbine (Table I), while in 6 rats, no analgesia was produced after TENS.

DISCUSSION

The first reported use of electrical stimulation in a brain structure, in order to induce analgesia was by Reynolds in 1969.[22] But the massive current use of peripheral stimulation of afferent fibers to control pain goes back to the publication in 1965 of Melzack & Wall's spinal gate control theory.[16] Since then, a great deal of investigation has been focused on the clinical and basic aspects of this kind of analgesia.

One study shows that dorsal horn neurons which can potentially transmit noxious information to supraspinal levels can have their cell activity decreased during TENS application to somatic receptive fields. This is consistent with the concept of the 'gate control theory of pain' in that less noxious information would be involved in the pain perception process.[7]

Previous studies in this department, using pharmacological approaches, have shown that the opiate and serotonergic systems are both involved in maintaining this kind of analgesia, and the antagonists of these systems would partially reverse the TENS-induced analgesia.[13,19] For further elucidation of the mechanism(s) responsible for this analgesia, the possibility of involvement of the noradrenergic system was investigated. For this reason, noradrenergic agonists and antagonists were introduced to different groups of rats.

At first, by using propranolol as a beta-adrenoceptor blocker, we ruled out the effect of these receptors in TENS analgesia. Phenoxybenzamine and phentolamine, which have prominent alpha-1 receptor affinity, did not alleviate this analgesia. Clonidine and yohimbine as alpha-2 adrenoceptor agonist and antagonists were the only drugs which significantly increased or decreased TENS analgesia. It has to be emphasized that yohimbine, at a dose of 1 mg/kg completely reversed the analgesia to baseline level. Such an effect was not seen in neither of the other opioid or serotonergic systems dealing with this kind of analgesia. Acupuncture-like or conventional TENS, too, had no significant difference in any of the obtained responses and this was in contrast with reports that suggest the usefulness and effect of hi TENS in acute and phasic pain[5,13] and lo TENS in tonic pain states, such as ischemia.[21]

Reserpinizing the rats causes depletion of the

catecholamine content of the corresponding nerve endings. In 8 out of 14 reserpinized rats, TENS successfully induced analgesia, in which yohimbine exerted no effect. The other six rats showed resistance to this procedure and developed no analgesic behavior. This might indicate that TENS is able to induce its effect through more than one single mechanism/system, but the exact reason for this different response in this groups of rats needs to be further evaluated.

Our present results, therefore, support the fact that the noradrenergic system, through its alpha-2 receptors, has a significant role in the maintenance of TENS-induced analgesia, and this effect is paralleled by and/or accompanied with other systems, such as the opiate and serotonergic systems. The effect of other neurotransmitters in this domain of analgesia remains to be investigated.

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