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Original Article

Rapid Eye Movement (REM) Sleep Deprivation Reduces Pain Perception in Sciatic Nerve-ligated Wistar Rats; Involvement of Muscarinic Autonomic Receptor(s)

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Abstract

Effects of chronic sleep deprivation was investigated in Wistar rats. Forty rats were divided equally into two study groups. Study 1 investigated the effects of REM sleep deprivation (REMSD) on neuropathic pain perception while study 2 investigated the involvement of autonomic receptors on the pain perception following REMSD. Chronic constriction injury (CCI) of the sciatic nerve was used to induce neuropathic pain and multiple platform method for the induction of REMSD. The results of the test group showed a significant ($p<0.05$) increase in the mean reaction time (hot plate test) to thermal hyperalgesia. Also, there was a significant ($p<0.05$) increase in tail withdrawal latency (tail immersion tests) in test group. Various autonomic receptor antagonists were administered in study 2 to investigate their possible involvement in the observed analgesic effect of REMSD. Both atropine and propranolol produced an increase in pain perception thus, abolishing the anti-nociceptive effect of REMSD on neuropathic pain but the effect of atropine was more pronounced. From this, it appears that atropine; a muscarinic receptor blocker has the maximum capacity to reverse the analgesic effect of REMSD on neuropathic pain thus, suggesting the involvement of muscarinic cholinergic system. From these results, we hypothesize that REMSD has hypoalgesic effect on neuropathic pain which is modulated by muscarinic-cholinergic receptor.

Introduction

Disturbed sleep and short sleep duration are not uncommon in patients with various chronic pain disorders. Also, rats with peripheral neuropathy induced by sciatic nerve constriction injury have poor quality of sleep with reduced sleep efficiency, although there are reports of studies using the Bennett model of sciatic nerve ligation that show no effects on sleep. Accumulating evidences suggest that insufficient sleep and poor sleep quality are risk factors for inflammation-related conditions (1).

Peripheral nerve injury arising from disease or trauma induces long-lasting pathological pain. The resulting pain which is termed neuropathic pain manifests as spontaneous pain, allodynia or hyperalgesia. Tactile allodynia is a cardinal symptom of neuropathic pain. Usually, a vicious cycle develops because pain interferes with sleep and inadequate sleep has an

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influence on pain perception. Thus, it is of paramount importance to better understand the interplay between pain and sleep, and its mechanism. Specifically, we wonder if sleep deprivation aggravates neuropathic pain.

According to Hicks et al., REMSD increased the sensitivity of rats to electrical stimuli for up to 24 hours. In another experiment, they showed that REMSD for 4 days decreased nociceptive thresholds for up to 96 hours after termination of the sleep deprivation procedure (2).

Relating sleep deprivation to neuropathic pain, Huang *et al.,* in their study reported that post-CCI total sleep deprivation increased microglial activation and aggravated nerve injury-induced neuropathic pain. They opined that post-injury sleep deprivation could exacerbate injury-induced ectopic discharges from the median nerve and enhance glutamate release that in turn aggravates the activation of microglia and the development of neuropathic pain (3).

It is true that a number of researches have been published on the existing relationship between sleep and pain. A larger part of the research reported hyperalgesic effect of sleep deprivation on pain perception, though most of these reports were on inflammatory pain. Hence, this research was designed to investigate the effect of REMSD on neuropathic pain and the probable involvement of autonomic receptors in accordance with a previous report (4).

Methods

Experimental Animals

Forty male Wistar rats (12 weeks old) weighing 160.5±15.4 g were used for the study. They were housed under standard laboratory condition, maintained at a temperature of 24±1ºC, relative humidity of 55±5% and on a 12 hours light-dark cycles. The rats were randomly divided into two study groups as shown in Table I. Study 1&2 investigated the effects of sleep deprivation on pain perception in sciatic nerve ligated rats and possible mechanism of action respectively.

Induction of REM sleep deprivation

REM sleep deprivation was induced in the animals according to the method described by van-Hulzen and Coenen (5). The Modified Multiple Platform Version of the Flower pot method was used. Briefly, metabolic cage containing rats were gently placed in a bowl of water. The rats swam and climbed the wire gauges on the cage which provided a dry platform upon which they could stay. When a rat entered the stage of paradoxical sleep, it fell inside water, due to muscle atonia, and had to swim back to hold the wire gauges for support and in order to stay alert. In this study, muscle atonia was only used as a marker of REM sleep.

Induction of neuropathic pain by sciatic nerve ligation in rats

Neuropathic pain was induced by chronic constriction injury (CCI) using the method of Bennett and Xie (6). Mechanism of action of REMSD was investigated by administration of autonomic nervous system blockers- Atropine, propranolol, prazosin and hexamethonium (Table I).

Experimental procedures

The experimental procedure and animal grouping are shown in Table I.

For the two studies, nociceptive threshold was assessed after 72 hours of sleep deprivation using the hot plate and tail immersion methods.

The data were statistically analyzed using one-way ANOVA followed by Duncan *post hoc* test. Mean±SEM is given for data in text and graphs. The level of significance was set as $p<0.05$

Results

Paw Heat (Hot plate) Test

Fig. 1A shows the nociceptive response of rats to hot plate test. In the test group, there was significant ($p<0.05$) increase in mean reaction time from 0.70±0.15 secs in ligated control group to 1.63±0.17

secs in test group. There was a significant ($p<0.05$) decrease in mean reaction time from 1.54±0.21 secs in non ligated control to 0.70±0.15 secs in ligated control. The anti-nociceptive effect of sleep deprivation in groups administered atropine and propanolol was significantly ($p<0.05$) reinforced compared with test group.

TABLE I: Animal grouping (study 1: A-D; study 2: E-H).

Groups (N)	*Sleep deprivation (SD)*	*CCI*	*Treatment*
A - Normal un-ligated control	Negative	Negative	
B - Sham control	Negative	Negative	
C - Ligated control	Negative	Positive	
D - Test (ligated sleep-deprived)	Positive	Positive	
E - Propranolol	Positive	Positive	30 mg/kg orally
F - Hexamethonium	Positive	Positive	10 mg/kg i.p
G - Atropine	Positive	Positive	2 mg/kg i.p
H - Prazosin	Positive	Positive	5 mg/kg orally

n=5 animals. CCI = chronic constriction injury. Positive animals were ligated,negative animals were not ligated. SD = Sleep deprivation. Positive animals were sleep deprived while negative animals were not sleep deprived. i.p. = intraperitoneally.

Cold Water Tail Immersion Test

In the cold water tail immersion test, CCI caused hyperalgesia in ligated control but the induction of sleep deprivation in the test group significantly ($p<0.05$) increased (9.73±0.67 secs) the TWL when compared with ligated control (3.08±0.67 secs). Compared with the test group, the mean TWL of atropine group (4.51±0.14 secs) was significantly lower while prazosin (13.76±0.50 secs) significantly reinforced the reduction of pain perception after sleep deprivation (Fig. 1B).

Tail Heat Hyperalgesia Test

As shown in Fig. 1C, Sleep deprivation produced a

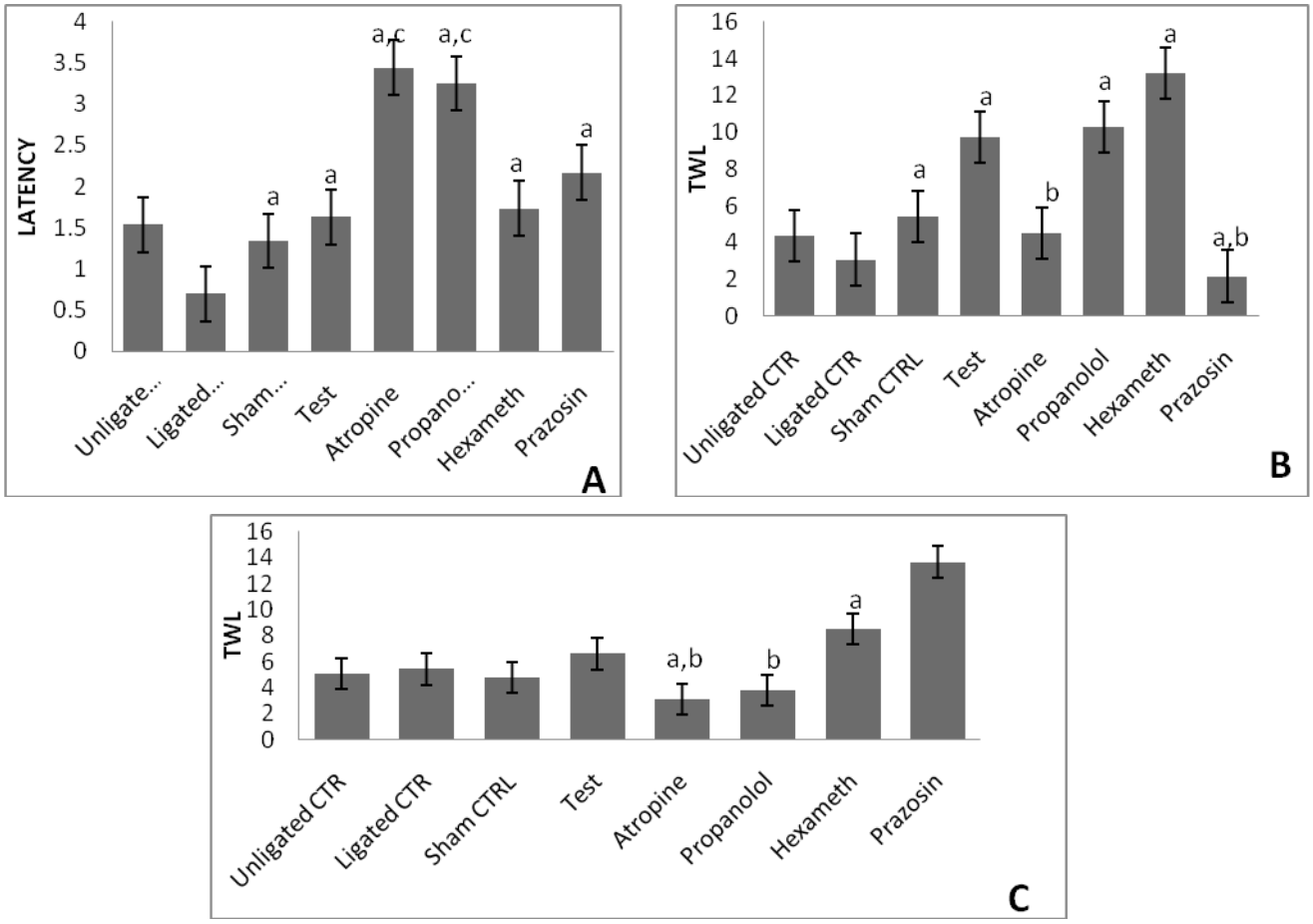

Fig. 1: Effect of Sleep deprivation on: (A) Paw heat hyperalgesia: [a]$p<0.05$ compared with ligated control group. [b]$p<0.05$ compared with normal control group. [c]$p<0.05$ compared with test. CTR - Control. Latency (sec). (B) Tail cold hyperalgesia: [a]$p<0.05$ compared with ligated control group. [b]$p<0.05$ compared with test group. CTR - Control. TWL - Tail withdrawal latency (sec). (C) Tail heat hyperalgesia: [a]$p<0.05$ compared with ligated control group. [b]$p<0.05$ compared with test group.

statistically insignificant increase in TWL in the test group (6.56±0.77 secs) compared with ligated control (5.39±0.65 secs). Also, the decrease in mean TWL observed in the ligated control (5.39±0.65 secs) when compared with non ligated control (5.06±1.03 secs) was insignificant. Atropine (3.07±0.28 secs) and propanolol (3.80±0.27 secs) produced significantly ($p<0.05$) different TWLs from test group (6.57±0.77 secs) but atropine abolished the anti-nociceptive effect of sleep deprivation than propanolol.

Discussion

Chronic constriction injury to the sciatic nerve as a method of inducing neuropathic pain produced a decrease in pain threshold in the ligated control as evidenced by decrease in mean reaction time and tail withdrawal latency. This is because chronic constriction injury causes spontaneous pain-related behaviour, allodynia and hyperalgesia there by confirming the report of Anderson and Colleagues (7). This also closely relates with findings observed in sleep-deprived mice. The anti-nociceptive effect of sleep deprivation observed in present study might be due to reduction in the sensitivity of the nerve fibres as a result of stress. The decrease in pain perception can also be attributed to alteration in the sleep-wake cycle which might have affected the serotonin status of the animals (8).

Various autonomic receptor antagonists were administered in this study to investigate their possible involvements in the observed analgesic effect of REMSD. Both atropine and propanolol produced a decrease in pain threshold thus, abolishing the anti-nociceptive effect of REMSD on neuropathic pain but the effect of atropine was more pronounced. From this, it appears that atropine; a muscarinic receptor blocker has a higher capacity to reverse the analgesic effect of REM sleep deprivation on neuropathic pain thus, suggesting the involvement of muscarinic cholinergic system. Analgesia via activation of muscarinic receptors has been described and it is known to be centrally mediated (9).

In conclusion, this study showed that REMSD attenuated hyperalgesia induced by chronic constriction injury of the sciatic nerve. This may probably involve the muscarinic-cholinergic system of the parasympathetic pathway.

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References

1. Kontinen VK, Ahnaou A, Drinkenburg WH, Meert TF. Sleep and EEG patterns in the chronic constriction injury model of neuropathic pain. *Physiol Behav* 2003; 78: 241–246.
2. Hicks RA, Coleman DD, Ferrante F, Sahatjian M, Hawkins J. Pain thresholds in rats during recovery from REM sleep deprivation. *Percept Mot Skills* 1979; 48: 687–690.
3. Huang C, Chiang RP, Chen C, Tsai Y. Sleep deprivation aggravates median nerve injury-induced neuropathic pain and enhances microglial activation by suppressing melatonin secretion. *Sleep* 2014; 37: 9.
4. Owoyele BV, Oladejo RO, Ajomole K, Ahmed RO, Mustapha A. Analgesic and anti-inflammatory effects of honey: The involvement of autonomic receptors. *Metab Brain Dis* 2014; 29: 167–173.
5. Van-Hulzen ZJ, Coenen AM. Paradoxical sleep deprivation and locomotor activity in rats. *Physiol Behav* 1981; 27: 741–744.
6. Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* 1988; 33: 87–107.
7. Andersen ML, Silva A, Kawakami R, Tufik S. The effects of sleep deprivation and sleep recovery on pain thresholds of rats with chronic pain. *Sleep Science* 2009; 2: 2.
8. Owolabi GO, Ajao OF, Akininiyi BK, Owoyele BV. (2011). Effects of rapid eye movement sleep deprivation on pain perception level in male wistar rats. *Asian Jour of Biomed and Pharma Sci* 2011; 1: 20–24.
9. Nunes GP, Tufik S, Nobrega JN. Decreased muscarinic receptor binding in rat brain after paradoxical sleep deprivation: an autoradiographic study. *Brain Res* 1994; 645: 247–252.