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Article

Exploring the role of cAMP in gabapentin-mediated pain attenuating effects in chronic constriction injury model in rats

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It has been shown that an increase in cAMP leads to pain sensitization and gabapentin is shown to decrease cAMP levels. However, the impact of drugs modulating cAMP levels on analgesic actions of gabapentin is not studied. The present study investigates the effect of milrinone on pain attenuating effects of gabapentin in chronic constriction injury (CCI). Neuropathic pain was induced by putting four loose ligatures around the sciatic nerve. The pain assessment was done by noting the paw withdrawal threshold in the pinprick test, paw withdrawal latency in hot plate test and paw withdrawal duration in acetone drop test before surgery and on 14th-day post-surgery. There was a significant development of cold allodynia, mechanical and heat hyperalgesia on 14th day in CCI rats. Gabapentin (100 mg/kg) treatment for 14 days significantly attenuated pain, while milrinone (50 mg/kg) treatment for 14 days significantly exacerbated neuropathic pain in CCI-subjected rats. Milrinone (30 and 50 mg/kg) also attenuated analgesic actions of gabapentin in CCI-subjected rats, suggests that gabapentin may abolish neuropathic pain by increasing the intracellular levels of cAMP in CCI-subjected rats.

Keywords: Neuropathic pain. Gabapentin. Milrinone. Chronic constriction injury. cAMP.

INTRODUCTION

International Association for the study of Pain (IASP) defined neuropathic pain as "Pain initiated or caused by a primary lesion or dysfunction in the nervous system'' (Burket *et al*., 2003). The development of peripheral, as well as central sensitization, is the key feature during the damage of the nervous system and sensitization of the nerves occurs due to the loss of inhibitory controls (Attal *et al*., 2006; Gwak *et al*., 2006). The symptoms due to nerve damage may include numbness, tingling, spontaneous pain, hyperalgesia, allodynia, dysesthesia and other sensory abnormalities (Jensen *et al*., 2011; Khangura *et al*., 2017). Depending on the location of the nerve damage, types of nerve affected and etiology, there are different types of neuropathies including peripheral neuropathy, cranial neuropathy, autonomic neuropathy, diabetic neuropathy, drug-induced neuropathy, and alcoholic neuropathy, etc. (Haga *et al*., 2015; Zeng *et al*., 2017). It is estimated that more than 30% of the general population is affected by persisting pain, which often becomes pathological and debilitating, and causes people to seek medical attention (Ji *et al*., 2014; Li *et al*., 2014) and around 7 in every 100 people over the world have chronic neuropathic pain (Colloca *et al*., 2017).

The therapeutic approaches of neuropathic pain include different classes of drugs including anti-convulsant (gabapentin, pregabalin, carbamazepine, and lamotrigine) (Backonja, 2002), anti-depressants (amitriptyline, nortriptyline, lofepramine duloxetine and venlafaxine) (Kim, Abdi, 2014), opioids (morphine, oxycodone, propoxyphene) (Navratilova *et al*., 2015), N-methyl-D-aspartate (NMDA) receptor antagonists (ketamine, methadone, amantadine, dextromethorphan,

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and memantine) (Gagnon *et al*., 2003; Carlsson *et al*., 2004) and topical agents (tramadol, codeine, and dihydrocodeine) (Jaggi, Singh, 2011; Stanos, Galluzzi, 2013). Various herbal preparations containing phytomedicines such as curcumin, sodium ferulate, safranal, tanshinone IIA, geniposidic Acid, limonoids, and vitamin E are also shown to ameliorate neurodegeneration and neuropathic pain (Tamaddonfard *et al*., 2014; Cao *et al*., 2015; Meng *et al*., 2015; Venkatesan *et al*., 2015; Chu *et al*., 2016).

Nevertheless, gabapentin is clinically proven, effective treatment for the management of neuropathic pain (Dworkin *et al*., 2007; Jang *et al*., 2018). Federal Drug Administration (FDA) and CDC guidelines also recommend gabapentinoids as the first-line drug for the treatment of neuropathic pain (Luo *et al*., 2017). Gabapentinoids include gabapentin and pregabalin and these have shown promising results in different types of neuropathies including diabetic neuropathy (Ramsay, 1994), postherpetic neuralgia (Backonja *et al*., 1998), migraine (Rowbotham *et al*., 1998) and pain associated with cancer and multiple sclerosis (Di Trapani *et al*., 2000). Studies have shown that gabapentin inhibits ectopic discharge from the injured peripheral nerves and attenuates spontaneous pain (Abdi, Lee, Chung, 1998; Pan *et al*., 1999; Luo *et al*., 2017). The broad spectrum of pharmacological activity of gabapentin has led several investigators to determine its mechanism of action. The neuropathic pain attenuating effects of gabapentin has been attributed to their actions on the central nervous system (at the spinal cord or the brain) due to enhanced inhibitory input on the GABA-mediated pathways, which leads to a reduction in the excitatory action potential (Cai *et al*., 2012). Furthermore, antagonism of NMDA receptors and blockade of calcium channels in the central system CNS or inhibition at the peripheral nervous system may also contribute in attenuating pain (Abdi, Lee, Chung, 1998; Dooley *et al*., 2000). Gabapentin has also been shown to modulate other targets including transient receptor potential channels, protein kinase C and inflammatory cytokines (Vellani, Giacomoni, 2017). It may also act on the supra-spinal region to stimulate noradrenaline-mediated descending inhibition, which contributes to attenuating neuropathic pain (Kukkar *et al*., 2013). However, its precise mechanisms are not clearly defined yet (Billie *et al*., 2006).

Milrinone is a biguanide compound and documented as a selective phosphodiesterase-3 inhibitor. It significantly inhibits cAMP phosphodiesterase activity, increasing intracellular cAMP levels (Alousi, Johnson, 1986). Owing to its action (selective increase in cAMP levels), it has been used in clinics as an inodilator agent in the management of congestive heart failure (CHF). However, its prolonged use is not beneficial in CHF patients; rather it increases the mortality rate in these patients. Accordingly, it is employed for short term management of CHF during the decompensation state (Hilleman, Forbes, 1989; Landmesser, Drexler, 2007).

Adenosine 3', 5'cyclic monophosphate (cAMP) is a key second messenger in various signal transduction pathways and it regulates numerous cellular functions, including cell growth and differentiation, gene transcription and protein expression (Yan *et al*., 2016). Studies have shown the key participation of cAMP in neuropathic pain and it has been shown that an increase in cAMP levels may be important in the development of neuropathic pain (Bie et al., 2005; Hugo, Levine, 2007). Moreover, drugs/interventions that decrease the levels of intracellular cAMP are shown to attenuate pain in different experimental models (Shao *et al*., 2016). There exists a relationship between cAMP and Ca^{2+} channel as an increased level of cAMP increase protein kinase A activity, which sequentially promotes the opening of the L-type calcium channel resulting in calcium entry into the cell (Earl, Linden, Weglicki, 1986). Many studies have implicated the cAMP-calcium-protein kinase A pathway in neuropathic pain conditions (Guindon *et al*., 2008).

It has been shown that gabapentin reduces intracellular calcium current in injured as well as in the control of primary afferent neurons (Sarantopoulos *et al*., 2002). Other studies have also shown that gabapentin decrease intracellular calcium levels (Martin *et al*., 2002). Gabapentin has been shown to attenuate the expression of phosphorylated cAMP response elements in the amygdala (Li *et al*., 2010) suggesting the possible role of cAMP in gabapentin mediated analgesic actions. However, there is no study reporting the influence of

cAMP modulating drugs on the neuropathic pain attenuating the actions of gabapentin. Owing to PDE-3 inhibitory activity, milrinone increases the cAMP levels. Accordingly, milrinone was employed as a pharmacological tool to investigate the role of cAMP in neuropathic pain attenuating actions of gabapentin in chronic constriction injury model in rats. In other words, to explore whether the decrease in cAMP levels plays an important role in gabapentin-mediated pain attenuating action, a pharmacological agent which increases the intracellular cAMP levels (milrinone) was employed in this study. The attenuation of beneficial effects of gabapentin in the presence of an agent that increases the cAMP levels (milrinone) may confer the importance of cAMP in gabapentin-mediated pain attenuating actions. Therefore, the present study was designed to investigate the possible role of cAMP in pain attenuating actions of gabapentin by employing milrinone as a pharmacological tool (as a cAMP elevating agent) in chronic constriction injury in rats.

MATERIAL AND METHODS

Experimental Animals

All experiments were performed as per approval by the Institutional Animal Ethics Committee (IAEC) (Reg. no.1407/PO/Re/S/11CPCSEA). Sprague Dawley rats of either sex, weighing 200–250 g were purchased from the Institute of Microbial Technology, Chandigarh. They were housed in the departmental animal house with standard laboratory conditions *i.e.* temperature 23± 2ºC (Baumans, Van Loo, 2013), chow diet and the normal cycle of 12 hours light and 12 hours dark. The care of the animals was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) Ministry of Environment and Forest, Government of India. (Approval Number: ATRC/04/18).

Drugs and Reagents

Gabapentin (Gabantin™, 500 mg) was purchased from Sun Pharmaceuticals Industries, India. Milrinone was obtained from Neon Laboratories limited, Mumbai. All the chemicals used in the present study were of analytical grade.

Induction of Neuropathy Pain by Chronic Constriction Injury (CCI)

Neuropathic pain was induced in rats by chronic constriction injury (Bennett, Xie, 1988). Rats were anesthetized by intraperitoneal injection of ketamine (80 mg/kg) and xylazine (10 mg/kg *i.p.*) (Wang *et al*., 2017). With proper surgical care, the hair of the rat's lower back and thigh region was shaved and the skin was sterilized with a 0.5% povidone solution. The left thigh was cut and a cut made directly through the biceps femoris muscle to expose the sciatic nerve. After exposure, the sciatic nerve was ligated with a silk 4-0 thread at four sites with a 1 mm gap. The care was taken to tie the ligatures around the nerve so that the nerve was barely constricted. The ligation affected approximately 6 mm of the nerve length (Sumizono *et al*., 2018). The muscle and skin were closed in two layers with the use of thread and topical antibiotic was applied. All surgical procedures were carried out under normal sterile conditions.

Behavioral Examination

Cold allodynia (acetone test)

The cold allodynia was assessed by spraying a 100 µL of acetone onto the surface of the paw of rat (placed over a wire mesh), without touching the skin. The response of rat to acetone was noted for the 20s and was graded to a 4-point scale as defined by Flatters and Bennett (Flatters, Bennett, 2004). 0: no reflex; 1: quick stamp, flick or withdrawal of the paw; 2: repeated flicking or prolonged withdrawal; and 3: repeated flicking with the licking of the paw. Acetone was applied three times to the hind paw, with a gap of 5 min between the acetone applications and the individual scores noted in a 20s interval were added to obtain a single score over a cumulative period of 60s. The minimum score was 0, while the maximum possible score was 9 (Kukkar *et al*., 2013).

Heat hyperalgesia (hot-plate test)

The heat hyperalgesia was assessed by Eddy's hot-plate, as an index of thermal hyperalgesia, by measuring the thermal nociceptive threshold. The animals were placed on the hot-plate at a temperature of 52.5 ± 1.0 °C and withdrawal latency, in terms of the licking of the hind paw, was recorded in seconds. The maintained cut-off time was 15 sec (Jain *et al*., 2009).

Mechanical hyperalgesia (pinprick test)

Mechanical hyperalgesia was measured by the pinprick test (Erichsen, Blackburn-Munro, 2002). The injured surface of the hind paw was touched with the point of a bent gauge needle (at 90° to the syringe) at the strength necessary to produce a reflex withdrawal response. The paw withdrawal duration (PWD) was recorded in seconds and the normal quick reflex withdrawal response was given the value of 0.6 s.

Experimental Protocol

Ten groups, each comprising five rats, were employed in the present study.

Group I: Normal Control

In the normal control group, rats were not subjected to any treatment. The different behavioral tests, including the heat hyperalgesia, cold allodynia, and mechanical hyperalgesia were employed on day 0 (a day before surgery) and 14th day (post-surgery).

Group II: Sham Control

In the sham control group, rats were subjected to the surgical procedure to expose the left sciatic nerve on day 1 without any nerve ligation. The behavioral tests including the heat hyperalgesia, cold allodynia, and mechanical hyperalgesia were conducted before doing surgery on day 0 (a day before surgery) and 14th day (post-surgery).

Group III: CCI Control

In this group, rats were subjected to the surgical procedure to expose and ligate the left sciatic nerve on day 1. The pain-related behavioral tests were performed at different time intervals as described in group I.

Group IV: Gabapentin (100 mg/kg) in CCI

In this group, gabapentin (100 mg/kg) was administered in CCI subjected rats for 14 days, starting from day 1 (day of surgery). The pain-related behavioral tests were performed at different time intervals as described in group I.

Groups V, VI and VII: Milrinone (10, 30 and 50 mg/kg i.p.) in Gabapentin + CCI

Milrinone (10, 30 and 50 mg/kg) was injected 30 min before gabapentin administration for 14 days in CCI-subjected rats, starting from day 1. The pain-related behavioral tests were performed at different time intervals as described in group I.

Group VIII: Milrinone (50 mg/kg) in CCI

Milrinone (50 mg/kg) was administered in CCI subjected rats for 14 days, starting from day 1 (post-surgery). The pain-related behavioral tests were performed at different time intervals as described in group I.

Group IX: Milrinone (50 mg/kg) per se

Milrinone was administered in normal rats for 14 days. The pain-related behavioral tests were performed at different time intervals as described in group I.

Group X: Gabapentin (100 mg/kg) per se

Gabapentin was administered in normal rats for 14 days. The pain-related behavioral tests were performed at different time intervals as described in group I.

Statistical Analysis

The results were expressed in mean ± S.D. The data of behavioral tests were analyzed using two-way ANOVA followed by Bonferonni's *post hoc* test, using Graph pad prism version-5.0 software. The *P-value*< 0.05 was considered to be statistically significant.

RESULTS

Effect of Pharmacological Interventions on Cold-Allodynia (Acetone Drop Test) in Chronic Constriction Injury-induced neuropathic pain

Chronic constriction injury resulted in significant development of cold allodynia on 14th-day post-surgery (Figure 1) as compared to the sham group, measured by acetone drop test. Administration of gabapentin (100 mg/kg *i.p.*) for 14 days significantly attenuated CCI-induced cold allodynia in comparison to the sham group. There was a significant decrease in allodynia scores in gabapentin treated CCI rats. Pretreatment with milrinone (30 and 50 mg/kg, *i.p.*), for 14 days, attenuated gabapentin-induced decrease in allodynia score in CCI-subjected rats. However, milrinone (10 mg/kg) did not show a significant effect on the allodynia gabapentin treated CCI rats. Pretreatment with milrinone (50 mg/kg *i.p.*) led to a significant increase in cold allodynia score in CCI-subjected rats. *Per se* administration of milrinone (50 mg/kg *i.p.*) and gabapentin did not modulate cold allodynia in normal rats.

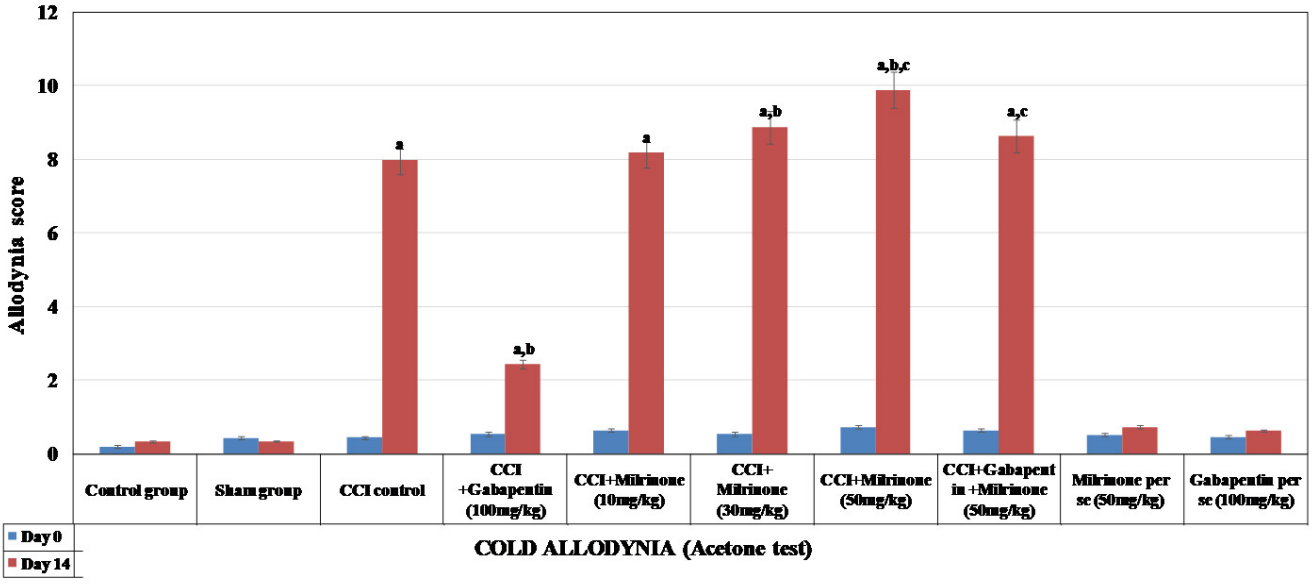

FIGURE 1 - Effect of pharmacological interventions on chronic constriction injury-induced paw cold allodynia assessed by acetone drop test. Values are given in mean ± S.D., n=5 rats per group; Two-way ANOVA followed by Bonferonni's *post hoc* test. [a]*P*<0.05 vs sham control, [b]*P*<0.05 vs chronic constriction injury, [c]*P*<0.05 vs gabapentin in CCI.

Effect of pharmacological interventions on heat-hyperalgesia (hot plate test) in chronic constriction injury-induced neuropathic pain

Chronic constriction injury significantly decreased paw withdrawal latency in a hot plate test as compared to the sham group (Figure 2), signifying the development of heat-hyperalgesia. Administration of gabapentin (100 mg/kg *i.p.*), for 14 days, attenuated chronic constriction injury-induced development of heat-hyperalgesia in a significant manner. Pretreatment with milrinone (30 mg/kg and 50 mg/kg *i.p.*) for 14 days abolished gabapentin-

induced decrease in paw withdrawal latency in CCI-subjected rats. However, milrinone (10 mg/kg) did not modulate the effects of gabapentin in CCI-subjected rats. Administration of milrinone (50 mg/kg *i.p.*) for fourteen days significantly decreased paw withdrawal latency in CCI-subjected rats. *Per se* administration of milrinone (50 mg/kg *i.p.*) and gabapentin did not modulate heat-related behavioral functions in normal rats.

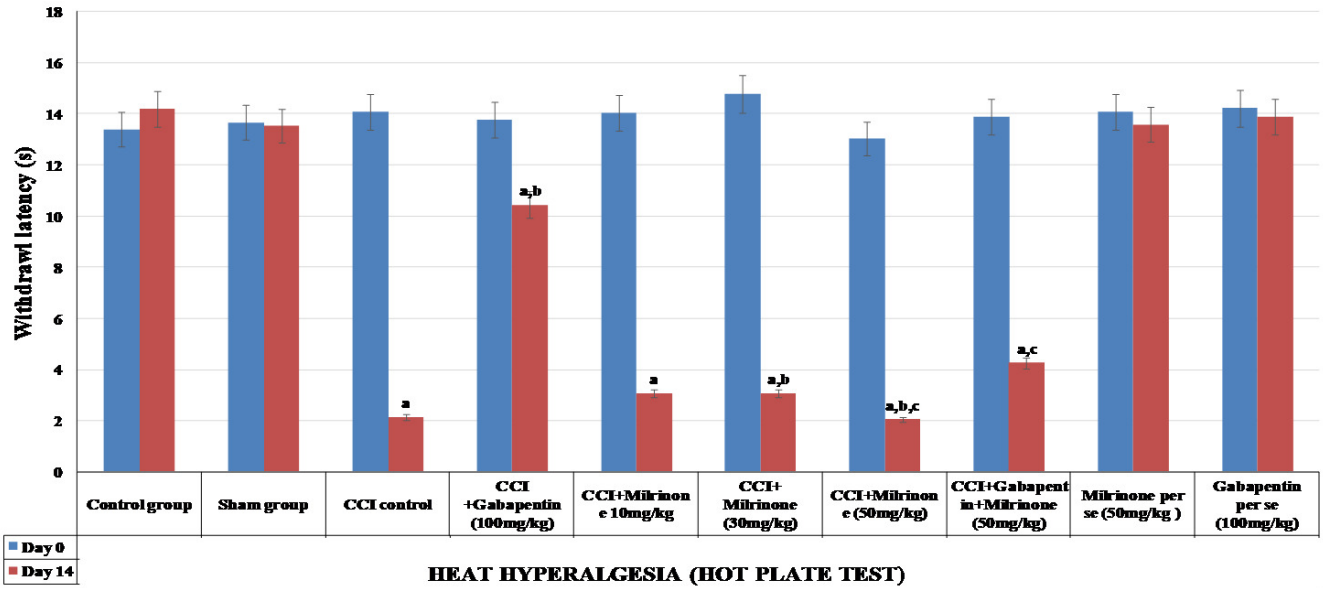

FIGURE 2 - Effect of pharmacological interventions on chronic constriction injury-induced heat hyperalgesia assessed by hot plate test. Values are given in mean ± S.D., n=5 rats per group; Two-way ANOVA followed by Bonferonni's *post hoc* test. [a]$P<0.05$ vs sham control, [b]$P<0.05$ vs chronic constriction injury, [c]$P<0.05$ vs gabapentin in CCI.

Effect of pharmacological intervention on mechanical hyperalgesia (pinprick test) in chronic constriction injury-induced neuropathic pain

Chronic constriction injury led to a significant increase in paw withdrawal duration in response to pin prick test, as compared to the sham group (Figure 3), suggesting the development of mechanical hyperalgesia. Treatment with gabapentin (100 mg/kg *i.p.*), for 14 days, attenuated CCI-induced increase in withdrawal duration in a significant manner. Pretreatment with milrinone (30 mg/kg and 50 mg/kg, *i.p.*), for 14 days, abrogated the effects of gabapentin on paw withdrawal duration in CCI-subjected rats. Milrinone (10 mg/kg) did not modulate the behavior of gabapentin in CCI-subjected rats. Treatment with milrinone (50 mg/kg *i.p.*) for 14 days significantly increased paw withdrawal duration in CCI-subjected rats. *Per se* administration of milrinone (50 mg/kg i.*p.*) and gabapentin did not modulate mechanical pain-related behavioral functions in normal rats.

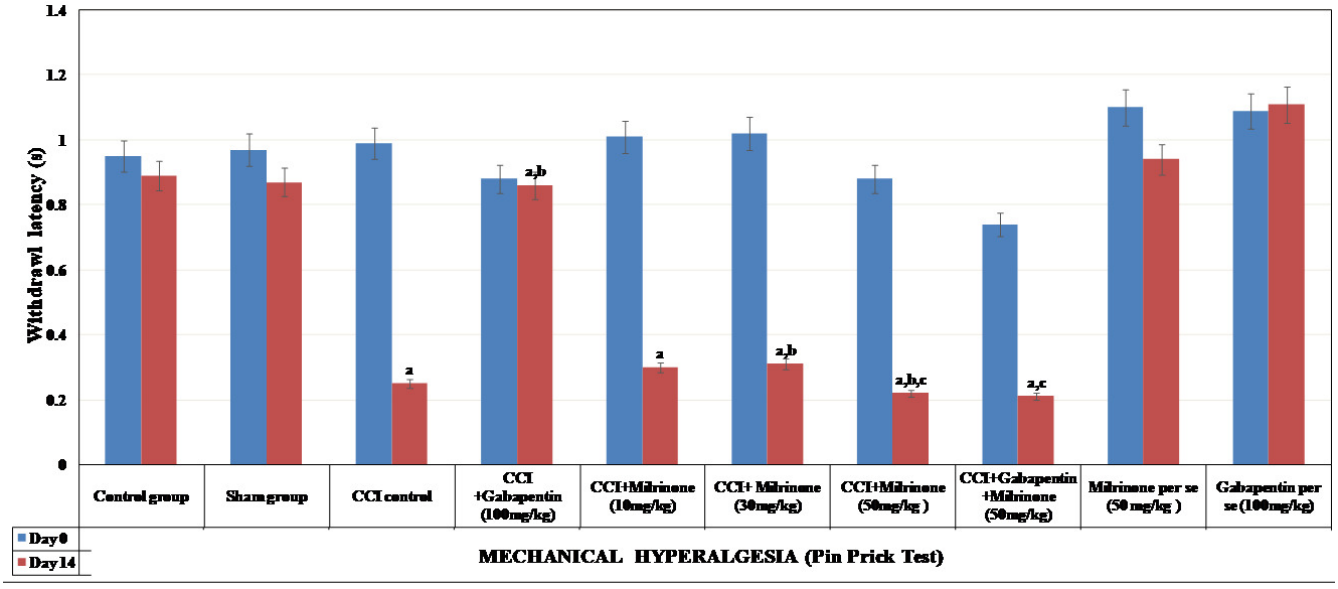

FIGURE 3 - Effect of pharmacological interventions on chronic constriction injury-induced Mechanical hyperalgesia assessed by pin prick test. Values are given in mean ± S.D., n=5 rats per group; Two-way ANOVA followed by Bonferonni's *post hoc* test. [a]$P<0.05$ vs sham control, [b]$P<0.05$ vs chronic constriction injury, [c]$P<0.05$ vs gabapentin in CCI.

DISCUSSION

The goal of the present study was to elucidate the role of cAMP in gabapentin-mediated pain attenuating effects in chronic constriction injury model in rats. To accomplish this, the present study employed the chronic constriction injury model to induce neuropathic pain (Bennett, Xie, 1998; Jaggi *et al*., 2011; Ko *et al*., 2015). In the present study, CCI-subjected rats showed significant enhancement in paw withdrawal in response to acetone application on injured paw. The observed cold allodynic response in the CCI-subjected rats in the form of increased withdrawal duration mimics the symptoms of cold allodynia in patients suffering from complex regional pain syndrome (Kemler, de Vet, 2000; Tahmoush *et al*., 2000). Furthermore, there was also a significant development of mechanical and heat hyperalgesia, observed on the 14th day after surgery. The development of mechanical hyperalgesia was observed by noting an increase in paw withdrawal duration in response to pin prick test, while the development of heat hyperalgesia was observed by noting a decrease in paw withdrawal latency in hot plate test. These results observed in the present study are in line with previous findings (Bennett, Xie, 1988; Flatters, Bennett, 2004; Khangura *et al*., 2017). Earlier studies have documented that the pain-related behavioral alterations are at peak on the 14th day in chronic constriction subjected rats (Jaggi, Singh, 2011; Kukkar *et al*., 2014).

In the present study, administration of gabapentin for 14 days significantly attenuated CCI-induced pain-related behavioral alterations including paw cold allodynia, mechanical and heat hyperalgesia. Earlier studies have also observed the beneficial effects of gabapentin on the behaviors of both thermal and mechanical stimulations in CCI-induced rats (Yeh *et al*., 2011; Kukkar *et al*., 2013). As per the FDA and CDC guidelines, gabapentinoids (pregabalin or gabapentin) are the first-line drugs for the treatment of neuropathic pain (Luo *et al*., 2017).

Gabapentin is a well established anti-epileptic and neuropathic pain attenuating drug, however, its underlying molecular mechanisms are not entirely clear (Billie *et al*., 2006). It has been reported that gabapentin decreases spontaneous neuronal activity by binding to the α2δ-1 subunit of voltage-gated calcium channels (VGCC), and thereby reducing neuronal calcium currents (Gee *et al*., 1996; Shimoyama *et al*., 2000). Gabapentin has also been shown to decrease cAMP levels in the brain

regions (Li *et al*., 2010). Considering the close association between cAMP and Ca^{2+} signaling (Hofer, 2012), it was hypothesized that there may be a key role of cAMP in gabapentin-mediated neuropathic pain attenuating actions. Therefore, to explore the role of cAMP in gabapentin-mediated pain attenuating effects, as a cAMP elevating agent, milrinone (as a pharmacological agent) was co-administered in gabapentin-treated CCI rats. In the present study, pretreatment with milrinone (30 mg/kg and 50 mg/kg) for 14 days significantly abolished neuropathic pain attenuating actions of gabapentin in CCI-subjected rats. Since milrinone administration leads to an increase in the cAMP levels, therefore, it is proposed that milrinone-induced increase in intracellular cAMP levels may contribute to abolishing the analgesic actions of gabapentin in the present study.

The cAMP is a key second messenger and it modulates numerous physiological as well pathophysiological functions in the body (Yan *et al*., 2016). Amongst different functions, studies have shown that an increase in the levels of cAMP increases the pain sensitization (Shao *et al*., 2016). It has been shown that an increase in pain sensitization during morphine withdrawal is due to the potentiation of a cAMP-linked signaling pathway (Bie *et al*., 2005). Many other studies have implicated that cAMP-protein kinase A pathway triggers hyperexcitability in sensory neurons of dorsal root ganglia (Guindon *et al*., 2008). Moreover, studies have also demonstrated that inhibition of cAMP attenuates neuropathic pain (Liou *et al*., 2007; Shao *et al*., 2016). The key role of cAMP in increasing pain sensitization was also supported by the results of the present study showing that treatment with milrinone (50 mg/kg) exacerbated pain intensity in CCI-subjected rats. However, milrinone did not modulate pain-related behavior parameters in normal rats suggesting that cAMP may selectively increase pain sensitization during nerve injury, without any significant alteration in non-injured conditions. Gabapentin has also been shown to decrease cAMP levels in the brain regions (Li *et al*., 2010). The results of the present study provide evidence that the analgesic actions of gabapentin are dependent on the intracellular cAMP levels and an increase in cAMP levels may attenuate the analgesic actions of gabapentin.

CONCLUSION

The attenuation of gabapentin-mediated analgesic actions in the presence of milrinone suggests that an increase in intracellular cAMP following milrinone administration significantly contributes to abolishing the analgesic actions of gabapentin in the CCI-subjected rats. In other words, a decrease in cAMP plays a key role in gabapentin-mediated pain attenuating actions in CCI-subjected rats.

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REFERENCES

Abdi S, Lee DH, Chung JM. The anti-allodynic effects of amitriptyline, gabapentin and lidocaine in a rat model of neuropathic pain. Anesth Analg.1998;87(6):1360-1366.

Alousi AA, Johnson DC. Pharmacology of the bipyridines: amrinone and milrinone. Circulation. 1986;73(3 Pt 2):III10-24.

Attal N, Cruccu G, Haanpaa M, Hansson P, Jensen TS, Nurmikko T, et al. EFNS guideline on pharmacological treatment of neuropathic pain. Eur J Neurol. 2006;13(11):1153-1169.

Backonja MM, Galer BS. Pain assessment and evaluation of patients who have neuropathic pain. Neurol Clin. 1998;16(4):775-790.

Backonja MM. Use of anticonvulsants for treatment of neuropathic pain. Neurology. 2002;59(5 Suppl):S14-59.

Baumans V, Van Loo PL. How to improve housing conditions of laboratory animals: the possibilities of environmental refinement. Vet J. 2013;195(1):24-32.

Bennett GJ, Xie Y-K. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain. 1988;33(1):87–107.

Bie B, Peng Y, Zhang Y, Pan ZZ. cAMP-mediated mechanisms for pain sensitization during opioid withdrawal. J Neurosci. 2005;25(15):3824-32.

Billie JK, Power I. The mechanism of action of gabapentin in neuropathic pain. Curr Opin Investig Drugs. 2006;7(1):33–39.

Burket LW, Greenberg MS, Glick M. Burkett's Textbook of Oral Medicine. 10th ed. Philadelphia, PA: Lippincott Martin S Greenberg, Michael Glick (Eds), BC Decker Inc., Hamilton, 2003, 658.

Cai K, Nanga RP, Lamprou L, Schinstine C, Elliott M, Hariharan H, et al. The impact of gabapentin administration on brain GABA and glutamate concentrations: a 7T ^{1}H-MRS study. Neuropsychopharmacology. 2012;37(13):2764-71.

Cao FL, Xu M, Wang Y, Gong KR, Zhang JT. Tanshinone IIA attenuates neuropathic pain via inhibition glial activation and immune response. Pharmacol Biochem Behav. 2015;128;1-7.

Carlsson KC, hoem NO, Moberg ER, Mathisen LC. Analgesic effect of dextromethorphan in neuropathic pain. Acta Anaesthesiol Scand. 2004;8(3):328-336.

Chu S, Liu S, Duan W, Cheng Y, Jiang X, Zhu C, et al. The anti-dementia drug candidate, (-)-clausenamide, improves memory impairment through its multi- target effect. Pharmacol Ther. 2016:S0163-7258;00003-00006.

Colloca L, Ludman T, Bouhassira D, Baron R, Dickenson AH, Yarnitsky D, et al. Neuropathic pain. Nat Rev Dis Primers. 2017;3:17002.

Di Trapani G, Mei D, Marra C, Mazza S, Capuano A. Gabapentin in the prophylaxis of migraine: a double-blind randomized placebo-controlled study. Clin Ter. 2000;151(3):145–148.

Dolan S, Nolan AM. Biphasic modulation of nociceptive processing by the cyclic AMP-protein kinase A signalingpathway in sheep spinal cord. Neurosci Lett. 2001;309(3):157–160.

Dooley DJ, Mieske CA, Borosky SK. Inhibition of K (+)-evoked glutamate release from rat neocortical and hippocampal slices by gabapentin. Neuroscience Lett. 2000;280(2):107-10.

Dworkin RH, O'Connor AB, Backonja M, Farrar JT, Finnerup NB, Jensen TS, et al. Pharmacologic management of neuropathic pain. Evidence-based recommendations Robert H. Pain. 2007;132(3):237–251.

Earl CQ, Linden J, Weglicki WB. Biochemical mechanism for the inotropic effect of the cardiotonic drug milrinone. J. Cardiovasc. Pharmacol. 1986;8(4):864-872.

Endoh M, Yamashita S, Taira N. Positive inotropic effect of amrinone in relation to cyclic nucleotide metabolism in the canine ventricular muscle. J Pharmacol Exp Ther. 1982;221(3):775-783.

Erichsen HK, Blackburn-Munro G. Pharmacological characterization of the spared nerve injury model of neuropathic pain. Pain. 2002;98(1-2):151e61.

Ferreira SH, Nakamura M. I. Prostaglandin hyperalgesia, a cAMP/Ca^{2+}dependent process. Prostaglandins. 1979;18(2):179–190.

Field MJ, Oles OJ, Lewis AS, McCleary S, Hughes J, Singh L. Gabapentin (Neurontin) and S-(+)-3-isobutyl GABA represent a novel class of selective antihyperalgesic agents. Br J Pharmacol. 1997;121(8):1513-22.

Flatters SJ, Bennett GJ. Ethosuximide reverses paclitaxel- and vincristine-induced painful peripheral neuropathy. Pain. 2004;109(1-2):150–161.

Fukami k, Sekiguchi F, Kawabata A. Hydrogen sulfide and T-Type Ca 2+ channels in pain processing, neuronal differentiation and neuroendocrine secretion. Pharmacology. 2017;99(3-4):196–203.

Gagnon N, Almahrezi A, Schreier G. Methadone in the treatment of neuropathic pain. Pain Res Manag. 2003;8(3):149-154.

Gee NS, Brown JP, Dissanayake VU, Offord J, Thurlow R, Woodruff GN. The novel anticonvulsant drug, gabapentin (Neurontin), binds to the alpha2delta subunit of a calcium channel. J Biol Chem. 1996;271(10):5768-76.

Goodman CW, Brett AS. Gabapentin and pregabalin for pain is increased prescribing a cause for concern? N Engl J Med. 2017;377(5):411-414.

Guindon J, Hohmann AG. Cannabinoid CB2 receptors: a therapeutic target for the treatment of inflammatory and neuropathic pain. Br J Pharmacol. 2008;153(2):319–334.

Gwak HV, Tan HY, Nam TS, Paik KS, Hulsebosch CE, Leem JW. Activation of spinal GABA receptors attenuates chronic central neuropathic pain after spinal cord injury. J Neurotrouma. 2006;23(7):1111-1124.

Haga N, Kubota M, Miwa Z; Japanese Research Group on Congenital Insensitivity to Pain. Hereditary sensory and autonomic neuropathy types IV and V in Japan. Pediatr Int. 2015;57(1):30-6.

Hofer AM. Interactions between calcium and cAMP signaling. Curr Med Chem. 2012;19(34):5768-73.

Honerjager P, Schafer-Korting M, Reiter M. Involvement of cyclic AMP in the direct inotropic action of amrinone. Biochemical and functional evidence. Naunyn-Schmiedebergs Arch Pharmacol. 1981:381(2);112-20.

Hucho T, Levine JD. Signaling pathways in sensitization: toward a nociceptor cell biology. Neuron. 2007;55(3):365-76.

Hilleman DE, Forbes WP. Role of milrinone in the management of congestive heart failure. DICP. 1989;23(5):357-362.

Jaggi AS, Singh N. Therapeutic target for the management of peripheral nerve injury-induced neuropathic pain. CNS Neurol Disord Drug Target. 2011;10(5):589-609.

Jaggi AS., Jain V, Singh N. Animal models of neuropathic pain. Fundam. Clin Pharmacol. 2011;25(1):1-28.

Jain V, Jaggi AS, Singh N. Ameliorative potential of rosiglitazone in tibial and sural nerve transection-induced painful neuropathy in rats. Pharmacol Res. 2009;59(6):385-392.

Jang JS, Kwon Y, Hwang SM, Kim JH, Yun T, Kim YS, et al. Comparison of the efficacy of a gabapentinoid with an opioid versus an opioid alone in patients with spinal cord stimulation. Pain Physician. 2018;21(4):E429-E434.

Jensen TS, Baron R, Haanpää M, Kalso E, Loeser JD, Rice AS, et al. A new definition of neuropathic pain. Pain. 2011;152(10):2204–2205.

Ji RR, Xu ZZ, Gao YJ. Emerging targets in neuroinflammation-driven chronic pain. Nat Rev Drug Discov. 2014;13(7):533–548.

Kemler MA, de Vet HC. Health-related quality of life in chronic refractory reflex sympathetic dystrophy (complex regional pain syndrome type I). J Pain Symptom Manage. 2000;20(1):68-76.

Khangura RK, Bali A, Kaur G, Singh N, Jaggi AS. Neuropathic pain attenuating effects of perampanel in an experimental model of chronic constriction injury in rats. Biomed Pharmacother. 2017;94:557-563.

Kim KH, Abdi S. Rediscovery of nefopam for the treatment of neuropathic pain. Korean J Pain. 2014;27(2):103-111.

Ko MH, Hsieh YL, Hsieh ST, Tseng TJ. Nerve demyelination increases metabotropic glutamate receptor subtype 5 expression in peripheral painful mononeuropathy. Int J Mol Sci. 2015;16(3):4642-4665.

Konstaninou K, Dunn KM. Sciatica: review of epidemiological studies and prevalence estimate. Spine. 2008;33(22):2464–2472.

Kukkar A, Bali A, Singh N, Jaggi AS. Implications and mechanism of action of gabapentin in neuropathic pain. Arch Pharm Res. 2013;36(3):237-51.

Kukkar A, Singh N, Jaggi AS. Attenuation of neuropathic pain by sodium butyrate in an experimental model of chronic constriction injury in rats. J Formos Med Assoc. 2014;113(12):921-8.

Kukkar A, Singh N, Jaggi AS. Neuropathic pain-attenuating potential of aliskiren in chronic constriction injury model in rats. J Renin Angiotensin Aldosterone Syst. 2013;14(2):116-123.

Laird MA, Gidal BE. Use of gabapentin in the treatment of neuropathic pain. Ann Pharmacother. 2000;34(6):802 –807.

Landmesser U, Drexler H. Update on inotropic therapy in the management of acute heart failure. Curr Treat Options Cardiovasc Med. 2007;9(6):443-449.

Li CQ, Zhang JW, Dai RP, Wang J, Luo XG, Zhou XF. Surgical incision induces anxiety-like behavior and amygdala sensitization: effects of morphine and gabapentin. Pain Res Treat. 2010;2010:705874.

Li SS, Zhang WS, Ji D, Zhou YL, Li H, Yang JL, et al. Involvement of spinal microglia and interleukin-18 in the anti-nociceptive effect of dexmedetomidine in rats subjected to CCI. Neurosci Lett. 2014;560:21–25.

Liou JT, Liu FC, Hsin ST, Yang CY, Lui PW. Inhibition of the cyclic adenosine monophosphate pathway attenuates neuropathic pain and reduces phosphorylation of cyclic adenosine monophosphate response element-binding in the spinal cord after partial sciatic nerve ligation in rats. Anesth Analg. 2007;105(6):1830-7.

Luo WJ, Yang F, Yang F, Sun W, Zheng W, Wang XL et al. Intervertebral foramen injection of ozone relieves mechanical allodynia and enhances analgesic effect of gabapentin in animal model of neuropathic pain. Pain Physician. 2017;20(5):E673-E685.

Luo ZD, Chaplan SR, Higuera ES, Sorkin LS, Stauderman KA, Williams ME, et al. Upregulation of dorsal root ganglion (alpha)2(delta) calcium channel subunit and its correlation with allodynia in spinal nerve-injured rats. J Neurosci. 2001;21(6):1868–1875.

Martin DJ, McClelland D, Herd MB, Sutton KG, Hall MD, Lee K, et al. Gabapentin-mediated inhibition of voltage-activated Ca2+ channel currents in cultured sensory neurones is dependent on culture conditions and channel subunit expression. Neuropharmacology. 2002;42(3):353-66.

Mellick GA, Mellicy LB, Mellick LB. Gabapentin in the management of reflex sympathetic dystrophy. J Pain Symptom Manage.1995;10(4):265–266.

Meng B, Shen LL, Shi XT, Gong YS, Fan XF, Li J, et al. Effects of curcumin on TTX-R sodium currents of dorsal root ganglion neurons in type 2 diabetics rats with diabetic neuropathic pain. Neurosci Lett. 2015:605:59-64.

Navratilova E, Xie JV, Meske D, Qu C, Morimaura K, Okun A, et al. Endogenous opioid activity in the anterior cingulate cortex is required for relief of pain. J Neurosci. 2015;35(18):7264-7271.

Ng GY, Bertrand S, Sullivan R, Ethier N, Wang J, Yergey J, et al. Gamma-aminobutyric acid B receptors with specific heterodimer composition and postsynaptic actions

in hippocampal neurons are targets of anticonvulsant gabapentin action. Mol Pharmacol. 2001;59(1):144–152.

Pan HL, Eisenach JC, Chen SR. Gabapentin suppresses ectopic nerve discharges and reverses allodynia in neuropathic rats. J Pharmacol Exp Ther. 1999;288(3):1026-30.

Ramsay, RE. Clinical efficacy and safety of gabapentin. Neurology. 1994;44(6 Suppl 5):S23-S30.

Rowbotham M, Harden N, Stacey B, Bernstein P, Magnus-Miller L. Gabapentin for the treatment of postherpetic neuralgia: a randomized controlled trial. JAMA. 1998;280(21):1837-1842.

Sarantopoulos C, McCallum B, Kwok WM, Hogan Q. Gabapentin decreases membrane calcium currents in injured as well as in control mammalian primary afferent neurons. Reg Anesth Pain Med. 2002;27(1):47-57.

Shao XM, Sun J, Jiang YL, Liu BY, Shen Z, Fang F, et al. Inhibition of the cAMP/PKA/CREB pathway contributes to the analgesic effects of electroacupuncture in the anterior cingulate cortex in a rat pain memory model. Neural Plast. 2016;2016:5320641.

Shimoyama M, Shimoyama N, Hori Y. Gabapentin affects glutamatergic excitatory neurotransmission in the rat dorsal horn. Pain. 2000;85(3):405-14.

Sluka KA. Stimulation of deep somatic tissue with capsaicin produces long-lasting mechanical allodynia and heat hypoalgesia that depends on early activation of the cAMP pathway. J Neurosci 2002;22(13):5687–5693.

Stanos SP, Galluzzi KE. Topical therapies in the management of chronic pain. Postgrad Med. 2013;125(Suppl 1):25-33.

Sumizono M, Sakakima H, Otsuka S, Terashi T, Nakanishi K, Ueda K et al. The effect of exercise frequency on neuropathic pain and pain-related cellular reactions in the spinal cord and midbrain in a rat sciatic nerve injury model. J Pain Res. 2018;11:281-291.

Surges R, Freiman TM, Feuerstein TJ. Gabapentin increases the hyperpolarization-activated cation current Ih in rat CA1 pyramidal cells. Epilepsia. 2003;44(2):150-6.

Tahmoush AJ, Schwartzman RJ, Hopp JL, Grothusen JR. Quantitative sensory studies in complex regional pain syndrome type 1/RSD. Clin J Pain. 2000;16(4):340-4.

Tamaddonfard E, Farshid AA, Maroufi S, Kazemi-Shojaei S, Erfanparast A, Asri-Rezaei S, et al. Effect of safranal, a constituent of saffron, and vitamin E on nerve functions and histopathology following crush injury of sciatic nerve in rats. Phytomedicine, 2014;21(5):717-723.

Vellani V, Giacomoni C. Gabapentin inhibits protein kinase c epsilon translocation in cultured sensory neurons with additive effects when coapplied with paracetamol (Acetaminophen). Sci World J. 2017;2017:3595903.

Venkatesan R, Ji E, Kim SY. Phytochemicals that regulate neurodegenerative disease by targeting neurotrophins: a comprehensive review. Biomed Res Int. 2015;2015:814068.

Wang Z, Wang J, Qin L, Zhang W. Tongluo Zhitong Prescription alleviates allodynia, hyperalgesia, and dyskinesia in the chronic constriction injury model of rats. Evid Based Complement Alternat Med. 2017;2017:8197281.

Yan K, Gao LN, Cui YL, Zhang Y, Zhou X. The cyclic AMP signaling pathway: Exploring targets for successful drug discovery (Review). Mol Med Rep. 2016;13(5):3715-3723.

Yeh CY, Chung SC, Tseng FL, Tsai YC, Liu YC. Biphasic effects of chronic intrathecal gabapentin administration on the expression of protein kinase C gamma in the spinal cord of neuropathic pain rats. Acta Anaesthesiol Taiwan. 2011;49(4):144-148.

Zeng L, Alongkronrusmee D, van Rijn RM. An integrated perspective on diabetic, alcoholic, and drug-induced neuropathy, etiology, and treatment in the US. J Pain Res. 2017;10:219-228.

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