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Agmatine attenuates neuropathic pain in sciatic nerve ligated rats: Modulation by hippocampal sigma receptors

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ABSTRACT

Present study investigated the influence of the sigma (σ_1 and σ_2) receptors within hippocampus on the agmatine induced antinociception in neuropathic rats. Animals were subjected to sciatic nerve ligation for induction of neuropathic pain and observed the paw withdrawal latency in response to thermal hyperalgesia, cold allodynia and the mechanical hyperalgesia. Intrahippocampal (i.h.) as well as intraperitoneal (i.p.) administration of agmatine attenuated neuropathic pain in sciatic nerve ligated rats. Intrahippocampal administration of σ_1 agonist (+)-pentazocine or σ_2 agonist PB28 sensitized whereas, σ_1 antagonist BD1063 or σ_2 antagonist SM21 potentiated antinociceptive effect of agmatine. The behavioral effects correlated with hippocampal tumor necrosis factor-α (TNF-α) levels observed by western blot analysis. These results suggest that both the σ_1 and σ_2 receptor subunits within hippocampus play an important role in antinociceptive action of agmatine against neuropathic pain.

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1. Introduction

Neuropathic pain is the most common form of chronic pain, caused by primary lesion or dysfunction of peripheral or central nervous system, characterized by allodynia and hyperalgesia (Gilron et al., 2006) and resistant to conventional analgesics. Evidences suggest that an endogenous neuromodulator, agmatine alleviated the neuropathic pain and proposed as potential substance for management of neuropathic pain (Karadag et al., 2003; Onal et al., 2003). Agmatine, an endogenous biogenic amine and NMDA receptor antagonist, is a novel neurotransmitter, synthesized following decarboxylation of L-arginine by arginine decarboxylase (ADC) in brain and other tissues. Besides NMDA receptors, it binds to α_2-adrenoceptors, imidazoline receptors as well as 5-HT receptors with lower affinity and inhibits nitric oxide synthase (NOS) (Reis and Regunathan, 2000; Raasch et al., 2001). Agmatine modulate morphine tolerance, dependence (Wu et al., 2008) and exhibits antiproliferative (Regunathan and Reis, 1997) and neuroprotective (Olmos et al., 1999) properties. Several studies found that both systemic and spinal administration of agmatine demonstrate significant analgesia in animal models of inflammatory pain (Paszcuk et al., 2007) as well as effective in alleviating hyperalgesia and/or allodynia in several chronic neuropathic pain models (Fairbanks et al., 2000; Karadag et al., 2003; Aricioglu-Kartal et al., 2003; Onal et al., 2003). Although agmatine does not appear to be an effective analgesic for acute phasic pain, studies have shown that it can potentiate the analgesic effects of opioids (Bhalla et al., 2011) and attenuate the streptozotocin-induced diabetic neuropathy in rats (Onal et al., 2003). In fact, a recent clinical trial confirm that agmatine is safe and effective for treating pain and improving quality of life in patients suffering from lumbar disk-associated radiculopathy (Keynan et al., 2010).

Several physiological, pharmacological and behavioral evidences suggest that the hippocampal formation play an important role in the affective and motivational components of pain perception. Evidences advocate its importance in the central perception of pain (Soleimannejad et al., 2006, 2007). TNF-α, a key pro-inflammatory cytokine, was substantially increased in hippocampus following peripheral nerve injury (Ren et al., 2011) and its administration within hippocampus produced neuropathic pain like symptoms (Martuscello et al., 2012). Agmatine is widely distributed in several brain regions including pyramidal cells of

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rat hippocampus (Otake et al., 1998). Although, co-localization of the agmatine and sigma receptors (σ_1 and σ_2R) is yet unknown, their abundant presence is identified within the hippocampus, a putative target in neuropathic pain (Betancourt et al., 2012). In rat hippocampal neurons, σ_1 receptors show subcellular presence in neuronal perikarya, mitochondrial membrane, endoplasmic reticulum and dendrites. Further, σ_1 receptors agents not only modulate opioid analgesia but also play an active role in nociception in the absence of opioid drugs in some behavioral models (See Review; Cobos et al., 2008). Although the exact molecular mechanism for the σ_1 receptor modulation of opioidergic pain and neuropathic pain is yet unclear, the plethora of evidences established the role σ_1 receptors in analgesia (Cobos et al., 2008; Maurice and Su, 2009).

Hence we investigated the possible interaction of agmatine with sigma (σ_1 and σ_2R) receptors within hippocampus in sciatic nerve ligated rats and its correlation with TNF-α.

2. Materials and Methods

2.1. Subjects

Adult Sprague-Dawley rats (220–260 g; NIN, Hyderabad, India) of either sex were housed in acrylic cages (24 × 17 × 12 cm) under controlled environmental conditions (24 ± 1 °C, 50 ± 20% Relative-Humidity), maintained at 12:12 h light/dark cycle (lights on 07:00–19:00 h). Food and water were available ad libitum. All experimental procedures employed were approved by Institutional Animal Ethical Committee of S. K. B. College of Pharmacy, Kamptee, (M.S.) India and carried out under strict compliance with Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Ministry of Environment and Forests; Government of India; New Delhi. Every possible effort was made to reduce the suffering of animals during experimental procedure.

2.2. Drugs and Solutions

Agmatine sulfate, (+)-Pentazocine HCl, PB28 dihydrochloride, L-arginine and D-arginine were obtained from Sigma-Aldrich Chemicals, St. Louis, USA. SM21 maleate and BD1063 dihydrochloride were obtained from Tocris Bioscience, Missouri, USA. All drugs were dissolved in aCSF (140 mM NaCl, 3.35 mM KCl, 1.15 mM $MgCl_2$, 1.26 mM $CaCl_2$, 1.2 mM Na_2HPO_4, and 0.3 mM NaH_2PO_4, pH 7.4) just before the experiments and infused via cannulae implanted in CA1 hippocampal area in volume of 1 μl/side bilaterally while, intraperitoneal (i.p.) administration of drugs were made in sterile saline in volume of 10 ml/kg.

2.3. Intra-hippocampal cannulae implantation

The intra-hippocampal (i.h.) cannulae implantation was performed to infuse drugs in CA1 hippocampal area according to brain atlas (Paxinos and Watson, 2005). Briefly, rats were anesthetized with an i.p. injection of ketamine HCl (50 mg/kg) (Troikaa Pharmaceutical Ltd, India) and xylazin HCl (10 mg/kg) (Indian Immunologicals Ltd., India). The 30-gauge stainless steel guide cannulae were implanted into the CA1 area of hippocampus using stereotaxic coordinates, −3.2 mm posterior, ±2.0 mm bilateral to midline and 3.0 mm ventral from bregma. A 28 gauge stainless steel dummy cannulae was inserted to occlude the guide cannulae when not in use. The procedure for cannulation surgery and its verification was similar as described earlier (Taksande et al., 2011).

2.4. Induction of neuropathic pain by partial sciatic nerve ligation

Sciatic nerve ligation model was used to induce neuropathic pain according to the protocol previously described (Seltzer et al., 1990). Rats were anesthetized with i.p. injection of ketamine HCl and xylazin HCl. Common sciatic nerve was exposed at the middle level of left thigh by blunt dissection through biceps femoris. Proximal to sciatic trifurcation, about 7 mm of nerve was freed from adhering tissue and 4 ligatures (4.0 chromic gut sutures, Suture India Ltd.) were tied loosely around it with about 1 mm spacing without disturbing the blood vessel. The skin was sutured using 5–0 silk suture (Suture India Ltd.) and cleaned with povidone iodine solution. The rats were treated prophylactically with oxytetracyclin (50 mg/kg, i.p.) and neosporin to avoid infection.

2.5. Evaluation of neuropathic pain-related behavior

Neuropathic pain was evaluated 7 days after the surgery and test sessions were carried out in sound proof room under controlled experimental conditions. Rats were randomly assigned to different groups (n=5–6) and received i.h./i.p. administration of drug or vehicle 15/30 min before the evaluation of individual rat for thermal hyperalgesia, cold allodynia and mechanical hyperalgesia. Sham operated rats served as control.

2.5.1. Thermal hyperalgesia

Thermal hyperalgesia refers to an increased sensitivity to heat stimuli assessed-by planter test based on withdrawal latency of injured ligated paw from heated metal or glass surface (Hargreaves et al., 1988). Apparatus consist of a transparent cylinder of glass [25 cm (diameter) X 40 cm (height)] with stainless steel metal plate at base electrically heated by a coil present below the plate. Rat was kept on heated surface of the plate maintained at 55 °C ± 1 °C. The latency (s) until the rat jumped or licked its hind paw was registered (cut-off time, 15 s).

2.5.2. Noxious cold allodynia

Allodynia is a response to a normally nonpainful stimulus. Withdrawal latencies from noxious cold stimulus in neuropathic rats were assessed as described by Cahill and Coderre (2002). Open ended clear plexiglass cylinder was placed in cold water bath maintained at 1 °C with a depth of 1 cm. Rats were placed into the bath and the latency to respond was measured. Neuropathic rats responded by elevating their injured paw out of contact with the water. A cut-off period of 15 s was inflicted to prevent tissue damage. Rats were removed from the cold stimulus after response or cut-off time.

2.5.3. Mechanical hyperalgesia

Pin prick test using a safety pin was performed to evaluate mechanical hyperalgesia. The lateral plantar surface of the hind-paw was touched with the point of safety pin at intensity sufficient to produce a reflex withdrawal response in normal unoperated rats and insufficient to penetrate the skin. Paw withdrawal latency (s) was recorded. Normal rats exhibited very short paw withdrawal latency and it was set arbitrarily as 0.5 s. A cut-off time of 15 s was implied (Gonzalez et al., 2000).

2.6. Western blot analysis

Hippocampi were homogenized in ice-cold lysis buffer as described previously (Shukla et al., 2011). Samples containing 40 μg protein were electrophoresed on 12% (w/v) SDS polyacrylamide gel and transferred onto polyvinylidene difluoride membranes for use with the antibody

against TNF-α (1:1000, Cell Signaling Technology, Danvers, MA). β-actin protein levels were measured in the same membranes using a monoclonal antibody (1:10,000, Sigma, St. Louis, MO). The optical density of each protein was normalized to the corresponding β-actin signal using Image J software (NIH).

2.7. Experimental design

2.7.1. Effect of agmatine (i.p. and i.h.), sigma receptor agonists, [(+)-pentazocine (σ1) and PB28 (σ2)] and antagonists [BD1063 (σ1) and SM21 (σ2)] on neuropathic pain

Different group of sciatic nerve ligated rats were injected with saline (1 ml/kg, i.p.), aCSF (1 μl/side/rat, i.h.), agmatine (40–120 mg/kg, i.p.) or (2.5–10 μg/side/rat, i.h.) 30 or 15 min i.p. or i.h. respectively prior to their evaluation for thermal hyperalgesia, mechanical hyperalgesia and cold allodynia as described in Section 2.5.

Separately, ligated rats were injected with σ1 receptor agonist, (+)-pentazocine (10–40 μg/side/rat, i.h.), antagonist, BD1063 (20–80 μg/side/rat, i.h.), σ2 receptor agonist, PB28 (1–10 μg/side/rat, i.h.), antagonist, SM21 (1–5 μg/side/rat, i.h.) or aCSF (1 μl/side/rat, i.h.), 15 min before being tested for thermal hyperalgesia, mechanical hyperalgesia and cold allodynia.

2.7.2. Simultaneous administration of subeffective doses of sigma receptor antagonists [BD1063 (σ1) and SM21 (σ2)] along with agmatine in neuropathic rats

Neuropathic rats received BD1063 (20 μg/side/rat, i.h.) or SM21 (1 μg/side/rat, i.h.) or aCSF (1 μl/side/rat, i.h.) 5 min before agmatine (2.5 μg/side/rat, i.h.) or aCSF (1 μl/side/rat, i.h.) injection. Paw withdrawal latency was determined in thermal hyperalgesia, mechanical hyperalgesia or cold allodynia 15 min after the agmatine injection.

2.7.3. Western blot analysis of hippocampal TNF-α levels in agmatine (i.h.), sigma receptor agonists and antagonists treated neuropathic rats

Selected groups of rats ($n=4$) from the above treatment groups were included for hippocampal western blot analysis of TNF-α levels. In first set of experiment, immediately after assessment of neuropathic pain, hippocampal tissue from sham operated or ligated rats injected with aCSF (1 μl/side/rat, i.h.), agmatine (10 μg/side/rat, i.h.), σ1 receptor agonist, (+)-pentazocine (40 μg/side/rat, i.h.), antagonist, BD1063 (80 μg/side/rat, i.h.), σ2 receptor agonist, PB28 (10 μg/side/rat, i.h.), antagonist, SM21 (5 μg/side/rat, i.h.) were processed for western blot analysis as discussed in Section 2.6.

In second set, sham operated or ligated rats injected with subeffective doses of BD1063 (20 μg/side/rat, i.h.), SM21 (1 μg/side/rat, i.h.) or aCSF (1 μl/side/rat, i.h.) alone or 5 min before subeffective dose of agmatine (2.5 μg/side/rat, i.h.) or aCSF (1 μl/side/rat, i.h.) injection were sacrificed immediately after neuropathic pain assessment and hippocampal excision was performed.

2.8. Data analysis

All data were presented as the mean ± S.E.M. Comparison of paw withdrawal response to thermal hyperalgesia, cold allodynia and mechanical hyperalgesia in sham operated and sciatic nerve ligated groups were carried out by unpaired student's t-test. Dose dependent study of different drugs was analyzed by one-way ANOVA followed by post-hoc Dunnett test. For multiple comparisons data were analyzed by one-way ANOVA followed by post-hoc Newman–Keuls test. Data of some (< 15%) animals, where guide cannulae were found incorrectly placed was not considered for statistical analysis.

3. Results

3.1. Intraperitoneal and intrahippocampal administration of agmatine attenuates neuropathic pain

Paw withdrawal latency in thermal hyperalgesia [$t=23.78$, $Df=10$, $P<0.001$], cold allodynia [$t=12.30$, $Df=10$, $P<0.001$],

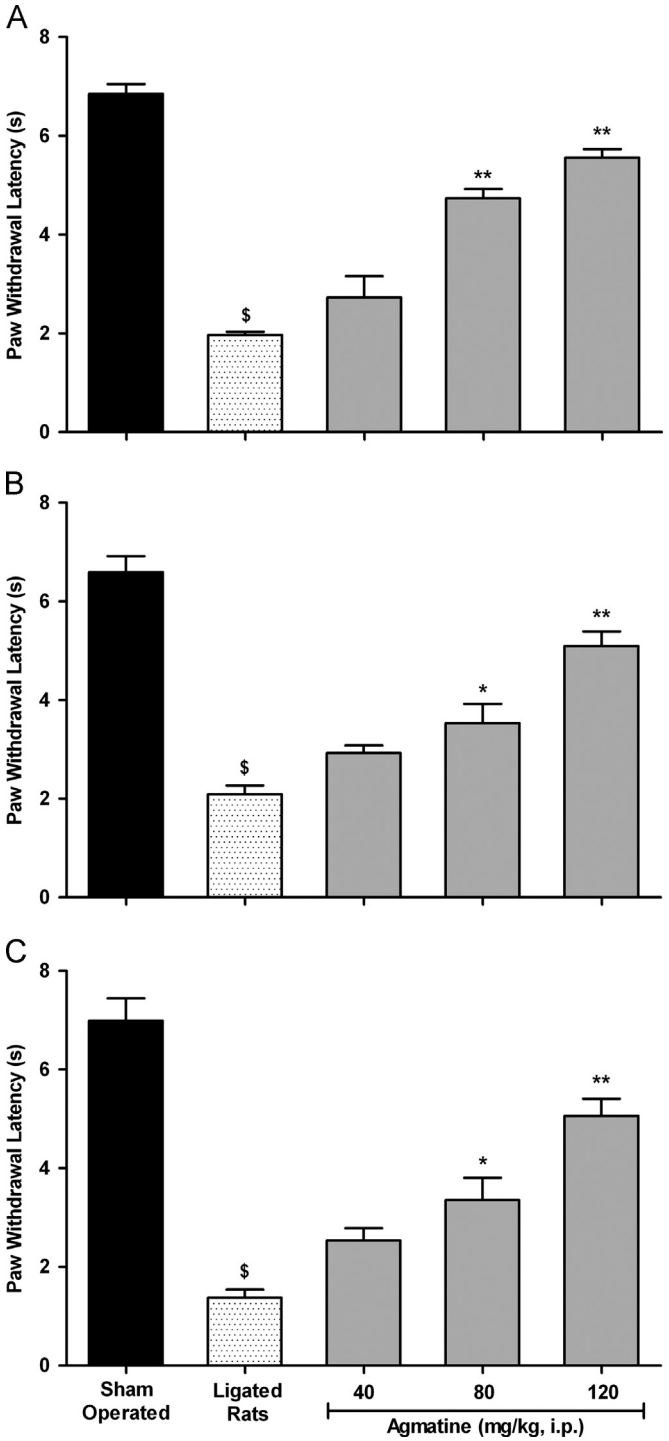

Fig. 1. Effect of agmatine on (A) thermal hyperalgesia, (B) cold allodynia and (C) mechanical hyperalgesia procedures in neuropathic rats. Each group of rats were treated with either saline (1 ml/kg, i.p.) or agmatine (40–120 mg/kg, i.p.) 30 min prior to assessment of thermal hyperalgesia, cold allodynia or mechanical hyperalgesia. Each bar represents the mean ± S.E.M. ($n=5$–6). Data analyzed by one-way ANOVA followed by post-hoc mean comparisons by Dunnett test. \$ $P<0.001$ vs. Sham operated rats, $^{*}P<0.01$, $^{**}P<0.001$ when compared against sciatic nerve ligated neuropathic rats.

and mechanical hyperalgesia [$t=11.69$, $Df=10$, $P<0.001$] were significantly decreased in sciatic nerve ligated neuropathic animals as compared to sham operated rats, suggesting that the neuropathic pain with hyperalgesia and allodynia was induced successfully by partial sciatic nerve ligation (Fig. 1).

Intraperitoneal administration of agmatine (80–120 mg/kg) attenuated the neuropathic pain as indicated by significant increase in the paw withdrawal latencies in thermal hyperalgesia [F (4, 29)= 60.53, $P<0.001$], cold allodynia [F (4, 29)=46.40, $P<0.001$] and mechanical hyperalgesia [F (4, 29)=43.41, $P<0.001$] in neuropathic rats as compared to saline treated ligated rats. Post hoc Dunnett test showed that agmatine 80 mg/kg ($P<0.001$, $P<0.01$, $P<0.01$) and 120 mg/kg ($P<0.001$, $P<0.001$, $P<0.001$) but not 40 mg/kg significantly increased the paw withdrawal latency in thermal hyperalgesia, cold allodynia and mechanical hyperalgesia evaluation tests respectively as compared to saline treated ligated rats.

Similarly, microinjection of agmatine (5 and 10 μg/side/rat, i.h.) also showed significant effect on thermal hyperalgesia [F (4, 28)= 71.99, $P<0.001$], cold allodynia [F (4, 28)=25.14, $P<0.001$] and mechanical hyperalgesia [F (4, 28)=27.82, $P<0.001$] in neuropathic rats as compared to aCSF treated ligated rats (Fig. 2). Post hoc analysis revealed that agmatine at a dose of 5 μg/rat, i.h. ($P<0.01$, $P<0.05$ and $P<0.01$) as well as 10 μg/side/rat, i.h. ($P<0.001$, $P<0.001$ and $P<0.001$) elevated the pain threshold in all three procedures respectively as compared to the neuropathic rats. However, low dose of agmatine (2.5 μg/side/rat, i.h.), failed to influence the basal paw withdrawal latency in ligated animals.

3.2. *Effect of sigma receptor agonists, [(+)-pentazocine (σ1) and PB28 (σ2)] and antagonists [BD1063 (σ1) and SM21 (σ2)] in neuropathic rats*

We found that σ1 receptor agonist, (+)-pentazocine (20–40 μg/side/rat, i.h.) and σ2 receptor agonist, PB-28 (1–10 μg/side/rat, i.h.) treated ligated rats dose dependently reduced the paw withdrawal latency (Fig. 3). One way ANOVA suggests significant effect of (+)-pentazocine (10–40 μg/side/rat, i.h.) [F (3, 24)=7.32, $P<0.01$] as well as PB-28 (1–10 μg/side/rat, i.h.) [F (3, 24)=11.74, $P<0.001$] on paw withdrawal latency in thermal hyperalgesia. Post hoc analysis indicated that (+)-pentazocine, 20 μg ($P<0.01$) and 40 μg/side/rat ($P<0.01$) as well as PB-28, 5 μg ($P<0.05$) and 10 μg/side/rat, i.h. ($P<0.001$) significantly decreased the paw withdrawal latency in thermal hyperalgesia (Fig. 3a). Further, selected doses of (+)-pentazocine (40 μg/side/rat) ($P<0.01$ and $P<0.01$ resp.) and PB-28 (10 μg/rat) ($P<0.001$ and $P<0.01$ resp.) also significantly reduced the paw withdrawal latency in cold allodynia [Pentazocine-F (3, 24)=5.31, $P<0.01$ and PB-28-F (3, 24)=8.32, $P<0.001$; Fig. 3b] and mechanical hyperalgesia [Pentazocine-F (3, 24)=5.78, $P<0.01$ and PB-28-F (3, 24)=6.21, $P<0.01$; Fig. 3c] respectively (One way ANOVA post hoc Dunnett mean comparisons). Lower doses of (+)-pentazocine and PB-28 failed to modify the basal reaction latency in cold allodynia and mechanical hyperalgesia as compared to aCSF treated neuropathic rats.

Administration of σ1 receptor antagonist, BD-1063 [40 and 80 but not 10 μg/side/rat] significantly increased the paw withdrawal latencies in thermal hyperalgesia [F (3, 22)=24.35, $P<0.001$], cold allodynia [F (3, 22)=12.46, $P<0.001$] and mechanical hyperalgesia [F (3, 22)=17.54, $P<0.001$] in neuropathic rats as compared to aCSF treated ligated rats (Fig. 3). Post hoc analysis demonstrated that BD1063 administered in the dose of 40 μg/side/rat, i.h. ($P<0.001$, $P<0.01$ and $P<0.01$)] and 80 μg/rat, i.h. ($P<0.001$, $P<0.001$ and $P<0.001$) significantly altered the paw withdrawal latencies in thermal hyperalgesia, cold allodynia and mechanical hyperalgesia respectively. Similarly i.h. microinjections of σ2 receptor antagonist, SM21 (5 but not 1 and 2.5 μg/side/rat) significantly increased the paw withdrawal latencies in thermal hyperalgesia [F (3, 23)=4.66, $P<0.05$], cold allodynia [F (3, 23)= 5.85, $P<0.01$] and mechanical hyperalgesia [F (3, 23)=6.15, $P<0.01$] in sciatic nerve ligated neuropathic animals as compared to control rats (Fig. 3). Post hoc comparison indicated that SM21 5 μg/side/rat, i.h. significantly increased the paw withdrawal latencies in the tests of thermal hyperalgesia ($P<0.05$), cold allodynia ($P<0.01$) and mechanical hyperalgesia ($P<0.01$).

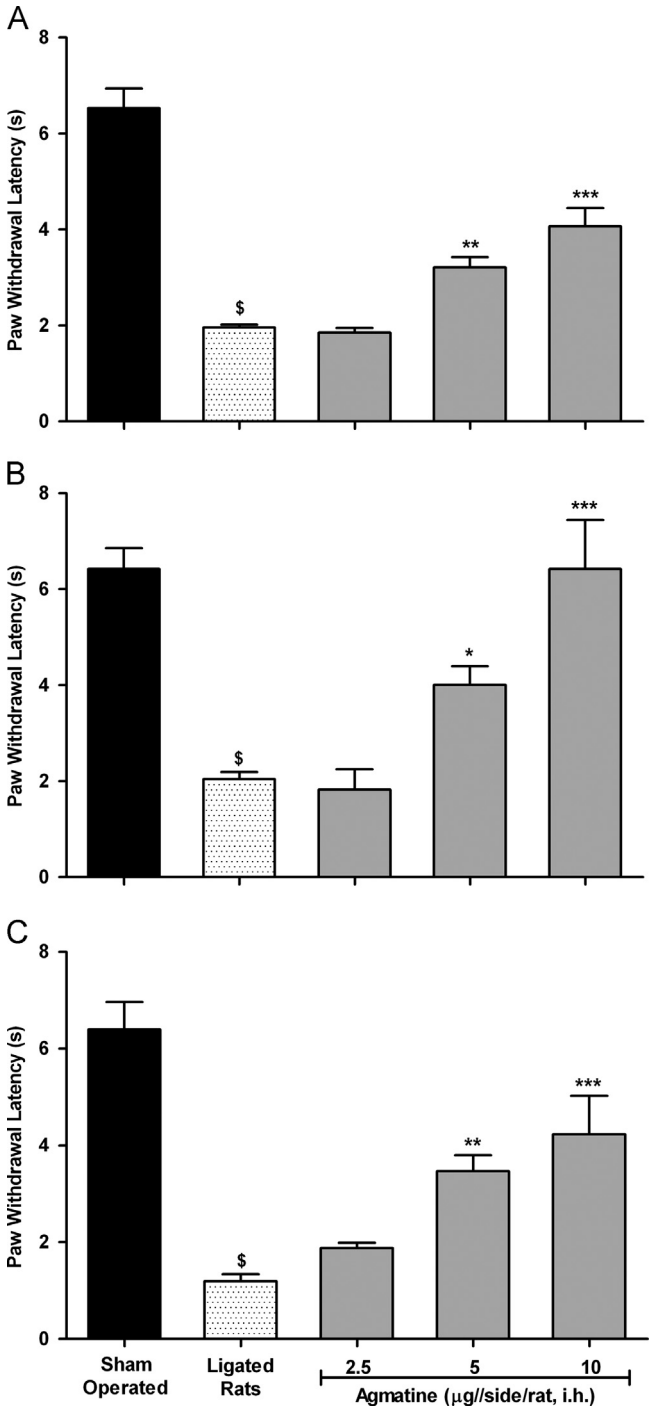

Fig. 2. Effect of intrahippocampal (i.h.) administration of agmatine on (A) thermal hyperalgesia, (B) cold allodynia and (C) mechanical hyperalgesia in neuropathic rats. Rats were treated with either aCSF (1 μl/side/rat, i.h.) or agmatine (2.5–10 μg/side/rat, i.h.) 15 min prior to assessment of thermal hyperalgesia, cold allodynia or mechanical hyperalgesia. Each bar represents the mean ± S.E.M. ($n=5$–6). Data analyzed by one-way ANOVA followed by post-hoc Dunnett test, \$ $P<0.001$ vs. Sham operated rats, *$P<0.05$, **$P<0.01$, ***$P<0.001$ when compared against sciatic nerve ligated neuropathic rats.

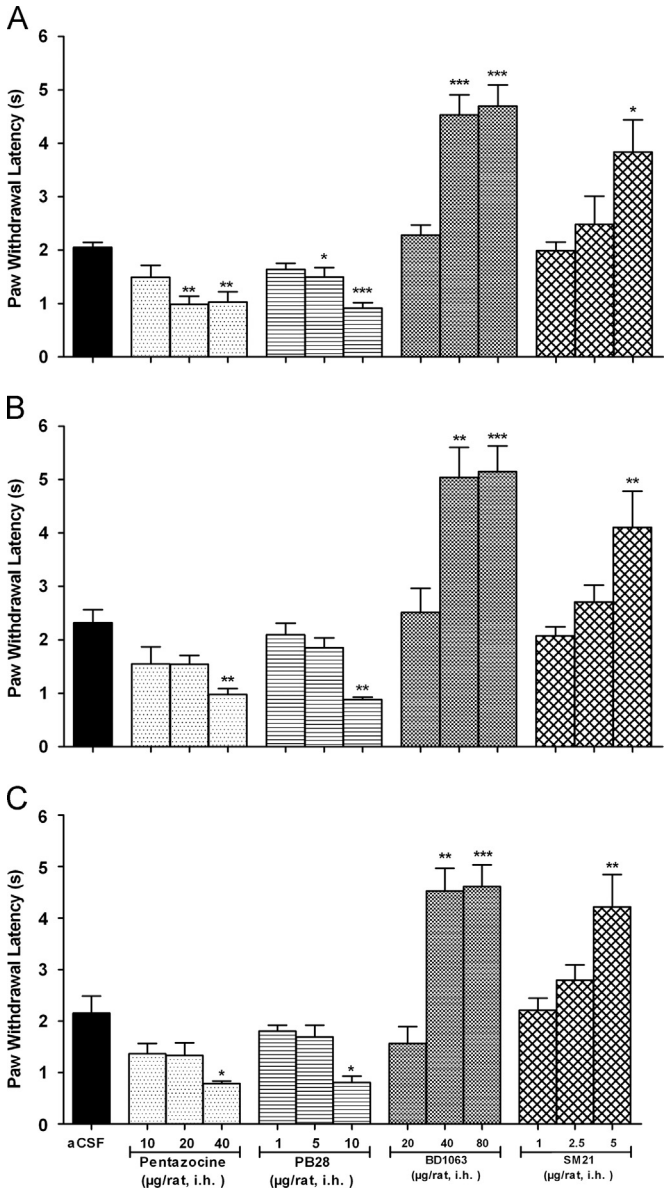

Fig. 3. Dose related effect of σ1 receptor agonist, (+)-pentazocine, σ2 receptor agonist, PB28, σ1 receptor antagonist, BD1063 and σ2 receptor antagonist, SM21 on (A) thermal hyperalgesia, (B) cold allodynia and (C) mechanical hyperalgesia in sciatic nerve neuropathic rats. Each group of rats were treated with either aCSF (1 µl/side/rat, i.h.) or (+)-pentazocine (10–40 µg/side/rat, i.h.) or PB28 (1–10 µg/side/rat, i.h.) or BD1063 (20–80 µg/side/rat, i.h.) or SM21 (1–5 µg/side/rat, i.h.) 15 min prior to assessment of neuropathic pain. Each bar represents the mean (n= 5–6) ± S.E.M. *$P<0.05$, **$P<0.$ 01, ***$P<0.001$ when compared against aCSF treated sciatic nerve ligated neuropathic rats (One-way ANOVA followed by post-hoc Dunnett test).

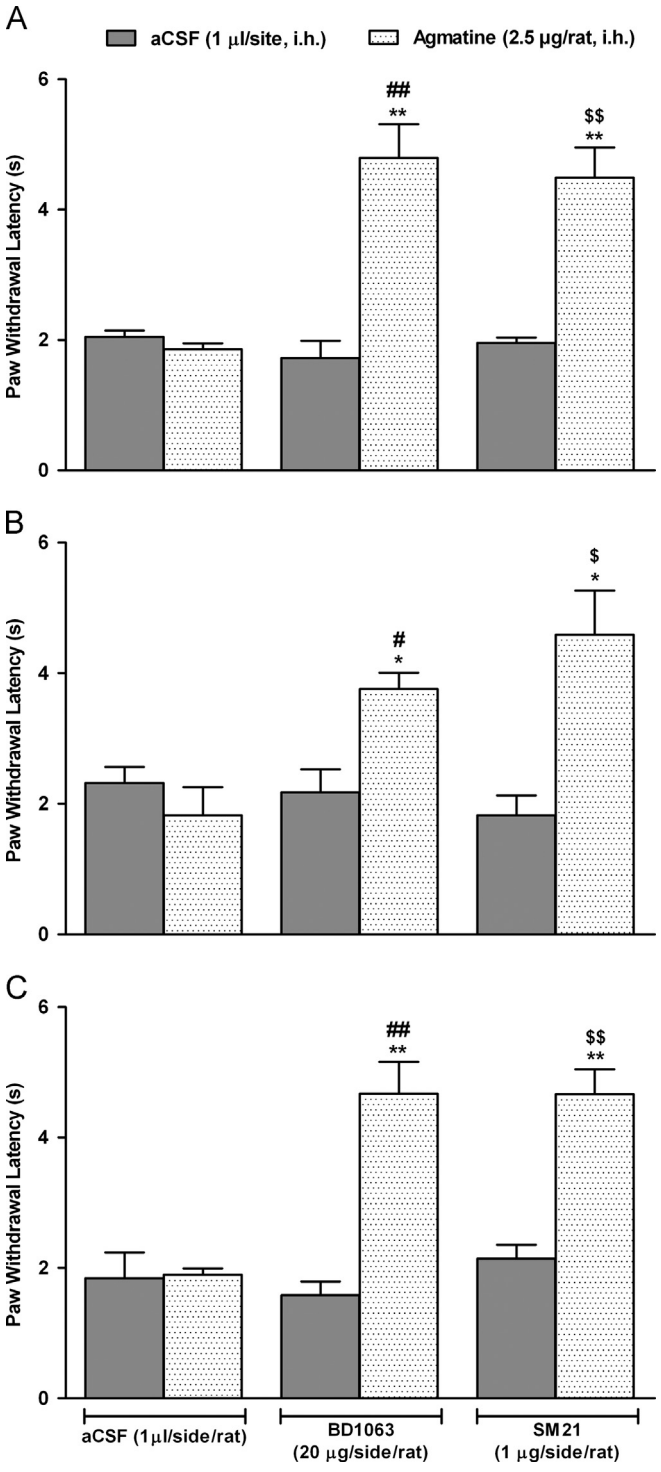

Fig. 4. Effect of σ1 receptor antagonist, BD1063 (20 µg/side/rat) or σ2 receptor antagonist, SM21 (2.5 µg/side/rat) on antinociceptive effect of agmatine in neuropathic rats. Each group of neuropathic rats were pre-treated with either aCSF (1 µl/side/rat, i.h.) or BD1063 (20 µg/side/rat, i.h.) or SM21 (1 µg/rat, i.h.) 15 min prior to the administration of agmatine (2.5 µg/rat, i.h.) or aCSF (1 µl/side/rat, i.h.) and were accessed for thermal hyperalgesia, cold allodynia and mechanical hyperalgesia. Each bar represents the mean and ± S.E.M (n=5=6). *$P<0.01$, **$P<0.001$ Vs agmatine control, # $P<0.01$ ## $P<0.001$ Vs BD1063 treatment, $-$P<0.01, $ $-$P<0.001 vs. PB28 treated neuropathic rats. Data analyzed by one-way ANOVA followed by post-hoc Newman–Keuls test.

SM21 administered in lower doses (1 and 2.5 µg/side/rat) did not influence paw withdrawal latencies (Fig. 3).

3.3. σ1 and σ2 receptor antagonists augmented the antinociceptive effect of agmatine in neuropathic rats

Administration of subthreshold doses of σ1 receptor antagonist, BD1063 or σ2 receptor antagonist, SM21, 5 min before subthreshold dose of agmatine potentiated its antinociceptive effect in neuropathic rats (Fig. 4). Pretreatment of subeffective doses of both BD1063 (20 µg/side/rat, i.h.) and agmatine (2.5 µg/side/rat, i.h.) significantly increased paw withdrawal latency in thermal hyperalgesia [F (3, 22)=33.71, $P<0.001$], cold allodynia [F (3, 22)=9.89, $P<0.001$] and mechanical hyperalgesia [F (3, 22)=25.74, $P<0.001$] tests in neuropathic rats. Moreover, i.h. administration of subthreshold doses of SM 21 (1 µg/side/rat, i.h.) in combination with agmatine (2.5 µg/rat, i.h.)

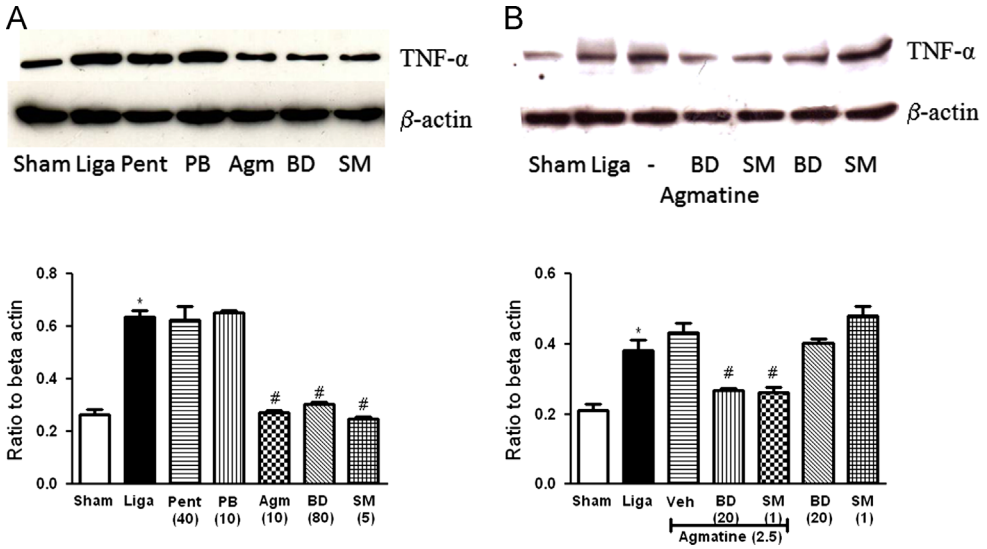

Fig. 5. Effect of sigma receptor (σ1 and σ2) agonists, antagonists and agmatine on hippocampal TNF-α expression. (A) Increased hippocampal TNF-α expression was found in ligated rats (Lane 2) treated with effective doses of σ1 receptor agonist (+)-pentazocine (40 μg/side/rat) (Lane 3), σ2 receptor agonist PB28 (10 μg/side/rat) (Lane 4), while TNF-α expression was decreased in ligated rats treated with effective doses of agmatine (10 μg/rat, i.h.) (Lane 5), σ1 receptor antagonist, BD1063 (80 μg/side/rat) (Lane 6) or σ2 receptor antagonist, SM21 (5 μg/side/rat) (Lane 7). (B) Increased hippocampal TNF-α expression in ligated rats (Lane 2) remained unaffected by sub-effective dose of agmatine (10 μg/rat, i.h.) (Lane 3), while it was reduced when combined with sub-effective dose of BD1063 (80 μg/side/rat) (Lane 4) as well as σ2 receptor antagonist, SM21 (5 μg/side/rat). The alone effect of sub-effective dose of σ1 receptor antagonist, BD1063 (80 μg/side/rat) (Lane 6) and σ2 receptor antagonist, SM21 (5 μg/side/rat) (Lane 7) are also shown here. Doses in parenthesis are given in mg/kg. Each bar represents the mean and ± S.E.M. (n=4–6). *$P<0.01$, **$P<0.001$ vs. agmatine control, # $P<0.01$ ## $P<0.001$ Vs BD1063 treatment, $-$P<0.01, $$-$P<0.001$ vs. PB28 treated neuropathic rats. Data analyzed by one-way ANOVA followed by post-hoc Newman–Keuls test.

also enhanced the antinociceptive effect of agmatine in thermal hyperalgesia [F (3, 23)=52.94, $P<0.001$], cold allodynia [F (3, 23)= 15.50, $P<0.001$] and mechanical hyperalgesia [F (3, 23)=22.26, $P<0.001$] in neuropathic rats. Post hoc analysis by Dunnett test demonstrated that BD1063 ($P<0.001$, $P<0.01$, $P<0.001$) or SM21 ($P<0.001$, $P<0.001$, $P<0.001$) pretreatment significantly increased antinociception in agmatine treated rats in thermal hyperalgesia, cold allodynia and mechanical hyperalgesia respectively as compared to agmatine (2.5 μg/side/rat, i.h.) alone treated neuropathic rats.

3.4. Sigma receptor agonists, [(+)-pentazocine (σ1) and PB28 (σ2)] increases while, antagonists [BD1063 (σ1) and SM21 (σ2)] and agmatine decreases hippocampal TNF-α levels

TNF-α level was found to be increased in the hippocampus of ligated animals as compared to the sham operated rats, suggesting that the neuropathic pain increases TNF-α levels (Fig. 5). These levels remained unchanged in ligated rats treated with effective dose of agonists, (+)-pentazocine (σ1) as well as PB28 (σ2) while antagonists BD1063 (σ1) and SM21 (σ2) treated ligated animals showed significant reduction in hippocampal TNF-α levels.

Further, we also found that the neuropathic rats treated with alone subeffective dose of antagonists BD1063 (σ1) and SM21 (σ2) did not showed ($P>0.05$) any changes in TNF-α levels as compared to ligated rats. However, neuropathic rats showed significant decrease ($P<0.0001$) in TNF-α levels after concomitant treatment of these agents with agmatine in subeffective doses [F (6, 27)= 23.48, $P<0.0001$]. This suggested that the alleviation of neuropathic pain by sigma receptor antagonists BD1063 and SM21 and agmatine was associated with decrease in TNF-α levels within hippocampus.

4. Discussion

Results suggest that, agmatine dose dependently increased the paw withdrawal latency in thermal hyperalgesia, cold allodynia and mechanical hyperalgesia procedures after intrahippocampal (1–10 μg/rat) as well as intraperitoneal (80–120 mg/kg) administration. We also found that its precursor, L-arginine (10–20 μg/side/rat) (Unpublished observation), showed antinociceptive activity in neuropathic rats which further supports the antinociceptive potential of agmatine. Indeed, earlier studies demonstrated the role of agmatine in attenuating neuropathic pain threshold after peripheral and central administration in rats (Onal et al., 2003; Aricioglu-Kartal et al., 2003; Paszcuk et al., 2007). In addition, intrathecal administration of agmatine reversed long-lasting hypersensitivity induced by neuropathic pain in rats (Fairbanks et al., 2000). However, the underlying mechanisms and neurotransmitters system involved in the antinociception activity of agmatine is poorly understood.

Extensive research suggests that sigma receptor plays potential role in mechanism and modulation of neuropathic pain signaling. Recent literature projects drugs acting on sigma receptor as rational targets in the treatment of neuropathic pain (See Review; Cobos et al., 2008; Maurice and Su, 2009). It is proposed that sigma-1 receptors regulate activity-induced spinal sensitization and neuropathic pain after peripheral nerve injury (Cobos et al., 2008; de la Puente et al., 2009). Activation of spinal σ_1 receptors by its agonists also attenuated morphine related antinociception. Interestingly, σ_1 receptor inhibition potentiates morphine-induced mechanical analgesia but not its acute side effects (Sanchez-Fernandez et al., 2013). On the contrary, σ_1 receptor antagonist BD1047 as well as NE100 decreased neuropathic pain threshold in rats (Kim et al., 2008). Moreover, BD1047 dose-dependently reduced mechanical allodynia and hyperalgesia in neuropathic rats, and also pain behaviors in nociceptive formalin test (Roh et al., 2008). Although the direct evidences for involvement of σ_2 receptors in antinociception are unavailable, recent in vitro studies have shown some efficacy of σ_2 agents against neurogenic pain (Diaz et al., 2012). In parallel to above studies, we found that intrahippocampal injections of σ_1 receptor antagonist BD1063 (5–50 μg/rat) and σ_2 receptor antagonist SM21 (1–5 μg/rat) increased while σ_1 receptor agonist (+)-pentazocine (10–40 μg/rat) and σ_2 receptor agonist PB28 (1–10 μg/rat) decreased paw withdrawal latency in all the three procedures like thermal hyperalgesia, cold allodynia and mechanical hyperalgesia in rats.

Evidences suggest a possible association between the hippocampus and neuropathic pain. As mentioned elsewhere, agmatine endogenously modulates and exogenously attenuates neuropathic pain. It is also suggested that imidazolines including agmatine may interact with σ_2 binding sites (Molderings et al., 1996). In addition, high densities of σ receptors are present in the hippocampus (Palacios et al., 2003) and also found to be localized in Schwann cells of rat sciatic nerve (Palacios et al., 2004). In this context, co-administration of sub-threshold doses of agmatine and σ_1 receptor antagonist BD1063 or σ_2 receptor antagonist SM21 within the hippocampus significantly potentiated the effect of agmatine on paw withdrawal latency. Thus, it can be suggested that both σ_1 and σ_2 receptors within hippocampus are at least partly involved in antinociceptive effect of agmatine in neuropathic rats. Moreover, this is the first study to demonstrate the behavioral evidence for involvement of σ_2 receptors in neuropathic pain. Further, it has been suggested that σ_1 ligands can modulate several neurotransmitter systems through NMDA receptors (Cobos et al., 2008), and this is an important aspect in the modulation of neurotransmission by σ_1 ligands. Interestingly, agmatine shows several pharmacological actions via NMDA receptors (Reis and Regunathan, 2000). Hence, the interaction of NMDA-sigma receptors in the antinociceptive activity of agmatine also cannot be denied.

Several reports suggest the involvement of TNF-α in neuropathic pain such that the specific elevation of TNF in the hippocampus induces pain-like symptoms (Martuscello et al., 2012) whereas over-production of TNF-α following peripheral nerve injury might lead to neuropathic pain (Ren et al., 2011). In this context, western blot analysis showed that agmatine, BD1063 and SM21, sigma (σ_1 and σ_2) receptor antagonist resp. treatment showed the low intensity bands of TNF-α as compared to ligated control rats indicating the reduction in TNF-α levels within hippocampus. Indeed, SSR125329A, a high affinity sigma receptor ligand exhibiting high affinity for sigma(1) and sigma(2) receptors inhibited TNF-α synthesis (Bourrié et al., 2002). Whereas, agmatine inhibited the TNF-α production of retinal ganglionic cells in hypoxic condition (Hong et al., 2008).

In summary present study confirms the role of sigma (σ_1 and σ_2) receptors within hippocampus in the antinociceptive effect of agmatine against the neuropathic pain in rats. Further, we also found that sigma (σ_1 and σ_2) receptors agents along with or without agmatine alters hippocampal TNF-α levels. Thus, present study proposes sigma (σ_1 and σ_2) agents as possible alternative to treat the neuropathic pain. However, future studies are required to prove their clinical efficacy.

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