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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.

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