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Chronic administration of [Pyr^1] apelin-13 attenuates neuropathic pain after compression spinal cord injury in rats

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ABSTRACT

Apelin is an endogenous ligand for apelin receptor (APJ) with analgesic effect on visceral, analgesic and proanalgesic influences on acute pains in animal models. The purpose of this study was to determine the possible analgesic effects of [Pyr^1] apelin-13 on chronic pain after spinal cord injury (SCI) in rats. Animals were randomly divided into three major groups as intact, sham and SCI. The SCI group randomly allocated to four subgroups as no treatment, vehicle-treatment (normal saline: 10 μl, intrathecally) and two subgroups with intrathecal injection (i.t) of 1 μg and 5 μg of [Pyr^1] apelin-13. After laminectomy at T6-T8 level, spinal cord compression injury was induced using an aneurysm clip. Vehicle or [Pyr^1] apelin-13 injected from day1 post SCI and continued for a week on a daily basis. Pain behaviors and locomotor activity were monitored up to 8 weeks. At the end of the experiments, intracardial paraformaldehyde perfusion was made under deep anesthesia in some animals for histological and immunohistochemistry evaluations. Western blot technique was also done to detect caspase-3 in fresh spinal cord tissues. SCI decreased nociceptive thresholds and locomotor scores. Administration of [Pyr^1] apelin-13 (1 μg and 5 μg) improved locomotor activity and reduced pain symptoms, cavity size and caspase-3 levels. Results showed long-term beneficial effects of [Pyr^1] apelin-13 on neuropathic pain and locomotion. Therefore, we may suggest [Pyr^1] apelin-13 as a new option for further neuropathic pain research and a suitable candidate for ensuing clinical trials in spinal cord injury arena.

1. Introduction

Neuropathic pain following spinal cord injury (SCI) is a significant clinical problem worldwide (Clark et al., 2010; Hu and Zhao, 2014; Yu et al., 2013). Traumatic SCI leads to long-lasting neurological deficits below the lesion level and is associated with paraplegia and neuropathic pain symptoms such as hyperalgesia and allodynia (Clark et al., 2010; Santos-Nogueira et al., 2012). These symptoms are serious complications post SCI that persist for long and provoke physical dependence, psychological and social problems with poor quality of life (Clark et al., 2010; Impellizzeri et al., 2012; Liu et al., 2015b; Talac et al., 2004). The exact mechanism underlying neuropathic pain is still obscure (Bruce et al., 2002; Chew et al., 2014; Sun et al., 2012). Spinal cord apoptosis and increased caspase-3 activity in the region may be one leading cause (Liu et al., 2015a; Sakurai et al., 2003; Vaquero et al., 2006). Today, optimal neuropathic pain management is a challenging issue (Clark et al., 2010; Talac et al., 2004). Although numerous analgesic drugs are available, appropriate treatment is still difficult to reach (Hu and Zhao, 2014; Jergova et al., 2012; Sun et al., 2012). Nowadays, SCI neuropathic pain researchers have focused on neuroprotective agents as a new therapeutic window targeting secondary injury events to protect the cells against apoptosis (Aziz et al., 2014; Bai et al., 2012; Kakinohana et al., 2011). Apelin, an endogenous ligand for APJ receptor, initially isolated from bovine stomach tissue (Chen et al., 2015; Zeng et al., 2010). This peptide is usually derived from a 77 amino acid precursor protein by angiotensin-converting enzyme II and cleaved into 13, 17 and 36 active amino acid forms (Chen et al., 2015; Zeng et al., 2012) with high binding affinity to APJ receptor (Hatzelmann et al., 2013). Among different apelin isoforms, apelin-13 and [Pyr^1] apelin-13 are more potent than others (Pope et al., 2012; Zeng et al., 2010). Efficient distribution of the apelinergic system in CNS pain-related sites may be a clue for apelin pain modulator role (Lv et al., 2012; Xu et al., 2009). In addition, in some studies apelin is shown to have analgesic effects in visceral (Lv et al., 2012) and some acute pain models (Turtay et al., 2015; Xu et al., 2009) in mice and rats. However, to the best of our

Abbreviations: SCI, spinal cord injury; i.t, intrathecal; SNL, spinal nerve ligation; MW, molecular weight; BBB, Basso Beattie, and Bresnahan; PyrAP13, pyroglutamyl apelin-13; WK, week; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PVDF, poly vinylidene fluoride; i.p, intraperitoneal; i.c.v, intracerebroventricular; CCI, chronic compression of the dorsal root ganglia; BSCB, blood spinal cord barrier; BMSCs, bone marrow-derived mesenchymal stem cells.

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knowledge, literature is completely empty, of papers on apelin and neuropathic pain post SCI in spite of bunch of articles showing apelin anti-apoptotic and neuroprotective effects (Chen et al., 2015; Zeng et al., 2012; Zeng et al., 2010; Zhao et al., 2011). For example, Zhao et al., have recently showed low apelin expression post SCI led to apoptosis induced neuronal death. They also depicted reduced sciatic nerve ligation (SNL) induced motor neuron apoptosis in the spinal anterior horn post apelin injection at proximal nerve stump (Zhao et al., 2011).

Based on our literature review, apelin with neuroprotective and anti-apoptotic properties may have potential to provide a robust therapeutic strategy for alleviation of neuropathic pain. There's no published paper on the effect of apelin in chronic pain situation post SCI. Therefore, in this study we investigated the analgesic and anti-apoptotic role of [Pyr^1] apelin-13 in a central neuropathic pain model.

2. Materials and methods

2.1. Experimental animals

Adult male Wistar rats (140–160 g) were kept at a room temperature of 22 ± 1 °C with free access to fresh food and water in a 12 h light/dark cycle. All experimental protocols performed in accordance with guidelines and policies of the International Association for the Study of Pain in conscious animals (Zimmermann, 1983) and approved by the ethics committee of animal study in Iran University of Medical Sciences (Approved number: 23621). Rats were randomly divided into three major groups as intact, sham and SCI groups. In SCI group, the animals were allocated into four subgroups as SCI, that underwent spinal cord compression injury, SCI + vehicle which received intrathecal injection of vehicle (0.9% saline solution) for one week and apelin treated groups, which received [Pyr^1] apelin-13 intrathecal injections in 1 μg and 5 μg doses for one week (n = 8).

2.2. Intrathecal catheter implantation

Intrathecal catheter implanted using a modified Yaksh and Rudy method (Yaksh and Rudy, 1976). Briefly, a 7 cm polyethylene (PE-10) catheter first immersed in 70% ethanol and then flushed with 0.9% sterile saline. Anesthesia was inducted with a mixture of ketamine/xylazine (80/10 mg/kg, i.p). Upon anesthesia completion, animals were placed in a stereotaxic instrument. A midline 1.5–2 cm rostral to caudal incision was made on the skull skin. After exposing atlanto-occipital membrane, dura punctured with the tip of insulin syringe. The catheter was then slowly conducted 2.5–3 cm into the subarachnoid space where its tip placed close to the injury site. The catheter was firmly fixed to the surrounding tissue. Muscles and skin were separately sutured with 3–0 chromic and silk surgical threads respectively. The catheter was then flushed with 10 μl of 0.9% saline solution and closed with heat at the external end. Animals were then returned to their cages for one week recovery period before spinal cord injury induction.

2.3. SCI induction procedure

For spinal cord injury induction, deep anesthesia was done first. Surgical area was clean shaved and disinfected with 70% ethanol and povidone iodine solution. A midline longitudinal incision was made at T6-T8 dorsal surfaces. Laminectomy was carried out at T6-T8 segments and the spinal cord then exposed. SCI was induced according to the method explained by Hama and Sagan (Hama and Sagen, 2012). Briefly, the spinal cord was extradurally compressed at T7–T8 level for 1 min using an aneurysm clip (Harvard Apparatus, MA) with 20 g closing force. Sham animals were only subjected to laminectomy without spinal cord compression. Urinary excretion was manually carried out twice daily until natural voiding reflex recovery.

2.4. Drug

[Pyr^1] apelin-13 was purchased from Biorbyt company (MW: 533.85). Stock solutions were prepared with 0.9% sterile saline and then stored at −20 °C until use. Stock solution thawed and diluted before injection on a daily basis (Xu et al., 2009).

2.5. Drug administration

Apelin and vehicle were intrathecally administered in a volume of 10 μl within 60 s using a Hamilton syringe (Ray et al., 2011) once a day from day 1 post SCI and continued for a week.

2.6. Behavioral tests

Behavioral tests were performed 30 min post acclimation period on a weekly basis by a person blinded to the experiment starting from the first week post SCI for 8 weeks.

2.6.1. Heat hyperalgesia

Noxious heat sensitivity assessed using a test apparatus consisting a portable heat source and a timer (Ugo Basile, Italy) according to Hargreaves et al. method (Hargreaves et al., 1988). In brief, rats were individually placed in chambers on a smooth glass surface. The heat source under the glass floor was positioned directly under the mid-plantar surface of hind paw. A 25 s cut-off time was used to prevent tissue damage. Withdrawal latencies were measured for both hind paws with at least three minutes interval between each. Three trials were carried out for each paw and the mean was calculated and used for the statistical analysis.

2.6.2. Mechanical allodynia

Mechanical allodynia was measured with a series of Von Frey filaments (Stoelting Co., USA). Rats were placed in a specific chamber on a metal mesh. The filaments were perpendicularly applied to the plantar area of the hind paw to buckle at a specific force. Fifty percent paw withdrawal threshold was determined with a set of eight specific filaments of 3.61, 3.84, 4.08, 4.31, 4.56, 4.74, 4.93 and 5.18 equivalent to 0.4, 0.6, 1, 2, 4, 6, 8, and 15 g respectively using up–down method (Chaplan et al., 1994). Abrupt withdrawal like lifting, licking and shaking considered as a positive response.

2.6.3. Cold allodynia

To assess cold allodynia, animals were placed on a wire mesh floor cage in specific chambers. Acetone (0.1 ml) was then sprayed to hind paw mid-plantar surface. Paw lift, lick or shake were considered as positive response. The test was performed five times with 5 min interval between each for each hind paw. Withdrawal responses were measured and reported as positive response percentage (Hosseini et al., 2014).

2.6.4. Mechanical hyperalgesia

Mechanical hyperalgesia was measured using Randall-Selitto analgesimeter (Ugo Basile, Italy) (Santos-Nogueira et al., 2012). Increasing mechanical pressure was used to determine the hind paw pressure threshold. Briefly, rats were immobilized with a towel and the stimuli applied with a dome shaped tip to the plantar surface of the hind paws. Test was done two times for each paw with 5 min interval. The force in gram at which the animal screams or withdraws the paw was measured. Threshold scores for two trials on each paw were averaged and used for statistical analysis.

2.7. Locomotor function testing

The Basso, Beattie, and Bresnahan (BBB) scoring method was used to assess locomotor activity (Basso et al., 1995). This test is a 21-point scale

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