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Research article

D-Aspartic acid ameliorates painful and neuropsychiatric changes and reduces β-amyloid $A\beta_{1-42}$ peptide in a long lasting model of neuropathic pain

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HIGHLIGHTS

- Increased levels of β-amyloid protein are shown in a long lasting model of neuropathic pain.
- Changed β-amyloid protein expression is associated with the appearance of behavioural dysfunctions.
- The D-Aspartic acid treatment reduces pain and pain-associated neurological dysfunctions together with a normalization of the β-amyloid levels.

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ABSTRACT

Depressive symptoms and other neuropsychiatric dysfunctions are common in neurodegenerative disorders, including chronic pain and dementia. A correlation between the β-amyloid protein accumulation and the development of depression has been suggested, however the underlying mechanisms are unknown. D-Aspartate (D-Asp) is a free D-amino acid found in the mammalian brain and involved in neurological and psychiatric processes, such as cognition and affective disorders. In this study we have investigated the effects of a repeated treatment with D-Asp in a long-lasting (12 months) model of neuropathic pain, the spared nerve injury (SNI), in mice. Specifically, we evaluated i) the pain sensitivity and related emotional/cognitive dysfunctions induced by SNI, ii) possible changes in the β-amyloid protein accumulation in specific brain regions involved in pain mechanisms ii) possible changes in steroids level in neuropathic animals with or without D-Asp in the same brain areas.

SNI mice showed an increase of the insoluble form of $A\beta_{1-42}$ at hippocampal level and displayed cognitive impairments, stereotypical and depressive-like behaviours. D-Asp treatment reduced abnormal behaviours and normalized the β-amyloid protein expression. Moreover, D-Asp dramatically increased steroids level measured in the prefrontal cortex and in the hippocampus. Our findings provide new insights into pain mechanisms and suggest a possible role of β-amyloid protein in neuropsychiatric dysfunctions associated with chronic pain.

1. Introduction

Spared nerve injury (SNI) is a model of neuropathic pain that allows for the analysis of spinal and supraspinal changes associated with the maintenance of hyperalgesia and allodynia [1]. Aside from pain sensitivity that is still detectable one year from neuropathy induction, several neuropsychiatric changes are also associated

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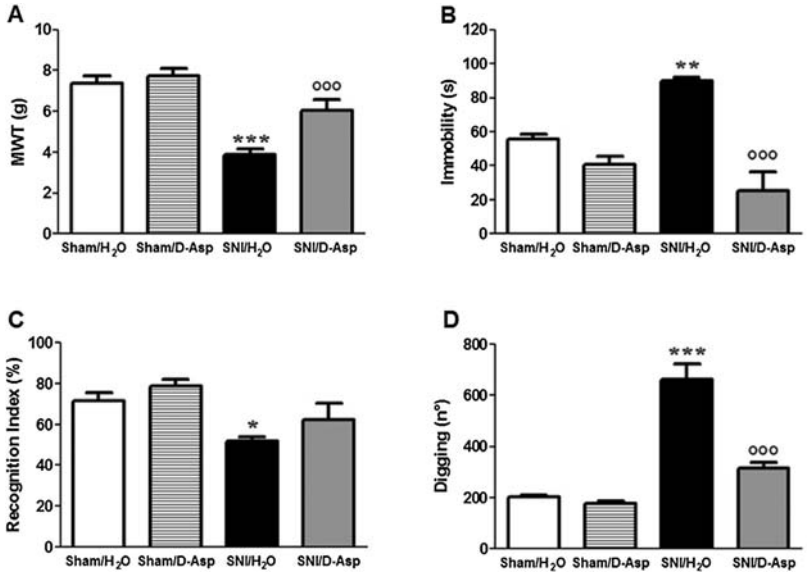

Fig 1. Effects of D-Asp treatment on sham and SNI-induced mechanical allodynia, obsessive-compulsive, and depressive-like behaviours.
(A) Mechanical withdrawal latency (g), (B) immobility time (s) during the tail suspension test, (C) recognition index (%) on the object-recognition test, and (D) the amount of digging in the marble burying test in H_2O- or D-Asp-treated sham or SNI mice, 1 year post injury. Data are expressed as mean ± SEM (One-way ANOVA followed by Bonferroni post-test; n = 6 * $p < 0.05$ ** $p < 0.01$ and *** $p < 0.001$ vs Sham/H_2O, and °°° $p < 0.001$ vs SNI/H_2O).

with SNI. It has been reported that animals with SNI develop depressive-like behaviours and cognitive impairments that are associated with alterations in neuronal activity of the hippocampus and prefrontal cortex [2,3].

It is well known that soluble forms of amyloid-β (Aß) peptide participate to synaptic dysfunctions in early stages of neurodegenerative dementias, including Alzheimer's disease [4]. In addition, a robust relationship between the Aß accumulation and depressive behaviour development has been suggested [5]. However, no studies investigating a possible relationship between chronic pain mood-related symptoms and β-amyloid metabolism have been performed.

D-Aspartic acid (D-Asp) is a natural amino acid present in all animal phyla, including humans [6]. It was first discovered in the brain of the mollusc *Octopus vulgaris* as a consequence of a search for a natural substrate for the enzyme, D-aspartate oxidase [7]. D-Asp occurs mainly in endocrine and nervous tissues [8,9], where it is involved in important physiological functions. In the central nervous system (CNS), D-Asp is involved in the neuromodulation of cell–cell signalling [10,11] and is capable of improving learning and memory in rats [12], as well as, increasing long term potentiation (LTP) synaptic plasticity [13], and the growth of neuronal dendrites in mice [14].

The beneficial effects of D-Asp in the CNS led us to investigate the effects of this amino acid on the cognitive behaviour of one year-sham and SNI mice. We also assessed whether treatment with D-Asp could reduce the soluble β-amyloid $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ peptides in the serum and of soluble and insoluble $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ in the prefrontal cortex and hippocampus of sham and neuropathic mice.

Previous studies have shown that steroid hormones exert a protective action against neurodegeneration in the mammalian brain, particularly in the metabolism of β-amyloid in Alzheimer's disease [15,16]. Specifically, pregnenolone and its derivatives, such as progesterone, testosterone and 17β-estradiol are known to affect neuronal and glial development, synaptic formation, synaptic plasticity, cognitive behaviour and depression [17–19]. On these grounds, we also measured the steroid levels in the prefrontal cortex and hippocampus in sham and SNI mice treated with vehicle or D-Asp.

2. Material and methods

2.1. Animals

Sham and neuropathic (SNI, *spared nerve injury*) male CD-1 mice (30–35 g) were housed three per cage under controlled conditions (12:12 h light/dark cycle; room, with temperature 20–22 °C, humidity 55–60%), with chow and tap water were available ad libitum. The surgery, performed as according to Decostered and Woolf [1] consisted of the legation and transaction of the tibial and peroneal nerves, leaving the sural nerve intact. Sham animals were anaesthetized and the sciatic nerve was exposed, but not transacted. Surgeries were carried out under anaesthesia (intraperitoneal injection of ketamine 60 mg/kg + xylazine 10 mg/kg), and all evaluations were performed at 12 months on both SNI and sham mice. Experimental procedures were approved by the Animal Ethics Committee of the University of Campania, Naples. Animal care was in observance with the IASP and European Community (E.C. L358/1 18/12/86) guidelines on the use and protection of animals in experimental research. All efforts were made to reduce animal suffering and the number of animals used.

2.2. D-Aspartate preparation and experimental conditions

D-Aspartic acid (Sigma Chemical Company) was prepared in a stock solution of 1 M Na-D-aspartate in distilled water while stirring. Then, while stirring a solution of 2 M NAOH, the D-aspartic acid/H_2O mixture was added 5–10 ml at a time until the D-aspartic acid was solubilised to form sodium D-aspartate. The solution was suspended in drinking water, filtrated on filter paper with 0.42 μm pores, divided into aliquots, and stored at −20 °C. For each mouse, a solution of 20 mM sodium D-aspartate, obtained by diluting the stock solution in drinking water, was given ad libitum. The solution was changed every 2–3 days. The concentration was selected based on our previous study where we established that this concentration was not dangerous to animals over the course of several months [14–20].

12 months-SNI mice received drinking water or D-Asp in drinking water for 30 days. Mice were treated as follows: 1)Sham mice