Neuroscience Letters 603 (2015) 42–47

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research paper

Neuropathic pain depends upon D-serine co-activation of spinal NMDA receptors in rats

Yan Lefèvre[a,b], Aurélie Amadio[a,b], Peggy Vincent[a,b], Amélie Descheemaeker[c], Stéphane H.R. Oliet[a,b], Radhouane Dallel[c], Daniel L. Voisin[a,b,*]

[a] Neurocentre Magendie, Inserm U862, F-33000 Bordeaux, France
[b] Univ. Bordeaux, F-33000 Bordeaux, France
[c] Clermont Université, Université d'Auvergne, NEURO-DOL, BP 10448, F-63000 Clermont-Ferrand, Inserm, U1107, F-63001 Clermont-Ferrand, France

HIGHLIGHTS

- We investigated the role of D-serine in pain after spinal nerve ligation in rats.
- Allodynia depended upon glial D-serine that co-activates spinal NMDA receptors.
- Spinal D-serine may be a target to treat mechanical allodynia in neuropathic pain.

ARTICLE INFO

Article history:
Received 1 April 2015
Received in revised form 20 June 2015
Accepted 5 July 2015
Available online 13 July 2015

Keywords:
Neuropathic pain
Astrocyte
NMDA receptor
d-Serine

ABSTRACT

Activation of *N*-methyl-D-aspartate (NMDA) receptors is critical for hypersensitivity in chronic neuropathic pain. Since astroglia can regulate NMDA receptor activation by releasing the NMDA receptor co-agonist D-serine, we investigated the role of NMDA receptor and D-serine in neuropathic chronic pain. Male Wistar rats underwent right L5–L6 spinal nerve ligation or sham surgery and were tested for mechanical allodynia and hyperalgesia after 14 days. Acute intrathecal administration of the NMDA receptor antagonist D-AP5 as well as chronic administration of the glia metabolism inhibitor fluoroacetate significantly reduced mechanical allodynia in neuropathic rats. The effect of fluoroacetate was reversed by acutely administered intrathecal D-serine. Degrading D-serine using acute intrathecal administration of D-aminoacid oxidase also reduced pain symptoms. Immunocytochemistry showed that about 70% of serine racemase, the synthesizing enzyme of D-serine, was expressed in astrocyte processes in the superficial laminae of L5 dorsal horn. Serine racemase expression was upregulated in astrocyte processes in neuropathic rats compared to sham rats. These results show that neuropathic pain depends upon glial D-serine that co-activates spinal NMDA receptors.

1. Introduction

Neuropathic pain is frequent, with prevalence that varies from 6.9 to 10% [1], has a disruptive influence on the quality of life of patients [2], and is difficult to treat successfully. It thus presents a major therapeutic challenge to healthcare professionals [3]. Although the exact mechanisms of neuropathic pain are not yet fully understood [4], it is established that central sensitization in the spinal cord through increased *N*-methyl-D-aspartate (NMDA) receptor activity contributes to its pathophysiology [5–8].

Astroglia, a major non-neuronal cell type in the central nervous system, potentially contribute to chronic pain. In response to trauma to, or inflammation of, peripheral nerves resulting in neuropathic pain, spinal cord astroglia are often activated [9]. This is reflected by increased expression of glial fibrillary acidic protein (GFAP) by astrocytes, which intensity correlates with behavioral hyperalgesia [10,11]. Conversely astroglial functional inhibition attenuates pain hypersensitivity [12]. Upon activation, astrocytes can release a variety of signaling molecules [9], and among the gliotransmitters that astrocytes can release D-serine deserves a special interest, since it serves as an endogenous ligand of NMDA receptors at their strychnine-insensitive glycine-binding site [13,14]. Indeed, activation of NMDA receptors requires binding of glutamate at their

Abbreviations: NMDA, N-methyl-D-aspartate; DAAO, D-aminoacid oxidase.

* Corresponding author at: Inserm U862, Neurocentre Magendie, Université de Bordeaux, 146 rue Léo-Saignat, F-33077 Bordeaux, France. Fax: +33 5 57 57 36 69.
E-mail address: daniel.voisin@inserm.fr (D.L. Voisin).

http://dx.doi.org/10.1016/j.neulet.2015.07.010
0304-3940/

glutamate-binding site, but also of a coagonist, such as D-serine. As a consequence, glia derived D-serine can control NMDA receptor activity and synaptic memory [15–17]. It could thus contribute to NMDA receptor dependent pain mechanisms [18,19]. In the present study, we investigated the role of NMDA receptors and spinal D-serine in a rat model of neuropathic pain.

2. Material and methods

2.1. Animals

Adult rats (150–175 g) were obtained from Janvier (France) and maintained in a controlled environment (lights on 07:00–19:00, 22 °C) with food and water freely available. They were housed 2 per cage. All efforts were made to minimize the number of animals used. The experiments followed the ethical guidelines of the International Association for the Study of Pain and the European Community Council directive of 22 September 2010 (2010/63/EU). The project was approved by Bordeaux Ethical Committee (CEEA50) under N° 50120169-A. Rats were acclimatized for 4 days to the animal facility and for 5 days to manipulations and devices prior to behavioral studies.

2.2. Drugs

All drugs were obtained from Sigma (Illkirch, France). Drugs were dissolved in artificial cerebrospinal fluid (aCSF) containing (mM): NaCl, 123; KCl, 2.5; $Na2HPO_4$, 1; $NaHCO_3$, 26.2; $MgCl_2$, 1.3; $CaCl_2$, 2.5 and glucose, 10 (pH 7.4, 295 mOsm kg^{-1}). In control experiments, only vehicle was injected. For experiments investigating the role of NMDA receptors, we used D-AP5, a competitive antagonist of NMDA receptors. D-AP5 (25 nmoles in 5 μL) was acutely injected 20 min before behavioral evaluation. Fluoroacetate, an inhibitor of glia metabolism (5 nmoles/h) was administered chronically for 14 days. For experiments investigating the effect of selective D-serine degradation, we used D-aminoacid oxidase (DAAO), an enzyme that selectively induces degradation of D-serine. DAAO (0.17 U in 5 μL aCSF with flavin adenine dinucleotide, FAD, the co-enzyme for DAAO), or vehicle alone (5 μL aCSF with FAD), were injected intrathecally acutely 45 min before behavioral evaluation. D-serine (100 μg in 5 μL) was acutely injected in animals under chronic glia inhibition or after chronic infusion of aCSF 5 min before behavioral evaluation.

2.3. Surgery

Surgery was performed on all rats under gaseous anesthesia with a mixture of isoflurane (5% for induction and 2% for maintenance) and a 1:1 flow ratio of air/O_2 (1 L/min for induction and 0.5 L/min for maintenance). Before skin incision and at the time of wound closing, a local anesthesia was also performed (2% lidocaine). The rats resumed normal activity within 30 min after termination of the gaseous anesthesia. All rats were treated with antibiotics at days 0, 1 and 2 (6.25% sulfadoxine + 1.25% trimethoprime, 100 μL IM).

Male Wistar rats were used in the spinal nerve ligation model experiments [20]. The right L5 and L6 spinal nerves were isolated and tightly ligated with a 6.0 polyamid thread (Ethicon, Issy les Moulineaux, France). After complete hemostasis, the incision was closed by using metal skin clips (AMS, Bordeaux, France).

Drugs were administered, through intrathecal catheters (32G intrathecal catheter, 003" Teflon coated stylet, Harvard Apparatus, USA) that were chronically implanted above the lumbar part of the spinal cord in animals at day 0. For acute drug administration, the external portion of the catheter ran under the skin up to the neck. Drugs were injected using a Hamilton syringe connected to the catheter and flushed with 5 μL of aCSF (dead volume of the catheter). For chronic drug administration an Alzet osmotic pump (mini-pompes osmotiques Alzet modèle 2002, Charles River laboratory) was filled with the drug, placed subcutaneously, and connected to the catheter. It released the drug at 0.5 μL/h. Incision was closed by using metal skin clips (AMS, Bordeaux, France).

2.4. Behavioral studies

Mechanical allodynia and hyperalgesia were measured at the hind paw using responses to von Frey filament stimulation according to the method described in Ducourneau et al. [21]. To quantify allodynia, we used shifts in nociceptive threshold (Fig. 1A). To quantify mechanical hyperalgesia, we used a hyperalgesia score calculated as the sum of the differences between values obtained for each von Frey hair after and before (day 0) surgery and taken from above the value of the initial nociceptive threshold (Fig. 1A).

2.5. Immunocytochemistry studies

Rats (4 with right L5–L6 spinal nerve ligation and 4 with sham surgery) were killed, tissues were prepared and immunodetection was performed as described in Ducourneau et al. [21]. The only changes were that the anesthesia was pentobarbital IP 200 mg/kg and free-floating L5 sections were placed in 2% normal goat serum for 1 h, before incubation at room temperature in a mixture of mouse primary antibody directed against GFAP (1:2,000, 24 h; Sigma, France) and rabbit primary antibody directed against serine racemase (1:1000, 24 h; Santa Cruz Biotechnology, CA), followed by immunoreactivity revelation using a mixture of Cy3 conjugated goat anti-mouse (1:200) and Cy2 conjugated goat anti-rabbit (1:200) secondary antibodies (1 h at room temperature; Jackson Immunoresearch, Newmarket, UK).

GFAP and serine racemase immunofluorescence in the superficial dorsal horn laminae was analyzed using a motorized Zeiss Axioplan 2 Imaging microscope coupled with a Hamamatsu C4742–95 digital camera, and a RITC or a FITC filter set respectively. Image processing and morphometric analysis were performed with the Metamorph 7.0 program as in Miraucourt et al. [22]. Mean relative values of total area superficies of immunofluorescence for the delineated areas were calculated from the sum of total area superficies measured from 3 different sections in each rat. Co-labelling of serine racemase and GFAP was quantified by measuring the percentage of area where serine racemase and GFAP colocalized.

2.6. Statistical analyses

All data are expressed as mean ± SEM. To assess changes after drug administration unpaired or paired (as appropriate) *t*-tests were performed. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Effects of drugs on mechanical allodynia and hyperalgesia in neuropathic rats

All animals displayed generally good health, with no signs of distress or abnormal weight increase during the post-surgery period. After spinal nerve ligation, nociceptive response-threshold (G) to von Frey hair stimulation on the ipsilateral hindpaw were dramatically reduced from day 0: 30.14 ± 4.93 (D-AP5 group, $n = 21$), 27,42 ± 4,60 (fluoroacetate group, $n = 24$), 28.19 ± 5.03 (DAAO group, $n = 16$) to day 14 (measured either before acute treatment of after chronic administration of solvent): 1.59 ± 0.48 ($n = 21$), 3.50 ± 0.74 ($n = 14$), 1.50 ± 0.50 ($n = 16$) respectively (Fig. 1). In all