ORIGINAL ARTICLE

Low doses of dextromethorphan have a beneficial effect in the treatment of neuropathic pain

Véronique Morel[a,b,c], Gisèle Pickering[a,b,c], Monique Etienne[a,b], Amandine Dupuis[a,b], Anne-Marie Privat[a,b], Maryse Chalus[a,b], Alain Eschalier[a,b], Laurence Daulhac[a,b]*

[a]*Facultés de Médecine/Pharmacie, Laboratoire de Pharmacologie, Clermont Université, Université d'Auvergne, Pharmacologie Fondamentale et Clinique de la Douleur, F-63000 Clermont-Ferrand, France*
[b]*Inserm, U1107 Neuro-Dol, F-63001 Clermont-Ferrand, France*
[c]*Centre de Pharmacologie Clinique, CHU Clermont-Ferrand, Inserm CIC 501, F-63003 Clermont-Ferrand, France*

ABSTRACT

N-methyl-D-aspartate receptor (NMDAR) antagonists may be given in persistent neuropathic pain, but adverse events especially with ketamine may limit their clinical use. Less central and cognitive adverse events are described with dextromethorphan and memantine. These molecules have been explored in many preclinical and clinical studies, but data are conflicting as regards neuropathic pain alleviation. Dextromethorphan and memantine have been administered to animals after spinal nerve ligation (SNL) to evaluate their antinociceptive/cognitive effects and associated molecular events, including the phosphorylation of several tyrosine ($pTyr^{1336}$, $pTyr^{1472}$) residues in the NR2B NMDAR subunit. Spinal nerve ligation and sham animals received dextromethorphan (10 mg/kg, i.p.), memantine (20 mg/kg, i.p.) or saline (1 mL/kg, i.p.). These drugs were administered once symptoms of allodynia and hyperalgesia had developed. Tests were carried out before and after surgery. Tactile allodynia, mechanical hyperalgesia and spatial memory were, respectively, evaluated by von Frey, Randall & Selitto and Y-maze tests and molecular events by Western blot analysis. Spinal nerve-ligated animals displayed nociception and impaired spatial memory. Dextromethorphan, but not memantine, reversed neuropathic pain (NP) symptoms, restored spatial memory integrity and decreased the expression of $pTyr^{1336}$NR2B. Following postoperative administration of dextromethorphan, this study has demonstrated for the first time a concordance between behaviour, cognitive function and molecular events *via* $pTyr^{1336}$NR2B for neuropathic pain alleviation. Confirmation of these findings in patients would constitute a major step forward in the treatment of neuropathic pain and in the improvement of cognitive function and quality of life.

INTRODUCTION

Chronic pain is a public health problem [1,2]. Neuropathic pain (NP) is generally characterized by a combination of positive and negative symptoms (i.e. pain, paraesthesia/dysesthesia and sensory deficits) with a plausible neurological distribution [3–6]. Pain with neuropathic characteristics has been estimated to affect 7–8% of the general population [1]. Pharmacological management remains the most common therapeutic option for NP, but results are still unsatisfactory, and many patients do not obtain sufficient pain relief [5]. Persistent and intractable pain is difficult to treat, and several studies indicate that N-methyl-D-aspartate

receptor (NMDAR) antagonists, such as ketamine, dextromethorphan or memantine [6–8], are potential drugs after therapeutic failure with recommended treatments and would prevent or treat painful symptoms [9–13]. In the context of NP, these molecules have been explored in many preclinical [11–21] and clinical [6–10,22–28] studies, but data are not conclusive. Limiting factors are psychodysleptic adverse events especially with ketamine [29]. Central and cognitive adverse events are less frequent with dextromethorphan, a low-affinity noncompetitive NMDAR, commonly used as an antitussive drug, and with memantine prescribed in Alzheimer's disease, another uncompetitive antagonist with moderate affinity, strong voltage dependency and rapid unblocking kinetics [30,31]. Dextromethorphan has been shown to be beneficial in preclinical [17,18] and clinical [6,10,23,24] NP studies or to have no effect [9,17,25]. In addition, memantine has been shown to reverse painful symptoms in animals [12,15,19–21] and in humans [26] or to have no effect on pain [12,22,27,28]. The effect of both these NMDAR antagonists depends on the aetiology and on the symptoms of the pain models. The cognitive impact of these antagonists has never been studied in the context of pain. We have recently shown that memantine prevents the increase of pTyr^{1472}NR2B in an animal NP model with concomitant improvement of NP symptoms and spatial memory [32]. However, molecular targets of dextromethorphan are so far unknown in animals.

The aim of this study is to evaluate in an animal neuropathic pain model the L5 spinal nerve ligation (SNL), the benefits of dextromethorphan on pain and cognitive function and the molecular targets involved via the NR2B subunit of the NMDAR, with a multidisciplinary approach including pharmacological, behavioural and biochemical techniques.

MATERIALS AND METHODS

Animals

All experiments were performed in adult male Sprague-Dawley rats (Janvier, Le Genet-St-Isle, France) initially weighing 125–150 g. They were housed five per cage under standard laboratory conditions and were allowed free access to food pellets and water. The ethical guidelines for investigation of experimental pain in conscious animals [33] were respected, and the experimental protocol was approved by the local ethics committee for animal experimentation (CEMEAA, authorization No CE6-11).

Surgical preparation

Experiments were carried out in 30 animals divided into two groups according to surgery, the L5 spinal nerve-ligated rats (SNL, $n = 15$) and sham-operated rats (SH, $n = 15$). Then, these two animal groups received, respectively, saline (SNLs, SHs), dextromethorphan (SNLdx, SHdx) or memantine (SNLm, SHm). Nerve surgery was carried out, according to the method described by Kim and Chung [34]. Using pentobarbital anaesthesia (6%, 1 mL/kg, intraperitoneal (i.p.) route), a dorsal midline incision was made from approximately L3–S2. The left L5 spinal nerve was isolated and tightly ligated with 6-0 silk (Ethicon®; Johnson Johnson; San Lorenzo, PR, USA). The surgical procedure for SH group was identical to that of the SNL group, except that the L5 spinal nerve was not ligated. Animals were then allowed to emerge from anaesthesia in an observation chamber under a warming light. Rats with a postoperative inability to flex the left hind limb, indicating some damage of the L4 nerve, were discarded from the study.

Drugs

Dextromethorphan (10 mg/kg, i.p.; Sigma, l'Isle d'Abeau, France), memantine hydrochloride (20 mg/kg, i.p.), was dissolved in physiological saline (NaCl 0.9%) and was prepared daily, just before the injections. Drugs were administered by i.p. route. Memantine hydrochloride was generously given by Lundbeck Laboratories (H. Lundbeck A/S, Copenhagen, Denmark).

Behavioural studies

Assessment of tactile allodynia
Rats were individually placed in a separate transparent plexi-glass chamber (25 × 20 × 25 cm) on a mesh floor. After a 20-min acclimation period, a series of calibrated von Frey filaments ranging from 1.4 to 26 g with a logarithmic increment was applied perpendicularly to the plantar surface of both hind paws with sufficient force to bend the filament for 6 s. Brisk withdrawal or paw flinching was considered as a positive response. In the absence of a response, the filament of the next greater force was applied. Following a response, the filament of the next lower force was applied. The tactile stimulus producing 50% likelihood of withdrawal response was calculated using the 'up and down' method of Dixon [35,36].

Assessment of mechanical hyperalgesia
Hypersensitivity to mechanical stimuli was determined by the paw pressure test, previously described by

Randall and Selitto [37]. Nociceptive thresholds, expressed in grams, were measured in rats using a Ugo Basil analgesimeter (Bioseb, Chaville, France) by applying a linearly increasing mechanical force to the left and right hind paws until a squeak was obtained. As this test involves animal handling, the experimenter got the rats used to being handled as follows: 3 days before the experiment, rats were held by the experimenter for 20 s without escaping, two or three times. On the day of the experiment, rats were again handled two or three times by the experimenter for 20 s, and simultaneously, the Ugo Basile apparatus was started to get the rat used to the noise of the apparatus. No animals showed aversive reactions during handling. Then, the paw of the rats was placed under the tip and the pressure progressively applied until the rat vocalized. The vocalization threshold was measured three times to obtain two consecutive values that differed <10%, with an interval of at least 10 min between two measures. The maximal pressure applied (cut-off) was 450 g.

Cognitive test: assessment of spatial memory

Rats were submitted to the Y-maze test, as previously described [38]. The maze was made of black-painted wood; each arm was 40 cm long, 12 cm high and 3 cm and 10 cm wide at the bottom and top, respectively. The test consisted of two trials separated by an intertrial interval. All rats were transported to the behavioural testing room in their home cage at least 1 h before testing. In the first training (acquisition) trial, each rat was placed at the centre of the apparatus and allowed to move freely through the maze during 8 min with one of the arms closed (novel arm). Animals were returned to their home cage during 5 min until the second retrieval trial, during which they could explore freely all three arms of the maze. The time spent in each arm and in the novel arm was measured and analysed from video recordings. The time spent in the novel arm was calculated during the 2-min retrieval trial as a percentage of time spent in the novel arm (s)/total time spent in y-maze (s) [38,39]. The total number of visited arms was obtained by counting how many times each animal moved in each arm during a period of 2 min.

Experimental design

Symptoms of allodynia and hyperalgesia are known to develop 7 days after surgery [34]. As described in the literature [18,19,40], on day 9 after surgery, the L5 spinal nerve-ligated or sham animals received once a day, during 7 days, either dextromethorphan (dx): SNLdx, SHdx; 10 mg/kg, i.p. route, or memantine (m): SNLm, SHm; 20 mg/kg, i.p. route or saline (s): SNLs, SHs; 1 mg/kg, i.p. route.

Tactile allodynia and mechanical hyperalgesia were measured, respectively, on day 2 and day 1 before surgery. After surgery, tactile allodynia was then evaluated on day 7 and day 16, and mechanical hyperalgesia was again performed on day 8 and day 17. Y-maze test was performed on day 18 after surgery. Each experiment was performed blindly using different animals in randomized blocks to assess the effects of different treatments: dextromethorphan, memantine or saline.

Western blot

Animals were rapidly sacrificed by decapitation, and ipsilateral or contralateral spinal lumbar enlargements of the spinal cords were removed on ice. The samples were then stored at -80 °C in collecting tubes until further processing. Lumbar spinal enlargements were homogenized on ice with the lysis buffer containing 50 mM HEPES, pH 7.5, 150 mM NaCl, 10 mM EDTA, 10 mM $Na_4P_2O_7$, 2 mM orthovanadate, 100 mM NaF, 1% Triton X-100, 0.5 mM phenylmethylsulfonylfluoride, 20 μM leupeptin and 100 IU/mL aprotinin (Sigma). Using phosphatase inhibitors as orthovanadate, the phosphorylated forms of proteins during the preparation of extracts for electrophoresis then blot transfer are protected from dephosphorylation. Protease inhibitors as PMSF, leupeptin and aprotinin allow to prevent the protein degradation. After sonication, these samples were centrifuged for 15 min at 16000 g, and the supernatants containing solubilized proteins were collected. Proteins resolved on polyacrylamide gels were transferred electrophoretically to nitrocellulose membranes (Millipore, Saint-Quentin-en-Yvelines, France) for 2 h in a buffer containing 25 mM Tris, pH 8.3, 190 mM glycine and 20% methanol. Membranes were blocked with 5% nonfat dry milk and blotted with the primary antibodies, according to the manufacturer's recommendations (anti-NR2B rabbit IgG, 1:500, cat#06-600, Upstate, Millipore; anti-pTyr[1472]NR2B rabbit IgG, 1:1000, cat#AB5403, Chemicon, Millipore, Saint-Quentin-en-Yvelines, France; anti-pTyr[1336]NR2B rabbit IgG, 1:1000, cat#AB9690, Chemicon), overnight at 4 °C and with a horseradish peroxidase-conjugated anti-rabbit antibody (1/10.000, Pierce, Rockford, IL, USA) for 1 h at room temperature. Immunoreactivity was detected with an enhanced chemiluminescence method (ECL detection reagent, Super Signal® West

Pico Chemiluminescent Substrate; Pierce). The intensity of immunoreactive bands was quantified using image analyser (Chemidoc™; XRS System, Bio-Rad Laboratories, Marnes-la-Coquette, France) coupled to Image Lab™ software and was expressed as the ratio of $pTyr^{1472}NR2B$ or $pTyr^{1336}NR2B$ over NR2B densities in the spinal cord. Results were expressed as the percentage change from the control, where control was the mean of at least five spinal cords of animals collected the day before surgery.

Statistical analysis

Data are expressed as mean ± SEM. Differences between groups are compared by a two-way analysis of variance (ANOVA) followed by a Tukey's test for von Frey and Randall & Selitto studies and by Student's *t*-test in unpaired series for Western blot studies. One-way ANOVA followed by Tukey's test is used for Y-maze analysis. Statistical analyses were run using InStat, GraphPad software, La Jolla, CA, USA. The significance level was set at $P < 0.05$.

RESULTS

All behavioural experiments were carried out on ipsilateral and contralateral side to nerve injury. On the contralateral side, no significant difference was observed on behaviour (*Figure 1*), whatever the group (sham or SNL rats) or the treatment (saline, dextromethorphan or memantine). Only results obtained on the ipsilateral side in SNL animals are reported thereafter. In sham rats, no variation of nociceptive thresholds was observed for both pain symptoms, tactile allodynia (*Figure 2a*) and mechanical hyperalgesia (*Figure 2b*), and for cognitive experiments (*Figure 3a, b*).

Dextromethorphan, but not memantine, reverses tactile allodynia

In SNL animals, thresholds of 50% response measured on day 7 after surgery decreased significantly compared with those obtained before surgery (SNL, before surgery: 32.7 ± 1.3 g; day 7: 6.7 ± 1.4 g; $P < 0.001$; *Figure 2a*). Dextromethorphan significantly increased nociceptive thresholds of 50% response measured on day 16 after surgery compared with those obtained on day 7 (SNL, day 7: 6.7 ± 1.4; SNLdx, day 16: 23.0 ± 4.0 g; $P < 0.001$; *Figure 2a*). Treatment with memantine and saline leads to pain scores on day 16, which are not significantly different from those obtained on day 7 (SNL, day 7: 6.7 ± 1.4 g; SNLm,
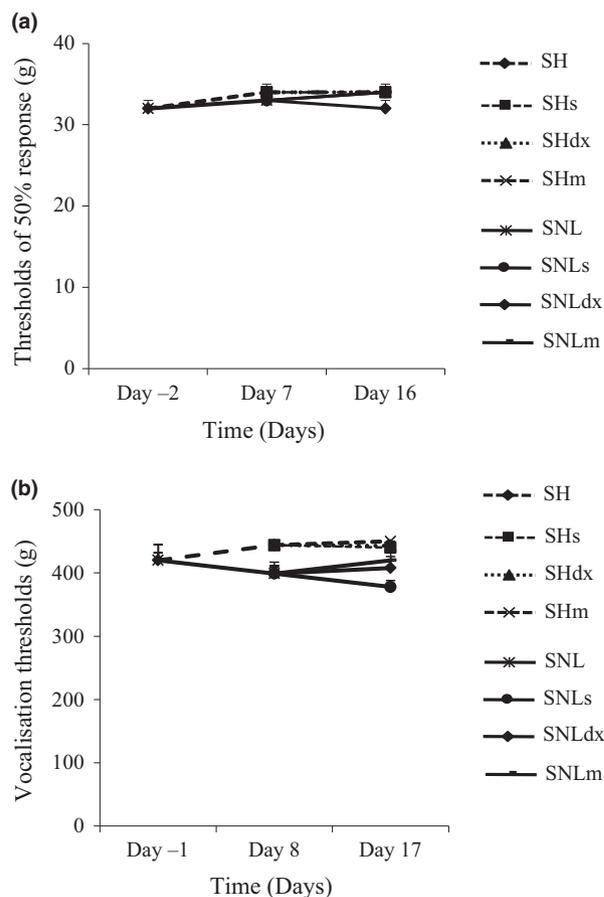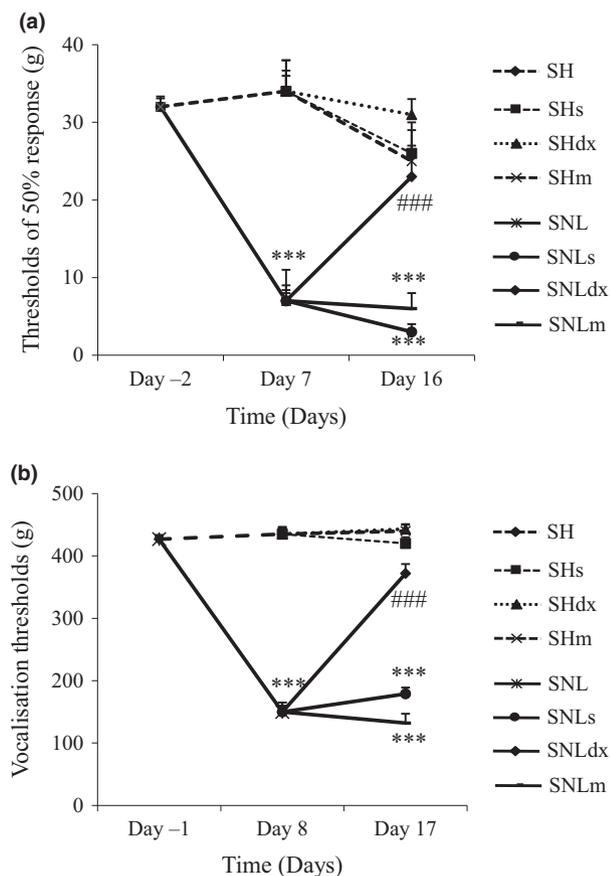

Figure 1 Spinal nerve ligation (SNL) does not induce tactile allodynia and mechanical hyperalgesia at the contralateral side of the lesion. Thresholds of 50% response (a) and vocalization thresholds (b) were measured at the contralateral side of the lesion before, and respectively, 7 or 8 days and 16 or 17 days in sham (SH) and SNL rats. Animals were treated for 7 days with dextromethorphan (dx: 10 mg/kg, i.p.), memantine (m: 20 mg/kg, i.p.) or saline (s: 1 mL/kg, i.p). Values are mean ± SEM ($n = 5$–8/group).

day 16: 5.5 ± 2.0 g; SNLs, day 16: 3.3 ± 1.0 g; NS; *Figure 2a*).

Dextromethorphan, but not memantine, reverses mechanical hyperalgesia

Vocalization thresholds measured on day 8 in ligated rats decreased significantly compared with those obtained before surgery (SNL, before surgery: 417.9 ± 4.0 g; day 8: 150.0 ± 7.0 g; $P < 0.001$; *Figure 2b*). Dextromethorphan significantly increased vocalization thresholds measured on day 17 after surgery compared with those obtained on day 8 (SNL, day 8: 150.0 ± 7.0; SNLdx, day 17: 372.0 ± 15.0 g;
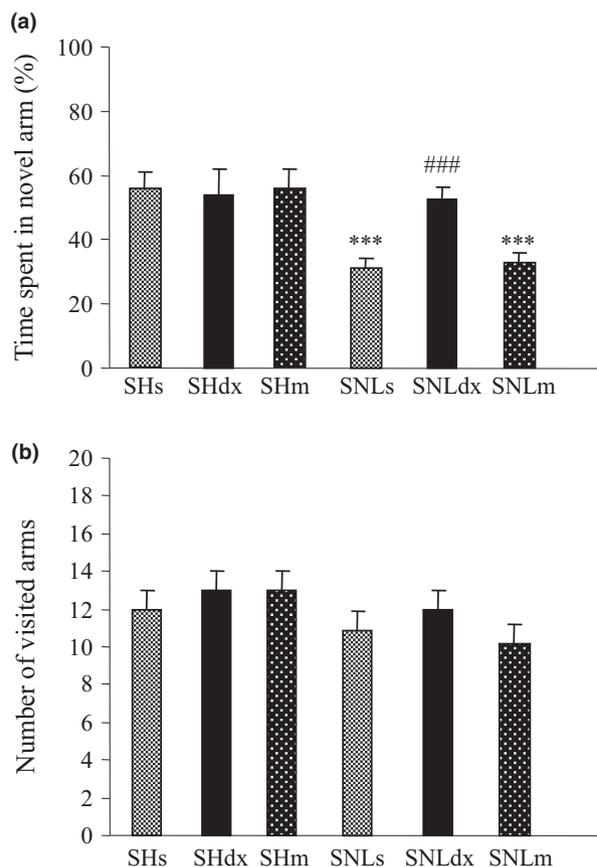
Figure 2 Repetitive dextromethorphan treatment reverses SNL-induced tactile allodynia and mechanical hyperalgesia at the ipsilateral side of the lesion. Thresholds of 50% response (a) and vocalization thresholds (b) were measured before, and respectively, 7 or 8 days and 16 or 17 days in sham (SH) and spinal nerve ligation (SNL)-operated rats at the ipsilateral side of the lesion. Animals were treated for 7 days with dextromethorphan (dx: 10 mg/kg, i.p.), memantine (m: 20 mg/kg, i.p.) or saline (s: 1 mL/kg, i.p.), starting on day 9. Values are mean $\pm$ SEM (n = 5–8/group). ***P < 0.001 vs. values obtained before surgery in SNL animals. ###P < 0.001 vs. values measured on day 7 for tactile allodynia and on day 8 for mechanical hyperalgesia in SNL animals.

P < 0.001; *Figure 2b*). On the contrary, treatment with memantine and saline did not improve painful thresholds on day 17 (SNL, day 8: 150.0 $\pm$ 7.0 g; SNLm, day 17: 132.0 $\pm$ 15.0 g; SNLs, day 17: 179.0 $\pm$ 10.0 g; NS; *Figure 2b*).

Dextromethorphan, but not memantine, restores spatial memory integrity

In SNL animals, treatment with saline (SNLs) or memantine (SNLm) leads to a significant decrease of the

Figure 3 Repetitive dextromethorphan treatment restores spatial memory integrity in painful animals. Sham (SH) or spinal nerve-ligated (SNL) rats were subjected to the Y-maze test. The percentage of time spent in the novel arm (a) and the total number of visited arms (b) were evaluated in animals treated with dextromethorphan (dx: 10 mg/kg, i.p.), memantine (m: 20 mg/kg, i.p.) or saline (s: 1 mL/kg, i.p.). Values are mean $\pm$ SEM (n = 5–8/group). ***P < 0.001 vs. corresponding group (SHs or SHm); ###P < 0.001 vs. SNL rats treated by saline (SNLs).

percentage of time spent in the novel arm compared with sham (SH) animals treated with the same drugs (SHs: 56.0 $\pm$ 5.0%; SNLs: 31.0 $\pm$ 3.0%; SHm: 56.0 $\pm$ 6.0%; SNLm: 33.0 $\pm$ 3.0%; P < 0.001; *Figure 3a*). However, SNL rats treated with dextromethorphan, SNLdx, showed a significant increase of the percentage of time spent in the novel arm compared with SNL animals treated with saline, SNLs (SNLdx: 52.0 $\pm$ 4.0%; SNLs: 31.0 $\pm$ 3.0%; P < 0.001; *Figure 3a*). No significant changes were observed in the total number of visited arms whatever the group, SH or SNL, or the treatment administered, saline, dextromethorphan or memantine (SHs: 12.0 $\pm$ 1.0; SHdx:

13.0 ± 1.0; SHm: 13.0 ± 1.0; SNLs: 11.0 ± 1.0; SNLdx: 12.0 ± 1.0; SNLm: 10.0 ± 1.7; NS; *Figure 3b*), suggesting that animals have no motor impairment.

Dextromethorphan, but not memantine, is associated with less NR2B-NMDAR subunits phosphorylation

The densitometric quantification of Western blots performed with specific anti-pTyr^{1336}NR2B antibody revealed a significant decrease of pTyr^{1336}NR2B ($P < 0.05$) in the spinal cord of SNLdx (125.0 ± 13%, $n = 5$) compared with SNLs animals (265.0 ± 18%, $n = 5$; *Figure 4a, b*). On the contrary, Western blots performed with specific anti-pTyr^{1472}NR2B showed that dextromethorphan did not affect significantly the phosphorylation of Tyr^{1472}NR2B (pTyr^{1472}NR2B, SNLs: 251.0 ± 14%; SNLdx: 244.0 ± 2%; NS; *Figure 4c, d*).

DISCUSSION

The findings of this study show in L5 spinal nerve-ligated (SNL) animals treated by dextromethorphan (SNLdx): (i) a suppression of NP symptoms such as tactile allodynia and mechanical hyperalgesia; (ii) a regaining of spatial memory integrity; and (iii) a decrease of spinal pTyr^{1336}NR2B expression.

Using this animal pain model, we demonstrate that a low dose of dextromethorphan (10 mg/kg, i.p. route) has a beneficial effect on NP symptoms such as tactile allodynia and mechanical hyperalgesia. In several pre-clinical studies, dextromethorphan has already been shown to alleviate thermal [17,18,21,41] and mechanical hypersensitivity symptoms [17,21,41], but these effects were observed with higher doses (30, 60 mg/kg) and were not reported at 15 mg/kg [17]. Our study confirms a beneficial effect on pain using mechanical stimuli [17,21,41], but with the low dose of 10 mg/kg. This difference in the results may be explained by the fact that the authors used a strain of rat different from ours, especially WKY rats that are more sensitive to pain, and a different NP model, the chronic constriction injury model.

Our results also show for the first time a concomitant beneficial effect of dextromethorphan on pain and
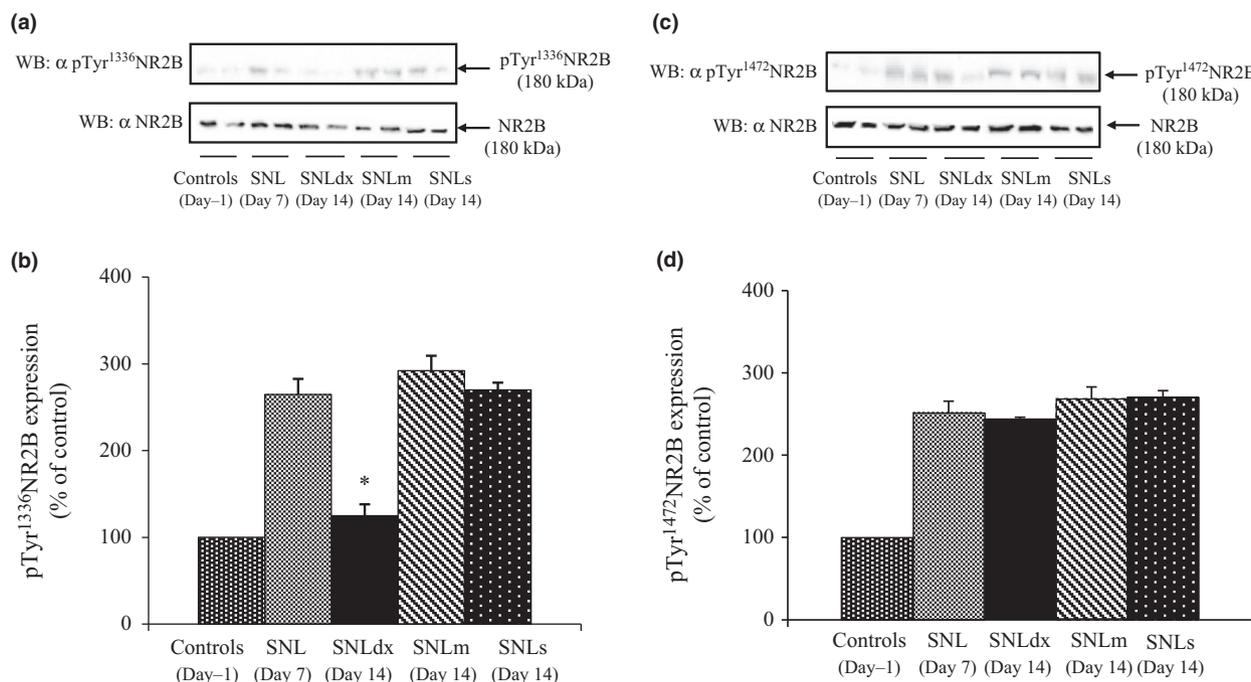
Figure 4 Repetitive dextromethorphan treatment is associated with less NR2B-NMDAR subunits phosphorylation. Representative Western blots were probed with antibodies that specifically recognize total NR2B (lower panel) or phosphorylation of NR2B (upper panel) on tyrosine 1336 (pTyr^{1336}NR2B): (a) and tyrosine 1472 (pTyr^{1472}NR2B): (c). Single bands that migrated at ~ 180 kDa were identified. (b, d) The autoradiograms were densitometrically analysed, and the ratio of pTyr^{1336}NR2B, pTyr^{1472}NR2B over NR2B densities in the spinal cord from at least five autoradiograms were plotted as a percentage of control (five spinal cords of animals collected the day before surgery) and presented as mean ± SEM ($n = 5$ rats/group). *$P < 0.05$ vs. SNL group on day 7 after surgery.

on cognition. The deleterious impact of chronic pain on cognitive and emotional domains has been largely reported in animal [42–46] and human studies [47–49]. In animals, pain-related cognitive impairment has been observed in spatial memory, decision-making, memory recognition and attentional performance [42,43]. A number of cerebral structures such as the prefrontal cortex, the amygdala, the anterior cingulate cortex, the insular cortex or the hippocampus play a crucial role in the interaction of pain and cognition [44]. These brain areas are associated with learning, memory, strategic planning or decision-making and also with emotional responses that are often impaired in chronic pain [43]. Hippocampal abnormalities induce short-term recognition memory impairment and deficits in long-term potentiation and may be involved in behaviour, learning and emotional deficits in neuropathic rats [45]. In an NP model, reduced hippocampus-prefrontal cortex connectivity [44], significant insular cortex lesions and a decreased insular volume [46] have been observed.

In humans, cognitive and brain imaging studies indicate that NP is associated with cognitive and emotional impairment [50]. Patients with NP perform poorly compared with controls in attention, executive function, learning or memory tests [51]. Cerebral areas of the pain matrix such as the somatosensory areas, the insular cortex, the prefrontal cortex, the anterior cingulate cortex, the amygdala, the thalamus and the hippocampus are also involved in cognitive and emotional information [52].

Our study indicates a beneficial effect of dextromethorphan on pain and on cognition and confirms the close functioning of pain and cognition at central level. Only one study [17] has so far demonstrated that dextromethorphan improves pain but not depression-like behaviour in the context of neuropathic pain; our study shows for the first time that dextromethorphan improves NP and cognition. Although our experiments do not allow to separate the effect of dextromethorphan on NP from those on the cognitive function, it is interesting to note that dextromethorphan has been proposed to have a dose-dependent impact on cognition [53]. While dextromethorphan impairs spatial learning at high doses (30, 40 mg/kg), it has been suggested to have a neuroprotective effect at a lower dose, such as the one we used (10 mg/kg). This latter effect has been demonstrated recently in a model of penetrating ballistic-like brain injury (PBBI) in the right frontal hemisphere. At 10 mg/kg, dextromethorphan reduced axonal fibre degeneration in the cingulate cortex and in the thalamus 72 h postinjury and was associated with an improvement of cognitive performance in a novel object recognition task 7 days post-PBBI [54].

Concerning memantine, another NMDAR antagonist used in this study, no effect on pain and on cognition has been observed when administered in postsurgery. These different findings with dextromethorphan and with memantine are confirmed by molecular findings. Postoperative administration of dextromethorphan in animals with chronic pain shows for the first time a decrease of $pTyr^{1336}NR2B$ with no variation of $pTyr^{1472}NR2B$ at spinal level, while postoperative memantine administration does not affect the phosphorylation of both residues. Spinal $pTyr^{1336}NR2B$ residue might be a specific pain target for the antagonistic action of dextromethorphan on NMDAR. Phosphorylation of $Tyr^{1336}NR2B$ residue possibly plays a major role of regulation in the alleviation of NP symptoms such as allodynia and hyperalgesia and also in cognitive function. Dextromethorphan and memantine display different molecular targets for pain alleviation. We showed in a recent study [32] that the molecular action of memantine when given before surgery involves the diminution of the $pTyr^{1472}NR2B$ expression, suggesting that this residue plays a crucial regulatory role in the development of NP symptoms and may prevent the massive influx of glutamate and consequent sensitization at spinal and supraspinal levels. Hence, dextromethorphan may have an action *via* spinal $pTyr^{1336}NR2B$ expression when NP is fully established, whereas memantine has no such action but acts preventively *via* spinal and supraspinal $pTyr^{1472}NR2B$ expression [32]. Supraspinal studies with both NMDAR antagonists will allow to confirm these different chronological impacts of dextromethorphan and memantine in NP. Few studies have so far reported the action of dextromethorphan on $pTyr^{1336}NR2B$ at spinal level and none at supraspinal level in the context of pain. NMDAR, especially NR2B subunits, is largely expressed in the brain in chronic pain [55]. This subunit is expressed in the insular cortex [46], in the anterior cingulate cortex [47], in the prefrontal cortex and in the hippocampus [44]. Only one study has identified a molecular target of dextromethorphan at central level, but it was in a context of ischaemia [48]. In our animal study, a significant decrease of $pSer^{831}$ expression in the NR1 subunit after administration of dextromethorphan was observed in the hippocampus.

A recent study [49] in mature hippocampal slices has demonstrated that phosphorylation of NR2B on Tyr^{1472} and Tyr^{1336} is, respectively, associated with synaptic and extrasynaptic expression. The authors evaluated the concentration of phosphatase and Src-family kinase (SFK) and reported that phosphatases are more concentrated extrasynaptically, while SFKs are more located synaptically. Synaptic or extrasynaptic distribution of NMDAR may contribute to the different functions of this receptor in various neurological disorders [56]. It may also contribute to different effects of dextromethorphan and memantine in chronic pain. The hypothesis of enhanced extrasynaptic signalling of dextromethorphan on Tyr^{1336} will be explored in future studies. Given these data, we can suggest that dextromethorphan could have an action *via* $pTyr^{1336}NR2B$ located in extrasynaptic NMDAR and memantine *via* $pTyr^{1472}NR2B$ expressed in synaptic NMDAR. Such a hypothesis could explain the different profiles of action of these two NMDAR antagonists and could help to prevent or treat neuropathic pain.

CONCLUSION

This study has demonstrated for the first time an improvement of behaviour and of cognitive function in chronic pain animals treated with low doses of dextromethorphan. It also showed that $pTyr^{1336}NR2B$ may be a molecular target of dextromethorphan in the treatment of postsurgery NP and in cognition. Future studies will focus on the molecular and cellular mechanisms of dextromethorphan at supraspinal level to determine the cerebral mechanisms involved in the context of pain. The development of randomized clinical trials with dextromethorphan vs. placebo will explore these findings in patients: similar results would constitute a major step forward in the treatment of NP and in the improvement of cognitive function and quality of life.

ACKNOWLEDGEMENT

ABBREVIATIONS LIST

NMDAR – N-methyl-D-aspartate receptor
SNL – Spinal nerve ligation
NP – Neuropathic pain
SH – Sham-operated rats

SHs/SHm/SHdx – Sham-operated rats received saline, memantine or dextromethorphan
SNLs/SNLm/SNLdx – Spinal nerve-ligated rats received saline, memantine or dextromethorphan
i.p. route – intraperitoneal route
WB – Western blot
$pTyr^{1336}NR2B$ and $pTyr^{1472}NR2B$ – phosphorylation of tyrosine 1336 and 1472 in the NR2B NMDAR subunit

REFERENCES

1 Bouhassira D., Lantéri-Minet M., Attal N., Laurent B., Touboul C. Prevalence of chronic pain with neuropathic characteristics in the general population. Pain (2008) **136** 380–387.

2 Attal N., Lanteri-Minet M., Laurent B., Fermanian J., Bouhassira D. The specific disease burden of neuropathic pain: results of a French nationwide survey. Pain (2011) **152** 2836–2843.

3 Jensen T.S., Gottrup H., Sindrup S.H., Bach F.W. The clinical picture of neuropathic pain. Eur. J. Pharmacol. (2001) **429** 1–11.

4 Baron R., Binder A., Wasner G. Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. Lancet Neurol. (2010) **9** 807–819.

5 Dworkin R.H., Backonja M., Rowbotham M.C. et al. Advances in neuropathic pain: diagnosis, mechanisms, and treatment recommendations. Arch. Neurol. (2003) **60** 1524–1534.

6 Sang C.N., Booher S., Gilron I., Parada S., Max M.B. Dextromethorphan and memantine in painful diabetic neuropathy and postherpetic neuralgia: efficacy and dose-response trials. Anesthesiology (2002) **96** 1053–1061.

7 Jørum E., Warncke T., Stubhaug A. Cold allodynia and hyperalgesia in neuropathic pain: the effect of N-methyl-D-aspartate (NMDA) receptor antagonist ketamine–a double-blind, cross-over comparison with alfentanil and placebo. Pain (2003) **101** 229–235.

8 Zhou H.Y., Chen S.R., Pan H.L. Targeting N-methyl-D-aspartate receptors for treatment of neuropathic pain. Expert Rev. Clin. Pharmacol. (2011) **4** 379–388.

9 Nelson K.A., Park K.M., Robinovitz E., Tsigos C., Max M.B. High-dose oral dextromethorphan versus placebo in painful diabetic neuropathy and postherpetic neuralgia. Neurology (1997) **48** 1212–1218.

10 Carlsson K.C., Hoem N.O., Moberg E.R., Mathisen L.C. Analgesic effect of dextromethorphan in neuropathic pain. Acta Anaesthesiol. Scand. (2004) **48** 328–336.

11 Wilson J.A., Garry E.M., Anderson H.A. et al. NMDA receptor antagonist treatment at the time of nerve injury prevents injury-induced changes in spinal NR1 and NR2B subunit expression and increases the sensitivity of residual pain behaviours to subsequently administered NMDA receptor antagonists. Pain (2005) **117** 421–432.

12 Takeda K., Muramatsu M., Chikuma T., Kato T. Effect of memantine on the levels of neuropeptides and microglial cells in the brain regions of rats with neuropathic pain. J. Mol. Neurosci. (2009) **39** 380–390.

13 Alexander J.K., DeVries A.C., Kigerl K.A., Dahlman J.M., Popovich P.G. Stress exacerbates neuropathic pain via glucocorticoid and NMDA receptor activation. Brain Behav. Immun. (2009) **23** 851–860.

14 Parsons C.G. NMDA receptors as targets for drug action in neuropathic pain. Eur. J. Pharmacol. (2001) **429** 71–78.

15 Suzuki R., Matthews E.A., Dickenson A.H. Comparison of the effects of MK-801, ketamine and memantine on responses of spinal dorsal horn neurones in a rat model of mononeuropathy. Pain (2001) **91** 101–109.

16 Christoph T., Schiene K., Englberger W., Parsons C.G., Chizh B.A. The antiallodynic effect of NMDA antagonists in neuropathic pain outlasts the duration of the in vivo NMDA antagonism. Neuropharmacology (2006) **51** 12–17.

17 Wang S., Zhang L., Lim G. et al. A combined effect of dextromethorphan and melatonin on neuropathic pain behavior in rats. Brain Res. (2009) **1288** 42–49.

18 Karimi G., Tabrizian K., Rezaee R. Evaluation of the analgesic effect of dextromethorphan and its interaction with nitric oxide on sciatic nerve ligated rats. J. Acupunct. Meridian Stud. (2010) **3** 38–42.

19 Carlton S.M., Hargett G.L. Treatment with the NMDA antagonist memantine attenuates nociceptive responses to mechanical stimulation in neuropathic rats. Neurosci. Lett. (1995) **198** 115–118.

20 Eisenberg E., Lacross S., Strassman A.M. The clinically tested N-methyl-D-aspartate receptor antagonist memantine blocks and reverses thermal hyperalgesia in a rat model painful mononeuropathy. Neurosci. Lett. (1995) **187** 17–20.

21 Chaplan S.R., Malmberg A.B., Yaksh T.L. Efficacy of spinal NMDA receptor antagonism in formalin hyperalgesia and nerve injury evoked allodynia in the rat. J. Pharmacol. Exp. Ther. (1997) **280** 829–838.

22 Nikolajsen L., Gottrup H., Anders G.D., Jensen T.S. Memantine (a N-methyl-D-aspartate receptor antagonist) in the treatment of neuropathic pain after amputation or surgery: a randomized, double-blind, cross-over study. Anesth. Analg. (2000) **91** 960–966.

23 Sang C.N. NMDA-receptor antagonists in neuropathic pain: experimental methods to clinical trials. J. Pain Symptom Manage. (2000) **19**(1 Suppl) S21–S25.

24 Shaibani A.I., Pope L.E., Thisted R., Hepner A. Efficacy and safety of dextromethorphan/quinidine at two dosage levels for diabetic neuropathic pain: a double-blind, placebo-controlled, multicenter study. Pain Med. (2012) **13** 243–254.

25 McQuay H.J., Carroll D., Jadad A.R. et al. Dextromethorphan for the treatment of neuropathic pain: a double-blind randomised controlled crossover trial with integral n-of-1 design. Pain (1994) **59** 127–133.

26 Hackworth R.J., Tokarz K.A., Fowler I.M., Wallace S.C., Stedje-Larsen E.T. Profound pain reduction after induction of

memantine treatment in two patients with severe phantom limb pain. Anesth. Analg. (2008) **107** 1377–1379.

27 Eisenberg E., Kleiser A., Dortort A., Haim T., Yarnitsky D. The NMDA (N-methyl-D-aspartate) receptor antagonist memantine in the treatment of postherpetic neuralgia: a double-blind, placebo-controlled study. Eur. J. Pain (1998) **2** 321–327.

28 Maier C., Dertwinkel R., Mansourian N. et al. Efficacy of the NMDA-receptor antagonist memantine in patients with chronic phantom limb pain-results of a randomized double-blinded, placebo-controlled trial. Pain (2003) **103** 277–283.

29 Cvrcek P. Side effects of ketamine in the long-term treatment of neuropathic pain. Pain Med. (2008) **9** 253–257.

30 Parsons C.G., Danysz W., Quack G. Memantine is a clinically well tolerated NMDA receptor antagonist-a review of preclinical data. Neuropharmacology (1999) **38** 735–767.

31 Jonhson J.W., Kotermanski S.E. Mechanism of action of memantine. Curr. Opin. Pharmacol. (2006) **6** 61–67.

32 Morel V., Etienne M., Wattiez A.S., et al. Memantine, a promising drug for the prevention of neuropathic pain in rat. Eur. J. Pharmacol. (2013) **721** 382–390.

33 Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain (1983) **16** 109–110.

34 Kim H.K., Chung J.M. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain (1992) **50** 355–363.

35 Dixon W.J. Efficient analysis of experimental observations. Annu. Rev. Pharmacol. Toxicol. (1980) **20** 441–462.

36 Chaplan S.R., Bach F.W., Pogrel J.W., Chung J.M., Yaksh T.L. Quantitative assessment of tactile allodynia in the rat paw. J. Neurosci. Methods (1994) **53** 55–63.

37 Randall L.O., Sellito J.J. A method for measurement of analgesic activity on inflamed tissue. Arch. Int. Pharmacodyn. Ther. (1957) **111** 409–419.

38 Dellu F., Mayo W., Cherkaoui J., Le Moal M., Simon H. A two-trial memory task with automated recording: study in young and aged rats. Brain Res. (1992) **588** 132–139.

39 Conrad C.D., Galea L.A., Kuroda Y., McEwen B.S. Chronic stress impairs rat spatial memory on the Y-Maze and this effect is blocked by tianeptine pre-treatment. Behav. Neurosci. (1996) **110** 1321–1334.

40 Réus G.Z., Abelaira H.M., Stringari R.B., Fries G.R., Kapczinski F., Quevedo J. Memantine treatment reverses anhedonia, normalizes corticosterone levels and increases BDNF levels in the prefrontal cortex induced by chronic mild stress in rats. Metab. Brain Dis. (2012) **27** 175–182.

41 Lynch J.J. 3rd, Wade C.L., Zhong C.M., Mikusa J.P., Honore P. Attenuation of mechanical allodynia by clinically utilized drugs in a rat chemotherapy-induced neuropathic pain model. Pain (2004) **110** 56–63.

42 Hu Y., Yang J., Hu Y., Wang Y., Li W. Amitriptyline rather thanlornoxicam ameliorates neuropathic pain-induced deficits in abilities of spatial learning and memory. Eur. J. Anaesthesiol. (2010) **27** 162–168.

43 Pais-Vieira M., Mendes-Pinto M.M., Lima D., Galhardo V. Cognitive impairment of prefrontal-dependent decision-making in rats after the onset of chronic pain. Neuroscience (2009) **161** 671–679.

44 Cardoso-Cruz H., Lima D., Galhardo V. Impaired spatial memory performance in a rat model of neuropathic pain is associated with reduced hippocampus-prefrontal cortex connectivity. J. Neurosci. (2013) **33** 2465–2480.

45 Ren W.J., Liu Y., Zhou L.J. et al. Peripheral nerve injury leads to working memory deficits and dysfunction of the hippocampus by upregulation of TNF-α in rodents. Neuropsychopharmacology (2011) **36** 979–992.

46 Coffeen U., Manuel Ortega-Legaspi J., López-Muñoz F.J., Simón-Arceo K., Jaimes O., Pellicer F. Insular cortex lesion diminishes neuropathic and inflammatory pain-like behaviours. Eur. J. Pain (2011) **15** 132–138.

47 Zhuo M. Plasticity of NMDA receptor NR2B subunit in memory and chronic pain. Mol. Brain (2009) **2** 4.

48 Fu X.Z., Zhang Q.G., Meng F.J., Zhang G.Y. NMDA receptor-mediated immediate Ser831 phosphorylation of GluR1 through CaMKIIalpha in rat hippocampus during early global ischemia. Neurosci. Res. (2004) **48** 85–91.

49 Goebel-Goody S.M., Davies K.D., Alvestad Linger R.M., Freund R.K., Browning M.D. Phospho-regulation of synaptic and extrasynaptic N-methyl-d-aspartate receptors in adult hippocampal slices. Neuroscience (2009) **158** 1446–1459.

50 Apkarian A.V., Sosa Y., Krauss B.R. et al. Chronic pain patients are impaired on an emotional decision-making task. Pain (2004) **108** 129–136.

51 Morel V., Pickering G. Impact de la douleur sur les processus cognitifs chez l'homme. Douleur et Analgésie (2013) **26** 11–16.

52 Moriarty O., McGuire B.E., Finn D.P. The effect of pain on cognitive function: a review of clinical and preclinical research. Prog. Neurobiol. (2011) **93** 385–404.

53 Bane A., Rojas D., Indermaur K., Bennett T., Avery D. Adverse effects of dextromethorphan on the spatial learning of rats in the Morris water maze. Eur. J. Pharmacol. (1996) **302** 7–12.

54 Shear D.A., Williams A.J., Sharrow K., Lu X.C., Tortella F.C. Neuroprotective profile of dextromethorphan in an experimental model of penetrating ballistic-like brain injury. Pharmacol. Biochem. Behav. (2009) **94** 56–62.

55 Wu L.J., Zhuo M. Targeting the NMDA receptor subunit NR2B for the treatment of neuropathic pain. Neurotherapeutics (2009) **6** 693–702.

56 Gladding C.M., Raymond L.A. Mechanisms underlying NMDA receptor synaptic/extrasynaptic distribution and function. Mol. Cell. Neurosci. (2011) **48** 308–320.