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Spinal administration of a δ opioid receptor agonist attenuates hyperalgesia and allodynia in a rat model of neuropathic pain

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Abstract

Neuropathic (NP) pain is a debilitating chronic pain disorder considered by some to be inherently resistant to therapy with traditional analgesics. Indeed, μ opioid receptor (OR) agonists show reduced therapeutic benefit and their long term use is hindered by the high incidence of adverse effects. However, pharmacological and physiological evidence increasingly suggests a role for δ OR agonists in modulating NP pain symptoms. In this study, we examined the antihyperalgesic and antiallodynic effects of the spinally administered δ OR agonist, D-[Ala², Glu⁴]deltorphin II (deltorphin II), as well as the changes in δ OR expression, in rats following chronic constriction injury (CCI) of the sciatic nerve. Rats with CCI exhibited cold hyperalgesia and mechanical allodynia over a 14-day testing period. Intrathecal administration of deltorphin II reversed cold hyperalgesia on day 14 and dose-dependently attenuated mechanical allodynia. The effects of deltorphin II were mediated via activation of the δ OR as the effect was antagonized by co-treatment with the δ -selective antagonist, naltrindole. Western blotting experiments revealed no changes in δ OR protein in the dorsal spinal cord following CCI. Taken together, these data demonstrate the antihyperalgesic and antiallodynic effectiveness of a spinally administered δ OR agonist following peripheral nerve injury and support further investigation of δ ORs as potential therapeutic targets in the treatment of NP pain.

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Keywords: Delta opioid receptor; Deltorphin; Neuropathic pain; Chronic constriction injury; Hyperalgesia; Allodynia

1. Introduction

Neuropathic (NP) pain is a debilitating chronic pain disorder involving a peripheral and/or central nervous system lesion. Characterized by the occurrence of allodynia (pain evoked by a normally innocuous stimulus), and hyperalgesia (increased sensitivity to noxious stimuli), NP pain is estimated to affect more than 2–3% of North Americans (Gilron et al., 2005). Yet despite its

prevalence and adverse impact on functionality and quality of life, it remains a challenge for physicians to treat. The clinically available opioids, such as morphine, are agonists at the μ opioid receptor (OR), and although partially effective in alleviating symptoms of NP pain (Gilron et al., 2005), they elicit several adverse effects such as gastro-intestinal disturbances and sedation (Shook et al., 1987). In contrast, pre-clinical studies suggest that δ OR agonists are capable of producing analgesia with a lower incidence of adverse effects (Porreca et al., 1984; Mika et al., 2001). Indeed, administration of δ OR agonists produces minimal induction of dependence (Cowan et al., 1988) with lower abuse potential (Mika et al., 2001). Together with a lower propensity

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for respiratory (Kiritsy-Roy et al., 1989; May et al., 1989; Szeto et al., 1999), cognitive (Sharif and Hughes, 1989), and gastro-intestinal (Shook et al., 1987) impairments than their μ counterparts, agents which selectively activate the δ OR represent a promising class of drugs for the treatment of chronic pain.

The analgesic effectiveness of selective δ OR agonists has been demonstrated in numerous pharmacological studies of acute and persistent pain (Stewart and Hammond, 1994; Glaum et al., 1994; Fraser et al., 2000); however studies examining the role of δ ORs in NP pain states encouragingly support further determination of the effectiveness of selective δ OR agonists in animal models of NP pain. Hence, administration of selective δ OR agonists was shown to alleviate allodynia and/or hyperalgesia in various rat models of neuropathic pain induced by nerve injury following spinal administration (Nichols et al., 1995; Mika et al., 2001). Furthermore, δ OR agonists elicited anti-hyperalgesic effects in rats with experimental diabetic neuropathy (Kamei et al., 1997; Chen and Pan, 2003). Nevertheless, there exist other studies that report the lack of effect of δ OR agonists in attenuating neuropathic pain symptoms, wherein mechanical allodynia induced by spinal nerve ligation was unaltered by selective δ OR agonists (Lee et al., 1995). Similarly, there are inconsistent reports on changes in δ OR expression following nerve injury. While autoradiographic binding (Stevens et al., 1991; Besse et al., 1992) and immunohistochemical (Zhang et al., 1998; Stone et al., 2004) studies report no change or decreased δ OR expression following nerve injury, Zaratin et al. (1998) observed an increase in δ OR mRNA. However, a recent study utilizing genetically modified mice reported enhanced neuropathy-induced hypersensitivity in δ OR knock-out mice compared to wild-type littermates (Nadal et al., 2006), suggesting a role of δ ORs in modulating neuropathic pain.

The antinociceptive potential of δ OR agonists, together with a lower incidence of adverse effects than μ OR agonists, makes the δ OR an attractive target in the pharmacological treatment of chronic pain syndromes. However, little is known about the efficacy of δ OR agonists in alleviating NP pain symptoms, nor about the functional changes in receptor expression, targeting, and pharmacology that may occur in NP pain states. It is therefore important to explore the functional role of the δ OR following nerve injury. In the present study, we aimed to investigate the anti-hyperalgesic and anti-allodynic effectiveness of a spinally administered δ OR agonist following sciatic nerve injury. Quantitative experiments aimed to determine protein expression were performed to assess nerve injury-induced changes in δ OR in the dorsal spinal cord. Some of the data have already been published in abstract form (Holdridge and Cahill, 2005).

2. Methods

2.1. Animals

Experiments were performed on adult male Sprague Dawley rats (225–250 g; Charles River, Que., Canada) housed in groups of two. Rats were maintained on a 12/12 h light/dark cycle and were given *ad libitum* access to food and water. Experiments were carried out during the light cycle according to protocols approved by the Queen's University Animal Care Committee and in accordance with guidelines set forth by the Canadian Council on Animal Care and the International Association for the Study of Pain Committee for Research and Ethical Issues.

2.2. Surgical procedures

2.2.1. Induction of chronic neuropathic pain

Chronic constriction injury (CCI) was accomplished by a slight modification of methods previously described by Mosconi and Kruger (1996). Rats were anaesthetized by isoflurane inhalation (induced at 5 l/min, maintained at 2 l/min) and their left hind legs and hips shaved clean of hair. A small incision was made in the left hind leg, distal to the hip, along its longitudinal axis. The underlying muscle was bluntly dissected to expose the sciatic nerve. The nerve was freed of connective tissue and a fixed diameter (2 mm) cuff assembled from polyethylene 90 tubing was placed around the nerve proximal to its bifurcation. The muscle and dermal wounds were closed with single non-continuous stitches using Monocryl 3-0 suture thread. The rats were given Tribissen injectable (0.02 ml/kg) antibiotic and 5 ml lactated ringer sub-cutaneously. Furacin antibiotic was applied topically to the incision site. Rats were monitored until awakening from the anesthetic and then returned to their home cages. This model is a modified version of that described by Bennett and Xie (1988) in which 4 chromic gut ligatures are tied loosely around the sciatic nerve. The modified version has been employed in the current study as it produces nociceptive behaviours reminiscent of those reported clinically, such as thermal hyperalgesia and mechanical allodynia, with reportedly less variability as compared to its predecessor. Indeed, the use of a cuff of fixed diameter ensures a consistent constriction of the nerve and negates the possibility of variation in tightness of chromic gut ligatures between individual ligatures and between operators. Furthermore, the Mosconi & Kruger model has been used extensively in the mechanistic and pharmacological characterization of neuropathic pain (Fisher et al., 1998; Cahill and Coderre, 2002; Cahill et al., 2003; Coull et al., 2003; Coull et al., 2005). Sham-operated rats received identical surgeries without manipulation of the nerve and were used, along with naïve rats,

as behavioural controls. Post-surgical behavioural testing revealed no differences between sham-operated and naïve rats ($p = 0.2262$ for cold hyperalgesia; $p = 0.1669$ for mechanical allodynia) and as such, data from the two groups were pooled to form the control group.

2.3. Behavioural testing

Separate groups of rats were used in each behavioural testing paradigm. All rats underwent pre-surgical behavioural testing in their respective paradigms, to establish baseline values to which post-surgical and post-drug values could be compared. Rats were then divided into three groups, those that underwent sciatic nerve constriction, those undergoing sham surgery, and naïve rats. Intrathecal (i.t.) administration of all drugs (30 μ l volume) was accomplished by way of lumbar puncture between the L4 and L5 vertebrae under brief halothane anesthesia. Successful drug placement was confirmed by a vigorous tail flick upon injection. Anti-allodynic and anti-hyperalgesic effects of the selective δ OR agonist D-[Ala², Glu⁴]deltorphin II (deltorphin II; Sigma, St. Louis, MO, USA) were assessed at 20 min following injection, as preliminary experiments in our laboratory have revealed this time point to correspond with peak analgesic effect. To assess the antinociceptive stress response following the lumbar puncture procedure, saline vehicle was administered and the effects observed at 20 min post-injection. Moreover, the receptor selectivity of deltorphin II-mediated antinociceptive effects was assessed by co-administration of deltorphin II with the δ OR-selective antagonist, naltrindole (Sigma) in a 1:2 molar ratio (10 μ g DELT: 11.52 μ g NALT).

2.3.1. Noxious cold testing

Withdrawal latencies from noxious cold were assessed in CCI and age-matched control rats as previously described (Cahill and Coderre, 2002). An open-ended clear plexi-glass cylinder was placed end-up into a 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 latency of 180 s was imposed to prevent tissue damage in the event that the rats did not respond. Rats were removed from the cold stimulus upon responding or reaching the cut-off latency.

2.3.2. Innocuous mechanical testing

Mechanical withdrawal responses to von Frey filament stimulation were assessed in CCI and control rats as previously described by Chaplan et al. (1994). Rats were placed under opaque Plexiglas® boxes positioned on a wire grid platform (5 mm \times 5 mm mesh), through which von Frey filaments were applied to the plantar surface of the hind paw in an up-down fashion. In brief,

filaments were applied in either ascending or descending force as necessary in order to most accurately determine the threshold of response. The intensity of stimuli ranged from 0.25 g to 15 g. From the resulting response patterns, the 50% response thresholds (g) were calculated. Paw withdrawal thresholds are expressed as 50% withdrawal thresholds or converted to % maximum possible effect (MPE) according to the following formula:

$$\%MPE = \frac{(\text{post-drug latency} - \text{baseline})}{(\text{cut-off latency} - \text{baseline})} \times 100$$

2.4. Molecular studies

2.4.1. Western blotting

Neuropathic and naïve rats were sacrificed by decapitation under light halothane anesthesia and their spinal cords were quickly removed by spinal ejection. The lumbar spinal cord was isolated and cut longitudinally into dorsal and ventral segments. The dorsal segment was then hemisected into ipsi- and contralateral segments and homogenized with a Polytron in buffer A containing 50 mM Trisma base, pH 7.4 and 4 mM ethylenediamine-tetraacetic acid (EDTA) with protease inhibitors (Complete™ Protease Inhibitor Tablets, Roche Molecular Biochemicals, Laval, Quebec, Canada; Phenylmethylsulfonyl Fluoride, Sigma-Aldrich, St. Louis, MO, USA). The samples were centrifuged at 4 °C for 10 min at 1000 rpm (Beckmann). The supernatant was collected and centrifuged at 4 °C for 30 min at 50,000 rpm. The pellets were resuspended in buffer B containing 50 mM Trisma base, pH 7.4 and 0.2 mM EDTA with protease inhibitors by vortexing and sonication for 5 s.

Protein content was determined (Bradford, 1976) and samples were denatured using 6x Laemmli sample buffer (0.375 mM Trisma base, pH 6.8, 30% v/v glycerol, 12% v/v 2- β -mercaptoethanol, 12% w/v sodium dodecyl sulfate (SDS), 0.2% w/v bromophenol blue) and then vortexed for 30 min at room temperature. Denatured samples were stored at –20 °C. Unused tissue samples were stored at –80 °C for denaturing at a later date.

Samples were loaded (45 μ g protein) and resolved using 8% Tris–Glycine pre-cast gels (Novex, San Diego, CA, USA) and the proteins were electroblotted onto nitrocellulose membranes (BioRad Laboratories, Richmond, CA, USA). A Biotinylated Protein Ladder (Cell Signalling Technology) and Kaleidoscope Prestained Standards (BioRad Laboratories, Richmond, CA, USA) were used to calibrate gel migration.

Nitrocellulose membranes were incubated for 1 h at room temperature (RT) with blocking solution (1% bovine albumin serum, 1% chicken ovalbumin in 26 mM Trisma buffered saline (TBS) containing 0.075% Tween 20) and then overnight with δ OR

antibody (Chemicon, Temecular, CA, USA; lots 23040417, 24040710) at 0.13 $\mu\text{g}/\text{ml}$ in blocking solution. An HRP-conjugated goat anti-rabbit secondary antibody (Amersham Pharmacia Biotech) diluted 1:4000 and an HRP-conjugated anti-biotin antibody (New England Biolabs) diluted 1:10,000 in 26 mM TBS containing 0.075% Tween 20 and 5% powdered milk, were used to visualize the bound primary antibodies and the biotinylated protein ladder, respectively. Secondary antibody incubation was carried out for 1 h at RT. The membranes were subsequently exposed to chemiluminescent reagents (Amersham Pharmacia Biotech) and developed onto hyperfilm. All membranes were routinely stripped and re-probed for β -actin to normalize the immunoreactive band density for minor differences in protein loading. Blots were digitized with a Hewlett-Packard 4570c Scanjet Scanner and formatted using Adobe Photoshop version 7.0 (Adobe Systems Inc., San Jose, CA, USA). Scion Image software (NIH) was used to measure integrated densities of immunoreactive bands. A calibration curve was calculated using the distance traveled by the biotinylated protein ladder and the molecular weights of immunoreactive bands were then estimated by extrapolation. The specificity of the Chemicon δOR antisera was confirmed by pre-adsorption of the antisera with an appropriate antigenic peptide (20 $\mu\text{g}/\text{ml}$ of peptide for 0.1 $\mu\text{g}/\text{ml}$ antibody).

2.5. Statistical analysis

All behavioural data are expressed as means \pm standard error of mean (s.e.m.) and molecular data as means \pm standard deviation (s.d.). Statistical analyses for were performed using one way analysis of variance (ANOVA) followed by the Tukey's multiple comparison test for post-hoc, or by unpaired *t*-test, as applicable. All graphs were generated and statistical analyses performed using GraphPad Prism software 3.01 (San Diego, CA, USA).

For gel electrophoresis experiments, immunoblots are representative of experiments performed on $N = 3$ per condition obtained from separate groups of animals. Additionally, each set of samples was run in duplicate and averaged to represent an N value of one. All behavioural data were performed on $N = 6$ –7 per group.

3. Results

3.1. Behavioural observations

Neuropathic rats developed characteristic postural manifestations which were evident in the ipsilateral hind leg only. These rats displayed cupped hind paws, which they held in what appeared to be a protective manner, bearing more of their body weight on the contralateral

side. Following surgery, CCI rats were not hindered in their ability to retrieve food and water or in their social interaction with cage mates. Sham-operated rats recovered quickly from surgery and displayed no postural manifestations. Furthermore, surgery rats displayed no obvious changes in weight gain or grooming behaviour compared to naive rats.

3.1.1. Anti-hyperalgesic effects of δOR agonist in cold testing

Fig. 1A illustrates the time course of cold-induced nociceptive responses in control and CCI rats. On days 7 and 14 following surgery, CCI rats displayed a significant decrease in the latency to withdraw the ipsilateral hind paw from a noxious cold stimulus, as compared with pre-surgical baseline values ($F_{3,15} = 9.128$, $p = 0.0011$). This decrease in cold thermal latency was interpreted as hyperalgesia and was exhibited in the ipsilateral hind paw, but not the contralateral side. Control animals showed no change in withdrawal thresholds throughout the 2-week testing period. Acute i.t. administration of deltorphin II (10 μg) on day 14 following surgery produced significant increases in withdrawal latencies in both control ($F_{3,18} = 11.28$, $p < 0.05$) and CCI ($F_{3,15} = 9.128$, $p < 0.01$) rats as compared to pre-drug values on the same day. Deltorphin II reversed cold hyperalgesia in CCI rats, producing latencies that were not significantly different from baseline values. Furthermore, CCI post-drug latencies were not significantly different from pre-drug values of control rats on day 14, indicating a return to normal nociceptive levels in CCI rats. When post-drug latency was converted to a % value of the pre-drug latency, Deltorphin II was shown to have a significantly greater effect in CCI rats compared to controls (unpaired *t*-test, $p = 0.0089$; Fig. 1B).

3.1.2. Anti-allodynic effects of δOR agonist in mechanical testing

Prior to surgery, all rats were unresponsive up to the maximum tactile force of 15.0 g, indicating the innocuous nature of the stimulus. Following surgery, CCI rats displayed a significant decrease in mechanical withdrawal thresholds in the ipsilateral hind paw interpreted as the development of mechanical allodynia, with no change in withdrawal thresholds on the contralateral hind paw over time ($F_{13,52} = 6.117$; Fig. 2A). Control animals remained unresponsive to the von Frey filament-stimulation throughout the 2-week testing period (data not shown). Acute i.t. administration of vehicle had no effect on withdrawal thresholds in CCI rats (Fig. 2C). In contrast, acute i.t. administration of deltorphin II (3–30 μg) on day 14 post-CCI surgery produced a dose-dependent increase in mechanical withdrawal thresholds in CCI rats (Fig. 2B). Post-drug 50% withdrawal thresholds were significantly higher than pre-

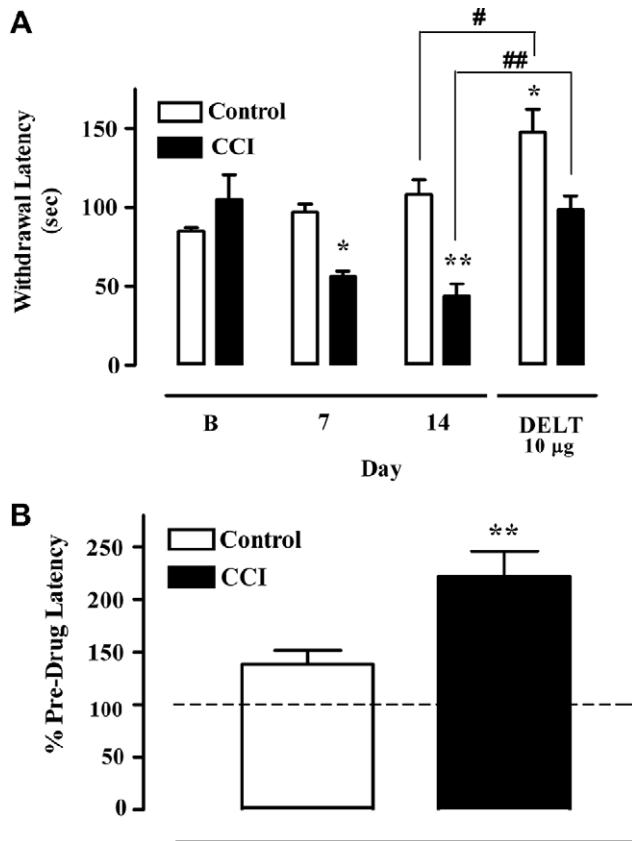


Fig. 1. (A) Cold withdrawal latencies using the noxious cold water test were assessed in control and CCI rats. (B) Anti-hyperalgesic effects of i.t. administration of deltorphin II (10 µg) were assessed at 20 min following injection on day 14 post-surgery in control and CCI rats. Mean cold withdrawal latencies following drug administration were converted to % values of respective pre-drug day 14 latencies. All data represent means \pm s.e.m. for $N = 6-7$ per group. Data in panel A were analyzed by a one-way ANOVA, followed by Tukey's *post hoc* multiple comparison test. Data in panel B were analyzed by a two-tailed, unpaired *t*-test. The *asterisk* denotes significant difference from respective baseline value (in A) or from control value (in B); * $P < 0.05$, ** $P < 0.01$. The number sign denotes significant difference from respective pre-drug value on day 14; # $P < 0.05$. DELT: deltorphin II; B: baseline.

drug values at doses of 10 µg or more ($F_{5,31} = 15.21$, $p < 0.05$ for 10 µg, $p < 0.01$ for 15, $p < 0.001$ for 30 µg). Co-administration of deltorphin II with the δ OR-selective antagonist, naltrindole, produced no significant effect on withdrawal thresholds, demonstrating that deltorphin II was mediating its anti-allodynic effects via activation of the δ OR (Fig. 2C).

3.2. Effect of chronic constriction injury on δ OR protein levels

To assess changes in δ OR expression in the dorsal spinal cord, western blotting techniques were used to quantify total protein in membranes prepared from lumbar spinal cord segments of naïve and day 14 CCI rats.

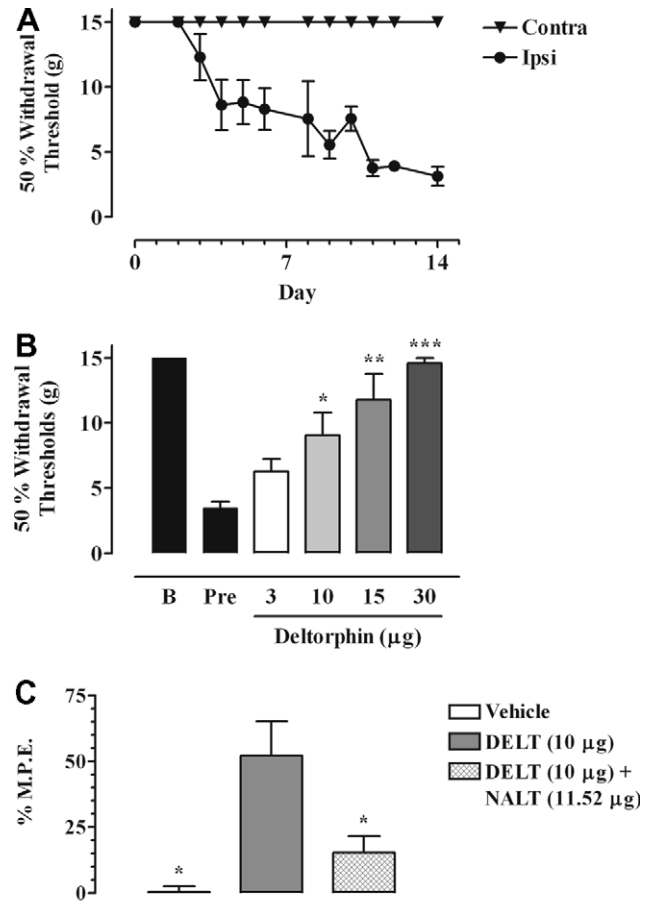


Fig. 2. (A) Mechanical withdrawal responses were assessed using calibrated von Frey filaments in CCI rats ($N = 7$). (B) Effects of i.t. deltorphin II were assessed on day 14 post-surgery in CCI rats (3, 10, 15, 30 µg; $N = 4-9$ per dose). Data were transformed to % maximum possible effect (MPE). (C) The effects of vehicle (saline) and co-administration of deltorphin II and the selective δ OR antagonist naltrindole (in a 1:2 DELT:NALT molar ratio; 10 µg DELT, 11.52 µg NALT) on mechanical withdrawal thresholds are presented. Mean 50% withdrawal thresholds were converted to % MPEs and compared to that of i.t. deltorphin II (30 µg) alone. Data represent means \pm s.e.m. Statistical analyses were performed by a one-way ANOVA, followed by Tukey's *post-hoc* multiple comparison test. The *asterisk* denotes significant difference from baseline value at day 0 (in A) or from deltorphin II (10 µg; in C); * $P < 0.05$, ** $P < 0.01$. Ipsi: ipsilateral; contra: contralateral; B: baseline; Pre: pre-drug; DELT: deltorphin II; NALT: naltrindole.

Immunoreactive bands were observed at estimated molecular weights of 52 and 75 kDa, consistent with earlier reports using the same antisera (Cahill et al., 2001). Immunoreactivity was confirmed by the absence of immunoreactive bands when the membrane was pre-incubated with antigenic peptide prior to antibody (data not shown). Following quantification of δ OR-immunoreactive bands, membranes were stripped and re-probed for β -actin, a housekeeping protein. To normalize differences in protein-loading, all data were expressed as δ OR/ β -actin ratios. No significant change in the

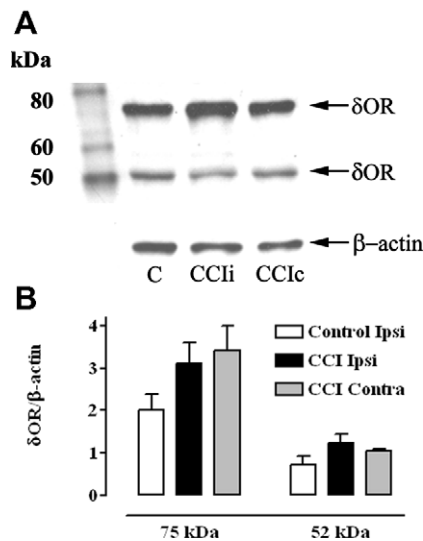


Fig. 3. (A) Identification of the δ OR protein by western blotting. Membranes from dorsal spinal cord tissues of control and CCI rats were isolated and proteins resolved using 8% Tris–Glycine gels followed by electroblotting onto nitrocellulose membranes. Immunoblot analysis reveals two specific immunoreactive bands with estimated molecular weights of approximately 52 and 75 kDa. Membranes were subsequently stripped and re probed for β -actin housekeeping protein. (B) Integrated density values of immunoreactive bands were converted to ratios of δ OR to β -actin and are expressed as means \pm s.d. for $N = 3$ per condition. One-way ANOVA analyses revealed no significant difference in δ OR immunoreactive band densities between CCI ipsilateral, CCI contralateral, and control. C: control; CCIi: ipsilateral; CCIc: contralateral.

integrated density ratios of either molecular weight band was observed at day 14 following nerve injury in the ipsilateral or contralateral spinal cord when compared to control ($F_{2,6} = 2.497$ for 52 kDa; $F_{2,6} = 2.340$ for 75 kDa; Fig. 3B).

4. Discussion

The present study reveals promising evidence for the use of δ OR-selective agonists in alleviating pain symptoms associated with peripheral nerve injury. The current behavioural experiments involving a noxious cold stimulus revealed that acute spinal administration of deltorphin II produced marked anti-hyperalgesic actions in CCI rats. Indeed, following surgery, CCI rats displayed significant reductions in withdrawal latencies in response to noxious cold water on days 7 and 14 while control rats showed no changes in latencies throughout the testing period. Intrathecal administration of the selective δ OR agonist deltorphin II returned withdrawal latencies in CCI rats to presurgical levels. These results complement previous observations that i.t. deltorphin II dose-dependently prolonged response latencies in the cold-water allodynia and the cold-water tail flick tests in rats following sciatic nerve crush (Mika et al.,

2001). Delta OR agonist-mediated anti-hyperalgesic effects have also been observed supraspinally where microinjection of [D-Pen², D-Pen⁵]-enkephalin (DPDPE) into the periaqueductal grey matter reversed cold allodynia following tight ligation of the tibial and sural nerves (Sohn et al., 2000). These findings contrast studies reporting that nerve injury resulted in a loss in potency of morphine (Kamei et al., 1992; Ossipov et al., 1995) and fentanyl (Zurek et al., 2001) following either systemic or spinal administration. Moreover, our data reveal a significantly enhanced effect of deltorphin II in CCI rats compared to controls. Granted, our experiments assessed the antinociceptive effects at only one dose, however the observation is not unique to our study. Kamei and colleagues (1995, 1997) reported enhanced δ OR-mediated analgesia in rats with experimental diabetic neuropathy as compared to control rats. While no changes in δ OR protein were observed in the spinal cord following CCI, a recent study from our laboratory reported a bilateral increase in δ OR protein in the DRGs of CCI rats compared to controls (Kabli and Cahill, in press). Deltorphin delivered intrathecally may diffuse toward and act at the DRG, which may in part explain the enhanced effects of spinally administered deltorphin following CCI. Furthermore, enhanced δ OR agonist effects have also been observed in other chronic pain models such as inflammation induced by complete Freund's adjuvant (CFA; Fraser et al., 2000; Hurley and Hammond, 2000; Cahill et al., 2003; Morinville et al., 2004) or by carrageenan (Hylden et al., 1991). Despite different induction methods, similar mechanisms may underlie the chronicity of both neuropathic and inflammatory pains including nociceptor activation-dependent changes (Dubner and Ruda, 1992; Woolf and Mannion, 1999; Woolf and Salter, 2000; Bridges et al., 2001), a mechanism also proposed to induce δ OR membrane-targeting and subsequently enhance δ OR agonist function (Gendron et al., 2006).

The anti-allodynic effects of deltorphin II were similarly assessed following nerve injury. Following surgery, CCI rats displayed significant decreases in mechanical withdrawal thresholds, indicating a hypersensitivity to innocuous light touch. The allodynic behaviour was observed in the ipsilateral hind paw only, and was absent in control animals. Spinal administration of deltorphin II dose-dependently reversed allodynia in a naltrindole-sensitive manner. Others similarly report a reversal of nerve injury-induced tactile allodynia following spinal (Nichols et al., 1995) and supraspinal (Sohn et al., 2000) administration of deltorphin II and DPDPE, respectively. Furthermore, Desmeules et al. (1993) observed a significant antinociceptive effect of the δ OR-selective agonist Tyr-D-Ser(O-*t*-butyl)-Gly-Phe-Leu-Thr (BUBU) in the paw pressure test in CCI rats. Intravenously administered BUBU produced anti-allodynic and analgesic effects, as exhibited by a return

to and increase from presurgical baseline vocalization thresholds. An additional interesting study employed the i.t. transplantation of immortalized rat astrocytic cells genetically modified by human preproenkephalin gene (An et al., 2005). Spinal injection of these cells on day 7 following spared nerve injury alleviated tactile allodynia as compared with control rats that received unmodified cells, and these effects persisted for 5 weeks. Moreover, several lines of evidence suggest a role for endogenous δ opioid peptides in modulating NP pain symptoms. Following ischemic spinal cord injury, most rats displayed tactile allodynia that was reversed with i.t. DPDPE (Hao et al., 1998a). Interestingly, those rats that did not display allodynia spontaneously, exhibited such behaviour upon administration of naltrindole, while this antagonist had no effect in naïve animals (Hao et al., 1998b). This suggests that following nerve injury, nociceptive levels may be under tonic inhibition via δ OR activation by endogenous peptides. Furthermore, δ OR knock-out mice displayed enhanced allodynic behaviour following partial sciatic nerve ligation, compared to wild type littermates (Nadal et al., 2006). Contrarily, Lee et al. (1995) reported no alleviation of neuropathic allodynia following i.t. or intracerebroventricular administration of DPDPE. The basis for these conflicting data is unclear at present. Nevertheless, δ OR agonists have also been shown to reverse allodynia induced by spinal administration of dynorphinA where (+)-4-[(α R)- α -(2S,5R)-4-Allyl-2,5-dimethyl-1-piperainyl]-3-methoxybenzyl]-N,N-diethylbenzamide (SNC80) dose-dependently alleviated tactile allodynia (Kawaraguchi et al., 2004).

Numerous studies suggest that μ OR agonists, like morphine, show decreased analgesic potency in NP states (Bian et al., 1999; Ossipov et al., 1995; Idänpään-Heikkilä and Guilbaud, 1999; Kim et al., 2003; Rashid et al., 2004). One proposed mechanism for the loss of opioid analgesia is the nerve injury-induced reduction in μ OR expression in the dorsal spinal cord (Stevens et al., 1991; Porreca et al., 1998; Kohno et al., 2005). Since the current behavioural experiments revealed no such loss in δ OR-mediated effects, we aimed to investigate changes in δ OR expression that may occur in response to nerve injury. Previous studies on the topic are inconclusive. While some groups report a decrease in δ OR-immunoreactivity (Stone et al., 2004) or binding (Stevens et al., 1991) following nerve injury, others report no change (Besse et al., 1992) or increased δ OR protein (Kabli and Cahill, in press) and mRNA (Zaratin et al., 1998). In the current study, spinal δ OR protein levels in naïve and CCI rats were assessed by western blotting techniques. The antisera utilized for δ OR immunodetection recognized an N-terminal sequence of the protein and revealed two specific immunoreactive bands at estimated molecular weights of 52 and 75 kDa. This was consistent with earlier reports using the same antibody

(Cahill et al., 2001), the specificity of which was confirmed as preadsorption of the antisera with an antigenic peptide abolished both immunoreactive bands. Different post-translational modifications of the δ OR such as homo- and heterodimerization (Cvejic and Devi, 1997; Jordan and Devi, 1999; George et al., 2000) and glycosylations (Belcheva et al., 1996; Miller, 1998) may account for δ OR variants detected at the protein level. The current protein analyses revealed no changes in δ OR protein expression in the dorsal spinal cord following CCI. Notwithstanding the minimal effects on δ OR expression, nerve injury may induce changes in receptor function and future studies will investigate this possibility.

In conclusion, we have demonstrated that spinal administration of deltorphin II effectively attenuates nerve injury-induced cold hyperalgesia and mechanical allodynia in the chronic constriction injury model while western blotting experiments revealed no alterations in δ OR protein biosynthesis. These data reveal a clinically relevant capacity of δ OR agonists to suppress hyperexcitable nociceptive transmission to normal physiological levels and suggest an important role of δ ORs in modulating pain symptoms associated with nerve injury, supporting further exploration of δ ORs as novel therapeutic targets in the treatment of NP pain.

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