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ABSTRACTS

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(The name of the author presenting the paper is shown in bold type. All authors have certified that, where appropriate, studies have been conducted with the approval of the relevant Human Ethics Committee or Animal Experimental Review Committee.)

Delta-opioid receptors modulate remifentanil-induced hyperalgesia by regulating *N*-methyl-D-aspartate receptor trafficking and function

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Numerous studies have demonstrated that intraoperative remifentanil infusion has been associated with postoperative opioid-induced hyperalgesia (OIH) and tolerance. Activation of delta-opioid receptor[1] (DOP) and augmentation of *N*-methyl-D-aspartate (NMDA) receptor[2] current may play an important role in the development of OIH. The aim of this study was to investigate the interactions of DOP and NMDA receptors in remifentanil-induced postinfusion hyperalgesia in a rat model of incision pain.

A total of 144 male Sprague–Dawley rats (260–280 g) with an intrathecal catheter sited were randomly divided into six groups (n=24): control (C), Incision pain (I), Remifentanil (R), Incision pain+remifentanil (RI), Incision pain+remifentanil+deltorphin II (RD), and Incision pain+remifentanil+naltrindole (RN). The model of Incision pain was established according to the Brennan method. Remifentanil was infused at a rate of 1 μg kg^{-1} h^{-1} for 1 h. Four nanomoles of deltorphin II or 30 nmol naltrindole in a total volume of 10 μl were infused through the intrathecal catheter in the RD and RN groups. Mechanical paw withdrawal threshold (PWT) and thermal withdrawal latency (PWL) were measured at 24 h before (T0) and at 2, 6, 24, and 48 h (T1–T4) after anaesthesia. Each animal was killed after the last measurement of PWT and PWL. Dorsal root ganglia were immediately removed for evaluation of the expression and trafficking of DOP receptors by immunofluorescence, western blot, and immune electron microscopy. Spinal dorsal horns (L4–L6) were prepared for determination of NMDA receptors and NR1, NR2A, and NR2B subunit levels in total and at the cell surface. A further 32 14- to 18-day-old male rats were divided into four groups (n=8): control (C); Remifentanil (R, 4 nmol litre^{-1}); Remifentanil+deltorphin II (RD, 4+10 nmol litre^{-1}); Remifentanil+naltrindole (RN, 4+1 nmol litre^{-1}). The lumbosacral spinal cord (L4–6) was prepared, and transverse sections were cut. Isolated spinal cord slices were moved into artificial cerebrospinal fluid and incubated for 60 min. After incubation, whole cell patch clamp was used to detect changes of the NMDA receptor-induced mEPSCs in dorsal horn neurones. Electrophysiological experiments were used to study whether DOP receptor activation or inhibition would change NMDA currents in dorsal horn neurones.

Compared with Group C, PWT significantly decreased and PWL significantly shortened. Expression and trafficking of DOP were up-regulated. Total and cell surface expression of NR1 and NR2B were significantly increased in Groups I, R, RI, and RD. NMDA receptor-induced mEPSCs in Groups R and RD showed a significant increase in amplitude and frequency compared with Group C in dorsal horn neurones. The amplitude and frequency in Group RN were significantly decreased compared with Group R.

Remifentanil infusion can enhance the expression and trafficking of DOP in DRG neurones, and subsequently enhance protein expression and physiological function of NMDA receptors in the spinal cord dorsal horn neurones. Inhibition of the expression and activity of the DOP receptor can prevent the occurrence of remifentanil-induced hyperalgesia.

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Spinal cord dorsal horn proteomics in bilateral chronic constriction injury-induced neuropathic pain in rats

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Unilateral chronic constriction injury (uCCI) of rat sciatic nerve is a common neuropathic animal model of pain. However, the extent to which uCCI actually mimics clinical neuropathic pain is uncertain. Recently, CCI of bilateral sciatic nerves in the rat has shown long-lasting increases in nocifensive responses to cold, which is similar to the human experience.[1 2] In this study, the behavioural changes to mechanical and cold stimuli in bilateral CCI rats were initially verified then followed by a proteomic analysis of lumbar spinal cord dorsal horn.

Experiments were performed with specific pathogen-free female Sprague–Dawley rats. Bilateral CCI of the sciatic nerves was performed under aseptic conditions with three loose ligatures of 4-0 chromic suture placed around the sciatic nerve. Both mechanical hyperalgesia and cold hyperalgesia were evaluated on three consecutive days before surgery and on postoperative days (PODs) 7 and 14, respectively. On POD 14, the nerve tissue of lumbar 4-6 spinal cord dorsal horn was dissected. Total protein were isolated, followed by reduction, alkylation, and trypsin digestion. Liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) was applied to characterize differentially expressed proteins. All proteins were analysed with Protein ANalysis THrough Evolutionary Relationships Version 8.0.

No motor deficit was observed in any bilateral CCI or sham rats. Both mechanical withdrawal threshold and cold hyperalgesia threshold decreased significantly on PODs 7 and 14, when compared with naïve or sham rats. Collectively, 1708, 1547, and 1663 proteins were characterized from the lumbar spinal cord dorsal horn of naïve, sham, and bilateral CCI rats, respectively. Twenty-five differentially expressed proteins associated with bilateral CCI were discovered, with 18 up-regulated and seven down-regulated (Table 1). These differentially expressed proteins are involved in several biological processes, such as apoptosis, cell adhesion, cell communication, cell cycle, cellular component organization, cellular process, developmental process, homeostatic process, immune system process, metabolic process, response to stimulus, system process, and transport. Among these differentially expressed proteins, PPP1CB (serine/threonine-protein phosphatase PP1-β catalytic subunit), is a direct inhibitor of CaMKIIa that may act as an inhibitor of long-term potentiation. MicroRNA-203, a modulator of PPP1CB expression, was found to be significantly down-regulated in our previous study,[3] and this correlation between miR-203 and PPP1CB may influence the course of neuropathic pain.

Table 1 Differentially expressed proteins associated with bilateral CCI

Accession	Protein name	Up- or down-regulated	Change folds
Acat3	Acetyl-CoA acetyltransferase, cytosolic	Up-regulated	>10
Actn4	α-Actinin-4	Up-regulated	>10
Aldoa	Fructose-bisphosphate aldolase A	Up-regulated	>10
Atp1a1	Sodium/potassium-transporting ATPase subunit α-1	Up-regulated	>10
Atp1a3	Sodium/potassium-transporting ATPase subunit α-3	Up-regulated	>10
Eno2	γ-Enolase	Up-regulated	>10
Hsp90aa1	Heat shock protein HSP 90-α	Up-regulated	>10
Ncam1	Neural cell adhesion molecule 1	Up-regulated	>10
Srprb	Srprb signal recognition particle receptor, B subunit	Up-regulated	>10
Tpm1	Tropomyosin α-1 chain isoform h	Up-regulated	>10
Tpm3	Isoform 2 of Tropomyosin α-3 chain	Up-regulated	>10
Tpm4	Tropomyosin α-4 chain	Up-regulated	>10
Ywhae	14-3-3 protein epsilon	Up-regulated	>10
Slc1a2	Isoform Glt1 of excitatory amino acid transporter 2	Up-regulated	2.166
Ppp1cb	Serine/threonine-protein phosphatase PP1-β catalytic subunit	Up-regulated	1.928
Atp1a2	Sodium/potassium-transporting ATPase subunit α-2	Up-regulated	1.926
Ndufa10	NADH dehydrogenase [ubiquinone] 1 α subcomplex subunit 10, mitochondrial	Up-regulated	1.822
Hsp90ab1	Heat shock protein HSP 90-β	Up-regulated	1.364
Pfkp	6-Phosphofructokinase type C	Down-regulated	−1.358
Ttn	Titin	Down-regulated	−1.505
Ap2a2	Adaptor-related protein complex 2, α 2 subunit	Down-regulated	−1.758
Myl1	Isoform MLC1 of myosin light chain 1/3, skeletal muscle isoform	Down-regulated	−2.068
Myl6l	Myosin light polypeptide 6	Down-regulated	−10
Tpm1	Tropomyosin α-1 chain isoform i	Down-regulated	−10
Tubb4	Tubulin, β 4	Down-regulated	>−10