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Plenary article

Opioid and noradrenergic contributions of tapentadol in experimental neuropathic pain

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HIGHLIGHTS

- Systemic tapentadol or duloxetine increases spinal norepinephrine levels.
- Tapentadol shows greater activity in nerve-injured rats relative to sham-operated rats.
- Systemic morphine reduces spinal norepinephrine levels.

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ABSTRACT

Tapentadol is a dual action molecule with mu opioid agonist and norepinephrine (NE) reuptake blocking activity that has recently been introduced for the treatment of moderate to severe pain. The effects of intraperitoneal (i.p.) morphine (10 mg/kg), tapentadol (10 or 30 mg/kg) or duloxetine (30 mg/kg), a norepinephrine/serotonin (NE/5HT) reuptake inhibitor, were evaluated in male, Sprague-Dawley rats with spinal nerve ligation (SNL) or sham surgery. Additionally, the effects of these drugs on spinal cerebrospinal fluid (CSF) NE levels were quantified. Response thresholds to von Frey filament stimulation decreased significantly from baseline in SNL, but not sham, operated rats. Duloxetine, tapentadol and morphine produced significant and time-related reversal of tactile hypersensitivity. Duloxetine significantly increased spinal CSF NE levels in both sham and SNL rats and no significant differences were observed in these groups. Tapentadol (10 mg/kg) produced a significant increase in spinal NE levels in SNL, but not in sham, rats. At the higher dose (30 mg/kg), tapentadol produced a significant increase in spinal CSF NE levels in both SNL and sham groups; however, spinal NE levels were elevated for an extended period in the SNL rats. This could be detected 30 min following tapentadol (30 mg/kg) in both sham and SNL groups. Surprisingly, while the dose of morphine studied reversed tactile hypersensitivity in nerve-injured rats, CSF NE levels were significantly reduced in both sham- and SNL rats. The data suggest that tapentadol elicits enhanced elevation in spinal NE levels in a model of experimental neuropathic pain offering a mechanistic correlate to observed clinical efficacy in this pain state.

1. Introduction

Pain is a complex experience with sensory, emotional and cognitive components [25]. The context in which nociceptors are activated plays an important role in the experience of pain [3,4]. Imaging studies performed with human volunteers receiving noxious stimuli under different experimental conditions have shown activation of brain areas known to process emotional responses, mood and attention [4,39]. Contextual engagement of multiple brain regions appear to participate in a "top-down" modulation of nociceptive circuits resulting in facilitation or inhibition of nociceptive inputs at the level of the spinal and trigeminal dorsal horn to elicit the pain experience [28].

The rostral ventromedial medulla (RVM) forms a final common relay in the descending modulation of nociception inputs [14]. This region has reciprocal connections with the periaqueductal gray (PAG) and together these loci form a key nexus of pain modulation [14]. These regions also have reciprocal connections with the locus coeruleus (A6) and the Kolliker–Fuse nucleus (A7), making up the pontine nuclei that are the main source of NE projections to the spinal cord [17,37,46]. Descending inhibition is believed to result, in part, from release of spinal NE [14,21,32,38]. Early animal studies had shown that the antinociceptive effect of supraspinal, but not spinal, morphine depends on activation of spinal α_2-adrenergic receptors suggesting an important role of descending noradrenergic projections [45]. Accordingly, the antinociceptive effect of

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opioids are blocked by systemic or spinal administration of α_2-adrenergic antagonists and enhanced by α_2-adrenergic agonists [26,29,31,44,45]. We, and others, have shown that the endogenous noradrenergic inhibitory system may protect against the development of signs of neuropathic pain in nerve-injured rats suggesting an important role of descending inhibition in chronic pain states [11,19,43]. Additionally, numerous pharmacological studies have demonstrated antinociceptive synergy between μ-opioid and α_2-adrenergic agonists at the spinal level [29,30].

Tapentadol was developed to mechanistically exploit the positive interaction between the opioid and the spinal NE system [34]. This compound exhibits weak affinity (i.e. 50-fold lower than morphine) at the μ-opioid receptor but additionally produces NE reuptake inhibition that is hypothesized to produce a synergistic μ-opioid/α_2-adrenergic mechanistic interaction with enhanced analgesic effects, particularly in neuropathic pain states [34]. As noted above, the antinociceptive effects of systemic and supraspinal morphine are blocked by spinal α_2-adrenergic receptor antagonists suggesting the release of spinal NE. However, few studies have measured spinal NE levels following morphine administration directly [5]. A recent study showed that morphine slightly reduced, rather than enhanced, spinal NE in the naïve rat [40].

In the present investigation, we compared the ability of tapentadol and morphine to reverse nerve-injury induced tactile hypersensitivity and to modulate spinal NE in rats with experimental neuropathic pain or in sham-operated controls. Duloxetine, a dual NE/5HT reuptake blocker was also studied for comparison.

2. Methods

2.1. Animals

Male, Sprague-Dawley rats (Harlan Laboratories Inc., Indianapolis, IN, USA) weighing 300–325 g at the time of testing were housed in a climate-controlled room on a 12 h light/dark cycle. Food and water were available at all times ad libitum. All experiments were performed in accordance to policies and procedures set forth by the International Association for the Study of Pain and the National Institutes of Health guidelines for the handling and use of laboratory animals. Approval was obtained from the Institutional Animal Care and Use Committee of the University of Arizona prior to all experimentation. Every effort was made to minimize animal pain and distress as well as to minimize the number of animals used. Experimenters were blinded to the treatment group in all behavioral experiments.

2.2. Spinal nerve ligation

As previously described by Kim and Chung, the L5/L6 surgical procedure was used to produce experimental chronic neuropathic pain [22]. Rats were anesthetized with isoflurane (2% mixed with room air; 2 L/min) and the lumbar vertebrae were exposed. The L5 and L6 spinal nerves were identified and tightly ligated with 4-O silk suture and the wound was closed. Sham-operated rats were prepared in the same manner as the SNL rats except the L5/L6 spinal nerves were not ligated. All rats were monitored for any visual signs of motor deficits, as well as for general health and weight maintenance.

2.3. CSF collection catheter preparation

The day before the surgery, 2 in. segments of PE-60 tubing (Scientific Commodities Inc., Lake Havasu, AZ, USA) were cut. Needle tips were removed from 23G syringes (BD Precision Glide, Franklin Lakes, NJ, USA). Using super glue, syringe needle, PE-60 tubing, and a gel loading pipette tip (Fisher Scientific, Pittsburgh, PA, USA) were securely fastened together to form a catheter. Catheters were allowed to dry overnight. On the day of the collection a P200 Pipetteman (Rainin, Columbus, OH, USA) was used with the prepared catheter to collect CSF.

2.4. Drug administration

The animals received an intraperitoneal (i.p.) injection of morphine (10 mg/kg) or tapentadol (10 and 30 mg/kg) dissolved in sterile saline or duloxetine (30 mg/kg) in distilled H_2O. Normal saline was used as the vehicle. Duloxetine was purchased from ChemPacific Corporation (Baltimore, MD, USA), morphine was provided by the NIDA Drug Supply Program and tapentadol was provided by Grunenthal Gmbh (Aachen, Germany).

2.5. Tactile hypersensitivity

Tactile withdrawal thresholds were determined 10–14 days following sham or SNL surgery. The rats were placed in suspended plastic chambers with wire mesh bottoms for 0.5 h prior to testing. A series of calibrated von Frey filaments was applied perpendicular to the plantar aspect of the ipsilateral hindpaw until the filament buckled [8,12,23]. The up-down method was used to determine the 50% withdrawal threshold with the Dixon nonparametric test as previously described [8,12,23]. Behavioral testing was performed approximately 10 min prior to initiation of CSF collection (i.e.; 20, 50 and 80 min after injection).

2.6. CSF collection

CSF was collected from naïve animals, or following sham- or SNL surgery. Rats were anesthetized with isoflurane (2% mixed with room air, 2 L/min) and placed in a stereotaxic frame. A 1.5 cm longitudinal incision from the back ridge of the skull to C1 was made and the muscles were retracted to expose the atlanto-occipital membrane. A prepared catheter and micropipette was used to puncture the membrane and collect the CSF (70–150 μl), free of blood, from the cisterna magna. The CSF was combined with an antioxidant cocktail (6.0 mM 1-cysteine, 2.0 mM oxalic acid, and 1.3% glacial acetic acid) and kept on ice in order to prevent the breakdown of catecholamines [18]. The samples were centrifuged (14,000 rpm) at 4 °C for 5 min. The amount of CSF collected was measured and added to a single catecholamine extraction tube. Catecholamine extraction kits were purchased from ESA, Inc., (Chelmsford, MA, USA) and the protocol was followed in full as per the provided manual.

2.7. HPLC with EC detector analysis

The HPLC system consisted of an Agilent 1100 quanternary pump and thermostated autosampler (Agilent Technologies, Palo Alto, CA, USA) coupled to an in-line Coulochem III electrochemical detector with model 5011A analytical cell (E1 −150 mV and E2 +250 mV) and model 5020 guard cell (+350 mV) (ESA Inc., Chelmsford, MA, USA). Using MD-TM mobile phase, at a flow rate of 0.400 ml/min, catecholamines were separated in samples using a MD-150 analytical column (3 mm × 15 cm) (ESA Inc., Chelmsford, MA, USA). Agilent ChemStation data acquisition software was used to analyze the chromatograms (Supplemental Fig. 1). Each sample of CSF was then spiked with a known amount of NE and re-injected into the HPLC system. The chromatograms were overlaid to confirm that the correct peak, indicated by a retention time of approximately 2.6 min, was collected. Additionally, a minimum of 3 separate series of sequentially varying amounts of NE in artificial CSF (aCSF) was injected into the HPLC system in order to generate a standard curve ($y = 0.8588x + 1.203$, $r^2 = 0.9998$). The lower limit