Neuropathic Pain Is Associated With MOR – Gs Coupling And Is Attenuated By Oxycodone + Ultra-low-dose Naltrexone

Pain Therapeutics, Inc.

H-Y. Wang[1]*, T.M. Largent[2], E. Friedman[1], Todd W. Vanderah[2], & L.H. Burns[3]

[1]Dept. of Physiology & Pharmacology, CUNY Medical School, New York, NY; [2]Dept. of Pharmacology, U. of Arizona, Tuscon, AZ; [3]Pain Therapeutics, Inc., South San Francisco, CA.

ABSTRACT

Both peripheral nerve injury and chronic opioid treatment result in hyperalgesia associated with enhanced excitatory neurotransmission and dynorphin release in spinal cord. We previously showed that chronic opioid administration causes a switch in mu opioid receptor (MOR) G protein coupling from Gi/o to Gs, and that ultra-low-dose opioid antagonist co-treatment reduces this switch. Using lumbar spinal cord from rats with L5/L6 spinal nerve ligation (SNL), we examined whether a similar switch in MOR coupling occurs in dorsal horn ipsilateral to the SNL injury. We also evaluated the effects of twice daily intrathecal delivery of 0.3 ng naltrexone (NTX) and 10 µg oxycodone over 7 days, both alone and in combination, on neuropathic pain behavior, spinal dynorphin levels, and on injury-induced changes in the MOR coupling profile in spinal dorsal horn. Oxycodone + NTX produced an anti-hyperalgesic effect over the week of testing and reduced allodynia for the first 4 days; oxycodone alone elicited a transient and weaker anti-hyperalgesia, and NTX alone had no effect. Oral drug delivery produced similar behavioral effects. The SNL injury induced MOR linkage to Gs in spinal dorsal horn and generally enhanced Gi/o coupling levels without affecting the expression of MOR or Gα proteins. Consistent with chronic morphine in uninjured rats, oxycodone treatment exacerbated the SNL-induced MOR – Gs coupling, and ultra-low-dose NTX co-treatment slightly attenuated the Gs coupling. The SNL-induced increase in spinal dynorphin was not clearly altered by any drug treatment in this study. These results suggest that the novel MOR – Gs coupling in response to SNL may contribute to the excitatory neurotransmission in spinal dorsal horn in neuropathic pain states. The anti-hyperalgesic and anti-allodynic effects of oxycodone + ultra-low-dose NTX (Oxytrex™) suggest a promising new treatment for neuropathic pain.

INTRODUCTION

We previously demonstrated that opioid analgesic tolerance involves a switch in G protein coupling by the MOR from Gi/o to Gs and that both tolerance and the associated coupling switch are suppressed by co-treatment with the ultra-low-dose opioid antagonist naloxone (Wang et al, 2005). We previously demonstrated that ultra-low-dose NTX co-treatment enhanced the efficacy of intrathecal morphine in the L5/L6 SNL model of neuropathic pain (Vanderah & Burns, 2004). Here we investigate both the behavioral and molecular pharmacological consequences of intrathecal oxycodone combined with ultra-low-dose NTX in the L5/L6 SNL model of neuropathic pain. Finally, since oxycodone + ultra-low-dose NTX is a novel investigational oral drug (Oxytrex), we also examined the anti-hyperalgesic and anti-allodynic effects of oral oxycodone + NTX in this model of neuropathic pain.

METHODS

Animals and Surgery

Male Sprague-Dawley 200g rats were tested for baseline thermal and tactile sensitivity prior to SNL surgery, a tight ligation of L5 and L6 spinal nerves. Thermal and tactile hypersensitivities were tested again 5 and 7 days later. Starting 8 days post-SNL, rats received twice daily doses, intrathecal (i.th.) or oral, for at least 7 days and were tested 20-30 min. after the a.m. dose for anti-allodynic and anti-hyperalgesic effects. For i.th. dosing, rats underwent i.th. catheterization and recovery prior to baseline testing. Spinal dorsal horn was harvested from Expt. 1 rats 16 hr after the last dose. Dose groups in each experiment are below.

Expt	Route	Dose groups	# animals
1	Intrathecal	Vehicle	6
		NTX (0.33ng)	11
		NTX (1 µg/kg)	7
		Oxycodone (10 µg)	19
		Oxy (10 µg) + NTX (0.33ng)	17
2	Oral	NTX (3 µg/kg)	6
		Oxycodone (10 mg/kg)	11
		Oxy (10 mg/kg) + NTX (0.03 µg/kg)	5
		Oxy (10 mg/kg) + NTX (1 µg/kg)	8
		Oxy (10 mg/kg) + NTX (3 µg/kg)	5

Measurement of Anti-hyperalgesia

Paw withdrawal latency to a thermal nociceptive stimulus was used to assess hyperalgesia. Rats were contained in individual Plexiglas compartments and a radiant heat source aimed at the plantar surface of the paw through a glass plate. The withdrawal latency is measured by a motion detector that halts both lamp and timer when the paw is withdrawn. Hyperalgesia was indicated by a decrease in latency from a baseline of ~20 s.

Measurement of Anti-allodynia

Allodynia or tactile hypersensitivity (i.e. sensitivity to non-noxious mechanical stimuli) was measured by the withdrawal threshold of the injured paw in response to probing with a series of Von Frey filaments with bending strengths from 0.41 to 15 g. Flinching at the lowest bending force indicates maximal allodynia. Mean withdrawal threshold was determined by sequentially increasing and decreasing the stimulus strength and analyzing responses using a Dixon non-parametric test.

Direct coupling protocol:

To investigate G protein coupling of MOR, synaptic membranes were prepared from spinal dorsal horn ipsilateral and contralateral to the SNL in rats from Expt. 1. Synaptic membranes (200 µg) were incubated with 1 µM morphine for 5 min at 37°C in Kreb's Ringer solution. Membranes were solubilized in immunoprecipitation buffer (25 mM HEPES, pH7.5; 200 mM NaCl, 2 mM MgCl2, 1 mM EDTA, 0.2% 2-mercaptoethanol, 50 µg/ml leupeptin, 25 µg/ml pepstatin A, 0.01 U/ml soybean trypsin inhibitor, 0.04 mM phenylmethylsulfonyl fluoride) containing 0.5% digitonin, 0.2% sodium cholate and 0.5% NP-40. G protein-coupled MOR were immunopurified using immobilized anti-Gα antibodies. MOR protein associated with specific Gα proteins was assessed by Western blotting using a specific antibody directed against the amino-terminal region of MOR (Santa Cruz Biotechnology). Statistical significance was determined by ANOVA followed by Neuman-Kuels comparisons.

RESULTS

MOR couples to Gs in SNL rats

SNL injury enhanced the recruitment of G proteins to MOR, shown by the significantly higher levels of coupling to MOR in spinal dorsal horn ipsilateral versus contralateral to the SNL injury (Fig. 1). These increases were more notable under morphine-stimulated conditions in vitro. In addition, SNL induced Gs coupling of MOR in spinal cord dorsal horn ipsilateral to the SNL that was not seen in contralateral spinal dorsal horn in rats treated with NTX alone (Fig. 1), nor in intact vehicle-treated rats in our previous work (Wang et al, 2005). Oxycodone treatment also contributed to this MOR – Gs coupling, since Gs coupling was observed in both ipsilateral and contralateral dorsal horn in oxycodone groups, but only in ipsilateral dorsal horn in rats treated with NTX alone. NTX co-treatment slightly but significantly attenuated the MOR – Gs coupling compared to oxycodone only treated rats in both ipsilateral and contralateral dorsal horn.

Fig. 1

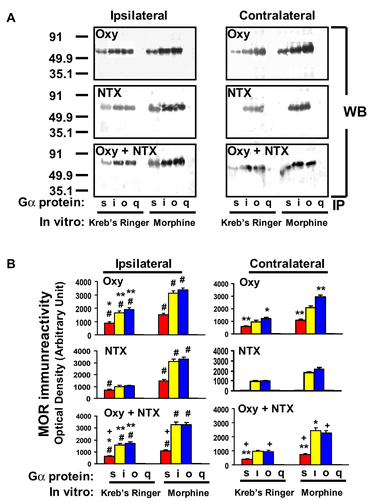

Fig. 1. **A**: Western blot of MORs co-immunoprecipitated with Gα proteins using specific antibodies against G αs, Gαi, Gαo and Gαq in membrane preparations from dorsal horn ipsilateral or contralateral to the SNL in rats treated with oxycodone, NTX or oxycodone + NTX. **B**: Densitometric quantitation of Western blots shown in A. # p<0.05 vs. respective value in contralateral side; * p<0.05 and ** p<0.01 vs. respective value in NTX group; + p<0.05 vs. respective value in oxycodone group.

Changes in G protein coupling were not due to changes in protein expression levels of MOR or G α proteins (Fig. 2).

Fig. 2

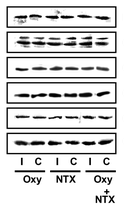

Fig. 2. Western blot showing no change in expression levels of MOR or Gα proteins from spinal dorsal horn of SNL injured rats. I = ipsilateral to SNL; C = contralateral to SNL.

Ultra-low-dose NTX enhances effects of i.th. oxycodone

Ultra-low-dose NTX greatly enhanced the anti-hyperalgesic effect of i.th. oxycodone in SNL animals (p<0.05 for AUC; Fig. 3). This combination produced minimal anti-hyperalgesic tolerance over the 11-day testing period. In the test of mechanical sensitivity, oxycodone + NTX produced a partial and transient anti-allodynic effect beyond the very subtle effect of oxycodone alone (p<0.05 for Days 1, 2 and 4; Fig. 4). I.th. NTX administered alone was without effect in both tests.

Fig. 3 Anti-hyperalgesic effect of intrathecal oxycodone +/- NTX

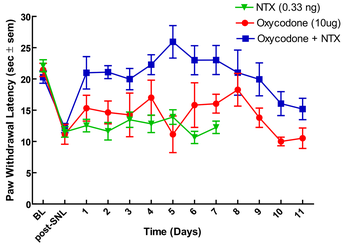

Fig. 3. The anti-hyperalgesic effect of twice daily i.th. injections of oxycodone (10 µg) + NTX (0.33 ng) was significantly greater than that of oxycodone (10 µg) alone in L5/L6 SNL rats.

Fig. 4 Anti-allodynic effect of intrathecal oxycodone +/- NTX

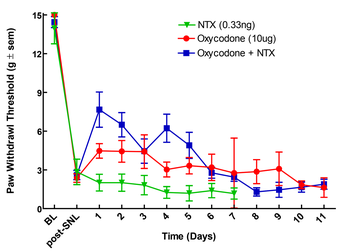

Fig. 4. Twice daily i.th. injections of oxycodone (10 µg) + NTX (0.33 ng) produced a partial and transient anti-allodynic effect compared to a much more subtle effect of oxycodone (10 µg) alone in L5/L6 SNL rats.

Ultra-low-dose NTX enhances effects of oral oxycodone

When dosed orally, NTX at 0.3, 1 or 3 µg/kg enhanced the anti-hyperalgesic effect of 10 mg/kg oral oxycodone to levels above or very near sham levels (Fig. 5). Comparing AUCs to the AUC for oxycodone alone, the anti-hyperalgesic effect of each oxycodone + NTX group was highly significant (p<0.0001 for 3 µg/kg NTX, p=0.001 for 1 µg/kg NTX, and p<0.001 for 0.3 µg/kg NTX). Some tolerance to this effect occurred on Day 7.

The anti-allodynic effects of these oral oxycodone + NTX treatments were more complete than after i.th. delivery, but again did not persist through Day 7 (Fig. 6). Again comparing AUCs to the AUC of the oxycodone alone group, the anti-allodynic effect over the 7 days of treatment was significant for oxycodone + the two highest NTX doses (p<0.0001 for µg/kg NTX and p<0.01 for 1 µg/kg NTX) and nearly significant for the 0.03 µg/kg NTX group (p=0.06).

Fig. 5 Anti-hyperalgesic effect of oral oxycodone +/- NTX

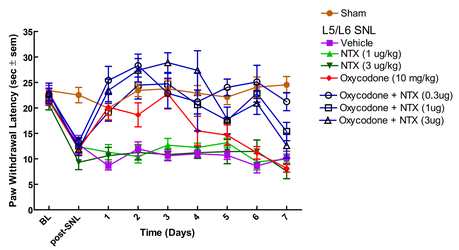

Fig. 5. Anti-hyperalgesic effects of oral oxycodone with or without NTX at various doses. The oxycodone alone group received 30 mg/kg on Day 1, and 10 mg/kg Days 2-7; all oxy + NTX groups received oxycodone at 10 mg/kg.

Fig. 6 Anti-allodynic effect of oral oxycodone +/- NTX

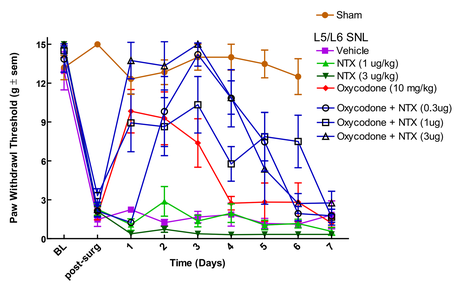

Fig. 6. Anti-allodynic effects of oral oxycodone with or without NTX at various doses. The oxycodone alone group received 30 mg/kg on Day 1, and 10 mg/kg Days 2-7; all oxy + NTX groups received oxycodone at 10 mg/kg.

DISCUSSION

The present work demonstrates that SNL injury induces MOR coupling to Gs proteins in the spinal dorsal horn ipsilateral to the ligation, suggesting excitatory signaling by these receptors instead of the usual inhibition. This excitatory signaling by MOR may contribute to the hyperalgesia associated with neuropathic injury. This aberrant G protein coupling occurred in dorsal horn ipsilateral but not contralateral to the SNL injury in animals not receiving oxycodone. In animals receiving oxycodone, MOR – Gs coupling was apparent in both ipsilateral and contralateral dorsal horn, suggesting that opioid treatment contributes to the altered coupling. Ultra-low-dose NTX co-treatment significantly attenuated MOR – Gs coupling on both sides. We previously demonstrated that MORs couple to Gs in CNS tissue from animals chronically treated with morphine, that ultra-low-dose naloxone co-treatment suppresses both the Gs coupling and the associated analgesic tolerance and physical dependence/withdrawal (Wang et al, 2005).

Even only slightly attenuating MOR – Gs coupling, ultra-low-dose NTX co-treatment enhanced the anti-hypersensitivities of oxycodone in this neuropathic pain model. An NTX dose of 0.33 ng injected i.th. strongly enhanced the anti-hyperalgesic effect and also attenuated the tolerance seen with 10 µg i.th. oxycodone alone. This NTX co-treatment also enhanced and prolonged the mild anti-allodynic effect of oxycodone.

To match the oral route of administration of the drug Oxytrex, we next tested oral oxycodone in combination with oral NTX at three doses. While there was no clear dose effect of oral NTX, all oral oxycodone/NTX combinations produced strong anti-hyperalgesia sustained at least through Day 6. Interestingly, oral delivery produced a much stronger anti-allodynic effect than i.th. dosing; the paw withdrawal threshold after oxycodone + 3 µg/kg NTX matched that of sham animals for the first three days. Although all oxycodone + NTX combinations lost this anti-allodynic effect on Day 6 or 7, these data are encouraging since opioid therapy typically does little to ameliorate the allodynia of neuropathic pain.

CONCLUSIONS

The present work demonstrates that SNL injury induces Gs coupling by MOR. The attenuation of this aberrant G protein coupling by ultra-low-dose NTX co-treatment was more mild in SNL animals than we previously showed in intact animals undergoing chronic opioid treatment; however, oxycodone + ultra-low-dose NTX produced profound anti-hypersensitivities in the L5/L6 SNL model. These data suggest a common molecular mechanism of action for ultra-low-dose opioid antagonist/opioid combinations in preventing analgesic tolerance and in alleviating hypersensitivities in SNL rats. The suppression of hyperalgesia and allodynia we observed suggests that Oxytrex may be a promising treatment for neuropathic pain.

REFERENCES

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