Published in final edited form as:

Naunyn-Schmiedeberg's Arch Pharmacol. 2013 April ; 386(4): 279–286. doi:10.1007/s00210-013-0833-8.

EFFECTS OF METHYLNALTREXONE ON GUINEA PIG GASTROINTESTINAL MOTILITY

Laura Anselmi^{1,2}, Jennifer Huynh¹, Gaia Vegezzi¹, and Catia Sternini^{1,2,*}

¹CURE Digestive Diseases Research Center, Digestive Diseases Division and Department of Medicine, University of California Los Angeles, David Geffen School of Medicine, Los Angeles, California 90095, USA

²Department of Neurobiology, University of California Los Angeles, David Geffen School of Medicine, Los Angeles, California 90095, USA

Abstract

The purpose of the present study was to compare the effects of methylnaltrexone (MNTX), a peripherally acting μ opioid receptor (μ OR) antagonist, on gastrointestinal (GI) motility in naïve vs. opiate-chronically treated guinea pigs *in vitro* and *in vivo*. We have used the electrically stimulated muscle twitch contractions of longitudinal muscle-myenteric plexus (LMMP) preparations and total GI transit as measure of GI motility. In LMMP preparations of naïve guinea pigs, MNTX (1–30 μ M) induced a significant, dose-response reduction of morphine-induced inhibition of electrically stimulated muscle twitch contractions, with an IC_{50} of 9.4×10^{-8} M. By contrast, MNTX abolished the inhibitory effect of acute morphine at any concentration tested (1–30 μ M) in the guinea pigs chronically treated with opiates. *In vivo*, MNTX (10–50 mg s.c.) did not affect GI transit in naïve guinea pigs when administered acutely or for 5 consecutive days, but reversed the GI transit delay induced by chronic morphine treatment. These findings show that MNTX is effective in reversing opiate-induced inhibition of GI motility acting at peripheral μ ORs, but does not exert a pharmacologic effect on GI transit in the absence of opiate stimulation.

Keywords

opioids; muscle twitch; transit; enteric nervous system

1. Introduction

Opioid analgesics such as morphine are the mainstay therapy for chronic malignant and nonmalignant pain (Schug et al., 1992). However, their administration is often accompanied by central and peripheral adverse effects, including the development of analgesic tolerance, physical dependence, sedation, respiratory depression and bowel dysfunction (Nestler, 2004; Becker et al., 2007). Opioid-induced bowel dysfunction (OBD) is a disabling condition characterized by a range of different gastrointestinal (GI) symptoms, including delayed gastric emptying, abdominal discomfort, bloating, gastroesophageal reflux, and constipation (Walsh, 1990; Pappagallo, 2001; Thorpe, 2001; Kurz and Sessler, 2003; Panchal et al., 2007; Holzer, 2010). The extreme discomfort due to severe constipation can become a limiting factor in the use of morphine compromising patient compliance and leading to the discontinuation of opiate treatment for pain control (Fallon and Hanks, 1999).

*Corresponding author: Catia Sternini, M.D., CURE, Digestive Diseases Research Center, Bldg. 115, Room 223, VAGLAHS, 11301 Wilshire Blvd., Los Angeles, CA, 90073, Tel: (310)-312-9477; Fax: 310-825-3133, csterini@ucla.edu.

Opioids receptors, including μ , δ , and κ are distributed throughout the central nervous system and periphery (Mansour et al., 1995; Sternini et al., 2004; Peng et al., 2012). μ opioid receptors (μ ORs) are the preferred targets for opiate drugs and mediate the development of OBD, which is predominantly due to the interaction of opiates with μ ORs in enteric neurons, resulting in inhibition of gastric emptying, and alteration of coordinated rhythmic contractions (De Luca and Coupar, 1996; Sternini, 2001). μ OR antagonists, such as naltrexone and naloxone, attenuate OBD symptoms, but they also reduce opiate-induced analgesia since these agents readily cross the blood brain barrier, and provoke opioid behavioral withdrawal syndrome (Culpepper-Morgan et al., 1992; Pappagallo, 2001). By contrast, methylnaltrexone (MNTX) and Alvimopan are opioid antagonists capable of selectively acting on peripheral μ ORs without affecting opiate central effects (DeHaven-Hudkins et al., 2008). MNTX is a quaternary N-methyl derivative of naltrexone, in which the addition of a methyl group increases the polarity reducing the ability to cross the blood-brain barrier (Brown and Goldberg, 1985; Yuan and Israel, 2006; Reichle and Conzen, 2008). MNTX preferentially acts at peripheral μ ORs and has been shown to be effective in ameliorating OBD symptoms without interfering with the central analgesic effect of opiates (Yuan et al., 1996; Deibert et al., 2010).

There have been numerous studies describing the pharmacological effects of MNTX on GI motility *in vitro* in guinea pigs, rodents and humans, and *in vivo* in rodents, which show its affinity for μ OR and its efficacy in blocking opiate effects at peripheral sites without affecting the analgesic effect. Given the variability among species, the purpose of the present study was to compare the effects of MNTX on GI motility in the presence or absence of opiate stimulation and with or without prolonged μ OR activation in the same species, using the guinea pig as a model system. We have compared the effect of MNTX on (1) morphine-induced inhibition of electrically stimulated muscle twitch contractions of longitudinal muscle-myenteric plexus (LMMP) preparations and (2) GI transit in guinea pigs treated chronically with morphine vs. naïve and normal animals without opiate stimulation. The results obtained from this study support the efficacy of MNTX in reversing morphine-induced inhibitory effect on motility, and show that MNTX is more effective following prolonged compared to acute μ OR activation, whereas it has no effect on GI transit *in vivo* in the absence of opiate stimulation.

2. Materials and methods

2.1 Animals

Animal care and procedures were in accordance with the National Institutes of Health recommendations for the humane use of animals. All experimental procedures were reviewed and approved by the appropriate Animal Care Committees at UCLA Los Angeles, CA, USA. Male albino Porcellus guinea pigs (150–250 g) (Hartley; Harlan Labs, San Diego, CA, USA) were used for these studies. Guinea pigs were divided in two groups: (a) animals, which did not receive any treatment or received subcutaneous injection of saline twice a day for 7 days (naïve); and (b) chronically treated animals that received subcutaneous injection of morphine sulfate twice a day for 7 days. Each morphine dose was increased progressively as follows: day 1–2, 10 mg/kg; day 3–4, 20 mg/kg; days 5–6, 40 mg/kg; and day 7, 80 mg/kg as described previously (Patierno et al., 2011). Animals were euthanized 30 min after the last morphine injection with an overdose of isoflurane (2.0 % v/v) in enclosed glass chamber.

2.2 Electrically induced contractile response of longitudinal muscle-myenteric plexus preparations

Segments of distal ileum were dissected from naïve and chronically morphine-treated guinea pigs and placed in standard Tyrode solution (mM: NaCl, 136.9; KCl 2.7; CaCl₂ 1.8; MgCl₂ 1.04; NaH₂PO₄ 0.4; NaHCO₃ 11.9; glucose 5.5) at 37°C. LMMP preparations were prepared by teasing the longitudinal muscle with the intact myenteric plexus from the underlying circular muscle (Sternini et al., 2000). Isometric contractions were recorded with a force-displacement transducer. After a 45-minutes equilibration period, strips were maximally stimulated (60 V, 0.1 Hz, 0.5 ms) using two platinum electrodes with a S44 stimulator (Grass Instrument Co., Quincy, MA). Concentration response-plots for the inhibitory effects of morphine (1nM–10 µM) on electrically induced contractions were constructed in a noncumulative fashion in the absence or in the presence of MNTX (100 nM–30 µM). Strips were washed for three 5-minutes periods after each agonist administration, and contractions were allowed to return to baseline before any subsequent pharmacological treatment. MNTX was left in contact with tissues for at least 20 min before agonist administration.

Concentration response plots were generated by plotting the percent inhibition of the twitch contraction versus the increasing logarithmic concentrations of morphine for both naïve guinea pigs and animals treated chronically with morphine. Individual concentration response plots were then generated for each concentration of MNTX (1–30 µM) and plotted. In order to determine the IC₅₀, the concentration required to produce a half-maximal inhibition of MNTX, a dose response curve was constructed by treating LMMP preparations with a single dose of morphine (100 nM) and increasing concentrations of the antagonist MNTX (1 nM to 30 µM). These data were fit to a theoretical sigmoidal binding equation: $B/B_{\max} = [1 + (c/EC_{50})^h]^{-1}$, where c is the concentration of MNTX tested, h is the Hill coefficient, and $B/B_{\max}$ is the fractional normalized response or percentage inhibition of the twitch contraction. The minimum and maximum values were fixed based on the regression value assigned by Prism (GraphPad ver. 5.0 Software, San Diego, CA). In the case of multiple comparisons, a one-way ANOVA in conjunction with the Bonferroni post hoc test was applied. Significance was set at $P < 0.05$, and variance was expressed as $\pm$ SE.

2.3 Gastrointestinal transit

GI transit was measured using a non-absorbable tracer fluorescein isothiocyanate-labeled dextran with an average molecular mass of 70 kD (FD70) (Moore, 2004; Patierno et al., 2011). Overnight fasted control animals (non treated) or animals chronically treated with morphine (chronic) were gavaged with 200 µL of FD70 dissolved in distilled water (2.5 mg/mL) and were euthanized 90 minutes later. The entire GI tract, from the stomach to distal colon was excised and divided into 15 segments: stomach, small intestine (divided into 10 segments of equal length), cecum, and colon (3 segments of equal length). The luminal content of each segment was collected, suspended in 1 mL of distilled water, vortexed, and centrifuged. The supernatants were collected and fluorometrically assayed for FD70 concentration using a multiwell fluorescent plate reader (Microplate Fluorescence Reader Flx 800; Bio-Tek, Winooski, VT). The wavelengths for detecting FD70 were 490 nm for excitation and 520 nm for emission; the slit width was 2.5 nm, and the integrated time was 1.0 second. The transit of FD70 along the GI tract was summarized by calculating the geometric center (GC) for the FD70 distribution (Miller et al., 1981). Control guinea pigs received either different doses of MNTX (10, 25 and 50 mg/kg) 30 min before the gavage or a daily dose of MNTX (25 mg/kg) for 5 consecutive days.

For chronically-morphine treated guinea pigs, a single dose of 25 mg/kg MNTX s.c. was administered 30 min after the last injection of morphine. Differences between means were

analyzed using one-way ANOVA with Bonferroni post hoc test for multiple comparisons. Significance was chosen as $P < 0.05$ and variance is reported as $\pm$ SE.

2.4 Drugs

Drugs used in this study were morphine sulfate from Baxter Healthcare Corporation (LA, CA) for *in vivo* injection, morphine sulfate from Sigma (St Louis, MO) for *in vitro* use, and MNTX (Wyeth Pharmaceuticals Inc., Philadelphia, and Progenics Pharmaceuticals, Tarrytown, NY). Drugs were dissolved in distilled water or 0.9% sodium chloride.

3. Results

3.1 Electrically induced contraction in LMMP preparations of the guinea pig ileum

Isolated LMMP preparations from either naïve or chronically morphine treated guinea pigs were stimulated electrically in the presence of increasing morphine concentrations (1 nM – 10 μ M) with or without MNTX (100 nM– 30 μ M). In naïve guinea pig LMMP preparations, morphine (1 nM–10 μ M) produced a concentration-dependent inhibition of electrically induced twitch contractions (Fig. 1). MNTX (1–10 μ M) had no effect on twitch basal contractions, however, we observed a transient increase in the magnitude of the twitch contraction during the first minute of administration of the highest concentration of MNTX (30 μ M) (data not shown). MNTX at the concentration of 1 μ M caused a rightward shift of the morphine inhibitory plot, without depression of its maximum response or efficacy (Fig. 1). With increasing concentrations of MNTX (3–30 μ M), the inhibitory effect of morphine was abolished. In order to determine the IC_{50} , each single dose of MNTX (1 nM–30 μ M) was tested in presence of 100 nM morphine. The inhibition of the twitch contraction induced by 100 nM morphine was significantly reduced by MNTX in a concentration dependent manner (1 nM –30 μ M), with an IC_{50} value of $9.4 \cdot 10^{-8}$ M for MNTX vs. morphine (Fig. 2).

In animals chronically treated with morphine for 7 days to make them tolerant, the morphine inhibitory concentration response curve was shifted rightward without reduction of the maximum response or efficacy when compared to untreated guinea pig LMMP preparations (Fig. 3), as previously reported (Chavkin and Goldstein, 1984; Sternini et al., 2000), and it was abolished by MNTX at every concentrations used, even the lowest one (1–30 μ M) (Fig. 3), suggesting increased antagonism following prolonged μ OR activation. Administration of MNTX (1–30 μ M) had no effect on basal contractions.

3.2 Gastrointestinal transit

In the control group, the orally administered fluorescent tracer was rapidly transported within the intestine such that the peak signal occurred in the cecum 90 minutes after administration. In naïve guinea pigs, there was no difference in the FD70 distribution, as assessed by calculating the GC with increasing doses of MNTX either injected i.p. or s.c. (control GC: 9.85 ± 0.46 ; 10 mg/kg MNTX i.p.: 9.47 ± 0.55 ; 25 mg/kg MNTX i.p.: 8.74 ± 1.01 ; 50 mg/kg MNTX i.p.: 7.33 ± 0.96 ; 25 mg/kg MNTX s.c.: 9.22 ± 0.48 ; 50 mg/kg MNTX s.c.: 9.18 ± 0.36). Moreover there was no difference in the GI transit time when 25 mg/kg of MNTX was injected s.c. for 5 days (9.30 ± 0.52 vs. 9.85 ± 0.46) (Fig. 4), indicating that MNTX has no pharmacological activity in the absence of opiate stimulation.

In contrast, in guinea pigs that were chronically treated with morphine, the distribution of the fluorescent tracer was shifted toward the more proximal segment of the GI tract, indicating a significant delay in GI transit time (3.83 ± 0.50 vs. 9.85 ± 0.46 , $p < 0.05$). Injection of 25 mg/kg of MNTX s.c. significantly reduced this delay of intestinal motility induced by chronic treatment with morphine (Fig. 4).

4. Discussion

In this study, we used the guinea pig as a model system to compare the effect of MNTX on GI motility *in vitro* and *in vivo* in normal conditions and following prolonged activation of μ ORs with morphine. This study showed that the inhibition of the twitch contraction induced by morphine (1 nM–10 μ M) was effectively antagonized by MNTX at various concentrations in either naïve or chronically morphine treated guinea pigs, extending previous observations. It also showed that MNTX antagonism of morphine-induced inhibition of muscle twitch was more effective following prolonged μ OR activation compared to acute stimulation. MNTX reversed the total GI transit delay induced by chronic treatment with morphine, whereas it had no effect on transit in naïve animals either administered through i.p. or s.c. routes, acutely or chronically.

Morphine and other opiates are the common treatment for cancer-related or non-related chronic pain, despite the many side effects that are limiting factors in pain management (Kurz and Sessler, 2003; Nestler, 2004; Panchal et al., 2007). OBD is one of the most distressing adverse effect associated with opiate administration (Kurz and Sessler, 2003) and is characterized by a range of GI symptoms, including abdominal pain, bloating and constipation (Pappagallo, 2001; Panchal et al., 2007). These symptoms can be so severe to induce patients to discontinue the narcotic therapy (Fallon and Hanks, 1999) compromising the management of pain and further lowering the quality of life of these patients. Opioid effects on GI functions are complex and involve μ ORs localized in neurons of the enteric nervous system. Enteric neurons expressing μ ORs are functionally distinct and include motor neurons and interneurons of the ascending and descending pathways controlling peristalsis and propulsion as well as secretomotor neurons regulating secretory functions (Bagnol et al., 1997; Ho et al., 2003; Sternini et al., 2004). Since μ ORs are coupled to inhibitory Gi/Go proteins, their activation results in the attenuation of neuronal activity by inhibiting neurotransmitter release (Sternini, 2001). Peripheral μ ORs are the main mediators of OBD symptoms, in particular constipation, thus antagonists preferentially acting at these μ ORs represent the best approach to reduce GI side effects in patients chronically treated with opiate analgesics. MNTX belongs to this category of drugs and it is characterized by poor lipid solubility resulting in limited ability to cross the blood brain barrier, with a consequent limited central nervous system activity. MNTX has been extensively tested in different species in both *in vivo* and *in vitro* systems and has been shown to be effective in OBD treatment in clinical trials (DeHaven-Hudkins et al., 2008; Viscusi et al., 2009; Garnock-Jones and McKeage, 2010). Interspecies variability needs to be taken into consideration when evaluating the inhibitory effects of opioid antagonists on intestinal motility, which vary from inhibition of propulsion to stimulation of non-propulsive or segmental motility pattern (Greenwood-Van Meerveld et al., 2004). The purpose of our study was to compare the potency of MNTX in blocking opiate effects on peripheral μ ORs in the presence or absence of prolonged μ OR activation vs. its pharmacological effect in the absence of opiate stimulation in a single species, the guinea pig, which has been extensively used to study opiate actions in the GI tract. The electrically-stimulated longitudinal muscle with the myenteric plexus attached from the guinea-pig small intestine is one of the most reliable preparations to study the effects of opiates and opioid peptides on cholinergic excitatory neuronal pathways that lead to neurogenic muscle contraction. Morphine inhibition of cholinergic transmission using these preparations has been reproduced in human ileum, making this animal model relevant to understand opioid biology in humans. Furthermore, MNTX blocked morphine inhibitory effect in both humans and guinea pig (Yuan et al., 1995). Moreover, opioid inhibition of cholinergic activity has also been described in human colon (Burleigh and Trout, 1986). A failure of morphine to inhibit cholinergic transmission in human intestine has been reported (Benko et al., 2010); however, differences in the experimental protocols, including different frequency and trains of

stimulation, might explain the discrepancies in results obtained with similar preparations (Yuan et al., 1995; Benko et al., 2010). It is also worthy to point out that inhibition of cholinergic transmission is not the only mechanism underlying the *in vivo* impairment of GI motility and that opioid effects on non-cholinergic-non-adrenergic neurons also play a role in the overall activities of opiates in the gut (Greenwood-Van Meerveld et al., 2004; Sanger and Tuladhar, 2004).

Our data showed that MNTX induced initial contraction of LMMPs probably reflecting an endogenous opioid tone and effectively antagonized the inhibitory effect of morphine on twitch contractions in electrically stimulated LMMPs, confirming previous observations in guinea pig and human isolated ileum (Yuan et al., 1995). This antagonism was observed in both naïve and chronically treated animals. However, in naïve animals, lower doses of MNTX decreased the morphine inhibitory effect without reducing the maximal response, typical example of competitive antagonism, with abolition of the response to morphine at higher concentrations. By contrast, in tolerant animals, even the lowest concentrations of MNTX completely abolished morphine inhibitory effect on muscle twitch. The increased efficiency of MNTX in blocking morphine effect on LMMP twitch might be due to the reduction of spare receptors (Chavkin and Goldstein, 1984) and to the increased efficiency of morphine to induce μ OR internalization observed in tolerant animals (Patierno et al., 2011). This suggests that in patients treated chronically with opiates, who have developed tolerance, lower doses of MNTX need to be used to block the opiate side effects on GI motility compared to patients receiving acute morphine.

In this study, we analyzed the effect of MNTX on total GI transit using different routes (s.c. and i.p.) and time courses of administration (only one injection or one injection for 5 days) and observed that MNTX did not affect the GI transit in normal animals in the absence of μ OR agonists in any of these conditions. Conversely, MNTX reversed morphine-induced peripherally mediated delay of GI transit. These findings are in agreement with previous reports showing efficacy of MNTX as a clinical adjuvant for treatment of chronic pain in patients (Greenwood-Van Meerveld et al., 2004; DeHaven-Hudkins et al., 2008), but indicate a lack of direct pharmacologic effect of MNTX on unstimulated μ ORs. In our study, we focused on the effect of MTNX on the opioid inhibitory activity in the ileum, which develops tolerance to chronic opiate treatment, unlike the colon, which does not become tolerant (Ross et al., 2008). The colon resistance to tolerance appears to be dependent on β arrestin 2 (Kang et al., 2012; Maguma et al., 2012), an intracellular protein that plays a role in ligand-induced μ OR internalization, a process that regulates the ability of the cells to respond to stimulation (Sternini, 2001). Whether MTNX antagonism at μ ORs varies in different regions of the gut remains to be elucidated. However, MNTX appears to reverse the agonist-induced impairment of GI transit and colorectal transit, indicating a similar effect of MNTX for the small bowel and colon (DeHaven-Hudkins et al., 2008). The effects of MNTX on opiate-induced inhibition of GI functions are overall similar to those described with Alvimopan, a peripherally acting μ OR antagonist, which has also extensively investigated in different species (DeHaven-Hudkins et al., 2008). Both compounds selectively antagonize GI functions mediated by μ ORs, without altering opiate analgesic effects and can reverse opioid inhibition of GI and colorectal transit, though Alvimopam has been reported to be overall more potent than MTNX (Zimmerman et al., 1994; Greenwood-Van Meerveld et al., 2004). Alvimopam has also been shown to improve postoperative ileus in rat when administered before surgery, whereas MTNX was not effective (Fukuda et al., 2006). Since abdominal surgery induces endogenous opioid release (Patierno et al., 2004), it has been suggested that Alvimopan amelioration of GI transit delay induced by surgery is mediated in part by inhibition of opioid release (Fukuda et al., 2006). However, several trials have now provided strong evidence that both MTNX and Alvimopan facilitate GI transit recovery in patients receiving opioids following abdominal surgery (Viscusi et al., 2009).

Whereas, the potency of MNTX on ameliorating the constipating effect of chronic opiates is now well established, it remains to be determined whether MNTX activity is affected by the level of expression and subcellular localization of peripheral μ ORs. Indeed, the observation of an effect of MNTX on endogenous opioid tone and its different potency on the neurogenic response in tolerant vs. naïve animals might reflect a different level of antagonism. This, in turn, might depend on the subcellular localization of the receptors, which can be affected by intracellular adaptation induced by prolonged receptor activation as well as by stress conditions such as abdominal surgery (Patierno et al., 2004; Patierno et al., 2011). It would also be of interest to determine the efficacy of MNTX on GI motility in conditions of inflammation that have been shown to be accompanied by increased expression of μ OR (Philippe et al., 2003; Philippe et al., 2006).

Acknowledgments

This study was supported by National Institutes of Health grants DK54155 and 41301 (Morphology and Cell Imaging Core) and a Wyeth/Pfizer grant.

References

- Bagnol D, Mansour A, Akil H, Watson SJ. Cellular localization and distribution of the cloned mu and kappa opioid receptors in rat gastrointestinal tract. *Neuroscience*. 1997; 81:579–591. [PubMed: 9300443]
- Becker G, Galandi D, Blum HE. Peripherally Acting Opioid Antagonists in the Treatment of Opiate-Related Constipation: A Systematic Review. *Journal of Pain and Symptom Management*. 2007; 34:547–565. [PubMed: 17900855]
- Benko R, Molnar Z, Nemes D, Dekany A, Kelemen D, Illenyi L, Cseke L, Papp A, Varga G, Bartho L. Unexpected insensitivity of the cholinergic motor responses to morphine in the human small intestine. *Pharmacology*. 2010; 86:145–148. [PubMed: 20699630]
- Brown DR, Goldberg LI. The use of quaternary narcotic antagonists in opiate research. *Neuropharmacology*. 1985; 24:181–191. [PubMed: 2986035]
- Burleigh DE, Trout SJ. Morphine attenuates cholinergic nerve activity in human isolated colonic muscle. *Br J Pharmacol*. 1986; 88:307–313. [PubMed: 2873856]
- Chavkin C, Goldstein A. Opioid receptor reserve in normal and morphine-tolerant guinea pig ileum myenteric plexus. *Proc Natl Acad Sci U S A*. 1984; 81:7253–7257. [PubMed: 6095280]
- Culpepper-Morgan JA, Inturrisi CE, Portenoy RK, Foley K, Houde RW, Marsh F, Kreek MJ. Treatment of opioid-induced constipation with oral naloxone: a pilot study. *Clin Pharmacol Ther*. 1992; 52:90–95. [PubMed: 1623695]
- De Luca A, Coupar IM. Insights into opioid action in the intestinal tract. *Pharmacol Ther*. 1996; 69:103–115. [PubMed: 8984506]
- DeHaven-Hudkins DL, DeHaven RN, Little PJ, Techner LM. The involvement of the mu-opioid receptor in gastrointestinal pathophysiology: therapeutic opportunities for antagonism at this receptor. *Pharmacol Ther*. 2008; 117:162–187. [PubMed: 18022696]
- Deibert P, Xander C, Blum HE, Becker G. Methylnaltrexone: the evidence for its use in the management of opioid-induced constipation. *Core Evid*. 2010; 4:247–258. [PubMed: 20694079]
- Fallon MT, Hanks GW. Morphine, constipation and performance status in advanced cancer patients. *Palliat Med*. 1999; 13:159–160. [PubMed: 10474699]
- Fukuda H, Suenaga K, Tsuchida D, Mantyh CR, Pappas TN, Hicks GA, Dehaven-Hudkins DL, Takahashi T. The selective mu opioid receptor antagonist, alvimopan, improves delayed GI transit of postoperative ileus in rats. *Brain Res*. 2006; 1102:63–70. [PubMed: 16797494]
- Garnock-Jones KP, McKeage K. Methylnaltrexone. *Drugs*. 2010; 70:919–928. [PubMed: 20426500]
- Greenwood-Van Meerveld B, Gardner C, Little P, Hicks G, Dehaven-Hudkins D. Preclinical studies of opioids and opioid antagonists on gastrointestinal function. *Neurogastroenterol and Motil*. 2004; 16:46–53.

- Ho A, Lievore A, Patierno S, Kohlmeier SE, Tonini M, Sternini C. Neurochemically distinct classes of myenteric neurons express the mu-opioid receptor in the guinea pig ileum. *J Comp Neurol*. 2003; 458:404–411. [PubMed: 12619074]
- Holzer P. Opioid antagonists for prevention and treatment of opioid-induced gastrointestinal effects. *Curr Opin Anaesthesiol*. 2010; 23:616–622. [PubMed: 20543677]
- Kang M, Maguma HT, Smith TH, Ross GR, Dewey WL, Akbarali HI. The role of beta-arrestin2 in the mechanism of morphine tolerance in the mouse and guinea pig gastrointestinal tract. *J Pharmacol Exp Ther*. 2012; 340:567–576. [PubMed: 22129596]
- Kurz A, Sessler DI. Opioid-induced bowel dysfunction: pathophysiology and potential new therapies. *Drugs*. 2003; 63:649–671. [PubMed: 12656645]
- Maguma HT, Dewey WL, Akbarali HI. Differences in the characteristics of tolerance to mu-opioid receptor agonists in the colon from wild type and beta-arrestin2 knockout mice. *Eur J Pharmacol*. 2012; 685:133–140. [PubMed: 22521552]
- Mansour A, Fox CA, Akil H, Watson SJ. Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. *Trends Neurosci*. 1995; 18:22–29. [PubMed: 7535487]
- Miller MS, Galligan JJ, Burks TF. Accurate measurement of intestinal transit in the rat. *J Pharmacol Methods*. 1981; 6:211–217. [PubMed: 7329070]
- Moore BA. Tyrphostin AG 126 inhibits development of postoperative ileus induced by surgical manipulation of murine colon. *AJP: Gastrointestinal and Liver Physiology*. 2004; 286:214G–224.
- Nestler EJ. Historical review: Molecular and cellular mechanisms of opiate and cocaine addiction. *Trends Pharmacol Sci*. 2004; 25:210–218. [PubMed: 15063085]
- Panchal SJ, Muller-Schwefe P, Wurzelmann JI. Opioid-induced bowel dysfunction: prevalence, pathophysiology and burden. *Int J Clin Pract*. 2007; 61:1181–1187. [PubMed: 17488292]
- Pappagallo M. Incidence, prevalence, and management of opioid bowel dysfunction. *Am J Surg*. 2001; 182:11S–18S. [PubMed: 11755892]
- Patierno S, Raybould HE, Sternini C. Abdominal surgery induces mu opioid receptor endocytosis in enteric neurons of the guinea-pig ileum. *Neuroscience*. 2004; 123:101–109. [PubMed: 14667445]
- Patierno S, Anselmi L, Jaramillo I, Scott D, Garcia R, Sternini C. Morphine induces mu opioid receptor endocytosis in guinea pig enteric neurons following prolonged receptor activation. *Gastroenterology*. 2011; 140:618–626. [PubMed: 21070774]
- Peng J, Sarkar S, Chang SL. Opioid receptor expression in human brain and peripheral tissues using absolute quantitative real-time RT-PCR. *Drug Alcohol Depend*. 2012; 124:223–228. [PubMed: 22356890]
- Philippe D, Dubuquoy L, Groux H, Brun V, Chuoi-Mariot MT, Gaveriaux-Ruff C, Colombel JF, Kieffer BL, Desreumaux P. Anti-inflammatory properties of the mu opioid receptor support its use in the treatment of colon inflammation. *J Clin Invest*. 2003; 111:1329–1338. [PubMed: 12727924]
- Philippe D, Chakass D, Thuru X, Zerbib P, Tsicopoulos A, Geboes K, Bulois P, Breisse M, Vorng H, Gay J, Colombel JF, Desreumaux P, Chamaillard M. Mu opioid receptor expression is increased in inflammatory bowel diseases: implications for homeostatic intestinal inflammation. *Gut*. 2006; 55:815–823. [PubMed: 16299031]
- Reichle FM, Conzen PF. Methylnaltrexone, a new peripheral mu-receptor antagonist for the prevention and treatment of opioid-induced extracerebral side effects. *Curr Opin Investig Drugs*. 2008; 9:90–100.
- Ross GR, Gabra BH, Dewey WL, Akbarali HI. Morphine tolerance in the mouse ileum and colon. *J Pharmacol Exp Ther*. 2008; 327:561–572. [PubMed: 18682567]
- Sanger GJ, Tuladhar BR. The role of endogenous opioids in the control of gastrointestinal motility: predictions from in vitro modelling. *Neurogastroenterol Motil*. 2004; 16(Suppl 2):38–45. [PubMed: 15357850]
- Schug SA, Zech D, Grond S, Jung H, Meuser T, Stobbe B. A long-term survey of morphine in cancer pain patients. *J Pain Symptom Manage*. 1992; 7:259–266. [PubMed: 1624812]
- Sternini C. Receptors and transmission in the brain-gut axis: potential for novel therapies. III. Mu-opioid receptors in the enteric nervous system. *Am J Physiol Gastrointest Liver Physiol*. 2001; 281:G8–15. [PubMed: 11408250]

- Sternini C, Patierno S, Selmer IS, Kirchgessner A. The opioid system in the gastrointestinal tract. *Neurogastroenterol Motil.* 2004; 16(Suppl 2):3–16. [PubMed: 15357847]
- Sternini C, Brecha NC, Minnis J, D'Agostino G, Balestra B, Fiori E, Tonini M. Role of agonist-dependent receptor internalization in the regulation of mu opioid receptors. *Neuroscience.* 2000; 98:233–241. [PubMed: 10854754]
- Thorpe DM. Management of opioid-induced constipation. *Curr Pain Headache Rep.* 2001; 5:237–240. [PubMed: 11309211]
- Viscusi ER, Gan TJ, Leslie JB, Foss JF, Talon MD, Du W, Owens G. Peripherally Acting Mu-Opioid Receptor Antagonists and Postoperative Ileus: Mechanisms of Action and Clinical Applicability. *Anesthesia & Analgesia.* 2009; 108:1811–1822. [PubMed: 19448206]
- Walsh TD. Prevention of opioid side effects. *J Pain Symptom Manage.* 1990; 5:362–367. [PubMed: 1980127]
- Yuan CS, Israel RJ. Methylnaltrexone, a novel peripheral opioid receptor antagonist for the treatment of opioid side effects. *Expert Opin Investig Drugs.* 2006; 15:541–552.
- Yuan CS, Foss JF, Moss J. Effects of methylnaltrexone on morphine-induced inhibition of contraction in isolated guinea-pig ileum and human intestine. *Eur J Pharmacol.* 1995; 276:107–111. [PubMed: 7781680]
- Yuan CS, Foss JF, O'Connor M, Toledano A, Roizen MF, Moss J. Methylnaltrexone prevents morphine-induced delay in oral-cecal transit time without affecting analgesia: a double-blind randomized placebo-controlled trial. *Clin Pharmacol Ther.* 1996; 59:469–475. [PubMed: 8612393]
- Zimmerman DM, Gidda JS, Cantrell BE, Schoepp DD, Johnson BG, Leander JD. Discovery of a potent, peripherally selective trans-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine opioid antagonist for the treatment of gastrointestinal motility disorders. *J Med Chem.* 1994; 37:2262–2265. [PubMed: 8057274]

Fig. 1. Morphine inhibitory curve in the absence (white circle) and presence of increasing concentrations (1 nM–30 μ M) of MNTX (black symbols). The electrically-evoked contractions are expressed as a percentage of the response prior to the administration of the drugs and are expressed as mean $\pm$ SE. All results are statistically significant vs. control ($p < 0.05$) ($n = 4-18$).

Fig. 2.
Inhibitory effect of 100 nM morphine on electrically stimulated twitch contractions of LMMP in presence of the μ OR antagonist, MTNX (1 nM 30 μ M). Values are expressed as mean $\pm$ SE. $n=4-18$. The graph represents a non linear regression for calculating the IC_{50} ($9.4 \cdot 10^{-8}M$).

Fig. 3.

This figure (a) compares the morphine inhibitory curve in LMMP preparations from naïve (white circle) and from morphine-chronically treated guinea pigs (black circle) in the absence of MNTX and (b) shows the effect of increasing concentrations of MTNX (1 μ M–30 μ M) (different black symbols) on morphine-inhibitory curve in LMMP preparations from guinea pigs chronically treated with morphine. The electrically-evoked contractions are expressed as percentage of the response prior to the administration of the drugs and are expressed as mean $\pm$ SE. All results are statistically significant vs. corresponding control (n=4–9).

Fig. 4.

Distribution of FD70 expressed as mean geometric center 90 minutes after gavage administration in control guinea pigs (white columns) and morphine-chronically treated guinea pigs (black columns). Note the lack of effect of MNTX in naïve animals either administered in single dose via different routes (i.p. and s.c.) and chronically for 5 consecutive days, and the reversal by a single dose of MNTX of the GI transit delay induced by chronic opiate administration (black columns). *** $P < 0.05$ vs control; ### $P < 0.05$ MNTX vs chronic control.