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Novel mixed NOP/MOP agonist BU08070 alleviates pain and inhibits gastrointestinal motility in mouse models mimicking diarrhea-predominant irritable bowel syndrome symptoms

Marta Sobczak¹, Gerta Cami-Kobeci², Maciej Sałaga¹, Stephen M. Husbands², and Jakub Fichna¹

¹Department of Biochemistry, Faculty of Medicine, Medical University of Lodz, Lodz, Poland

²Department of Pharmacy and Pharmacology, University of Bath, Bath, UK

Abstract

Background—The opioid and nociceptin systems play a crucial role in the maintenance of homeostasis in the gastrointestinal (GI) tract. The aim of this study was to characterize the effect of BU08070, a novel mixed MOP/NOP agonist, on mouse intestinal contractility *in vitro* and GI motility *in vivo* in physiological conditions and in animal models mimicking symptoms of irritable bowel syndrome (IBS), including diarrhea and abdominal pain.

Methods—The effect of BU08070 on muscle contractility *in vitro* was characterized in the ileum and colon. To assess the effect of BU08070 *in vivo*, the following parameters were assessed: whole GI transit, gastric emptying, geometric center, colonic bead expulsion, fecal pellet output and time to castor oil-induced diarrhea. The antinociceptive activity of BU08070 was characterized in the mustard oil (MO)-induced abdominal pain model and the writhing test, alone and in the presence of MOP and NOP antagonists.

Results—*In vitro*, BU08070 (10^{-10} – 10^{-6} M) inhibited colonic and ileal smooth muscle contractions in a concentration-dependent manner. *In vivo*, BU08070 prolonged the whole GI transit and inhibited colonic bead expulsion. The antitransit and antidiarrheal effect of BU08070 was observed already at the dose of 0.1 mg/kg (i.p.). BU08070 reversed hypermotility and reduced pain in mouse models mimicking IBS-D symptoms.

Conclusion—Our results suggest that BU08070 has a potential of becoming an efficient drug in IBS-D therapy. Here we also validate mixed NOP/MOP receptor targeting as possible future treatment of functional GI diseases.

*To whom correspondence should be addressed: Jakub Fichna, PhD, Department of Biomolecular Chemistry, Faculty of Medicine, Medical University of Lodz, Mazowiecka 6/8, 92-215 Lodz, Poland, Phone: ++48 42 272 57 07, Fax: ++48 42 272 56 94, jakub.fichna@umed.lodz.pl.

Chemical compounds studied in this article

β-Funaltrexamine (PubChem CID: 5311018); Buprenorphine (PubChem CID: 5311033); J-113397 (PubChem CID: 5311194); Nociceptin (PubChem CID: 56947112); Thebaine (PubChem CID: 5324289); UFP-101 (PubChem CID: 25081457)

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Keywords

abdominal pain; buprenorphine; gastrointestinal motility; irritable bowel syndrome; mixed agonist

Introduction

Irritable bowel syndrome (IBS) is a functional gastrointestinal (GI) disorder, which is diagnosed in 4–22% of population, depending on the study (for review, see: (Fichna and Storr, 2012)). The most common symptoms of IBS include abdominal pain and altered bowel habits. Three types of IBS are distinguished in accordance with Rome III criteria, including constipation-predominant (IBS-C), diarrhea-predominant (IBS-D) and IBS-A with alternating bowel habits, in which periods of diarrhea and constipation are observed (Fashner and Gitu, 2013). The pathophysiology of IBS remains unknown and since there are no well-defined causes of the disease, the therapy is limited to mitigation of the major symptoms. However, the multiplicity of endogenous mechanisms disturbed during IBS supports the concept of a multi-targeted therapy and encourages the development of novel drug candidates (Anastasi et al., 2013).

Numerous reports suggest that the endogenous nociceptin and opioid systems, which comprise the nociceptin (NOP) and opioid (MOP, KOP, DOP) receptors, respectively, and their endogenous peptide ligands may be involved in the pathophysiology of IBS-D (Sobczak, et al., 2013; Sobczak et al., 2014). Notably, NOP and opioid receptors are widely distributed in the central and peripheral nervous system and peripheral tissues, mainly in the GI tract and participate in many physiological processes, including alleviation of abdominal pain and inhibition of GI motility. NOP and MOP seem to play the most important role in the maintenance of homeostasis of the GI tract and therefore may constitute an attractive target in IBS-D therapy.

Recently, bifunctional compounds displaying NOP and MOP affinity have gained much attention as non-addictive analgesics (Dietis et al., 2009; Lin and Ko, 2013). One of the most common mixed NOP/MOP ligands is buprenorphine, a potent antinociceptive agent with unique physicochemical properties and greater analgesic potency than morphine, which has been used for several years in chronic to severe pain therapy (Cowan et al., 1977; Cowan et al., 1977). Buprenorphine is a semi-synthetic derivative of a morphine alkaloid thebaine, which binds to NOP and opioid receptors (Pergolizzi et al., 2010). Interestingly, being KOP antagonist, buprenorphine may limit spinal analgesia, sedation and psychotomimetic action (Johnson et al., 2005). The effect of buprenorphine in the GI tract has also been studied. *In vitro*, buprenorphine inhibited ileal muscle contraction induced by acetylcholine (10^{-6} M) in a concentration-dependent manner. Injected subcutaneously in mice, buprenorphine inhibited GI transit by about 50–60% in a wide range of doses (1.0–20.0 mg/kg) (Zhou et al., 2013).

Our group has recently synthesized a series of buprenorphine derivatives with the aim of increasing affinity for the NOP receptors. One of the obtained compounds, BU08070, displayed good binding affinity at NOP and opioid receptors, higher than that of the parent compound as evidenced in the *in vitro* binding studies (Cami-Kobeci et al., 2011). Namely,

the binding affinity (K_i /nM) of BU08070 at cloned human receptors was 18.45 ± 0.68 for NOP (vs. 77.42 ± 16.09 for buprenorphine) and 2.95 ± 0.65 for MOP (vs. 1.50 ± 0.80 for buprenorphine).

The aim of this study was to characterize the effect of BU08070 on mouse intestinal contractility *in vitro* and GI motility *in vivo* in physiological conditions and animal models mimicking symptoms of IBS, including diarrhea and abdominal pain. Our results suggest that BU08070 has a significant potential of becoming an efficient drug in the treatment of IBS-D.

1. Materials and Methods

1.1. Animals

Experimentally naive male albino mice (BALB/c) weighing 22–26 g were obtained from Nofer Institute of Occupational Medicine, Lodz, Poland. The animals were housed at a constant temperature (22–23 °C) and maintained under a 12-h light/dark cycle in sawdust-lined transparent plastic cages with free access to chow pellets and tap water. All experiments were performed in accordance with institutional animal ethics committee guidelines and approved by the Local Ethics Committee at the Medical University of Lodz (#589/2012).

1.2. In Vitro Organ Bath studies

All experiments were performed as described earlier (Fichna et al., 2009). Mice were sacrificed by cervical dislocation. Full-thickness fragments (0.5–1 cm) of the ileum and distal colon were isolated and kept in Krebs solution (NaCl 115 mM, KCl 8.0 mM, KH_2PO_4 2.0 mM, NaHCO_3 25 mM, MgCl_2 2.4 mM, CaCl_2 1.3 mM, and glucose 10 mM). The contents of the intestines were gently removed. One end of each intestinal segment was attached to the bottom of the individual organ bath, the other end to a FT03 isometric force displacement transducer (Grass Technologies, West Warwick, RI, USA) using a silk thread. Each intestinal segment was placed between two platinum electrodes in organ baths containing Krebs solution (25 ml) oxygenated with 95% O_2 and 5% CO_2 at 37 °C. The changes in tension were amplified by a P11T amplifier (Grass Technologies, West Warwick, RI, USA) and recorded on a personal computer using the POLYVIEW software (Polybytes Inc., Cedar Rapids, IA, USA). Electrical field stimulation was applied by a S88X stimulator (Grass Technologies, EFS, 8 Hz, 60 V, pulse duration 0.5 ms, train duration 10 s), and delivered through electrodes placed around the tissue.

The contractile responses were characterized in the presence of increasing concentrations of buprenorphine and BU08070 (10^{-10} – 10^{-6} M), with the contact time for each concentration of 8 min. Before the addition of drugs, the mean amplitude of four twitch contractions was expressed, and treated as an internal control. Changes in contractions were reported as the percentage of the internal control. In control experiments, the effect of the vehicle (dimethyl sulfoxide) was characterized. To characterize the involvement of MOP and NOP receptors, selective receptor antagonists were added into the organ baths 10 min prior to BU08070: β -funaltrexamine (β -FNA, 10^{-6} M, MOP antagonist) and J-113397 (10^{-6} M, NOP antagonist).

1.3. In vivo motility studies

Whole Gastrointestinal Transit—Whole GI transit time test evaluates the time of passage of the non-absorbable color dye (150 μ L of liquid, consisting of 5% Evans blue and 5% Arabic gum) through the GI tract (Fichna et al., 2013). Vehicle or BU08070 (0.1 mg/kg or 1 mg/kg) was administered i.p. 15 min before oral administration of the dye. Immediately after dye instillation, mice were transferred into individual cages, which were placed on a white piece of paper facilitating recognition of colored pellets. The whole GI transit time was evaluated from injection of the dye until excretion of the coloured pellet.

Gastric Emptying and Geometric Centre of Upper Gastrointestinal Transit—Gastric emptying (GE) and geometric centre (GC) experiments were performed as described earlier (Fichna et al. 2009; Kamysz et al., 2013). Briefly, mice were fasted overnight with free access to tap water. On the day of the experiment, vehicle or BU08070 (0.1 mg/kg) was injected i.p. and 15 min later 200 μ L of a marker solution (50 mg phenol red in 100 mL 1.5% methylcellulose) was administered orally. Twenty min after administration of the marker solution mice were sacrificed. The stomach and the small intestine were carefully isolated. The stomach was opened longitudinally and its contents transferred to a tube containing 4 mL of distilled water and gently vortexed. After 20 min of sedimentation, 1 mL of supernatant was transferred to another tube containing 1 mL of 1 M NaOH. The solutions (200 μ L) were colorimetrically assayed with a spectrophotometer (iMARK Microplate Reader, Biorad, United Kingdom) at 560 nm. Gastric emptying (%) was calculated according to the formula:

$$GE = 100 \times \left(1 - \frac{\text{amount of phenol red after 20 min}}{\text{amount of phenol red after 0 min}} \right)$$

In the GC experiments, 20 min after oral administration of a marker solution, the small intestine with its content was isolated, measured and divided equally into 10 segments. The intestinal matter of each bowel segment was transferred to tubes containing 2 mL of distilled water, vortexed and left for 20 min of sedimentation, after which 0.5 mL of supernatant was transferred to another tube containing 0.5 mL of 1 M NaOH to develop the maximum intensity of the colour. The solutions were colorimetrically measured with a spectrophotometer (iMARK Microplate Reader, Biorad, United Kingdom) at 560 nm. GC of small intestinal transit was calculated according to the formula:

$$GC = \sum (\%A \text{ per segment} \times \text{segment number}) / 100$$

GC ranged from 1 (minimal motility) to 10 (maximal motility).

Colonic Bead Expulsion Test—The colonic expulsion was performed as described previously (Fichna et al., 2009). Briefly, animals were fasted overnight. On the day of the experiment, vehicle or BU08070 (0.1 mg/kg) were administered i.p. and 10, 60 and 120 min later a prewarmed (37°C) glass bead (2 mm) was inserted into the distal colon 2.5 cm proximally to the anus using a silicone catheter. After instillation of the bead, mice were

placed in individual cages and the time to bead expulsion was assessed. The NOP and MOP antagonists (J-113397 and β -funaltrexamine, respectively) were injected i.p. 15 min before BU08070 administration.

Mouse Model of Castor Oil-induced Diarrhea—Animals were fasted overnight with free access to tap water. On the day of experiment, vehicle or BU08070 (0.1 mg/kg) was administered i.p. 15 min before the administration of the castor oil. Diarrhea was induced by oral administration of 200 μ L of castor oil and immediately after the animals were transferred into individual cages. The time between administration of castor oil and the appearance of symptoms of diarrhea (excretion of liquid faeces) was assessed and compared between groups (Fichna et al., 2009).

Mouse Models of Hypermotility—The fecal pellet output (FPO) was assessed in non-fasted animals (Fichna et al., 2011). In physiological conditions, mice were separated into individual cages for habituation one day before experiment. In stress conditions (new environment) mice were separated 15 min after vehicle or BU08070 (0.1 mg/kg) injection. The number of fecal pellets excreted over a 60 min was counted as a measure of GI tract motility.

1.4. Behavioural response to pain

The writhing test was performed as described previously (Gach et al., 2010). Mice were injected i.p. with acetic acid solution (10 ml/kg of 0.75%, vol/vol in saline). After the injection, mice were placed in transparent plastic cages and 5 min after recovery the total number of writhes was counted for 15 min. The writhing response was characterized by elongation of the body and the development of tension in the abdominal muscles and hind paws.

The behavioral responses to pain induced by i.c. administration of mustard oil (MO, allyl isothiocyanate) were determined as described previously (Eijkelkamp et al., 2007; Laird, et al., 2001). Briefly, 50 μ L of MO (1% in 70% ethanol) was injected i.c. under isoflurane anesthesia, then mice were separated into transparent plastic boxes (20 $\times$ 20 $\times$ 15 cm). After 5 min of recovery, spontaneous behaviors, including licking of the abdomen, abdominal retractions, squashing of lower abdomen against the floor, and stretching the abdomen, were observed for 20 min and counted as 1.

BU08070 (0.1 mg/kg) was injected i.p. 15 min before the MO or acetic acid instillation. Selective MOP (β -FNA, 1 mg/kg i.p.) and NOP (J-113397, 12 mg/kg, i.p.) receptor antagonists were administered 15 min before BU08070.

1.5. Drugs

All drugs and reagents, unless otherwise stated, were purchased from Sigma-Aldrich (Poznan, Poland). BU08070 was synthesized at the Department of Pharmacy and Pharmacology, University of Bath, Bath, Great Britain as described earlier. β -Funaltrexamine hydrochloride and J-113397 (($\pm$)-1-[(3*R**,4*R**)-1-(cyclooctylmethyl)-3-(hydroxymethyl)-4-piperidinyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one) were

purchased from Tocris Bioscience (Ellisville, MO, USA). In the *in vitro* experiments, all drugs were dissolved in dimethyl sulfoxide. In the *in vivo* tests, drugs were dissolved in 5% dimethyl sulfoxide in 0.9% NaCl, which was used as vehicle in control experiments and had no effects on the observed parameters.

1.6. Statistics

In the *in vitro* experiments *n* denotes the number of individual tissues. Statistical analyses were performed using PRISM 5.0 (GraphPad Software Inc., La Jolla, CA, USA). The data are expressed as means $\pm$ SEM. Student's *t*-test was used to compare single treatment means with control means. Analysis of variance (ANOVA) followed by Newman-Keuls post hoc test was used for analysis of multiple treatment means. *P* values ≤ 0.05 were considered statistically significant.

2. Results

2.1. BU08070 inhibits smooth muscle contractility in vitro

To assess the effect of buprenorphine and BU08070 on smooth muscle contractility in the mouse ileum and colon, organ bath studies on isolated tissue segments were performed. Buprenorphine and BU08070 (10^{-10} – 10^{-6} M) inhibited the EFS-induced twitch contractions in the ileum and colon in a concentration-dependent manner (Fig 1). As shown in Fig. 1C, the effect of BU08070 in the ileum was significantly inhibited by the NOP antagonist J-113397 (10^{-6} M) and blocked by the MOP antagonist β -FNA (10^{-6} M), what confirmed mixed activity of BU08070 at respective receptors. The inhibitory action of BU08070 in the colon was not changed by the NOP antagonist J-113397 (10^{-6} M), but it was partially reversed by the MOP antagonist β -FNA (10^{-6} M) (Fig. 1D), suggesting the involvement of MOP in the action of BU08070.

2.2. BU08070 has potent inhibitory effect on gastrointestinal motility in vivo

To extend our findings from the *in vitro* studies into the *in vivo* conditions, standardized assays to assess the effect of BU08070 on mouse GI motility were performed.

The action of BU08070 in the GI tract was characterized in several animal models mimicking physiological conditions. As shown in Fig. 2, BU08070 injected i.p. at the dose of 0.1 and 1 mg/kg produced a potent, dose-dependent inhibitory effect on whole GI transit time. To further elucidate this observation, the effect of BU08070 on upper and lower GI tract motility was examined. The i.p. injection of BU08070 at the dose of 0.1 mg/kg did not influence gastric emptying (Fig. 3A) and the upper GI transit, as indicated by no change in the geometric center (Fig. 3B). However, BU08070 (0.1 mg/kg, i.p.) produced a significant inhibitory effect on colonic expulsion up to 120 min after administration (Fig. 3C). The effect of BU08070 was non-significantly reversed by the NOP antagonist, J-113397 (12 mg/kg, i.p.) and blocked by the MOP antagonist, β -FNA (1 mg/kg, i.p.) (Fig. 4).

2.3. In vivo effects of BU08070 in mouse models mimicking IBS-D

To characterize the influence of BU08070 on the GI tract in pathophysiological conditions, two mouse models of hypermotility were used. In the first model, mice regarded as non-

stressed animals were placed in individual cages to acclimatize. On the day of the experiment, all experimental mice were injected with vehicle or BU08070 (i.p.) and the number of fecal pellets was counted during 60 min. We observed that the number of pellets was significantly decreased in mice injected with BU08070 in comparison with vehicle-treated animals (Fig. 5A). Stress (the change of the environment by separation of mice immediately before the experiment) induced a significant increase in fecal pellet output compared with non-stressed animals. BU08070 (0.1 mg/kg) injected i.p. in mice exposed to novel environment reversed stress-induced changes in GI motor activity (Fig. 5A).

The antidiarrheal potential of BU08070 was characterized in the mouse model of castor oil-induced diarrhea. Castor oil gavage induces an accumulation of water and electrolytes in the mouse intestine, causing the acute diarrhea in animals. BU08070 (0.1 mg/kg i.p.) significantly delayed time to appearance of liquid feces compared to vehicle-treated mice (Fig. 5B).

2.4. BU08070 exerts antinociceptive activity in mouse models of abdominal pain

Two mouse models of abdominal pain were used to characterize the antinociceptive effect of BU08070. In the writhing test, the i.p. administration of BU08070 (0.1 mg/kg) resulted in a significant reduction of the number of writhes (Fig. 6A). The antinociceptive effect of BU08070 was blocked by the MOP antagonist β -FNA (1 mg/kg, i.p.), but not the NOP antagonist, J-113397 (12 mg/kg, i.p.).

In mouse model of abdominal pain induced by the i.c. injection of MO solution, BU08070 (0.1 mg/kg, i.p.) significantly decreased pain-induced behaviors. Similarly to the writhing test, the MOP antagonist β -FNA (1 mg/kg, i.p.) blocked the action of BU08070 (0.1 mg/kg, i.p.) (Fig. 6B). The NOP antagonist, J-113397 (12 mg/kg, i.p.) did not reverse the action of BU08070 (0.1 mg/kg, i.p.). Interestingly, the antinociceptive effect of BU08070 was potentiated by the NOP antagonist.

3. Discussion

In this study we characterized the action of the novel buprenorphine analog, BU08070, as a potential antidiarrheal and antinociceptive agent. We found that BU08070, which is a mixed NOP/MOP ligand, significantly inhibits GI motility *in vitro* and *in vivo* and displays antinociceptive action in mouse models mimicking the symptoms of IBS-D. Earlier reports suggest that the novel compound has no adverse side effects in the central nervous system, typical for potent opioid agonists. Our results indicate that BU08070 may serve as an attractive template in the design of future anti-IBS-D treatments.

For thousands of years opioids have been applied as drugs to treat moderate to severe pain and diarrhea. However, the chronic application of opioids is associated with several side effects mediated primarily by MOP, which include tolerance, physical dependence, and respiratory depression. Furthermore, activation of KOP by non-selective opioids may result in sedative, dysphoric and psychotomimetic effects, while convulsions are the adverse effects typical for DOP agonists (for review see: (Schiller, 2010)). Chronic use of opioids may also challenge the physiology of the GI tract. The most common adverse effects include

opioid-induced bowel dysfunction and opioid-induced constipation, which occur in up to 10–20 % of adolescents and adults around the world.

Because of high efficiency of opioids, targeting opioid receptors still remains one of very few available strategies to limit pain and diarrhea in IBS-D patients. However, since biological activity at a single receptor is often insufficient and the activation of multiple opioid sites may produce adverse side effects, multitargeted ligands have recently attracted much attention. One of the multitargeted ligands is buprenorphine, which was shown to display a good affinity at NOP and MOP receptors (Cami-Kobeci et al., 2011). In this study, we found that buprenorphine significantly inhibits smooth muscle contractility in the mouse ileum and colon *in vitro* in a concentration-dependent manner and displays good anti-diarrheal potential. Buprenorphine was also characterized as an antinociceptive agent in several animal models of pain, for example writhing, hot plate, tail flick, formalin-induced inflammatory pain and mustard-oil-induced spontaneous pain tests (Christoph et al., 2005). Interestingly, the antinociceptive activity of buprenorphine was also demonstrated using visceromotor response to colorectal distension assay (Larauche et al., 2010). However, earlier studies, showing that buprenorphine may produce side effects typical for classical opioids, such as respiratory depression, dizziness or nausea (Boom et al., 2012), indicate the need to design the derivatives with similar pharmacological profile, but deprived of side effects typical for the parent compound.

The novel derivative of buprenorphine, a mixed NOP/MOP ligand BU08070 produced a potent inhibitory effect on mouse intestinal contractility *in vitro* and *in vivo* in physiological conditions and this effect was mediated by NOP and MOP receptors. BU08070 also displayed an anti-diarrheal action in mouse models mimicking the symptoms of IBS-D. The inhibitory action of BU08070 in the GI tract was similar to that of thienorphine (N-cyclopropylmethyl-7-[1-(R)-1-hydroxy-1-methyl-3-(thien-2-yl) propyl]-6,14-endo-ethanotetrahydro-oripavine), another buprenorphine analogue, designed by the group of Gong et al. (Yu, et al., 2006). However, thienorphine showed weaker inhibition on contractile amplitude in comparison with buprenorphine and only moderately inhibited small intestinal transit in mice. Interestingly, some indirect non-opioid pathways were shown to participate in thienorphine action, which need further investigation (Zhou et al., 2013).

Here we also found that BU08070 produced a potent antinociceptive effect in mouse models of abdominal pain, which was mediated by the MOP, but not NOP receptors. This is an interesting observation, as it is in line with the results obtained with SR14150, a mixed NOP/MOP ligand, whose antinociceptive effect was reversed mainly by naloxone (Toll et al., 2009). Moreover, another NOP/MOP agonist, SR14150 produced antinociception, which was reversed by naloxone, but not the NOP antagonist, UFP-101 (Spagnolo et al., 2008).

Noteworthy, in the MO-induced pain model we did not observe any effect of the NOP antagonist, J-113397 on the antinociceptive action of BU08070. However, in the writhing test the antinociceptive effect of BU08070 was increased in the presence of J-113397. Our results are in line with the findings of Lutfy et al. (Lutfy et al., 2003), who demonstrated that the antinociceptive activity of buprenorphine in the tail-flick assay was potentiated by the

NOP receptor antagonist J-113397. Similarly, other mixed NOP/MOP ligands, such as SR16435 and SR16507, produced a potent antinociceptive effect in the tail flick test that was potentiated by the NOP receptor antagonist, SB-612111 (Khroyan et al., 2009). These observations require further investigation.

Finally, our results show that BU08070 displays a measurable NOP agonist activity in the GI tract, what is surprising given the GTP γ S binding profile of this compound, suggesting an opposite effect at NOP receptors. This only confirms the captious action of nociceptin system.

4. Conclusion

BU08070, a novel derivative of buprenorphine, is a mixed NOP/MOP ligand with potent action in the GI tract *in vitro* and *in vivo*. BU08070 produces a significant anti-diarrheal and antinociceptive effect in mouse models mimicking IBS-D, therefore BU08070 is a very promising drug candidate and a potential alternative for currently used treatments.

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Figure 1.

Effect of buprenorphine and BU08070 (10^{-10} , 10^{-8} and 10^{-6} M) on EFS (8 Hz, 60 V, pulse duration 0.5 ms, train duration 10 s)-stimulated longitudinal smooth muscle contractions in mouse ileum (A and C) and colon (B and D). The effect of BU08070 was assessed in the presence of the NOP antagonist J-113397 (10^{-6} M) and the MOP antagonist β -funaltrexamine (β -FNA, 10^{-6} M). Representative original tracings of the effects of BU08070 on EFS stimulation in absence or presence of each antagonist in the mouse colon are shown in panel E. Data represent mean $\pm$ SEM for n=4–7 experiments. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ as compared to control. # $p<0.05$, ## $p<0.01$, ### $p<0.001$, vs. BU08070 alone.

Figure 2.

The inhibitory effect of BU08070 (0.1 and 1 mg/kg i.p.) on the whole gastrointestinal transit time in mice. Results are shown as mean $\pm$ SEM of $n=6$ mice per group. *** $p<0.001$, as compared to control (vehicle-treated mice).

Figure 3. Effect of BU08070 (0.1 mg/kg, i.p.) on gastric emptying (A), geometric center (B) and colonic bead expulsion time in mice (C). Data represent mean $\pm$ SEM of $n = 6$ mice per group. ** $p < 0.01$, as compared to control (vehicle-treated mice).

Figure 4.

The inhibitory effect of BU08070 (0.1 mg/kg, i.p.) on the colonic bead expulsion time alone and in co-administration with the NOP antagonist J-113397 (12 mg/kg, i.p.) or MOP antagonist β -funaltrexamine (β -FNA, 1 mg/kg i.p.). Data represent mean $\pm$ SEM of $n = 6$ mice per group. ** $p < 0.01$, as compared to control (vehicle-treated mice), # $p < 0.05$ BU08070 alone.

Figure 5.

The effect of BU08070 (0.1 mg/kg, i.p.) on fecal pellet output in mice (A) and time to castor oil-induced diarrhea (B). Data represent mean $\pm$ SEM for $n=6$ mice per group. *** $p<0.001$, as compared to control (vehicle-treated mice).

Figure 6.

The effect of BU08070 (0.1 mg/kg, i.p.) alone and in the presence of J-113397 (12 mg/kg, i.p.) or β -funaltrexamine (β -FNA, 1 mg/kg, i.p.) on a total number of behavioral pain responses induced by the i.p. administration of 0.75% acetic acid solution in the writhing test (A) and the i.c. administration of mustard oil solution (B) in mice. Data represent mean $\pm$ SEM for n=6 mice per group. ***p<0.001, as compared to control. ###p<0.001 vs. BU08070 alone.