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Further investigation of the rapid-onset and short-duration action of the G protein-biased μ-ligand oliceridine

Tomohisa Mori [a, 1], Yoshinori Takemura [b, 1], Takamichi Arima [c], Yoshiyuki Iwase [a], Michiko Narita [d, e], Kanako Miyano [e], Yusuke Hamada [a, e], Yukari Suda [a, e], Akinobu Matsuzawa [f], Kazuyuki Sugita [f], Shoki Matsumura [g], Shigeru Sasaki [g], Takayasu Yamauchi [g], Kimio Higashiyama [g], Yasuhito Uezono [h], Mitsuaki Yamazaki [b], Naoko Kuzumaki [a, **], Minoru Narita [a, e, *]

[a] Department of Pharmacology, Hoshi University School of Pharmacy and Pharmaceutical Sciences, 2-4-41, Ebara, Shinagawa-ku, Tokyo, 142-8501, Japan
[b] Department of Anesthesiology, Faculty of Medicine, University of Toyama, 2630 Sugitani, Toyama-shi, Toyama, 930-0194, Japan
[c] Department of Pharmacy, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo, 104-0045, Japan
[d] Department of Molecular and Cellular Medicine, Institute of Medical Science, Tokyo Medical University, 6-7-1 Nishi-Shinjuku, Shinjuku-ku, Tokyo, 160-0023, Japan
[e] Division of Cancer Pathophysiology, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo, 104-0045, Japan
[f] Department of Synthetic Medicinal Chemistry, Hoshi University School of Pharmacy and Pharmaceutical Sciences, 2-4-41, Ebara, Shinagawa-ku, Tokyo, 142-8501, Japan
[g] Institute of Medicinal Chemistry, Hoshi University School of Pharmacy and Pharmaceutical Sciences, 2-4-41, Ebara, Shinagawa-ku, Tokyo, 142-8501, Japan
[h] Department of Pain Control Research, The Jikei University School of Medicine, 3-25-8 Nishi-Shimbashi, Minato-ku, Tokyo, 105-8461, Japan

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ABSTRACT

TRV130 (oliceridine), a G protein-biased ligand for μ-opioid receptor, has recently been synthesized. It is considered to have strong antinociceptive effects and only minor adverse effects. However, whether or not oliceridine actually exhibits an ideal pharmacological profile as an analgesic has not yet been fully clarified in animal studies. This study examined the pharmacological profile of oliceridine in cells and animals. Oliceridine (10 μM) did not produce any μ-opioid receptor internalization in cells even though it increased impedance, which reflects the activation of Gi protein using the CellKey™ system, and inhibited the formation of cAMP. In mice, oliceridine (0.3–10 mg/kg) produced a dose-dependent antinociceptive effect with a rapid-onset and short-duration action in the hot-plate test, as well as antihyperalgesia after sciatic nerve ligation without the development of antinociceptive tolerance using the thermal hyperalgesia test. On the other hand, oliceridine inhibited gastrointestinal transit. Furthermore, oliceridine produced rapid-onset hyperlocomotion at antinociceptive doses; sensitization developed in mice and an emetic effect was observed in ferrets. These results indicate that, although oliceridine may produce dopamine-related behaviors even through selective stimulation of the G-protein-biased μ-opioid receptor pathway, it still offers advantages for breakthrough pain without antinociceptive tolerance with adequate doses.

1. Introduction

Pharmacological agents including receptor agonists and antagonists have been developed to accelerate and decelerate signal transduction mainly through G protein-coupled receptors (GPCRs). For the past four decades, such pharmacological agents have been synthesized based on an “on-off” system that involves sequential transduction for each GPCR. On the other hand, a biased ligand theory, in which diverse signaling can be provoked even after the

Abbreviations: GPCR, G protein-coupled receptors; QOL, Quality of life; HEK, Human embryonic kidney; PTX, Pertussis toxin.

* Corresponding author. Department of Pharmacology, Hoshi University School of Pharmacy and Pharmaceutical Sciences, 2-4-41 Ebara, Shinagawa-ku, Tokyo, 142-8501, Japan.
** Corresponding author.
E-mail addresses: n-kuzumaki@hoshi.ac.jp (N. Kuzumaki), narita@hoshi.ac.jp (M. Narita).
[1] These authors contributed equally to this work.

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activation of GPCR by ligands, has recently been proposed. In this theory, various pharmacological actions may be triggered through the regulation of different pathways [1].

It is well known that μ-opioid receptor agonists are effective at mitigating moderate to severe pain caused by cancer. However, adverse effects of μ-opioid receptor agonists such as constipation, emesis, drowsiness, hallucination, delirium and analgesic tolerance limit their utility and reduce the quality of life (QOL) of patients [2]. Animal models have been developed and used to predict the usefulness and safety of medicines before and during their development for use in humans. Many of the pharmacological effects of μ-opioid receptor agonists have been well documented in animal models.

Both a G protein-coupled signaling pathway and a β-arrestin recruitment pathway have been observed after the activation of μ-opioid receptor [3,4]. TRV130 (oliceridine) [5] and PZM21 [6], which selectively activate the G protein-coupled signaling pathway of μ-opioid receptors, have recently been synthetized. A growing body of evidence obtained using β-arrestin-2 knockout mice has suggested that selective activation of a G protein-coupled signaling pathway produces a potent antinociceptive effect without inducing severe constipation or respiratory dysfunction [5,7,8]. In fact, in animal studies, both oliceridine and PZM21 have been shown to exhibit weaker adverse effects than their respective antinociceptive effects [6,9]. Therefore, these biased ligands could be a safer option for controlling pain. However, the inducibility of antinociceptive effects and adverse side effects after selective activation of the G protein-coupled signaling pathway by its specific ligand have not been fully investigated. In this basic study, we further investigated the pharmacological utility of oliceridine as an analgesic.

2. Materials and methods

2.1. In vitro experiments

cAMP assay. After 24 h culturing of HEK293-MOR-FLAG cells (1.5×10^4 cells per well in 96-well plates) transfected with Glosensor cAMP plasmid, GloSensor cAMP assay (Promega, Madison, WI, USA) was performed to measure G protein activation according to the manufacturer's protocol [10]. After treatment with forskolin (10 μM), activator of adenylate cyclase cAMP system for 5 min, morphine, fentanyl and oliceridine were treated to each well for 30 min.

Electrical Impedance-based Biosensors (CellKey™ Assay). The CellKey™ assay has been described previously [11,12]. In brief, HEK293 cells stably expressing N-terminal Flag-Tag®-fused human μ-opioid receptors were cultured at 5.0×10^4 cells/well on a standard CellKey™ 96-well microplate. In this study, baseline data were recorded for 5 min before drug application. After drug application, ΔZ was measured every 10 s. Changes in ΔZ are expressed in terms of (ΔZ maximum-ΔZ maximum) after drug application.

Quantitative analysis of receptor internalization. The μ-opioid receptor internalization assay has been described previously [13]. In brief, we obtained cDNA for N-terminal Halotag®-fused human μ-opioid receptors from Kazusa DNA Research Institute (Kisaragi, Chiba, Japan). The clone was stably expressed in HEK293 cells. Halotag-μ-opioid receptor internalization was quantified using a single confocal image that included the nucleus and a large area of the cytoplasm as described previously [13]. The percentage of fluorescence in the cytoplasm (Δ % internalization) was calculated as the fluorescence intensity in the cytoplasmic region divided by the total fluorescence intensity.

Identification of internalized μ-opioid receptor-induced spots. The HaloTag® pH Sensor Ligand was also used to investigate μ-opioid receptor internalization. As described previously [13], in HEK293 cells expressing N-terminal Halotag®-fused human μ-opioid receptors, HaloTag® pH Sensor Ligand binds to Halotag protein on the cell membrane, but does not emit fluorescence under the conditions of Excitation/Emission: 534/562 nm. When HaloTag® pH Sensor Ligand-bound receptors internalize to endosomes by endocytosis, HaloTag® pH Sensor Ligand elicits red fluorescence in acidic vesicles. Therefore, the fluorescent intensity reflects the amount of the internalized-μ opioid receptor. μ-Opioid receptor internalization was quantified by counting the numbers of red fluorescent spots per cell with a Cellomics ArrayScan VTI (Thermo Fisher Scientific, Waltham, MA).

2.2. In vivo experiments

Animals. Male Institute of Cancer Research (ICR) mice (20–25 g) (Tokyo Laboratory Animals Science Co. Ltd., Tokyo, Japan) were used. Food and water were available *ad libitum* in their home cages. Male ferrets weighing 1–1.5 kg were obtained from Marshall Research Labs (North Rose, NY) and housed individually in cages. They were given a standard cat diet (70–80 g/animal, Oriental Yeast Co. Ltd., Chiba, Japan) and allowed free access to water. All animals were housed in a room maintained at 22 ± 1 °C with a 12-h light-dark cycle (light on 8:00 a.m. to 8:00 p.m.). The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals at Hoshi University, as adopted by the Committee on Animal Research of Hoshi University. Every effort was made to minimize the numbers and any suffering of animals used in the following experiments.

Hot-plate test. Antinociceptive effects were measured after the administration of oliceridine (0.3–10 mg/kg, s.c.) by recording the latency to paw-licking or -tapping in the hot-plate test (55 ± 0.5 °C; Muromachi Kikai Co., Ltd., Tokyo, Japan) as described previously [14]. Antinociception was calculated as a percentage of the maximum possible effect (% antinociception) according to the following formula: % antinociception = (test latency - predrug latency)/(cut-off time - predrug latency) × 100. To prevent tissue damage, we established a 30-s cut-off time.

Neuropathic pain model and measurement of thermal hyperalgesia. Partial sciatic nerve injury was applied by tying a tight ligature with a 8-0 silk suture around approximately one-third to one-half the diameter of the sciatic nerve on the right side (ipsilateral side) under a light microscope (SD30, Olympus, Tokyo, Japan) and under 3% isoflurane anesthesia as described previously in mice [15].

The sensitivity to thermal stimulation was tested as described previously [13]. In brief, each of the hind paws of mice was individually tested using a thermal stimulus apparatus (UGO-BASILE, Biological Research Apparatus, VA, Italy). The intensity of the thermal stimulus was adjusted to achieve an average baseline paw-withdrawal latency of approximately 8–10 s in naive mice.

Gastrointestinal transit. Gastrointestinal transit was determined based on a previous method [16,17]. Briefly, at 10 min after oliceridine (0.3–10 mg/kg, s.c.) injection, blue ink (0.3 ml/mouse; Pilot Co. Ltd., Tokyo, Japan) was administered orally. The percentage inhibition of gastrointestinal transit was calculated as follows: (distance traveled by the ink/distance from the pylorus to the cecum) × 100.

Evaluation of the emetic response. The number of retching and vomiting behaviors (emesis) in ferrets was evaluated after the administration of oliceridine (0.06–0.3 mg/kg, s.c.) as described previously [17]. Retching was defined as a rhythmic abdominal contraction without expulsion, and vomiting was the oral expulsion of solid or liquid from the gastrointestinal tract. After the injection of oliceridine, the number of emetic behaviors was assessed for

30 min. Tests were conducted at an interval of at least 7 days to allow for drug washout and to minimize the development of tolerance for emesis.

Locomotor assay. As described previously [18], the locomotor activity of mice was measured by an ambulometer, where any slight tilt of the activity cage caused by horizontal movement of the mouse was detected by microswitches (ANB-M20, O'Hara Co., Ltd., Tokyo, Japan). The total activity counts in each 10-min block were automatically recorded for 30 min prior to the injections and for 120 min following saline and drug administration. To evaluate behavioral sensitization to oliceridine, mice were given intermittent injections of oliceridine (0.3–3 mg/kg, s.c.), once every 72–96 h for 10 days.

Drugs. The drugs used in the present study were oliceridine (synthesized by us), morphine hydrochloride (Daiichi-Sankyo Co., Ltd, Tokyo, Japan) and fentanyl citrate (Daiichi Sankyo Propharma, Co. Ltd. Tokyo, Japan). All drugs were dissolved in saline and administered in a volume of 10 ml/kg for mice and 0.25 ml/kg for ferrets.

Statistical analysis. The 50% effective dose (ED_{50}) values were determined using an analysis of variance and linear regression techniques. A one-way and two-way analysis of variance (ANOVA) followed by the Tukey test and Bonferroni multiple comparisons test, respectively, were used for statistical analysis. All statistical analyses were performed using Prism software (Version 5.0a; GraphPad Software, Inc., La Jolla, CA). A P value of 0.05 was considered to reflect significance.

3. Results

3.1. Activation of Gi protein-dependent pathway by μ-opioid receptor agonists

To determine whether oliceridine and other opioids including morphine and fentanyl could suppress signal transduction through the G-protein-biased pathway, we measured the effects of μ-opioids on the forskolin-induced accumulation of cAMP in HEK293 cells that stably expressed μ-opioid receptors. In this study, the accumulated cAMP induced by forskolin was dose-dependently suppressed by oliceridine, morphine and fentanyl (10^{-10}-10^{-5}) (Fig. 1A).

The CellKey™ assay system is used to ascertain the activation of GPCRs by measuring the impedance (ΔZ) based on cellular dielectric spectroscopy (CDS) technology [11]. In this study, fentanyl (10^{-11}-10^{-6}) and oliceridine (10^{-10}-10^{-5}) dose-dependently induced an increase in impedance (ΔZ) in HEK293 cells that stably expressed μ-opioid receptors (Fig. 1B). In antagonism tests, the increase in impedance induced by oliceridine (10 μM) was significantly suppressed by pertussis toxin (an inhibitor of Gi-proteins: 10 ng/ml, 100 ng/ml) (Fig. 1C).

3.2. Lack of effects of oliceridine on the internalization of μ-opioid receptors in HEK293 cells that overexpressed μ-opioid receptors

We recently demonstrated that oliceridine (10^{-8}-10^{-5} M) scarcely affects the recruitment of β-arrestin-2, whereas fentanyl (10^{-9}-10^{-6} M) produces an increase in luminescence that reflects the recruitment of β-arrestin-2 in cells that stably expressed μ-opioid receptors [13]. Related to this phenomenon, the apparent internalization of μ-opioid receptors was observed after treatment with fentanyl (1 μM), while oliceridine (10 μM) failed to produce a significant internalization of μ-opioid receptors as reflected by spot counts inside cells that stably expressed μ-opioid receptors [13]. In the present study, neither oliceridine (10 μM) nor morphine (10 μM) increased fluorescence signals in the cytoplasm or spot fluorescence inside the cells, which reflect μ-opioid receptor internalization (Fig. 1D–E). In contrast, fentanyl (1 μM) produced the apparent internalization of μ-opioid receptors (Fig. 1E).

3.3. Rapid-onset antinociceptive effect and lack of development of antinociceptive tolerance induced by oliceridine in mice

To further characterize the pharmacological profile of oliceridine, we focused on the onset and duration of action of oliceridine. In this study, we measured the antinociceptive effects induced by oliceridine (3 mg/kg) every min after the administration of oliceridine. As a result, the antinociceptive effect of oliceridine peaked at 5 min after administration, and gradually decreased thereafter (Fig. 2A). Oliceridine (0.3–10 mg/kg) produced a dose-dependent antinociception based on the results of the mouse hot-plate test (Fig. 2B).

In partial sciatic nerve-ligated mice, neuropathic pain-like hypersensitivity was only observed on the ipsilateral side at 7 days after nerve ligation, and persistent pain resulting from sciatic nerve ligation lasted at least 28 days (Fig. 2D). Under these conditions, oliceridine (1 and 3 mg/kg) produced a significant antinociceptive effect for hyperalgesia (Fig. 2C). More importantly, tolerance to this antinociceptive effect induced by oliceridine (3 mg/kg) was not clearly observed for 28 days, at least under the neuropathic pain condition based on the measurement of thermal hyperalgesia (Fig. 2D).

3.4. Adverse effects induced by oliceridine in animals

We previously demonstrated that clinically prescribed μ-opioids have different likelihoods of inducing antinociceptive effects versus reducing gastrointestinal transit [16]. As shown in Fig. 3A, oliceridine at 0.3–10 mg/kg dose-dependently inhibited gastrointestinal transit in mice, and the potencies for inducing these effects (ED_{50}) of oliceridine were in the order: inhibition of gastrointestinal transit (6.140 mg/kg) > antinociceptive effects (3.534 mg/kg) (Figs. 2A and 3A).

Morphine induces emetic responses accompanied by motor impairment or sedation in ferrets [17]. In the present study, oliceridine (0.3 mg/kg, s.c.) produced significant retching (Fig. 3B), whereas vomiting was observed in only 2 of 7 ferrets (once in each ferret, data not shown).

As shown in Fig. 4A, oliceridine produced dose-dependent hyperlocomotion with the typical Straub's tail reaction (unpublished observation), and this effect was significant at 3 mg/kg. Furthermore, we examined whether intermittent administration of oliceridine could produce the sensitization of locomotor activity. After repeated treatment with oliceridine (0.3–3.0 mg/kg), 3 mg/kg of oliceridine produced a significant increase in locomotor activity in comparison with that at day 1 (Fig. 4B).

4. Discussion

If breakthrough pain, which is defined as a sudden-onset and transient exacerbation of generally well-controlled background pain [19], is not adequately treated, the patient's physical and mental distress increase and their quality of life decreases. Therefore, the control of breakthrough pain contributes to high-quality pain management. In current clinical practice, breakthrough pain is generally treated with a rapid-onset opioid, oral transmucosal fentanyl citrate [20]. Even if fentanyl is used appropriately, with long-term use, its analgesic effect can sometimes decrease early; i.e., analgesic tolerance to fentanyl may occur [21].

In August 2020, the U.S. Food and Drug Administration approved oliceridine as a novel opioid. In the present study, we demonstrated

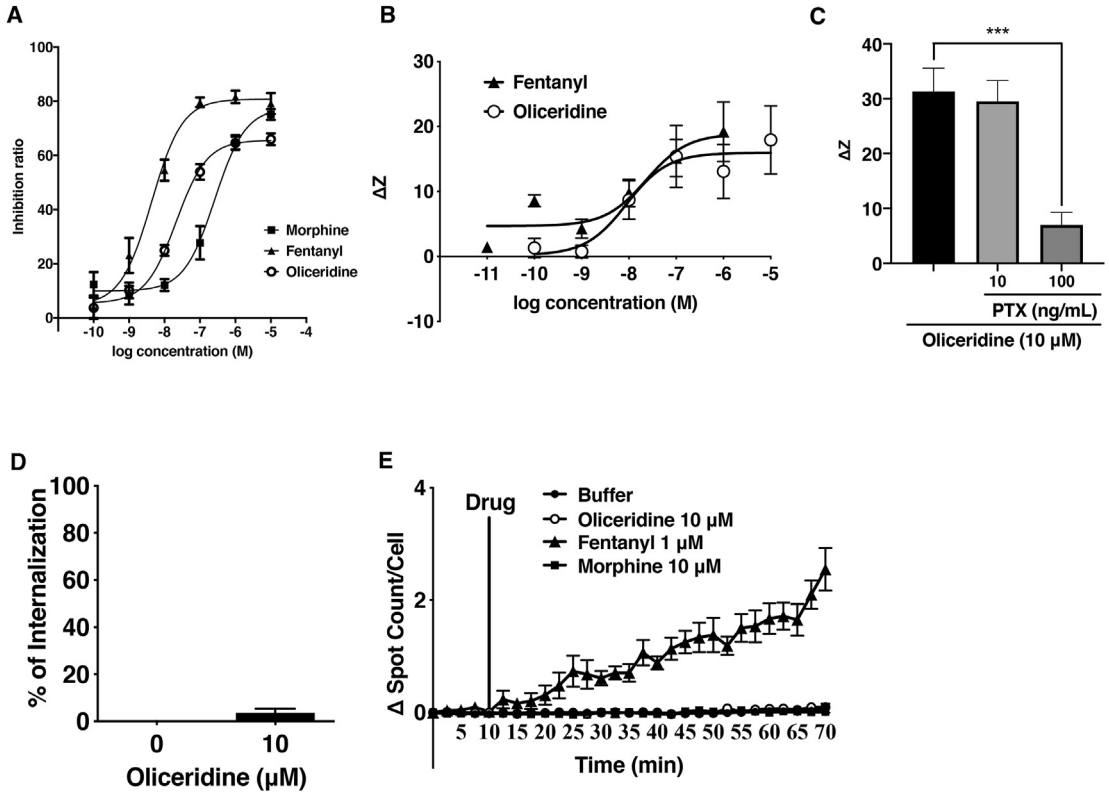

Fig. 1. Measurement of μ-opioid receptor agonist-induced intracellular events in cells that stably overexpressed μ-opioid receptor. (A) Effects of oliceridine, morphine and fentanyl on the forskolin-induced increase in intracellular cAMP levels in HEK293 cells that overexpressed human μ-opioid receptors. (B) CellKey™ measurements of the μ-opioid receptor response elicited by oliceridine and fentany. HEK293 cells transfected with μ-opioid receptor were treated with different doses of oliceridine and fentanyl. The data were corrected for the baseline response as a change in impedance. (C) Antagonism by pertussis toxin (PTX; 10 ng/ml, 100 ng/ml) of the oliceridine-induced activation of μ-opioid receptors. (D) Measurement of % internalization of μ-opioid receptors by treatment with oliceridine. (E) Δ in spotted μ-opioid receptors after the administration of opioids.

that oliceridine produced a rapid-onset and short-acting antinociceptive effect without the development of tolerance. This finding strongly suggests that oliceridine could be useful for reducing breakthrough pain.

In the present *in vitro* studies, we confirmed that oliceridine induced the activation of GPCRs in a pertussis toxin-reversible manner as well as the suppression of cAMP formation in μ-opioid receptors. Oliceridine also produced nearly undetectable levels of internalization of μ-opioid receptors, whereas we previously reported that a β-arrestin-preferred ligand fentanyl potently induced the recruitment of β-arrestin accompanied by the internalization of μ-opioid receptors [13]. Taken together, these findings provide further evidence that oliceridine may act as a G-protein-preferred ligand. In the present *in vivo* studies, we found that oliceridine significantly delayed gastrointestinal transit. A previous study demonstrated that oliceridine produced a potent analgesic effect, and caused less gastrointestinal dysfunction and respiratory suppression than morphine at equianalgesic doses [5]. The dose-response curves of fentanyl and morphine for delaying gastrointestinal transit are considered to be steep, and these opioids maximally delay gastrointestinal transit at antinociceptive doses [16]. In contrast, we demonstrated here that the dose-response curve for the inhibition of gastrointestinal transit induced by oliceridine was blunt, and treatment with oliceridine at its antinociceptive dose did not maximally inhibit gastrointestinal transit. Based on our present as well as previous results [16,22], we calculated the relative ratio (ED_{50} for antinociception divided by those for inhibition of gastrointestinal transit) between induction of antinociception and constipation caused by oliceridine (0.58), morphine (neutral-μ-agonist: 2.27), fentanyl (β-arrestin-biased μ-agonist: 0.67), and methadone (β-arrestin-biased μ-agonist: 1.14). These findings suggest that the inhibition of gastrointestinal transit, which is one of the unpleasant side-effects of μ-opioids, is not always correlated with stimulation of a β-arrestin-biased signaling pathway. It is widely accepted that morphine has relatively limited capability to cross the blood brain barrier, whereas oliceridine is likely to have a faster-onset action after injection than morphine as shown in the present study. It is well known that activation of supraspinal as well as spinal μ-opioid receptors is essential for the antinociceptive effects of μ-opioid receptor agonists. In contrast, activation of peripheral μ-opioid receptors mainly contributes to the inhibition of gastrointestinal transit by μ-opioid receptor agonists [23–26]. This background clearly indicates that the ability to cross the blood-brain barrier, namely the rapid onset of action induced by oliceridine, may be related to a low inducibility for constipation induced by μ-opioid receptor agonists.

It has been demonstrated that fentanyl induced respiratory depression without inducing retching or vomiting in ferrets as observed in previous study [27] with unpublished observation. In contrast, although oliceridine produced retching and vomiting, it was less effective at inducing emesis than morphine, as demonstrated in our previous [17] and present studies. Since the dopaminergic pathway is considered to be associated with opioid-induced emesis, activation of a G-protein-dependent pathway as well as a β-arrestin pathway through μ-opioid receptors may modulate central dopaminergic transmission.

It is well known that an increase in locomotor activity and sensitization to locomotor activity are mediated by activation of the

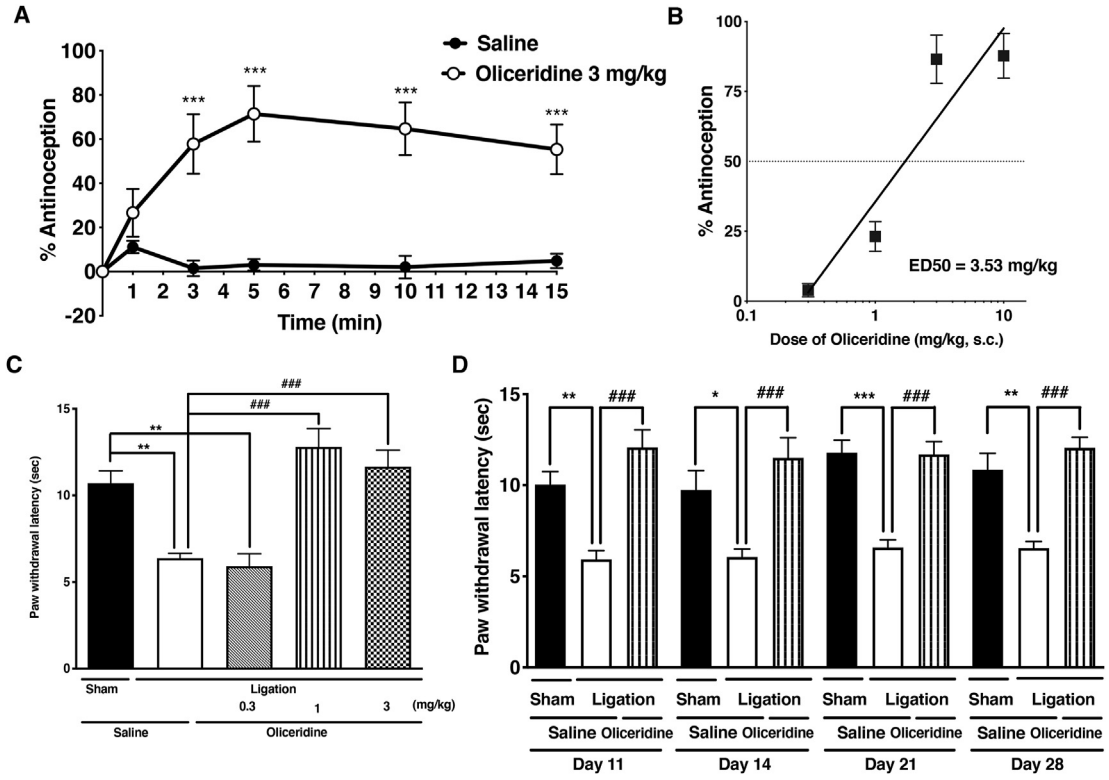

Fig. 2. Evaluation of oliceridine-induced pharmacological effects in animals. (A) Time course (0–15 min) of antinociceptive effects of oliceridine (3 mg/kg, s.c.) using the hot-plate test. (B) Dose-response (0.3–10 mg/kg, s.c.) of the antinociceptive effects of oliceridine using the hot-plate test. (C, D) Antinociceptive effects (C) and antinociceptive tolerance (D) induced by oliceridine under a state of neuropathic pain. Antinociceptive effects were measured 5 min after the injection of oliceridine (0.3–3 mg/kg, s.c.) in sciatic nerve ligated mice. Oliceridine (3 mg/kg, s.c.) was administered daily for 28 days. Each column or point represents the mean ± S.E.M. of 6–7 animals. *p < 0.05, **p < 0.01, ***p < 0.001 vs. saline group, ###p < 0.001 vs. ligation-saline group.

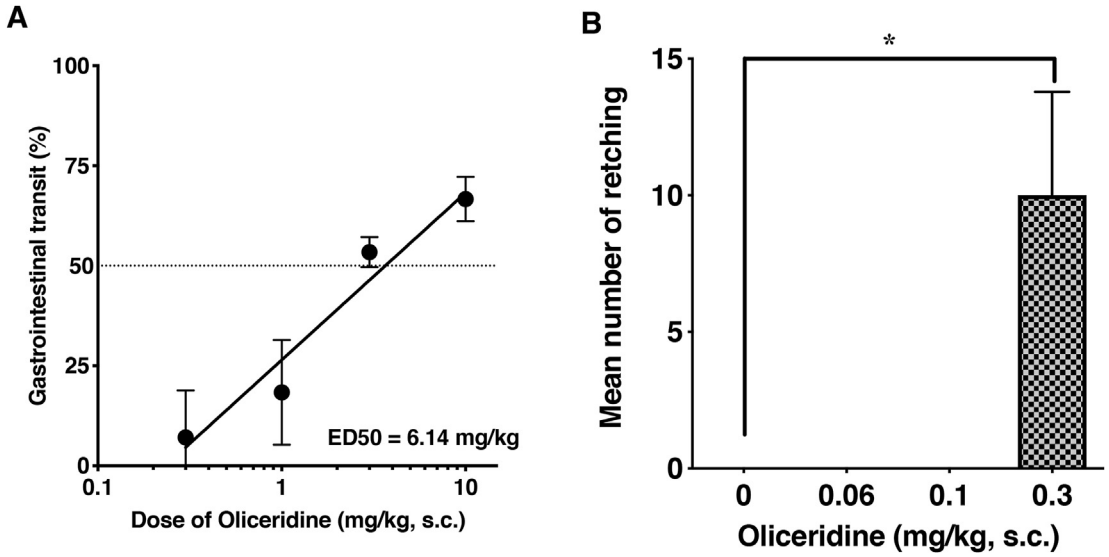

Fig. 3. Evaluation of oliceridine-induced adverse side-effects in animals. (A) Inhibitory effects of oliceridine (0.3–10 mg/kg, s.c.) on gastrointestinal transit in mice. Each point represents the mean ± S.E.M. of 6–7 animals. (B) Induction of oliceridine (0.06–0.3 mg/kg, s.c.) on retching behavior in ferrets. The number of retching behavior was monitored for 30 min. Each column represents the mean ± S.E.M. of 4–7 animals. *p < 0.05 vs. saline group.

mesolimbic dopaminergic system [18], and can be used to predict a drug's abuse potential. It has been reported that the potent G protein-biased ligand PZM21 produces neither hyperlocomotion nor a conditioned place preference in mice [6], suggesting that activation of a β-arrestin pathway dominantly contributes to the abuse potential through μ-receptors. However, in the present study, we found that oliceridine, like morphine [28,29], induced a significant increase in locomotor activity as well as sensitization to

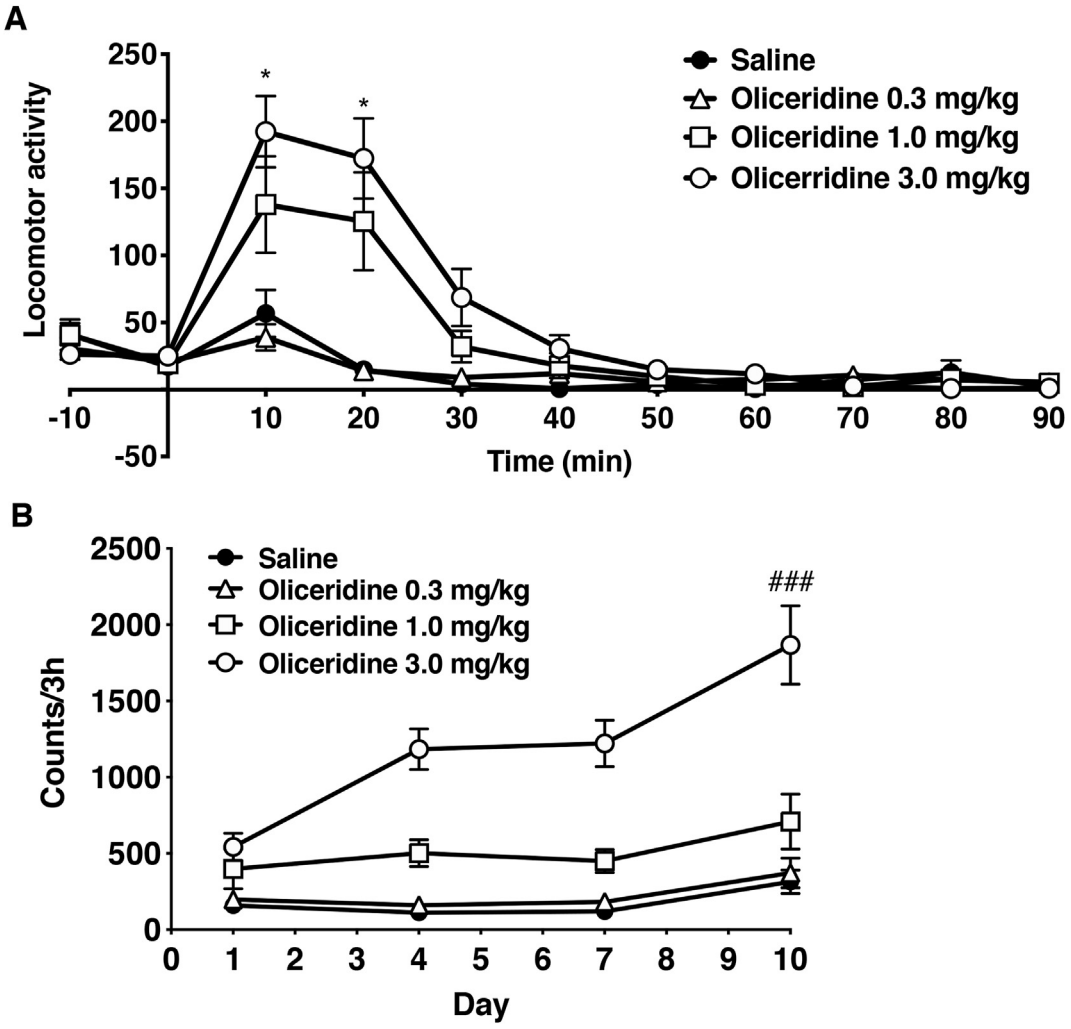

Fig. 4. Evaluation of oliceridine-induced hyperlocomotion. (A) Effect of oliceridine (single administration; 0.3–3.0 mg/kg, s.c.) on spontaneous locomotor activity. (B) Effect of intermittent treatment with oliceridine on the development of behavioral sensitization. Animals received intermittent injection of oliceridine (0.3–3 mg/kg, s.c.) or saline, with one injection every 72 h for 10 days. Each point represents the mean ± S.E.M. of 6 animals. *$p < 0.05$ vs. saline group, $^{###}p < 0.001$ vs. 1st drug administration.

locomotor activity in mice, but this activity was weaker than that of morphine. At this time, we cannot clearly explain the discrepancy between other reports, which may suggest that G-protein biased ligands have no activating effect on central dopaminergic network, and our present study. Although further examinations are needed to directly demonstrate whether the G-protein-biased pathway could be responsible for abuse potential through μ-opioid receptors, the present findings may provide a caution that activation of a G-protein-dependent signaling pathway by its selective ligands does not always produce analgesia without unpleasant effects related to central dopaminergic regulation.

In summary, although we cannot deny the possibility that oliceridine may produce some undesirable effects even through a G-protein-dependent pathway, it still offers advantages for treating severe pain, especially breakthrough pain, without antinociceptive tolerance by adequate doses due to its rapid-onset and short-duration action.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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