Full Paper

Disability of Development of Tolerance to Morphine and U-50,488H, a Selective κ -Opioid Receptor Agonist, in Neuropathic Pain Model Mice

Sourisak Sounvoravong¹, Masakatsu Takahashi^{2,*}, Mihoko N. Nakashima¹, and Kennichiro Nakashima¹

¹Department of Analytical Research for Pharmacoinformatics, Graduate School of Pharmaceutical Sciences;

²Division of Higher Educational Research, Research and Development Center for Higher Education;
Nagasaki University, 1-14, Bunkyo-machi, Nagasaki 852-8521, Japan

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Abstract. We examined the analgesic and anti-allodynic effects of morphine and U-50,488H (*trans*-($\pm$)-3,4-dichloro-*N*-methyl-*N*-(2-[1-pyrrolidinyl]-cyclohexyl)-benzeneacetamide methane-sulfonate salt), a selective κ -opioid receptor agonist, and the development of tolerance to their effects in neuropathic pain model mice induced by sciatic nerve ligation (SNL). In the tail-pinch method, morphine at 10 mg/kg, s.c. produced a weak analgesic effect in SNL mice; however, U-50,488H at 5 mg/kg, s.c. produced an analgesic effect equipotent to that in normal mice. In contrast, morphine produced an adequate analgesic effect when given either intracerebro-ventricularly (i.c.v.) or intrathecally (i.t.), but U-50,488H only produced analgesia when given i.t. Repeated administration of morphine (either i.c.v. or i.t.) or U-50,488H (either s.c. or i.t.), did not induce tolerance to the effect. In the static allodynia test with an application of von Frey filaments, both compounds given s.c. suppressed the allodynic effect, but in the dynamic allodynia test involving lightly stroking the plantar surface with a cotton bud, only U-50,488H produced an anti-allodynic effect. Repeated administrations of both compounds did not develop tolerance to these anti-allodynic effects. Thus, U-50,488H was found to be a highly effective at blocking hyperalgesia and allodynia in nerve injury, and these findings suggest that κ -opioid receptor agonists are attractive pharmacological targets for the control of patients with neuropathic pain.

Keywords: neuropathic pain, opioid, allodynia, tolerance, analgesia

Introduction

Abnormal pain states induced by peripheral nerve injury are commonly referred to as neuropathic pain (1). Traditionally, this pain is considered to consist of hyperalgesia (an increased sensitivity to painful stimuli) and allodynia (pain evoked by stimuli that are innocuous in the normal state). Two types of mechanical allodynia clearly discerned by clinical criteria, i.e., a static type, elicitable by application of gentle pressure on the symptomatic skin, and a dynamic allodynia, triggered by lightly stroking the surface of the skin, can be detected in patients suffering from neuropathic pain (2). Since neuropathic pain is often refractory to treatment with conventional analgesics such as opiates, increased mor-

phine doses are needed to produce the same analgesic effect in patients with chronic pain; that is, there is development of tolerance to the drug. A substantial advance in our understanding of the mechanisms that underlie neuropathic pain states has come from the development of animal models, including chronic sciatic nerve constriction injury, which produces severe damage to myelinated fibers (3), and tight ligation of approximately 1/2 to 1/3 of the sciatic nerve (1). Although the effectiveness of opioids in the management of neuropathic pain is still controversial (4, 5), the clinical use of opioids is continuing.

In fact, numerous reports have revealed that morphine produces attenuated analgesic effects in neuropathic pain (6, 7); and furthermore, the effects of κ -receptor agonists are enhanced in this pain state (8–10). However, our knowledge about the effectiveness of opioids

*Corresponding author. FAX: +81-95-819-2090
E-mail: takahasi@net.nagasaki-u.ac.jp

depending on the route of administration and studies of the development of tolerance in this neuropathy model are limited since the effectiveness of opioids may depend on the local expression of their receptors.

The aim of this study was to clarify the analgesic and anti-allodynic effects of morphine and U-50-488H given by various routes of administration in the neuropathic pain model mice induced by sciatic nerve ligation (SNL) according to the method of Seltzer et al. (1). In addition, since evidence (11–15) has been accumulated showing that patients with chronic pain or an animal model of inflammatory pain induced by formalin did not develop or showed delayed development of analgesic tolerance to morphine, whether tolerance to these compounds develops or not in this animal model was also examined.

Materials and Methods

Animals

Male ddY strain mice weighing 16–18 g (Otsubo Exp. Animals, Nagasaki) were purchased and were housed in groups of 6–8 animals. They were maintained at an ambient temperature ($22 \pm 1^\circ\text{C}$) and relative humidity ($55 \pm 5\%$) with free access to laboratory diet (MF; Oriental Yeast, Tokyo) and tap water. After reaching 23 to 28 g, they were used for the experiments. All procedures used in this study were approved by the Nagasaki University Animal Care and Use Committee.

Drugs and methods of administration

U-50,488H (*trans*-($\pm$)-3,4-dichloro-*N*-methyl-*N*-(2-[1-pyrrolidinyl]-cyclohexyl)-benzeneacetamide methane-sulfonate salt), a selective κ -opioid receptor agonist (Sigma Chemical Co., St. Louis, MO, USA) and morphine HCl (Takeda Pharm. Co., Osaka) were used. Morphine and U-50,488H were administered at a volume of 0.1 ml/10 g of body weight by subcutaneous (s.c.) administration and at a volume of 10 μl /mouse by intrathecal (i.t.) and intracerebroventricular (i.c.v.) administration.

Induction of neuropathic pain

Neuropathic pain model animals were prepared by sciatic nerve ligation (1). Briefly, all animals were anesthetized by intravenous (i.v.) injection of pentobarbital sodium (Nembutal, 50 mg/kg), and the common sciatic nerve was exposed unilaterally (right side) at the mid-thigh level, taking care to avoid the interruption of epineural circulation. Two silk ligatures (No. 8) were placed around the nerve. The ligatures were tied until they elicited a brief twitch in the high limb. The muscle layers and incision were closed and sutured with silk (No. 3). For sham-operated mice, the same procedure

without the ligation of the sciatic nerve was performed.

Measurement of antinociceptive effect

The antinociceptive effect was measured according to the modified Haffner's method, the tail-pinch test (16). Forceps with adjusted pressure were used to elicit a response time of 0.3–1.0 s on naive or sham-operated animals with a cut-off time of 10 s to avoid any damage to the tail, every 15 min after s.c. administration of morphine and U50488H and at 5, 10 min, and every 15 min after i.c.v. or i.t. administration of these compounds for a period of 90 min.

Measurement of dynamic allodynia (touching test)

The measurement of dynamic allodynia was conducted according to the method of Field et al. (8). Briefly, mice were individually placed in a clear plastic cage with a wire mesh bottom (1-cm² perforations). Dynamic allodynia was assessed by lightly stroking the plantar surface of the hind paw of the mice with a cotton bud. Model animals with less than 5-s latency of withdrawal response were selected for the test. When model animals did not show the response for at least 15 s after lightly stroking, the anti-dynamic allodynic effect was regarded as positive.

Measurement of the static allodynia

The measurement of static allodynia was performed as previously reported by Chaplan et al. (17). Mice were individually placed in a clear plastic cage with a wire mesh bottom (1-cm² perforations). The von Frey filament (VFF) (North Coast Medical, Inc., Morgan Hill, CA, USA) was applied as evaluator size of 0.04, 0.07, 0.16, 0.4, 0.6, 1.0, 1.4, 2.0, and 4.0 g. The VFFs were applied to the plantar surface of each hind paw from below the mesh floor (The filament was pressed against the plantar surface at a 90° angle until it bent). Paw withdrawal threshold was defined as the minimum evaluator size required to elicit a withdrawal reflex of the paw at least one time in five trials.

Measurement of static allodynia was conducted three times. The first measurement was performed before surgery (to determine the cut-off value score: 2 g), the second measurement was performed 10 days after surgery (to determine the baseline score), and the last measurement was conducted after drug administration (to determine the post-treatment score). The anti-static allodynic effects of drugs were estimated at 15-min intervals for a period of 90 min after s.c. administration of drugs and at 5-, 10-, and 15-min intervals for 90 min for i.t. and i.c.v. administration.

The evaluator size of the filament to elicit a withdrawal reflex of the paw for each size was converted

to individual percent maximum possible effect (%MPE) using the following formula:

$$\%MPE = \frac{(\text{post-treatment score}) - (\text{baseline score})}{(\text{cut-off value}) - (\text{baseline score})} \times 100$$

Statistical analyses

Values are expressed as the mean $\pm$ S.E.M. Data from single administrations of drugs were analyzed by Student's *t*-test, and data from daily administration of drugs were analyzed by one-way analysis of variance (ANOVA) followed by a post hoc multiple modified *t*-test (Dunnett's method). $P < 0.05$ was considered significant.

Results

Induction of neuropathic pain model mice

Ten days after the operation, sciatic nerve-injured mice clearly exhibited abnormal pain, as shown by increases of dynamic and static allodynia of more than 90%, respectively, in these mice at the injured paw compared with those in sham-operated mice. The mice showing allodynia were used for the experiments: these mice showed lower thresholds of VFFs, 0.07–0.6 g, compared to that of the sham-operated mice, 1.4 to 2.0 g (static); and these mice showed a less than 5-s latency of withdrawal response to the stroke of a cotton bud (dynamic).

Antinociceptive effect of morphine and U-50,488H in the tail-pinch test

In sham-operated mice, administration of morphine

at 10 mg/kg, s.c. produced a substantial analgesic effect; however, in SNL mice, only weak analgesia was observed. In contrast, U-50,488H given s.c. at 5 mg/kg produced a strong analgesic effect in SLM mice, which was equipotent to that observed in sham-operated mice (Fig. 1).

Morphine given i.c.v. or i.t. at 10 μ g/mouse produced analgesic effects in both SNL mice and sham-operated mice (Fig. 2). On the other hand, U-50,488H given i.c.v. produced analgesia in sham-operated mice but not in SNL mice; however, i.t. administration of the compound produced analgesic effects in both SNL mice and sham-operated mice.

Development of analgesic tolerance to morphine and U-50,488H in SNL mice

Daily treatment with morphine or U50,488H rapidly induced tolerance in sham-operated mice. In contrast, SNL mice did not develop tolerance to U-50488H (5 mg/kg, s.c) for 5 days (Fig. 3).

Effects of morphine and U-50488H on allodynia

Morphine, given s.c. at 10 mg/kg, failed to block dynamic allodynia. However, both i.t. and i.c.v. administration of morphine produced appreciable anti-dynamic allodynic effects. Morphine given s.c., i.t., and i.c.v. produced anti-static allodynia. On the other hand, U-50,488H given s.c. produced anti-dynamic and anti-static allodynia. However, when given i.c.v., it failed to block dynamic and static allodynia (Fig. 4). Repeated s.c. administration of U-50,488H did not induce tolerance to either anti-static or anti-dynamic allodynia, and it appears likely that tolerance to the anti-static

Fig. 1. Antinociceptive effect of morphine and U-50,488H in sciatic nerve ligation (SNL) mice. Animals were administered morphine, s.c. at 10 mg/kg (squares) (A) or U-50,488H, s.c. at 5 mg/kg (triangles) (B). Control groups were treated with saline (circles). Open and closed symbols indicate sham-operated mice and SNL mice, respectively. The antinociceptive effect was measured by the tail-pinch method every 15 min after injection of compounds. Each point indicates the mean $\pm$ S.E.M. of 8–16 animals. ** $P < 0.01$, compared with the saline group and ## $P < 0.01$, compared with the sham-operated mice group.

Fig. 2. Antinociceptive effect of morphine and U-50,488H in sciatic nerve ligation (SNL) mice. Morphine (10 μ g/mouse, squares) or U-50,488H (20 μ g/mouse, triangles or 10 μ g/mouse, diamonds) was administered i.c.v. (upper panel) or i.t. (lower panel) to SNL mice (closed symbols) or sham-operated mice (open symbols). The control groups (circles) received saline instead of compounds as control groups. After injection of compounds, the antinociceptive effect was measured by the tail-pinch method at 5, 10, and 15 min and then at intervals of 15 min for the following 45 min. Each point indicates the mean $\pm$ S.E.M. of 6 or 7 animals. $^{##}P<0.01$, compared with the sham-operated group. $^{*}P<0.05$, $^{**}P<0.01$, compared with the corresponding value before the injection.

Fig. 3. Development of antinociceptive tolerance to morphine and U-50,488H given by various routes in sciatic nerve ligation (SNL) mice. Morphine (10 μ g/mouse, i.c.v. or i.t.; squares), or U-50,488H (5 mg/kg, s.c. or 20 μ g/mouse, i.t.; triangles) was given daily for 5 days. Closed and open symbols indicate SNL mice and sham-operated mice, respectively. The antinociceptive effect measured by the tail-pinch method is transformed into the area under the curve (AUC) by plotting the increase in response time (s) on the ordinate and the time intervals (min) on the abscissa. Each point indicates the mean $\pm$ S.E.M. of 6 or 7 animals. $^{#}P<0.05$, $^{##}P<0.01$, compared with the sham-operated group. $^{*}P<0.05$, $^{**}P<0.01$, compared with the corresponding value of the 1st day.

Fig. 4. Anti-allodynic effect of morphine and U-50,488H in sciatic nerve ligation (SNL) mice. SNL mice received morphine (10 mg/kg, s.c.; 10 μ g/mouse, i.c.v. or i.t.; square) or U-50,488H (5 mg/kg, s.c.; 20 μ g/mouse, i.c.v. or i.t.; triangle), or saline (circle). The anti-allodynic effect was measured by suppression of allodynia using a cotton pad (dynamic allodynia) and a von Frey filament (static allodynia) every 15 min for 90 min or at 5, 10, and 15 min and then at intervals of 15 min for the following 75 min. Each point indicates the mean value $\pm$ S.E.M. of 6 or 7 animals. * P <0.05, ** P <0.01, *** P <0.001, compared with the sham-operated control group.

Fig. 5. Lack of the development of tolerance to suppressive effect of daily morphine and U-50,488H on the allodynia in sciatic nerve ligation (SNL) mice. SNL mice were given daily morphine, 10 mg/kg, s.c. (square); U-50,488H, 5 mg/kg, s.c. (triangle); or saline (circle) for 5 days. The anti-allodynic effect measured by suppression of allodynia using a cotton pad (dynamic allodynia) and a von Frey filament (static allodynia) is transformed into the area under the curve (AUC) by plotting the increase in response time (s) on the ordinate and the time intervals (min) on the abscissa. Each point indicates the mean value $\pm$ S.E.M. of 8–16 animals. *** P <0.001, compared with the group of animals that received saline.

Table 1. Summary for antinociceptive and anti-allodynic effect of morphine and U50,488H given by various routes of administration in neuropathic pain model mice

Compounds	Routes	Analgesia	Anti-allodynic effect	
			Dynamic allodynia	Static allodynia
Morphine	s.c.	↓	–	+
	i.t.	+	+	+
	i.c.v.	+	+	+
U-50,488H	s.c.	+	+	+
	i.t.	+	–	–
	i.c.v.	–	–	–

+: effective, –: ineffective, ↓: attenuate.

allodynic effect of repeated s.c. administration of morphine did not develop (Fig. 5). The antinociceptive and anti-allodynic effects of morphine and U50,488H in SNL mice are summarized in Table 1.

Discussion

Using Seltzer's method (1), we found that 10 days after the surgery, mice were in the state of mechanical allodynia, exhibiting a decreased threshold of nociceptive sensation. The results of this study also showed that both static and dynamic allodynia, symptoms that are also found in clinical patients with neuropathic pain (1, 2), could be detected in SNL.

Although the mechanism of the allodynic process remains unclear, numerous studies have demonstrated that allodynia results from nerve injury that induces changes in the spinal cord, and that maintenance after nerve injury is required for its mediation (17–19).

Dynamic and static allodynia have been suggested to be mediated by different sensory neurons; that is, dynamic allodynia is mediated by large diameter, A β -fibers, and static allodynia is mediated by small diameter, C and A δ fibers (8). Thus, the light stroking of the plantar surface of the hind paw of SNL mice with a cotton bud evoked dynamic allodynia that is mediated by A β -fibers, whereas static allodynia evoked by stimulation of VFF is mediated by C or A δ fibers (8).

Our results revealed that morphine given s.c. produced anti-static allodynic effects but not anti-dynamic allodynic effects, whereas U-50,488H given s.c. produced both anti-dynamic and anti-static allodynia. It has been suggested that μ - and κ -opioid receptors are located in the superficial laminae (I and II) of spinal dorsal horn neurons (20) and that these sites are the regions where terminals of nociceptive C- and A δ -fibers of peripheral afferent nerves are located (21). Therefore, our results support the idea that morphine and U-

50,488H could block static-allodynia.

In these experiments, U-50,488H but not morphine produced anti-dynamic allodynia. It has been reported that the A β -fibers, the dysfunction of which seems related to the production of dynamic allodynia, terminate in laminae III and IV (22). It has been suggested that the expression of μ -opioid receptor mRNA in this area is weak, while the expression of κ -opioid receptor mRNA is intense in the deeper layers of the dorsal horn (22). These morphological differences in the distribution of the two receptor types may, in part, provide an explanation for our findings that dynamic allodynia was blocked by administration of a κ - but not μ -opioid receptor agonist. Our results are also in accord with electrophysiological studies showing that κ - but not μ -opioid receptor agonists can inhibit the nociceptive responses of multi-receptive cells located in laminae III and IV (23).

It has been reported that spinal administration of κ -receptor agonists antagonized anti-nociception induced by morphine or other μ -opioid receptor agonists (24, 25), suggesting that two opioid receptor types, μ and κ , mutually compensate for the pain regulation in the spinal cord. This concept is likely to support our results that U-50,488H failed to suppress an allodynic effect, while morphine exerted an anti-allodynic effect when given i.t. In fact, Hao et al. has reported that i.t. U-50,488H did not alleviate the allodynia-like behavior, but rather enhanced it (26).

Interestingly, morphine given s.c. also did not induce tolerance to static or dynamic anti-allodynia; furthermore, U-50,488H given s.c. also did not induce tolerance to either the static or dynamic inhibitory effect of allodynia in SNL mice.

Although the mechanisms underlying the lack of induction of anti-allodynic tolerance to these opioids remain unclear, it is likely that, similar to the involvement of κ -opioid receptors in the suppression of the

development of morphine tolerance (27, 28), κ -opioid receptor mechanisms underlie the blockade of the development of anti-allodynic tolerance to these opioids. It may be plausible that the mutual interaction between μ - and κ -opioid receptors play an important role in the suppression of anti-allodynic tolerance development as well as in the blockade of the anti-allodynic effect of morphine by the κ -agonist as mentioned above (26).

In spite of the lack of analgesia in response to s.c. administration in SNL mice, i.t. and i.c.v. administration of morphine produced antinociceptive effects like that shown in sham-operated mice. Meanwhile, U-50488H given s.c. produced potent antinociceptive effects; however, when given i.t. at a lower dose of 10 μ g/mouse or i.c.v. at 20 μ g/mouse, it failed to produce antinociceptive effects. These results suggest that κ -opioid receptor agonists seem to play a prominent role in blocking nociceptive transmission in peripheral sites of neuropathic pain mice. In fact, it has been reported that the κ -opioid receptor is found in the peripheral tissues, including afferent fibers (20).

In our experiments, central administration of morphine, both i.t. and i.c.v., produced antinociceptive effects in SNL mice equipotent to those in the sham-operated mice.

In accord with our results, Suzuki et al. reported that i.t. administration of morphine produced a greater antinociceptive effect in spinal-nerve-ligated rats, suggesting the possible therapeutic value of i.t. morphine in clinical conditions in which systemic morphine fails to provide adequate analgesia (5). In contrast, it has been demonstrated that i.t. administration of morphine produces decreased efficacy against thermal hyperalgesia in spinal-nerve-ligated rats as compared with sham-operated rats (29). These discrepancies can not be explained clearly, but the differences in the analgesic effects of morphine may depend on the method of pain assessment. Finally, our results suggest the possibility that opiates would be effective compounds for management of neuropathic pain, depending on the route of administration.

Daily morphine given i.c.v. and i.t. and U-50,488H given s.c. and i.t. rapidly induced the development of tolerance to their analgesic effects in sham-operated mice, but these treatments failed to induce tolerance in SNL mice. The mechanism underlying the lack of development of tolerance to these analgesics has not yet been defined, but it may be partially due to κ -opioid receptor mechanism(s) or the pain-associated anxiety related to the receptor mechanism. In a previous report, we demonstrated that the effect of morphine given daily i.t. was suppressed by the concomitant exposure to socio-psychological stress (30) or a subanalgesic dose

of U-50,488H (27, 28), both of which were antagonized by nor-binaltorphimine, implicating the κ -receptor mechanism in the spine. The result shown here that SNL mice showed a persistent antinociceptive effect of U-50,488H when it was given i.t. but not i.c.v. may support the assumed relation of the neuropathic pain mechanism with κ -receptors in the spine, as suggested above.

In conclusion, our present findings indicate that opiates are not ineffective compounds for the suppression of neuropathic pain, although there is some variation in the production of analgesic and anti-allodynic effects induced by μ - and κ -opioid agonists, which may be due to the variations or alteration of the location and content of their receptors after nerve injury. Our results imply that κ -opioid receptor agonists blocking mechanical allodynia possess potential advantages over μ -opioid receptor agonists. Furthermore, κ -opioid receptor agonists lack the serious side effects associated with μ -opioid receptor agonists such as constipation, sedation, respiratory depression, and dependence. The κ -agonists seem to have quite high potential for the management of neuropathic pain, although their effectiveness will depend on the use of appropriate doses and routes of administration.

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