Effects of \( \kappa \)-Agonist on the Antinociception and Locomotor Enhancing Action Induced by Morphine in Mice

Minoru Narita\(^1\), Yuki Takahashi\(^1\), Kazuaki Takamori\(^1\), Masahiko Funada\(^1\), Tsutomu Suzuki\(^1\)*, Miwa Misawa\(^1\) and Hiroshi Nagase\(^2\)

\(^1\)Department of Pharmacology, School of Pharmacy, Hoshi University, Shinagawa-ku, Tokyo 142, Japan
\(^2\)Basic Research Laboratories, Toray Industries, Inc., Kamakura 248, Japan

Received November 24, 1992 Accepted January 22, 1993

ABSTRACT—The antinociception of intracerebroventricular injection (i.c.v.) of morphine was markedly abolished by pretreatment with naloxonazine (\( \mu \)-antagonist), s.c.; \( \beta \)-funaltrexamine (\( \mu \)/\( \kappa \)-antagonist), i.c.v.; or \( \beta \)-chlorophenylalanine (serotonin synthesis inhibitor), s.c. in the mouse 55°C hot-plate assay. Pretreatment with nor-binaltorphimine (\( \kappa \)-antagonist), i.c.v. or PCPA, s.c. drastically blocked the \( \kappa \)-agonist U-50,488H-induced supraspinal antinociception. These findings indicate either noradrenergic or serotonergic involvement in the mediation of the antinociception of i.c.v.-morphine through \( \mu \)-receptors. On the contrary, the antinociception of i.c.v.- U-50,488H through \( \kappa \)-receptors appears to depend on the serotonergic but not noradrenergic systems. The antinociceptive interaction between the i.c.v.-morphine and U-50,488H was an additive effect. On the other hand, i.c.v.-morphine dose-dependently increased the locomotion in mice, and this hyperlocomotion of morphine was drastically blocked by pretreatment with either \( \beta \)-funaltrexamine, i.c.v. or 6-hydroxydopamine (dopamine depletor), i.c.v. I.c.v.-U-50,488H dose-dependently reduced the increasing locomotion of i.c.v.-morphine, but not that of s.c.-apomorphine (dopamine receptor agonist), and this effect of U-50,488H was completely reversed by pretreatment with nor-binaltorphimine, i.c.v. These results suggest that coadministration of \( \kappa \)-agonists can suppress the dopamine-related hyperlocomotion of \( \mu \)-agonists without decreasing the antinociception of \( \mu \)-agonists in mice.

Keywords: Opioid interaction, Morphine, Locomotion, Antinociception, Straub tail

The \( \mu \) and \( \delta \)-agonists are known to have similar effects in several pharmacological actions, whereas \( \mu \) and \( \kappa \)-agonists appear to have different and/or opposite effects. Spinal and supraspinal \( \alpha \)-adrenergic systems play an important role in the antinociceptive effects of the \( \mu \)-agonist morphine (1). In contrast, the 5-HTergic but not the NAergic system is involved in mediating the antinociceptive action of U-50,488H, a selective \( \kappa \)-agonist (2). Whereas \( \mu \) and \( \delta \)-opoid receptors are coupled to \( K^- \) channels, \( \kappa \)-receptors are linked to voltage-dependent \( Ca^{2+} \)-channels in the mouse dorsal root ganglion neurons (3).

Abbreviations used are: 5-HT, serotonin; NA, noradrenaline; U-50,488H, (trans-dl)-3,4-dichloro-N-methyl-N-(2-(1-pyrroldinyl))cyclohexyl-benzenacetamide)methanesulfonate hydrate; DA, dopamine; i.c.v., intracerebroventricular injection; Nlz, naloxazine hydrochloride; \( \beta \)-FNA, \( \beta \)-funaltrexamine hydrochloride; BNTX, 7-benzylidine-7-dehydroxaloxone; NBt, naltrexone methanesulphonate hydrate; NTI, naltirindole methanesulphonate hydrate; nor-BNI, nor-binaltorphimine hydrochloride; DSP-4, N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine; PCPA, \( \beta \)-chlorophenylalanine methylester; DFPDE, [d-Pen\(^2\),d-Pen\(^3\)]enkephalin; 6-OHDA, 6-hydroxydopamine hydrochloride; E-2076, [N-methyl-Tyr\(^3\), N-\( \alpha \)-methyl-Arg\(^2\), d-Leu\(^4\)] dynorphin A (1-8) ethylamide; SCH23390, [(R, +)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzoazepine-OH]hydrochloride; DOPAC; 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid.
self-stimulation (11–13). In the place-conditioning paradigm, mice and rats prefer an environment associated with administration of \( \mu \) - and \( \delta \)-agonists but, avoid an environment associated with the administration of \( \kappa \)-agonists (14–16), suggesting that either \( \mu \) - or \( \delta \)-agonists exert a euphoric effect, while \( \kappa \)-agonists appear to be disphoric.

Activation of DAergic systems after administration of morphine and other \( \mu \)-opoids can elicit not only a motor-activating effect but also a reinforcing effect (17). Furthermore, Koob (18) proposed that the mesolimbic DAergic projection is a key component of the opioid reward mechanism. These findings indicate that although both \( \mu \) - and \( \kappa \)-agonists produce the antinociceptive effects through different mechanisms, there may be opposing properties of \( \mu \) - and \( \kappa \)-agonists on some pharmacological actions, especially the DA-related actions such as motor-activating and reinforcing effects. Thus, we hypothesized that one of the most important investigations for opioids is to determine if there is a negative modulation by \( \kappa \)-agonists on the morphine-induced facilitation of central DAergic systems. Regarding the hypothesis that there may be useful interactions among opioid receptors, we investigated the effects of \( \kappa \)-agonists on the morphine-induced supraspinal antinociception and locomotor enhancing action in mice.

MATERIALS AND METHODS

Animals

Male ddY mice (18–25 g) were obtained from Tokyo Animal Laboratories, Inc. (Tokyo). The mice were housed at a room temperature of 22±1°C with a 12-hr light-dark cycle (lights on 8:00 AM to 8:00 PM). Food and water were available ad libitum.

Antinociceptive assay

The antinociceptive test used in this study was determined by using a 55°C hot-plate as the nociceptive stimulus. The latency to paw-tap, paw-lick or an attempt to escape by jumping taken as the endpoint. Prior to drug administration, control latencies were determined. To prevent tissue damage, the mice that showed no response within 60 sec (cut-off time) were removed from the hot-plate. The percent of antinociception was calculated according to the following formula: % antinociception = 100 \( \times \) (test latency – control latency)/(60 sec – control latency). Morphine or U-50,488H was given into the lateral cerebral ventricle of unanesthetized mice. The unilateral injection site was approximately 2 mm from either side of the midline on a line drawn through the anterior roots of the ears (19). The injection was made with a 2-mm double-needle (Natsume Seisakusho Co., Ltd., Tokyo) attached to a 25-\( \mu \)l Hamilton microsyringe. Solutions were injected in a volume of 5–10 \( \mu \)l per mouse over a period of 10 sec. Antinociception was tested 10 min (peak time) after treatment with morphine or U-50,488H, i.c.v. Nlz (35 mg/kg, s.c.; \( \mu \)-agonist), \( \beta \)-FNA (1 \( \mu \)g, i.c.v.; \( \mu \)/\( \mu \)-agonist), BNTX (3 ng, i.c.v.; \( \delta \)-agonist), NTB (12 ng, i.c.v.; \( \delta \)-agonist), NTI (5 ng; \( \delta \)/\( \delta \)-agonist) and nor-BNI (1 \( \mu \)g, i.c.v.; \( \kappa \)-agonist) were given to mice 24 hr, 24 hr, 20 min, 20 min and 30 min prior to the morphine treatment, respectively, according to the previous reports (25–30). In combination studies, U-50,488H was coadministered with morphine. Mice were pretreated s.c. with DSP-4 (a selective denervation tool for NAergic nerves (20)) in a dose of 80 mg/kg 72 hr prior to treatment with morphine or U-50,488H, i.c.v. The 5-HT synthesis inhibitor PCPA was given s.c. at a dose of 200 mg/kg/day for 3 days; the antinociceptive assay was performed on the day 4.

Locomotor assay

The locomotor activity of mice was measured by an ambulometer (ANB-M20, O'hara Co., Ltd., Tokyo). The principle of the device and the measurement method have been described in detail by Hirabayashi and Alam (21). Briefly, a mouse was placed in a tilting-type round activity cage (20 cm in diameter and 19 cm in height). Any slight tilt of the activity cage caused by horizontal movement of the animal was detected by microswitches. Total activity counts in each 10-min segment were automatically recorded for 30 min prior to the injections and for 180 min following morphine, U-50,488H or DPDPE administration. U-50,488H (i.c.v.) was coadministered with morphine or DPDPE, i.c.v. \( \beta \)-FNA at a dose of 1.0 \( \mu \)g and nor-BNI at a dose of 1.0 \( \mu \)g were given i.c.v. to mice 24 hr and 30 min prior to morphine treatment, respectively. To produce the destruction of central DAergic neurons, mice were treated with a 25 \( \mu \)g dose of 6-OHDA, i.c.v. 72 hr prior to i.c.v.-administered morphine. Additionally, desipramine (25 mg/kg, s.c.) was given to mice 10 min prior to the 6-OHDA (i.c.v.) to block the uptake of 6-OHDA into NAergic terminals (22). Furthermore, we investigated the influence of s.c.-administered \( \kappa \)-agonists in the s.c. morphine-induced locomotion. Saline (0.1 ml/10 g of body weight); U-50,488H; or E-2078, a systemically active dy-

16 M. Narita et al.
apomorphine (s.c.).

**Drugs**

Morphine hydrochloride was obtained from Sankyo Co. (Tokyo). U-50,488H was supplied by Toray Research Center (Kamakura). DPDPE was purchased from Peninsula Laboratories, Inc. (Cambridge, MA, USA). DSP-4, β-FNA and SCH23390 were purchased from Research Biochemicals, Inc. (Wayland, MA, USA). Nlz, BNTX, NTB, NTI and nor-BNI were synthesized by us. PCPA, 6-OHDA, desipramine hydrochloride and apomorphine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sulpiride (Dogmatyl®) was obtained from Fujisawa Ind., Co. (Osaka). E-2078 was a gift from Eisai Co., Ltd. (Tsukuba). 6-OHDA was dissolved in 0.9% NaCl containing 10 mM ascorbic acid. Other drugs were dissolved in 0.9% NaCl. All doses refer to the salt forms of the drugs.

**Statistical analysis**

The data were presented as the mean ± S.E.M. One-way repeated analysis of variance (ANOVA) followed by the Newman-Keuls multiple comparison test was used for statistical evaluations. ED_{50} values of an opioid to induce antinociception and its 95% confidence limits were determined by the linear regression lines. To investigate the antinociceptive interaction between i.c.v.-morphine and -U-50,488H, we used isobolographic analysis (24, 25). The ED_{50} value of i.c.v.-morphine was plotted on the ordinate and the ED_{50} value of i.c.v.-U-50,488H, on the abscissa. A theoretical line of the additive interaction was drawn by connecting the ED_{50} value for i.c.v.-morphine and -U-50,488H. For each morphine-U-50,488H combination, the ED_{50} value along with the 95% confidence limit was calculated for each mixture. For each combination, there also exists a theoretical additive ED_{50} value that would be expected if the drug interaction was additive. The position of the point representing i.c.v.-morphine plus i.c.v.-U-50,488H above and below the theoretical line indicates the antagonistic and potentiative interactions between i.c.v.-morphine and -U-50,488H, respectively.

The potency ratio for the i.c.v.-morphine-induced antinociception in the presence of i.c.v.-U-50,488H (3 or 10 μg) was calculated as 1 for the % antinociception of 10 μg morphine alone according to the following formula: Antinociceptive ratio = [(% antinociception of i.c.v.-morphine at a dose of 10 μg in the presence of i.c.v.-U-50,488H) − (% antinociception of i.c.v.-saline)] / [(% antinociception of 10 μg morphine alone) − (% antinociception of i.c.v.-saline)]. Furthermore, the potency ratio for locomotion was calculated according to the following formula: Locomotor ratio = [(total activity counts for 3 hr of i.c.v.-morphine) / ((total activity counts for 3 hr of 10 μg morphine (i.c.v.) alone) − (total activity counts for 3 hr of i.c.v.-saline)]. On the other hand, we previously reported the blockade of i.c.v.-morphine-induced Straub tail in mice by U-50,488H, and the inhibitory effect of U-50,488H was completely reversed by nor-BNI (26). Therefore, in the present study, we calculated the potency ratio for the i.c.v.-morphine-induced Straub tail in the presence of i.c.v.-U-50,488H (3 or 10 μg) as 1 for the Straub tail scores (according to the scores of Narita et al. (26)) of 15 μg morphine (i.c.v.) alone.

**RESULTS**

Figure 1 shows the effects of μ-, δ- and κ-receptors on the morphine-induced supraspinal antinociception. Pretreatment with Nlz and β-FNA, but not BNTX, NTB, NTI and nor-BNI, significantly antagonized the morphine-induced antinociception. In contrast, i.c.v.-U-50,488H-induced antinociceptive activity was much milder than that of i.c.v.-morphine. Pretreatment with nor-BNI, but not β-FNA and NTI, completely abolished the antinociceptive activity of 50 μg U-50,488H (Fig. 2).

As shown in Table 1, ED_{50} values of i.c.v.-morphine-induced antinociception in either DSP-4- or PCPA-treated

---

**Fig. 1.** Role of μ-, δ- and κ-receptors in i.c.v.-morphine-induced antinociception. Each column represents % antinociception induced by 10 μg (i.c.v.) morphine. Nlz (35 mg/kg, s.c.; μ-receptor antagonist), β-FNA (FNA: 1 μg, i.c.v.; μ/δ-receptor antagonist), BNTX (3 ng, i.c.v.; δ-receptor antagonist), NTB (12 ng, i.c.v.; δ-receptor antagonist), NTI (5 ng; δ/κ-receptor antagonist) and nor-BNI (BNI: 1 μg, i.c.v.; κ-receptor antagonist) were given to mice 24 hr, 24 hr, 20 min, 20 min, 20 min and 30 min prior to the morphine treatment, respectively. Each group used 8–10 mice. *P < 0.05, **P < 0.01 vs. 10 μg morphine alone.
mice were significantly increased in comparison with those in the control non-pretreated animals. The inhibitory potency of DSP-4 on the antinociception of morphine was greater than that of PCPA.

The antinociceptive effect produced by i.c.v.-U-50,488H was significantly blocked by PCPA, while DSP-4 had no effect on the antinociception of i.c.v.-U-50,488H (Fig. 2).

The ED$_{50}$ value (with its 95% confidence limits) of i.c.v.-U-50,488H was 79.68 (63.24–108.18) $\mu$g. The antinociceptive activity of i.c.v.-morphine in combination with i.c.v.-U-50,488H statistically showed the additive effect (Fig. 3).

Figure 4 shows that i.c.v.-morphine but not U-50,488H

![Table 1. Effect of DSP-4 or PCPA pretreatment on the antinociceptive action of i.c.v.-morphine](image)

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>ED$_{50}$ (µg) of morphine (95% confidence limits)</th>
<th>Shift ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2.04 (1.00–4.18)</td>
<td>–</td>
</tr>
<tr>
<td>DSP-4</td>
<td>11.78 (7.04–19.70)*</td>
<td>5.77</td>
</tr>
<tr>
<td>PCPA</td>
<td>7.98 (4.48–14.21)*</td>
<td>3.91</td>
</tr>
</tbody>
</table>

ED$_{50}$ values were determined using the analysis of variance and linear regression techniques. To calculate ED$_{50}$ values, at least 3 drug doses were used and 7–10 mice were used for each dose. *$P<0.05$ vs. morphine alone.

![Fig. 2. Monoaminergic involvement in the antinociceptive action of the $\kappa$-opioid receptor agonist U-50,488H. $\beta$-FNA (FNA: 1 $\mu$g, i.c.v.; $\mu_\alpha/\mu_\gamma$-antagonist), NTI (5 ng; $\delta/\delta_2$-antagonist) and nor-BNI (BNI: 1 ng, i.c.v.; $\kappa$-antagonist) were given to mice 24 hr, 20 min, and 30 min prior to 50 $\mu$g U-50,488H (i.c.v.), respectively. Mice were pretreated with NA neurotoxin DSP-4 at a dose of 80 mg/kg (s.c.) 72 hr prior to i.c.v.-treatment with U-50,488H. A 5-HT synthesis inhibitor PCPA was given s.c. at a dose of 200 mg/kg/day for 3 days; the antinociceptive assay was performed on day 4. Each group used 8–10 mice. S: Saline (i.c.v.). **$P<0.01$ vs. saline; ***$P<0.01$ vs. 50 $\mu$g U-50,488H alone.](image)

![Fig. 3. Isobologram for the ED$_{50}$ of the antinociceptive activity of morphine plotted against U-50,488H after i.c.v.-injection in the mouse 55°C hot-plate test. The slope line between the points (■) representing ED$_{50}$ values of the antinociceptive activity induced by morphine alone and U-50,488H alone would theoretically indicate an additive interaction between i.c.v.-morphine and -U-50,488H. The position of the points representing i.c.v.-morphine plus -U-50,488H is not significantly below but close to the slope line, indicating the additive interaction between i.c.v.-morphine and -U-50,488H. Each group consisted of 8–10 mice.](image)
produced a dose-dependent increase in locomotion, and this locomotor enhancing action by i.c.v.-morphine was drastically antagonized by pretreatment with \( \beta \)-FNA. In addition, pretreatment with i.c.v.-6-OHDA in combination with s.c.-desipramine markedly suppressed the increasing locomotion induced by i.c.v.-morphine. As shown in Fig. 5, coadministration of i.c.v.-U-50,488H dose-dependently decreased the locomotor activity of 10 \( \mu \)g morphine, and the inhibitory effect of U-50,488H at a dose of 10 \( \mu \)g was completely reversed by pretreatment with i.c.v.-nor-BNI. Furthermore, either s.c.-U-50,488H or s.c.-E-2078 produced a dose-dependent attenuation in the hyperlocomotion of s.c.-morphine (10 mg/kg) in combination with s.c.-saline (Fig. 6).

In our additional studies, i.c.v.-administration of DPDPE, a selective \( \delta \)-agonist produced a dose-dependent locomotion (188.1 ± 57.1, 519.1 ± 97.7 and 643.2 ± 174.8 counts/3 hr for 1, 5 and 25 \( \mu \)g DPDPE, respectively), and this hyperlocomotion of i.c.v.-DPDPE was markedly antagonized by pretreatment with i.c.v.-NTI (51.3 ± 20.9 and 141.8 ± 64.4 counts/3 hr for 5 \( \mu \)g NTI plus 5 \( \mu \)g DPDPE and 5 \( \mu \)g NTI plus 25 \( \mu \)g DPDPE, respectively). The i.c.v.-DPDPE-induced locomotor enhancing action was significantly attenuated by i.c.v.-U-50,488H (170.9 ± 32.5 counts/3 hr for 10 \( \mu \)g U-50,488H plus 5 \( \mu \)g DPDPE; significantly different (P < 0.05) from 5 \( \mu \)g DPDPE alone).

On the other hand, the locomotor activity induced by s.c.-apomorphine was dose-dependently increased. The mean total counts after treatments with 0.5 and 1.0 mg/kg apomorphine in combination with i.c.v.-saline were 194.7 ± 27.1 and 329.5 ± 28.7 counts/3 hr, respectively. Both SCH23390 and sulpiride exerted a significant inhibiting effect on the locomotor enhancing action induced by 1.0 mg/kg apomorphine in combination with i.c.v.-saline (P < 0.01, Fig. 7). However, i.c.v.-U-

---

**Fig. 4.** Role of the \( \mu \)-receptors and DAergic systems in the i.c.v.-morphine-induced locomotor enhancing action in mice. Mice were treated with 1.0 \( \mu \)g \( \beta \)-FNA (i.c.v.) and 25 \( \mu \)g 6-OHDA (i.c.v.) 24 hr and 72 hr prior to i.c.v.-treatment with morphine, respectively. Desipramine (25 mg/kg, s.c.) was given 10 min prior to i.c.v.-treatment with 6-OHDA. Each column represents the mean total activity counts with S.E.M. for 3 hr after i.c.v.-administration of saline, U-50,488H (U-50: 10 \( \mu \)g) or morphine (1, 5 or 10 \( \mu \)g). Each group used 8–35 mice. *P < 0.05, **P < 0.01 vs. saline; ***P < 0.01 vs. 10 \( \mu \)g morphine alone.

**Fig. 5.** Effects of i.c.v.-U-50,488H on the i.c.v.-morphine-induced locomotor enhancing action in mice. U-50,488H (U-50: 1, 3 or 10 \( \mu \)g, i.c.v.) was coadministered with i.c.v.-morphine at a dose of 10 \( \mu \)g. Nor-BNI (1 \( \mu \)g) was given i.c.v. 30 min prior to i.c.v.-treatment with morphine. Each group used 12–19 mice. *P < 0.05 vs. 10 \( \mu \)g morphine; **P < 0.05 vs. 10 \( \mu \)g morphine plus 10 \( \mu \)g U-50,488H.

**Fig. 6.** Effects of s.c.-U-50,488H and -E-2078 on the s.c.-morphine-induced locomotor enhancing action in mice. Mice were treated with either s.c.-U-50,488H or -E-2078 10 min prior to 10 mg/kg morphine (s.c.). Each column represents the mean total counts with S.E.M. for 3 hr. Each group corresponded to a group of 10–20 mice. Asterisks indicate the significance of difference from morphine plus saline (*P < 0.05, ***P < 0.01).
50,488H failed to inhibit the s.c.-apomorphine-induced locomotor enhancing action (Fig. 7).

As shown in Fig. 8, U-50,488H at i.c.v.-doses that alone exerted little or no antinociceptive effects markedly suppressed the i.c.v.-morphine-induced locomotor enhancing action and Straub tail without decreasing the antinociception of morphine. The locomotor ratios in the presence of 3 and 10 pg U-50,488H were 0.540 and 0.372, respectively. The potency ratios for Straub tail in combination with 3 and 10 pg U-50,488H were 0.175 and 0.044, respectively.

DISCUSSION

There is evidence for four main types of opioid receptors in the central nervous system, designated as \( \mu \), \( \delta \), \( \kappa \) and \( \epsilon \). Among these opioid receptor types, analgesia is mainly caused via the stimulation of \( \mu \), \( \delta \) and \( \kappa \)-receptors (27, 28). It is essential to have highly selective opioid antagonists as pharmacological tools to investigate the pharmacological effects that are mediated by different types of opioid receptors. Pasternak’s group has proposed that \( \beta \)-FNA is a mixed \( \mu_1/\mu_2 \)-antagonist, whereas Nlz selectively acts at the \( \mu_1 \)-receptors (29). Furthermore, a series of highly selective, non-peptide \( \delta \)-receptor antagonists, such as NTI, BNTX and NTB, have been recently synthesized (30–32). Based on the antinociceptive studies, it is proposed that BNTX and NTB selectively act at the \( \delta_1 \) and \( \delta_2 \) receptors, respectively, while NTI is a non-selective \( \delta \)-antagonist (31–33). Moreover, Takemori et al. (34) have reported that nor-BNI is a highly selective \( \kappa \)-antagonist. Under the present conditions, the treatment with each antagonist hardly affected the naive mice and shows a high selectivity for each opioid receptor (29–34).

Our present findings suggest that i.c.v.-morphine-induced supraspinal antinociception against the nociceptive stimulus of a 55°C hot-plate may be mediated through \( \mu_1 \) and \( \mu_2 \)-receptors, but not \( \delta \) and \( \kappa \)-receptors. In contrast, the expression of the i.c.v.-U-50,488H-induced antinociception was due to central \( \kappa \)-receptors, but not \( \mu \)- and \( \delta \)-receptors.
Systemic injection of DSP-4 is known to cause a selective degeneration of the central NAergic pathway. Indeed, Nakazawa et al. (20) have reported that s.c.-DSP-4 completely reduced the levels of NA in the mouse spinal cord without modifying the levels of DA and 5-HT. Additionally, we also observed the NA depletion of the mouse whole brain by DSP-4 (M. Narita et al., unpublished data). In the present study, we found that the antinociceptive activity induced by i.c.v.-morphine was drastically inhibited by pretreatment with s.c.-DSP-4, whereas DSP-4 had no effect on the i.c.v.-U-50,488H-induced antinociception. On the contrary, PCPA, a 5-HT synthesis inhibitor (20), was capable of suppressing either i.c.v.-morphine- or -U-50,488H-induced antinociception. Our results further suggest both NAergic and 5-HTergic involvements in the mediation of the antinociceptive action of morphine administered i.c.v. In contrast, the i.c.v.-U-50,488H-induced antinociceptive effect appears to depend on 5-HTergic but not NAergic mechanisms. Although both NAergic and 5-HTergic systems have been reported to be involved in the antinociceptive action of μ-agonists such as morphine (35, 36), NAergic mechanisms may be more important in the mediation of antinociception by morphine (37). In the present study, the inhibitory potency of PCPA on the antinociception of morphine was weaker than that of DSP-4, indicating that in the mediation of morphine-induced antinociception, the NA systems are more important than the 5-HTergic systems. On the other hand, Ho and Takemori (2, 38, 39) have shown that the antinociceptive action of U-50,488H is probably mediated by the release of 5-HT in the brain and spinal cord. However, they have suggested that the antinociceptive action of U-50,488H is not mediated by the NAergic system because NA receptor antagonists failed to reverse the effect of U-50,488H. These reports support our data. Furthermore, the 5-HTergic involvement in the i.c.v.-morphine-induced antinociception may not be explained by the interaction of morphine with the κ-systems. Indeed, the i.c.v.-morphine-induced antinociceptive action was drastically blocked by the μ-antagonist β-FNA, but not the κ-antagonist nor-BNI. These results indicate that morphine administered i.c.v. may produce its supraspinal antinociceptive action through selective activation of central μ-receptors by both NAergic and 5-HTergic systems.

As shown in the combination studies, the antinociceptive interaction between i.c.v.-morphine and -U-50,488H was an additive effect. It is presently unclear what factors contribute to the additive effect. We expected these combinations to produce a synergic or antagonistic effect because the antinociception of morphine and U-50,488H are mediated through different mechanisms. This additive effect appears to result from the intricate interactions between NAergic and 5-HTergic systems in the mouse brain.

The opioid analgesics, of which the prototype is morphine, are of importance in the control of moderate to severe pain. Unfortunately, most of them possess high dependence liability (40). An enduring aim in opioid research has been to develop strong analgesics devoid of morphine-like side effects. In the previous study, we found that inactivation of the κ-opioid system may potentiate the development of tolerance to morphine antinociception in mice and may aggravate the naloxone-precipitated body weight loss in morphine-dependent mice and rats (41). From the above findings, we therefore hypothesized that there may be an inhibitory κ-opioid mechanism serving as negative modulation against the activation of μ-receptors. Furthermore, these observations support the probability that coadministration of κ-agonists may be a good means for producing a potent antinociceptive effect with decreasing some side-effects of morphine.

The present results clearly demonstrated that the activation of central μ-receptors by i.c.v.-morphine produced the potent locomotor enhancing action, and this effect was mainly mediated through activation of presynaptic DA-containing neurons. On the other hand, it has been proposed by our previous report that the expression of i.c.v.-morphine-induced Straub tail may also be due to the activation of central DAergic systems (26). More importantly, we presently found through the interactions between μ- and κ-agonists that administration of U-50,488H could attenuate the morphine-induced locomotor enhancing action as well as Straub tail, but not supraspinal antinociception. These findings further suggest that tonic activation of κ-receptors inhibits the morphine-induced DA-related behaviors without blocking the antinociceptive activity of morphine.

DPDPE, a selective δ-agonist, administered i.c.v. produced a dose-dependent locomotion, and this hyperlocomotion of i.c.v.-DPDPE was markedly antagonized by i.c.v.-pretreatment with NTI. Interestingly, i.c.v.-U-50,488H significantly abolished the i.c.v.-DPDPE-induced locomotor enhancing action. Additionally, our previous reports indicate that δ-receptors play a partial role in the morphine-induced hyperlocomotion and excitation of DA systems, but not antinociception (42, 43). From these findings, δ-receptors as well as μ-receptors play an important role as modulators of the expression of opioid-induced hyperlocomotion related to DA transmission, and the increased activity of κ-opioid systems can lead to suppression of the hyperlocomotion of μ/δ-agonists.

An interesting neurochemical interaction between morphine and U-50,488H was also found. We observed at 90 min after the i.c.v.-treatment that 10 μg morphine elevat-
ed the levels of DOPAC and HVA in the mouse whole brain, and these elevations were drastically abolished by coadministration of 10 μg U-50,488H without changing DA steady-state levels (44). Furthermore, activation of central μ/δ-opioid receptors by treatment with systemic morphine increased the DA-turnover in the mouse limbic forebrain (42, 45), in which the main field of the mesolimbic DA terminal field exists, and the enhancement of the DA-turnover by morphine was dose-dependently abolished by pretreatment with systemic U-50,488H (45). These findings suggest that an increased activity of κ-opioid systems may result in a suppression of the μ/δ-agonist-induced DA release.

A low dose of apomorphine stimulates presynaptic DA autoreceptors and inhibits the release of DA (46). However, a high dose of apomorphine administered systemically stimulates the postsynaptic D₂-receptors (47). Furthermore, it has been recently reported that the locomotor enhancing action induced by s.c.-apomorphine in a dose of 1 mg/kg was suppressed by pretreatment with the selective D₂-antagonist SCH23390 in male ddY mice (48). In the present study, we found either SCH23390 or sulpiride (D₂-antagonist) was capable of suppressing the locomotor enhancing action induced by s.c.-apomorphine at this dosage may be mediated by the activation of the postsynaptic D₂- and D₃-receptors. Under these conditions, i.c.v.-U-50,488H failed to suppress the s.c.-apomorphine-induced locomotor enhancing action. These findings provide further evidence that activation of endogenous κ-opioid systems may result in a suppression of the DA-related behaviors of i.c.v.-morphine by directly and/or indirectly inhibiting the release of DA induced by activation of central μ/δ receptors.

It is well known that the release of DA in the nucleus accumbens after μ-agonist administration can elicit not only a motor-activating effect, but also a positive reinforcing effect in the rodent (17). Therefore, the possible blockade of the reinforcing effects elicited by opioids and psychostimulants may result from, at least in part, an increased activity of κ-opioid systems. Indeed, we have the data that tonic activation of κ-receptors blocks the reinforcing effects of cocaine (49) and morphine (unpublished data).

In conclusion, the selective κ-agonist U-50,488H at i.c.v.-doses that alone exerted little or no antinociceptive effects can strongly attenuate the i.c.v.-morphine-induced DA-related behaviors, such as locomotor enhancing action and Straub tail, without decreasing the antinociceptive activity. In view of the present work, including our previous studies, it is tempting to speculate that the adequate coactivation of μ- and κ-receptors may produce a potent antinociceptive activity along with a decrease in some side-effects of opioids.

Acknowledgments

We wish to thank Seiich Oka and Minoru Tsuji for their technical assistance.

REFERENCES

Interaction between \(\mu\)- and \(\kappa\)-Agonists

23


48 Matsumoto, K., Cai, B., Ohra, H., Imamura, L. and