The Abilities of Specific $\kappa$-Opioid Agonists, U-50,488H and U-62,066E, to Cause Antitussive Tolerance Were Lower than That of Morphine

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ABSTRACT—We examined whether the chronic administration of selective $\kappa$-opioid agonists could produce antitussive tolerance, in a comparison with the $\mu$-opioid morphine. A certain degree of tolerance to the antitussive effects of morphine appeared in rats treated chronically with this drug. However, chronic administration of U-50,488H and U-62,066E, highly selective agonists for the $\kappa$-opioid receptor, does not result in the development of tolerance to their respective antitussive effects. These results suggest that the ability of $\kappa$-opioid agonists to cause tolerance to their respective antitussive effects was lower than that of a $\mu$-opioid agonist.

There is evidence that specific $\kappa$-opioid agonists, such as U-50,488H and U-62,066E, not only have potent analgesic effects (1) but also potent antitussive effects (2). One of the potential disadvantages to the analgesic use of U-50,488H and U-62,066E is that these drugs induce tolerance (1, 3). Thus, it is possible that chronic administration of $\kappa$-opioid agonists causes the development of tolerance to their respective antitussive actions. Therefore, we examined whether the chronic administration of selective $\kappa$-opioid agonists, U-50,488H and U-62,066E, could produce antitussive tolerance, in a comparison with the $\mu$-opioid morphine.

Previously, we have reported that tolerance to the antitussive effects of opiates is thought to result from the development of serotonin receptors subsensitivity (4). Furthermore, Ho and Takemori (5) suggested that the reduction of the affinity of serotonin receptors in the spinal cord may be involved in the development of tolerance to the antinociceptive action of U-50,488H. To ascertain the specificity of the involvement of serotonin receptors, the binding of $[\text{3H}]$serotonin to receptors on the brainstem membranes of rats chronically treated with U-62,066E or morphine were also examined.

Male Sprague-Dawley rats (Tokyo Animal Laboratory, Inc., Tokyo, Japan), weighing about 250 g, were used. Rats were injected with U-50,488H (10 mg/kg, i.p.), U-62,066E (10 mg/kg, i.p.), morphine (1 mg/kg, i.p.) or vehicle (1 ml/kg) twice a day (10:00 and 17:00), for four days. On day five, tolerance to the antitussive action of these drugs was determined by the previously described method (6). In brief, animals were exposed to a nebulized solution of capsaicin (30 $\mu$mol) under identical conditions, using a body plethysmograph. The number of coughs produced per 5-min period of exposure to capsaicin was counted. The rats were exposed to capsaicin 30 min before the injection of drugs to determine the frequency of control coughs; then they were exposed to
capsaicin 15 min after i.p. injection of drugs or vehicle. The number of coughs produced 15 min after the injection of drugs was compared with the number produced by vehicle-injected control rats. The antitussive effect was expressed in terms of the percentage reduction in number of coughs relative to the number of control coughs.

The serotonin binding assay was performed using the brainstem (medulla oblongata and pons) in 50 mM Tris buffer containing 10 μM pargyline and 4 mM CaCl₂ (pH 7.4), according to the method used by Bennet and Snyder (7). Samples of membrane preparations (60 μg protein/tube) were put in test tubes. [3H]Serotonin was added at concentrations over the range of 1.5 to 50 nM, and 10 μM unlabelled serotonin was used to determine nonspecific binding. Incubations were performed in a volume of 1 ml at 22°C for 30 min. The incubation was terminated by adding of 5 ml of ice-cold Tris buffer and rapid filtration through Whatman GF/C filters. The filters were washed twice with 5 ml Tris buffer and transferred to scintillation vials and then counted in a Packard Tri-Carb liquid scintillation spectrometer in 5 ml aqueous scintillant (Echonoflow, New England Nuclear).

U-50,488H and U-62,066E were generously supplied by Upjohn Company, Kalamazoo, MI, U.S.A. Morphine hydrochloride was purchased from Sankyo Co., Ltd., Tokyo, Japan. 5-Hydroxy(G-3H)tryptamine creatinine sulfate was obtained from Amersham International (Amersham, Buckinghamshire, U.K.). All antitussive drugs were dissolved in 0.9% saline immediately before use.

Data are expressed as the mean ± S.E.M. for a given number of rats. The statistical significance of differences was assessed by Student’s t-test after an analysis of variance (significance level was set at P = 0.05).

Morphine injection (1 mg/kg, i.p.) into vehicle-treated rats had a typical antitussive effect. The percent inhibition of the number of coughs, 15 min after the administration of morphine, was 84.4 ± 4.3%. Administration of U-50,488H (10 mg/kg, i.p.) and U-62,066E (10 mg/kg, i.p.) also significantly increased the percent inhibition of the number of coughs in rats treated chronically with vehicle (vehicle, 4.8 ± 2.9%; U-50,488H, 73.2 ± 5.0%; U-62,066E, 80.8 ± 5.7%). A certain degree of tolerance to the antitussive effects of morphine appeared in rats treated chronically with morphine. Thus, the antitussive response to morphine (1.0 mg/kg, i.p.) in chronically morphine-treated rats was only half that observed in rats treated chronically with vehicle (Table 1). As can be seen from Table 1, however, the percent inhibition of the number of coughs in chronically U-50,488H- or U-62,066E-treated rats in response to a challenge dose of morphine was similar to that observed in chronically vehicle-treated rats. In chronically U-50,488H-treated rats, the antitussive action induced by U-50,488H was similar to that observed in chronically vehicle-treated rats. There was no significant difference in the U-50,488H-induced antitussive effect between chronically vehicle-treated and chronically morphine- or U-62,066E-treated rats, respectively. The antitussive effects of U-62,066E in rats chronically treated with U-62,066E were similar to those in chronically vehicle-treated rats. Furthermore, when U-62,066E was given to chronically morphine- or U-50,488H-treated rats, the antitussive effects of U-62,066E were also not significantly different from the antitussive effect of U-62,066E in chronically vehicle-treated rats.

Table 2 shows the results of binding of [3H]serotonin to brainstem membranes from rats chronically treated with morphine or U-62,066E. There were no significant changes in both the Bₘₐₓ and apparent Kₛₐ values in brainstem tissues of rats chronically treated with either morphine or U-62,066E as compared to the vehicle-treated animals.

The present study clearly demonstrates that chronic administration of U-50,488H and U-62,066E, highly selective agonists for the α-opioid receptor, does not result in the development of tolerance to their respective antitussive effects. There is, furthermore, no cross-tolerance between U-50,488H and U-
Table 1. Development of tolerance to and cross-tolerance between morphine, U-50,488H and U-62,066E with respect to antitussive activity

| Chronically treatment drug (mg/kg) | Test drug (mg/kg) | % inhibition of the number of coughs (n) |
|------------------------------------|------------------|----------------------------------------|
| vehicle                            | morphine (1)     | 84.4 ± 4.3 (5)                         |
| morphine (1)                       | morphine (1)     | 50.9 ± 6.9 (5)*                        |
| U-50,488H (10)                     | morphine (1)     | 86.6 ± 10.2 (5)                        |
| U-62,066E (10)                     | morphine (1)     | 82.5 ± 6.1 (5)                         |
| vehicle                            | U-50,488H (10)   | 73.2 ± 5.0 (6)                         |
| U-50,488H (10)                     | U-50,488H (10)   | 73.8 ± 5.6 (6)                         |
| morphine (1)                       | U-50,488H (10)   | 74.4 ± 7.8 (5)                         |
| U-62,066E (10)                     | U-50,488H (10)   | 76.9 ± 8.9 (5)                         |
| vehicle                            | U-62,066E (10)   | 80.8 ± 5.7 (6)                         |
| U-62,066E (10)                     | U-62,066E (10)   | 78.3 ± 8.1 (7)                         |
| morphine (1)                       | U-62,066E (10)   | 75.8 ± 3.9 (6)                         |
| U-50,488H (10)                     | U-62,066E (10)   | 92.9 ± 4.6 (6)                         |

The data shown are the means ± S.E. of the percent reductions in the numbers of coughs. For the chronic treatment, rats received i.p. injection of morphine (1.0 mg/kg), U-50,488H (10 mg/kg) or U-62,066E (10 mg/kg), twice a day for four days. *=P < 0.05, with respect to the value for the vehicle treated group.

Table 2. 3H-Serotonin binding sites in the brainstem of rats chronically treated U-62,066E or morphine

| Chronic treatment | B_{max} (pmoles/mg protein) | K_a (nM) |
|-------------------|-------------------------------|----------|
| saline            | 2.1 ± 0.9                     | 16.4 ± 3.5 |
| U-62,066E         | 1.6 ± 0.4                     | 14.6 ± 2.3 |
| morphine          | 2.5 ± 0.3                     | 15.6 ± 1.1 |

B_{max} and K_a values were calculated by Scatchard plot analysis of the binding data. Values are expressed as the mean ± S.E. for five separate experiments. Rats were injected i.p. with U-62,066E (10 mg/kg), morphine (1 mg/kg) or saline twice a day for four days.

In the present study, no changes in affinity and density of serotonin receptors were observed in the brainstem of rats chronically treated with U-62,066E as compared to the vehicle-treated animals. Although we did not, in this study, assess the effects of U-50,488H (10 mg/kg, i.p.) and U-62,066E (10 mg/kg, i.p.) each reduced the number of coughs by about 80%, respectively. Moreover, chronic administration of an analgesic dose of U-50,488H (25 mg/kg, i.p.), on the same treatment schedule as that in the present study, resulted in the development of tolerance with respect to the analgesic effect of the drug (8). Thus, the ability of these \( \kappa \)-opioid agonists to cause tolerance to their respective antitussive effects was lower than that of a \( \mu \)-opioid agonist.

In the present study, no changes in affinity and density of serotonin receptors were observed in the brainstem of rats chronically treated with U-62,066E as compared to the vehicle-treated animals. Although we did not, in this study, assess the effects of U-50,488H on the functions of serotonin receptors because of the small quantity of sample, there is reason to believe that also no changes in affinity and density of serotonin receptors might have occurred in the brainstem of rats chronically treated with U-50,488H. Indeed, U-50,488H has the same antitussive potency as U-62,066E (2). In addition, the antitussive
effects of U-50,488H and U-62,066E are mediated by the same modes of interaction at the serotonin receptors (2). Ho and Takemori (5) reported that antinociceptive tolerance to U-50,488H was accompanied by a decrease in the affinity of [3H]serotonin to serotonin receptors in the spinal cord, but not in the supraspinal regions. Most opioid and nonopioid antitussive drugs have their site of action at the supraspinal level. Furthermore, U-50,488H and U-62,066E have their site of action at the supraspinal level (2). It seems likely, therefore, that the differential ability of x-opioid receptor agonists to cause tolerance to their own antinociceptive effects and to their own antitussive effects may be due to differences between their modes of action on the serotonergic systems at the supraspinal level and at the spinal level. Furthermore, it is possible that the mechanisms involved in the development of tolerance to the analgesic and antitussive effects may be different.

The development of tolerance to the antitussive effect of morphine was demonstrated in the present study. However, no changes in affinity and density of serotonin receptors were observed in the brainstem of rats chronically treated with morphine as compared to the vehicle-treated animals. Previously, we have reported that tolerance to the antitussive effects of opiates may result from the development of a down regulation, with unaltered affinity, of the brainstem serotonin receptors (4). This discrepancy may be due to differences in the method of tolerance induction or the duration of treatment. Thus, it is reasonable to speculate that the development of tolerance to the antinociceptive effect of morphine may be caused by the changes in functions of brainstem µ-opioid receptors, but not by changes in the functions of serotonin receptors. The decrease in the affinity of U-50,488H and nor-binaltorphimine to x-opioid receptors in both supraspinal and spinal cord tissues and no changes in receptor density has been reported in rats made tolerant to the antinociceptive effects of U-50,488H (5). However, greater changes in receptor affinity of the x-opioid receptor were found in the spinal cord than in the other brain regions (5). Several lines of evidence indicate that the spinal cord is the more important antinociceptive locus for the action of U-50,488H (9–11). By contrast, the supraspinal brain regions, particularly the brainstem, are the most important antitussive locus for x-opioid agonists, as mentioned above. Taken together, both these studies and the present results, it can be speculated that the subtype of receptors for the x-opioid agonist responsible for the regulation of antitussive effects in the brainstem may be different from that responsible for the antinociceptive effects in the spinal cord.

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