Further Evidence for the Implication of a \( \kappa \)-Opioid Receptor Mechanism in the Production of Psychological Stress-Induced Analgesia

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Abstract—The analgesic effect induced by exposure to psychological stress, using a communication box (psychological stress-induced analgesia, PSY-SIA), was completely antagonized by 10 min pretreatment with 0.5, 1 and 2 mg/kg of nor-binaltorphimine and with 0.5 and 1 mg/kg of Mr2266, selective \( \kappa \)-opioid receptor antagonists, in the tail pinch method. Neither footshock (FS)- nor forced swimming (SW)-SIA was affected by these antagonists. The selective \( \delta \)-opioid receptor antagonist naltrindole, at doses up to 20 mg/kg, had no appreciable effect on PSY-SIA. Daily morphine treatment, 10 mg/kg, s.c., resulted in tolerance to the analgesic effect, and concurrent exposure to PSY-stress suppressed the development of morphine tolerance. The substitution of treatment with U-50,488H for PSY-stress still resulted in analgesia on the initial day; and likewise, the suppression by U-50,488H of the development of morphine tolerance was replicated by PSY-stress. Pretreatment with nor-binaltorphimine antagonized the suppressive effect of PSY-stress on the development of morphine tolerance without affecting the analgesic effect of morphine per se. These results provide further evidence that PSY-SIA involves the mediation by \( \kappa \)-opioid receptor mechanisms.

Studies on the neuropharmacological action mechanisms of the analgesic effect following an environmental stressor has revealed that there are two types of mechanisms, opioid and non-opioid, in the production of such stress-induced analgesia (SIA) (1–5). We performed studies to characterize various SIA using the tail pinch and tail flick methods to assess the effect, and also found that footshock (FS)-SIA was antagonized by naloxone, while swimming (SW)-stress was insensitive to the antagonist in both methods, suggesting the mediation of opioid and non-opioid mechanisms, respectively (4, 6). These findings were broadly in agreement with several reports from other laboratories (1, 5). On the contrary, the antagonism of psychological (PSY)-SIA using a communication box was recognized in the tail pinch method but not in the tail flick method, and the similar antagonism by naloxone of U-50,488H-induced analgesic effect, a selective \( \kappa \)-opioid receptor agonist, was observed, suggesting the possible involvement of \( \kappa \)-opioid receptor mechanisms and to a lesser extent, \( \mu \)-receptor mechanisms in the production of PSY-SIA (6).

Meanwhile, it was reported that an analgesic effect induced by PSY-stress in rats was reversed by naloxone using the tail flick method (7). These data implicate that the endogenous opioid system may participate in PSY-SIA; however, it is yet unknown which of the major type of opioid receptors is involved.

The present study was designed to characterize more fully the involvement of the \( \kappa \)-receptor mechanism in the production of PSY-SIA, and also in the suppression by PSY-stress exposure of the development of mor-
phenine tolerance (8), because U-50,488H also suppressed the development of morphine tolerance (9).

Materials and Methods

Animals: Male mice of the ddY strain weighing 18–20 g (Otsubo Exp. Animals, Nagasaki) were purchased and housed as a group of 20 animals in plastic cages. They were kept in a room maintained at an ambient temperature of 22±1 °C and given normal laboratory diet and tap water ad libitum. After reaching 23–25 g, they were employed for the experiments.

Exposure to PSY-stress: Using the communication box, the animals were exposed to PSY-stress for 5 min. Briefly, the mice were placed individually into the 9 compartments, and electric footshock was delivered through the floor grids. Animals placed in a compartment in which the floor was covered with a plastic plate are prevented from receiving the shock, but they were exposed to PSY-stress by watching and hearing the struggle, jumping and vocalization of shocked animals. Details of the PSY exposure stress have been described elsewhere (6). A 5-min exposure to PSY-stress was started 15 min after morphine administration for 5 days; and then from the 6th day, U-50,488H instead of PSY-stress was given i.p. 15 min after the morphine injection, and vice versa.

Exposure to FS and SW-stresses: Animals were exposed to an inescapable and un-signalized FS (2 mA, 0.2 Hz, 1 sec duration) through the floor grid for 30 min (FS-stress) or mice were forced to swim in a water bath at 20°C for 5 min (SW-stress).

Compounds and administration schedules: Mr2266 ((-) -2-(3-furylmethyl)-norethazocine, a gift from Boehringer Ingelheim), U-50,488H (trans-3,4-dichloro-N-methyl-N-[2 -(1-pyrrolidinyl) -cyclohexyl] -benzeneacetamide methansulfonate hydrate, supplied by Upjohn), nor-binaltorphimine and naltrindole (a gift from Dr. H. Nagase, Toray), morphine (Takeda Pharm. Co.), and naloxone (Sigma Pharm. Co.) were used. Mr2266 was dissolved in 0.1 N HCl followed by adjusting the pH to 4–5 with an appropriate amount of NaOH solution. All other drugs were freshly prepared with saline. They were administered in a volume of 0.1 ml/10 g of body weight. Mr2266, nor-binaltorphimine or naltrindole was injected 10 min before the exposure to PSY-, FS- and SW-stresses.

Measurement of antinociception: The analgesic effect was measured by the modified Haffner’s method (10) or tail pinch test (TP), using a 6-sec cut-off time to avoid tissue damage due to longer application, every 5 min from immediately after the termination of stress exposure. The measurement of the antinociceptive effect was started from 30 min after morphine injection and then done at intervals of 15 min for the following 60 min. The analgesic effect expressed as the area under the curve (AUC) was obtained by plotting the increase in response time (sec) on the ordinate and the time intervals on the abscissa.

Statistical analysis: The results were expressed as the mean±S.E. Following two-way analysis of variance for repeated measures of the overall data to assess statistical significance, differences between the individual mean values in different groups were analyzed by Dunnett’s test.

Results

Effect of Mr2266, nor-binaltorphimine and naltrindole on PSY-, FS- and SW-SIAs: The analgesic effect induced by PSY-stress was completely antagonized by 10 min pretreatment with 0.5, 1 and 2 mg/kg of nor-binaltorphimine (Fig. 1), and likewise suppressed by 0.5 and 1 mg/kg of Mr2266 in a similar manner (Fig. 2). In contrast, neither FS-SIA nor SW-SIA was antagonized by the pretreatment with nor-binaltorphimine or with Mr-2266. The selective δ-antagonist naltrindole (11), up to the doses of 20 mg/kg, had no appreciable effect on PSY-SIA or SW-SIA. FS-SIA was partially but significantly counteracted by 20 mg/kg of naltrindole (Fig. 3). Throughout the experiments, nor-binaltorphimine, Mr2266 or naltrindole at the dose employed here did not affect the response time before the exposure to these stresses (data not shown).

Blockade by U-50,488H and PSY-stress of the development of morphine tolerance: Daily injection of morphine at 10 mg/kg, i.p., easily developed tolerance to the analgesic effect. PSY-stress exposure and 2 mg/kg of
Fig. 1. Comparison of the antagonism by nor-binaltorphimine of PSY-, FS- and SW-stress-induced analgesia. Mice were exposed to psychological (PSY)-, footshock (FS)- and forced swimming (SW)-stress for 5, 30 and 5 min, respectively. The analgesic effect was measured every 5 min from immediately after the termination of the stress exposure. Nor-binaltorphimine (○, 0.5 mg/kg; △, 1 mg/kg; □, 2 mg/kg), i.p., was administered 10 min before the stress exposure. The control group was given saline instead of the compound (○). Each point is the mean±S.E. of 16-18 mice. Dotted area indicates the mean±S.E. response time before exposure to stress. *P<0.05, **P<0.01, compared with the respective saline pretreatment groups.

U-50,488H had no effect on the analgesic effect of morphine in naive mice. Concurrent exposure to daily PSY-stress suppressed the development of morphine tolerance, and the analgesic effect on the initial day was maintained for 5 days. Substitution of treatment with U-50,488H for PSY-stress for 5 more days still resulted in analgesia on the initial day. Likewise, the blockade by U-50,488H of the development of morphine tolerance was continued by the substitution of the compound for PSY-stress. From the 6th day, when the combination with PSY-stress or U-50,488H was omitted, tolerance developed progressively as in the control animals with morphine alone. In the animals with morphine tolerance induced by 5 daily treatments, the attenuated analgesia of morphine was not influenced by the combination with either PSY-stress or U-50,488H for a further 5 days (Fig. 4).

Nullification by nor-binaltorphimine of PSY-stress effect on the development of morphine tolerance: Daily pretreatment with 1 and 2 mg/kg of nor-binaltorphimine, i.p., 10 min before morphine nullified the suppressive effect of PSY-stress on the development of tolerance to morphine without affecting the analgesic effect per se, as shown on the initial day (Fig. 5). Such a pretreatment with nor-binaltorphimine had no effect on the morphine analgesia and its tolerance development (data not shown).

Discussion

It has been reported by Takemori et al. that nor-binaltorphimine possesses high selectivity for κ-opioid receptors in vivo by antagonizing the analgesia of a κ-opioid agonist, U-50,488H and ethylketocyclazocine (12), in addition to having the same selectivity in vitro (13). This potent κ-opioid antagonist nor-binaltorphimine completely suppressed the PSY-SIA at a low dose of 0.5 mg/kg, but not FS- or SW-SIA at doses up to 2 mg/kg. Similar results were obtained with Mr2266, a
Fig. 2. Comparison of the antagonism by Mr2266 of PSY-, FS- and SW-stress-induced analgesia. Mr2266 (○, 0.5 mg/kg; △, 1 mg/kg), i.p., was administered 10 min before the stress exposure. The data are shown in the same way as in Fig. 1. Each point is the mean±S.E. of 16 mice. *P<0.05, **P<0.01, compared with the respective vehicle pretreatment groups. For abbreviations, see the legend of Fig. 1.
Fig. 4. Suppressive effect of concurrent PSY-stress exposure and U-50,488H and the substitution of the treatment on the development of analgesic tolerance to morphine. Mice were daily exposed to 5-min psychological (PSY)-stress 15 min after 10 mg/kg of morphine, s.c. (△, left), or were daily treated with 2 mg/kg of U-50,488H, i.p., 5 min after the injection of morphine (○, right), for 5 days. Control mice received morphine alone without PSY-stress (○, left) or received morphine with saline instead of U-50,488H (○, right). For another 5 days from the 6th day, in one half of the animals of each treatment group, the combination was substituted, U-50,488H for PSY-stress (○, left), PSY-stress for U-50,488H (△, right); the rest of the animals in each group were treated with morphine alone without the exposure to PSY-stress (△, left) and without the treatment with U-50,488H (○, right); and in each control group, U-50,488H (○, left) or PSY-stress (△, right) was given. Daily changes of the analgesic effect were expressed as the area under the curve (AUC). Each point is the mean±S.E. of 10–20 mice. *P<0.05, **P<0.01, compared with the corresponding value on the 1st day.

Yamamoto et al. reported that concurrent administration of U-50,488H at low doses suppressed the development of tolerance to morphine analgesia, suggesting the activation of the κ-opioid system in the underlying mechanism (9). Likewise, we reported concurrent treatment with PSY-stress exposure abolished the development of morphine tolerance, and we also suggested that the κ-opioid mechanism was related to the blocking effect of PSY-stress in the underlying mechanism (8). If this is the case, both PSY-stress exposure and U-50,488H treatment could be possibly substitutable for each other to maintain the suppression of morphine tolerance by these treatments. In fact, substitution of U-50,488H treatment for PSY-stress still resulted in analgesia on the initial day; and likewise, the suppression by U-50,488H of

less selective κ-receptor antagonist. Meanwhile, PSY-SIA and SW-SIA were not influenced by naltrindole at doses up to 20 mg/kg, whereas FS-SIA was partially antagonized by the δ-antagonist, suggesting the participation of δ-opioid receptors in the production of this SIA. Thus, the present findings, in line with our earlier suggestion (6), provide further evidence that the analgesic effect induced by the exposure to the PSY-stress using a communication box may be related to κ-opioid receptor mechanism, differing from the mechanism involved in FS- and SW-SIA.

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the development of morphine tolerance was replicated by PSY-stress. These results suggest that PSY-stress and U-50,488H may share a common mechanism with each other to suppress morphine tolerance.

On the other hand, it is well-known that numerous drugs or conditioning affect the development of morphine tolerance. For example, we have previously reported that phentolamine, propranolol (14), cycloheximide (15) and FS-stress exposure (8) abolished the tolerance development. However, we could not find any substitutable effect between α- and β-blockers on the suppression (16), presumably resulting from the lack of a common mechanism between these blockers. This may confirm the assumption that a common mechanism between substitutes is required for production of such an exchangeable inhibitory effect on the tolerance development, as shown between PSY-stress and U-50,488H.

Evidence further supporting the essential role of a κ-receptor mechanism in the effect of PSY-stress would be provided by the findings that the suppressive effect of PSY-stress on the development of morphine tolerance was abolished by the pretreatment with norbinaltorphimine.

Meanwhile, Jensen and Smith reported that a significant increase in tail-flick latency occurred in rats that only witnessed foot-shock, and such an analgesic effect was antagonized by naloxone at 10 mg/kg (7). This conflicting result may be attributed to the high dose of naloxone, which may affect not only μ- but also δ- and κ-opioid receptors. Additionally, the receptor subtype involved in pro-
ducing the analgesia was not clarified.

On the other hand, emotional factors such as fear and anxiety seem to play an essential role in the production of PSY-SIA; and as expected diazepam, an antianxiety drug, abolished the analgesia (17). This may suggest the existence of an alternative mechanism, i.e., emotional factors induced by PSY-stress, through which the PSY-stress is capable of producing analgesia. Although the literature on the direct relationship between a κ-opioid receptor mechanism and GABA-benzodiazepine receptor complex mechanism is much less substantial, there is some evidence that, for example, analgesia through κ-opioid receptors was attenuated by muscimol, a GABA<sub>A</sub> agonist (M. Satoh, personal communication). The analgesic effect induced by the benzodiazepine antagonist Ro-15-1788 was antagonized by the opioid antagonist naloxone (18). Thus, the possibility that both a κ-opioid mechanism and emotional factors are closely related to each other for the production of PSY-SIA may still remain.

In conclusion, we provide further evidence that a κ-opioid receptor mechanism plays an essential role in the production of analgesia and the pharmacological effect induced by PSY-stress.

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