Implication of Endogenous Opioid Mechanism in the Production of the Antinociceptive Effect Induced by Psychological Stress in Mice

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Abstract—Psychological (PSY) stress using the communication box produced a short-lasting antinociceptive effect which was less potent than that induced by physical stress such as footshock (FS) and forced swimming (SW) in mice. Naloxone completely antagonized PSY-stress induced analgesia (SIA) when the analgesia was measured by the tail pinch (TP) method; however, the antagonist did not reverse the effect in the tail flick (TF) assay. On the other hand, FS-SIA was antagonized by naloxone in both methods, while naloxone failed to reverse SW-SIA in either TF or TP assessment. Daily exposure to psychological stress developed tolerance to the analgesia. One-way cross-tolerance between PSY-SIA and morphine and the naloxone antagonism of PSY-SIA by the tail pinch method lead to the suggestion that an endogenous opioid system may be involved in the underlying mechanism for its production. On the contrary, from the findings of cross-tolerance between SW- or FS-SIA and the lack of naloxone antagonism in the TF method, the involvement of a more complicated mechanism is suggested in PSY-SIA. In both tests, U-50488H, a selective κ-agonist, produced profound analgesia; however, no appreciable antagonism of naloxone was found in the TF test, whereas the effect was completely blocked by naloxone in the TP test. From the similarity in naloxone antagonism of PSY-stress and U-50488H induced analgesia, the participation of a common mechanism which may be mediated by κ-opioid receptors, is suggested in the production of PSY-SIA.

It is well established that various physical stressful procedures such as footshock (1–4), immobilization (5, 6), immobilized water-immersion (4) and forced swimming (7) induce a significant analgesic effect in experimental animals (stress-induced analgesia, SIA). These physical stresses might involve some psychological (PSY) factors, i.e., anxiety, fear and despair; however, few reports (8, 9) have appeared on the biochemical or humoral changes induced by pure PSY stress because of the technical difficulties, and as far as we know, there has only been one report on PSY-SIA in the rat (10). On the other hand, SIAs induced by physical stresses were divided into two types, opioid or non-opioid mediated, by the naloxone sensitivity or the cross-tolerance with morphine (4).

Recently, evidence has accumulated to support the existence of multiple opiate receptors, namely μ, κ, δ and σ types (11, 12). Tyers (13) differentiated the opiate receptors which participate in the production of the analgesic effect by the pain applied for evaluation: one was mechanical noxious stimuli induced pain which was inhibited by μ- and κ-opiate agonist, and the other was induced by heat stimuli and was blocked by only μ-agonist.

In the present study, we examined the characteristics of the antinociceptive effect induced by pure PSY stress, especially the contribution of an opioid mechanism and types of receptors which may participate in the mechanism, by comparing the results obtained with different types of physical stress such as footshock (FS)-, forced...
swimming (SW)-induced analgesia and morphine analgesia.

Materials and Methods

Animals: Male mice of the ddY strain, weighing 18-20 g, 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.

FS stress: Animals placed on the grid floor received inescapable and unsignaled FS (2 mA, 0.2 Hz, 1 sec duration) for 30 min. Details of this procedure have been described elsewhere (14).

SW stress: Mice were forced to swim in a water bath, 40(L)x35(W)x20(H) cm, with 15 cm of water in depth at 20°C for 3 min. On removal from the water, animals were immediately wiped to dryness with absorbent paper.

PSY stress: The chamber (30 x 30 x 30 cm) with a grid floor composed of 1.5 mm stainless steel rods 0.7 cm apart from each other was divided into 9 compartments (10 x 10 x 30 cm) with transparent plastic walls (Fig. 1). A scrambled electric shock (2 mA, 0.2 Hz, 1 sec duration), by which significant FS-SIA was produced, was delivered through the floor grid. Plastic plates were placed on the grids of five compartments to prevent the animals from receiving direct footshock, but they were exposed to psychological stress by watching and hearing the struggle, jumping and vocalization of shocked animals in the adjacent compartments which were daily provided with naive animals. Mice exposed to PSY stress showed restlessness, alertness and even squeal in accordance with the behavior of mice exposed to direct FS.

Drugs and administration schedules: The following compounds were used: Morphine-HCl (Takeda Pharm., Co.), naloxone-HCl (Sigma, U.S.A.) and U-50488H (trans-3,4-dichloro-N-methyl-N-(2-(1-pyrrolidinyl)-cyclohexyl)-benzeneacetamide methanesulfonate hydrate, gift from Dr. T. Yamamoto, Kyushu University). These drugs were dissolved in saline and administered i.p. in a volume of 0.1 ml/10 g of body weight. Naloxone, 2 mg/kg, was injected 10 min before the exposure to the stress. U-50488H, 30 mg/kg, or morphine, 10 mg/kg, was administered i.p. The doses are expressed in terms of the salts.

Measurement of antinociception: The analgesic effect was measured by a modified Haffner's method (15), tail pinch test (TP), using a 6 sec cut-off time, and by a modified D'Amour and Smith method (16), tail flick method (TF), with a maximal response time of 10 sec, every 5 min from immediately after the termination of stress exposure or every 15 min after the injection of morphine or U-50488H.

Evaluation of tolerance and cross-tolerance: The analgesic effect induced by 10 mg/kg morphine or each stress was

Fig. 1. Communication box to expose the animal to psychological stress. Mice were placed individually into the 9 compartments, and electric footshock was delivered through the floor grids. Animals placed in the compartment in which the floor is covered with plastic plate, shown as the white area, are prevented from receiving the shock.
measured daily and expressed as the change of area under the curve (AUC) by plotting the increase in response time (sec) on the ordinate and the time intervals (min) on the abscissa. In the animals rendered tolerant by 3 daily treatments with morphine or each stress, the analgesic effect induced by each stress or morphine was estimated on the 4th day in order to assess the development of cross-tolerance between them.

**Statistical analysis:** The significance of difference was evaluated by Student's t-test (two-tails).

**Results**

**Naloxone antagonism:** The exposure to psychological stress produced short-lasting analgesia in both TP and TF tests. The intensity was maximal immediately after the termination of stress exposure, then diminished rapidly and no appreciable analgesia was observed 10 min later (Fig. 2). The analgesic effect could not be obtained in the animals placed in the apparatus but not exposed to the PSY stress. PSY-SIA was completely antagonized by the pretreatment with 2 mg/kg of naloxone when the analgesic effect was measured by the TP method; however, the antagonist did not reverse the effect in TF assessment (Fig. 2).

FS-induced analgesic effect was completely antagonized by naloxone pretreatment in both TF and TP methods (Fig. 3); however, naloxone failed to reverse the analgesia produced by SW in either TF or TP (Fig. 4).

**Tolerance and cross-tolerance:** Daily exposure to PSY stress resulted in the gradual loss of analgesic effect, and the effect was decreased to about one forth of the initial value on the 3rd day indicating the development of tolerance to the effect (Fig. 5). Repetition of exposure to FS or SW-stress also developed tolerance to the effect (Fig. 5). Animals tolerant to PSY-SIA were tolerant to SW-SIA, but not to FS-SIA. In both FS- and SW-SIA tolerant mice, PSY-SIA was significantly attenuated. On the other hand, the intensity of either SW-SIA in FS-SIA tolerant animals or that of FS-SIA in SW-SIA tolerant mice was not reduced (Fig. 5).

Daily injection of 10 mg/kg morphine, i.p.,

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**Fig. 2.** Psychological stress induced analgesia (PSY-SIA) and its naloxone antagonism. Mice were exposed to psychological stress for 5 min. Analgesic effect (response time) was measured by the tail pinch method (TP, a cut-off time of 6 sec) and the tail flick method (TF, a cut-off time of 10 sec), every 5 min from immediately after the termination of the stress exposure. Naloxone, 2 mg/kg, or saline was injected intraperitoneally 10 min before exposure to the stress. Saline-PSY-stress (△—△). Naloxone +PSY-stress (▲—▲). Each point is the mean±S.E. of 18 animals. Dotted area indicates the mean ±S.E. response time before exposure to stress. **P<0.01, *P<0.05, compared with the respective saline pretreated group.
Footshock stress induced analgesia (FS-SIA) and its naloxone antagonism. Mice were exposed to FS-stress for 30 min. Saline+FS-stress (○—○), Naloxone+FS-stress (●—●). Each point is the mean±S.E. of 8 to 18 animals. ***P<0.001, compared with the respective saline pretreated group. For other details, refer to Fig. 2.

Forced swimming stress induced analgesia (SW-SIA) and its naloxone antagonism. Mice were exposed to SW-stress for 3 min. Saline+SW-stress (□—□), Naloxone+SW-stress (■—■). Each point is the mean±S.E. of 10 to 14 animals. For other details, refer to Fig. 2.

also easily developed tolerance to the analgesic effect. In morphine tolerant animals, PSY-SIA was reduced significantly, although in PSY-SIA tolerant animals, morphine produced significant analgesia as in naive animals (Fig. 6).

Effect of naloxone on U-50488H and morphine analgesia: Thirty mg/kg of i.p. U-50488H produced an analgesic effect in both TP and TF tests, which was nearly equipotent
Fig. 5. Development of tolerance to each SIA and cross-tolerance between them. Analgesic effect measured by the TP method was expressed as the area under the curve (AUC) by plotting the increase in response time (sec) on the ordinate and the time intervals on the abscissa. Left: Daily changes of the intensity of each SIA. PSY-SIA (○—○). FS-SIA (△—△). SW-SIA (□—□). Each point is the mean±S.E. of at least 12 animals. Analgesic effect was expressed as the percent of the effect on the 1st day. Right: Cross-tolerance between SIAs. Animals were rendered tolerant by 3 daily treatments with each stress, and the cross-tolerance between each SIA was estimated on the 4th day. PSY, FS and SW: tolerant animals to PSY-, FS- and SW-SIA, respectively. PSY (●●●), FS (□□□□□) and SW (‖TT‖); analgesic effect of PSY-, FS- and SW-SIA. Values are the mean±S.E. of at least 12 animals and expressed as the percent of the effect of the control group. **P<0.01, *P<0.05, compared with the control.

Psychological stress produced an analgesic effect, as well as physical stress such as FS and SW (4) in mice, although the intensity was less potent to some extent than that induced by physical stresses.

FS-SIA was antagonized by naloxone in both the TP method and TF methods, suggesting the participation of an opioid-mediated mechanism in this SIA. In contrast, naloxone failed to reverse SW-SIA in either TF or TP assessment, implying the involvement of a non-opioid system in the production. Lack of cross-tolerance between FS- and SW-SIA provides additional evidence that the mechanisms by which antinociceptive action is caused are distinct from each other. On the other hand, PSY-SIA was antagonized by naloxone in the TP test but not in TF assessment. Furthermore, PSY-SIA was partially attenuated in both FS-SIA and SW-SIA tolerant animals, indicating the development of cross-tolerance between PSY-SIA and SW- or FS-SIA. These findings suggest that PSY-SIA might share the common mechanism with FS- and SW-SIA, opioid and non-opioid form, respectively.

Morphine and naloxone are a typical agonist and a typical antagonist of opioid
Fig. 6. Development of tolerance to morphine and cross-tolerance between morphine and PSY-SIA. Left: Daily changes of the analgesic effect of morphine (10 mg/kg/day, i.p.). Each point is the mean±S.E. of 39 animals. *P<0.01, compared with the value on the 1st day. Right: Cross-tolerance between morphine and PSY-SIA. The analgesic effect induced by morphine, 10 mg/kg, or the stress was estimated on the 4th day. Mor and PSY: tolerant animals to morphine and PSY-SIA, respectively. Mor (□□□□) and PSY (••••): analgesic effect of morphine and PSY-SIA. Values are the mean±S.E. of 11 to 12 animals. **P<0.01, compared with the control. For other details, refer to Fig. 5.

Fig. 7. Analgesic action of U-50488H and its naloxone antagonism in TP and TF methods. Analgesic effect was measured by the TP method or TF method, every 15 min after U-50488H injection for 60 min. Naloxone (2 mg/kg, ■——■) or saline (○——○) was injected i.p. 10 min before U-50488H (30 mg/kg). Each point is the mean±S.E. of at least 10 animals.
Fig. 8. Analgesic effect of morphine and its naloxone antagonism in TP and TF methods. Analgesic effect was measured by the TP method or TF method, every 15 min after morphine injection for 90 min. Naloxone (2 mg/kg, •—•) or saline (○—○) was injected i.p. 10 min before morphine (10 mg/kg). Each point is the mean±S.E. of 10 animals.

In the TP method, morphine induced significant analgesia, which was antagonized by naloxone. In the TF method, however, the analgesic effect was not significantly different between the naloxone and saline groups.

The analgesic effect of morphine is detectable in both methods, but naloxone antagonizes the analgesic effect only in the TP method. This suggests that the mechanism of analgesia induced by morphine is different in the two methods.

On the other hand, U-50488H, a selective κ-opioid agonist, also induced analgesia in both tests; however, the analgesic effect was not significantly different between the naloxone and saline groups in the TF method, whereas it was antagonized by naloxone in the TP method.

The facts that cross-tolerance between PSY-SIA and morphine was one-way, a morphine tolerant animal was also tolerant to PSY-SIA but a PSY-SIA tolerant animal was not tolerant to morphine, and PSY-SIA was antagonized by naloxone only in the TP method suggested that the production of PSY-SIA involved at least two different mechanisms, opioid and non-opioid forms. As the non-opioid mechanism, catecholaminergic (5, 14, 22), serotonergic (6, 22, 23) and cholinergic (24, 25) mechanisms and in some cases, in combination with an opioid mechanism (5, 14, 22, 25) are concerned in the production of various physical SIA.

Actually, we have reported previously that the FS- and immobilized-water immersion stress-induced analgesia was significantly reduced in reserpinized animals, suggesting the involvement of a catecholaminergic mechanism in the process (14). In our preliminary experiment, we found that PSY-
SIA is also significantly reduced in reserpinized animals. PSY-stress consists of various emotional factors such as fear and anxiety, so that the analgesia induced by PSY-stress may be antagonized by antianxiety drugs rather than an opioid antagonist. Tifluadom which is derived from benzodiazepine, a minor tranquilizer, exerts its analgesic effect through κ-receptors (26), and the sedative effect of opioids is also mediated by κ-opioid receptors (11). These results may suggest the important role of a κ-opioid receptor mediated mechanism in the production of PSY-SIA.

References


