Blockade of the Development of Analgesic Tolerance to Morphine by Concurrent Treatment with Opioid- But Not Non-Opioid-Mediated Stress in Mice

Masakatsu TAKAHASHI, Yoshiki DEGUCHI and Hiroshi KANETO
Department of Pharmacology, Faculty of Pharmaceutical Sciences,
Nagasaki University, Bunkyo-machi, Nagasaki 852, Japan

Accepted September 24, 1987

Abstract—Studies have been carried out to determine how the analgesic effect of morphine and the development of tolerance to the effect would be influenced by concurrent exposure to stresses in mice. Application of footshock (FS) stress, which produces analgesia mediated by opioid \( \mu \)-receptors, or psychological (PSY) stress, which produces analgesia in a manner more closely related to opioid \( \kappa \)-receptors, did not affect the analgesic effect of morphine, but completely blocked the development of tolerance during 5 daily concomitant treatments. On the other hand, forced swimming (SW) stress induced analgesia (SIA), which was not antagonized by naloxone, suppressed morphine analgesia, but failed to block the tolerance development. The blockade of the development of tolerance to morphine analgesia by stresses may not be attributed to the analgesic effect induced by the stresses because a combination of weak FS stress, which induces no analgesia, also effectively suppressed the development of morphine tolerance. In addition to the opioid mechanism, an adrenergic mechanism can not be excluded because of the reserpine antagonism of these SIAs.

We have previously shown that stressful stimuli such as footshock (FS) and immobilization-water immersion (IW) increased the nociceptive thresholds in mice and these stress-induced analgesia (SIA) are distinguished into opioid- and non-opioid mediated forms by the underlying mechanisms for their production, from the naloxone antagonism and cross-tolerance to morphine (1). We found that forced swimming (SW) in cold water and psychological (PSY) stress also produced an analgesic effect; and the effect of the former was resistant to naloxone, and that of the latter was only insensitive to naloxone when the analgesic effect was measured by the tail-flick method (2).

It is generally accepted that the catecholaminergic function plays an important role in the production of the analgesic effect of morphine and the development of tolerance to the effect (3-5). Similarly, the production of the FS- or IW-induced SIAs are completely suppressed by the pretreatment with reserpine, indicating the participation of the catecholaminergic mechanism in the production (6).

These results urged us to study the interaction of opioid and catecholaminergic mechanisms in the production of morphine analgesia and also in the development of tolerance to the effect. In the present experiment, the effects of concurrent exposure to various stresses, opioid and non-opioid forms, on morphine analgesia and the development of tolerance were investigated.

Materials and Methods

Animals: Male mice of the dd strain weighing 18-20 g were purchased and housed as a group of 20 animals in a plastic cage. 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.

Drug: Morphine-HCl (Takeda Pharm. Co.)
and naloxone-HCl (Sigma Pharm. Co.) were dissolved in saline and administered i.p. in a volume of 0.1 ml/10 g of body weight. The doses are expressed in terms of the respective salt. Reserpine (Apoplon, Daiichi Pharm. Co.) was diluted with saline and administered as the other drugs.

**Exposure to stresses:** 1) FS-stress: Animals were exposed to an inescapable and unsignaled FS (2 mA or 0.5 mA, 0.2 Hz, 1 sec duration) through the floor grid for 15 min. 2) SW-stress: Mice were forced to swim in a water bath at 20°C for 5 min. 3) PSY-stress: Using the communication box, animals were exposed to psychological (PSY) stress for 5 min. Briefly, mice were placed individually into the 9 compartments (10×10×30 cm), and electric footshock was delivered through the floor grids. Animals placed in the compartments in which the floor is 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 the shocked animals. Mice exposed to PSY stress showed restlessness, alertness and even squeal in accordance with the behavior of mice exposed to direct FS. FS-stress was applied from 5 min after morphine injection, and exposure to other stresses were started immediately after the 1st measure of the analgesic effect of morphine, 15 min after injection. Details of the exposure to each stress have been described elsewhere (1, 2).

**Assessment of analgesic effect:** Analgesic effect was measured at the interval of 15 min after i.p. injection of 10 mg/kg of morphine for 90 min by the modified Haffner’s method (7), with a cut-off time of 6 sec to avoid tissue damage due to longer application. The effect was expressed as the area under the curve (AUC) by plotting the increase in response time (sec) on the ordinate and the time intervals (min) on the abscissa.

**Evaluation of the degree of tolerance:** Injection of morphine and concomitant exposure to stress was repeated for 5 days, and then the injection of morphine was continued for a further 5 days. The analgesic effect was measured daily and compared with the effect of the 1st treatment.

**Statistical analysis:** The significance of difference was determined by Student’s t-test.

**Results**

**Effect of stress exposure on the analgesic effect of morphine:** As for the overall analgesic effect for 90 min, FS-stress, 2 mA and 0.5 mA, and PSY-stress did not modify the analgesic effect of morphine, whereas SW-stress significantly inhibited the morphine analgesia (Fig. 1).

**Effect of stress exposure on the development of tolerance:** Daily injection of morphine developed tolerance to the analgesic effect; and after 5 repetitions, the effect of morphine was remarkably reduced. During daily combined treatment with morphine and FS-stress, 2 and 0.5 mA, or PSY-stress, the development of tolerance was suppressed and the analgesic effect on the initial day was maintained for 5 days; on the contrary, daily concomitant exposure to SW-stress did not prevent the development of morphine tolerance (Figs. 2 and 3).

In the mice treated daily with morphine and FS- or PSY-stress exposure, the analgesic effect of the same dose of morphine on the 6th day was maintained substantially at the same level as the initial effect, and further treatment with morphine developed tolerance as rapidly as that in naive animals (Figs. 2 and 3) which were daily treated with only morphine.

**Effect of naloxone and reserpine on SIAs:** Pretreatment with 2 mg/kg naloxone 10 min before stress exposure, completely suppressed the FS- and PSY-SIA; however, it did not antagonize the SW-SIA (Fig. 4a). On the contrary, 2.5 mg/kg reserpine 24 hr before stress exposure antagonized all FS-, PSY- and SW-SIAs (Fig. 4b).

**Discussion**

Based on the fact that opioid and catecholaminergic mechanisms are involved in the production of morphine- and stress-induced analgesia (3–6), an investigation on the effect of stress exposure on morphine analgesia and the development of tolerance to the effect was made in mice. Our expectation was the potentiation of morphine analgesia by the concurrent exposure to the
Fig. 1. Effect of concomitant stress exposure on morphine analgesia. Animals were exposed to a) FS-stress 5 min after morphine injection (10 mg/kg, i.p.) for 15 min or b) PSY- or SW-stress 15 min after morphine injection for 5 min, as shown by the horizontal bars, and the analgesic effect was measured by the TP method. Morphine alone (●—●), morphine+FS (2 mA) (○—○), morphine+FS (0.5 mA) (○—○—○), morphine+SW (□—□), morphine+PSY (△—△). Each point indicates the mean±S.E. of 26–37 animals. ***P<0.001, **P<0.01, *P<0.05, compared with the group treated with morphine alone.

Fig. 2. Effect of daily FS-stress on the development of tolerance to morphine analgesia. Daily changes of analgesic effect 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. Morphine alone (○—○), morphine in combination with FS, 2 mA (□—□) or FS, 0.5 mA (△—△) for 5 days, and the application of stress was withdrawn from the 6th day (closed symbols). ***P<0.001, **P<0.01, *P<0.05, compared with the corresponding value on the 1st day.
Fig. 3. Effect of SW or PSY-stress on the development of tolerance to morphine. Daily changes of analgesic effect of morphine alone (○—○) or morphine in combination with SW (□—□) or PSY (△—△). For other details, refer to the footnote of Fig. 2. ***P<0.001, **P<0.01, *P<0.05, compared with the corresponding value on the 1st day. ~P<0.05, compared with the effect of morphine in naive control animals.

Fig. 4. Comparison of the naloxone and reserpine antagonism on FS-, SW- and PSY-stress induced analgesia. Mice were exposed to FS-, SW- or PSY-stress for 30, 5 or 5 min, respectively. Analgesic effect was measured every 5 min from immediately after the termination of the stress exposure. Naloxone, 2 mg/kg, 10 min before (upper chart) or reserpine, 2.5 mg/kg, 24 hr before (lower chart) the exposure to each stress was administered i.p. Control groups (given saline instead of drugs, open symbols), naloxone or reserpine treated group (closed symbols). Each point is the mean±S.E. of at least 20 mice. Dotted area indicates the mean±S.E. response time before exposure to stress. ***P<0.001, **P<0.01, *P<0.05, compared with the respective saline pretreated group.
stresses. The results of the experiment, however, have shown that morphine analgesia was attenuated by the concomitant treatment with SW-stress and not substantially altered by FS- and PSY-stress. Thus, the effect of stressful procedure does not produce any additive or synergistic effect but is rather antagonistic to morphine-induced analgesia.

The development of tolerance to the analgesic effect of morphine was completely suppressed by the combination with FS or PSY stress, but not with SW-stress. The analgesic effects induced by FS- and PSY-stress are produced via a \( \mu \)- and \( \kappa \)-opioid receptor-mediated mechanism, respectively. In contrast, the analgesia induced by SW-stress is mediated through a non-opioid mechanism as evidenced by naloxone antagonism. Thus, the participation of an opioid mechanism in the production of the SIAs might be essential for the blockade of the tolerance development. In addition, the blockade of the tolerance development may not be concerned with the production of analgesia, since a weak footshock stress of 0.5 mA, which produced no appreciable analgesia (data not shown), could prevent the development of tolerance.

The treatment with morphine alone on the 6th day after 5 daily concomitant exposures to stress and morphine produced an analgesic effect comparable to the effect in naïve control animals. This result suggests that the suppressive effect of stress exposure on the development of morphine tolerance is not due to the potentiation of morphine analgesia by the stress exposure. Actually, in the animals treated daily with morphine plus FS- or PSY-stress, the elimination of concurrent exposure to stress resulted in the rapid development of tolerance.

On the other hand, in the animals pretreated with reserpine, the production of SIAs irrespective of the underlying mechanisms, opioid or non-opioid forms, were suppressed, suggesting the possible participation of adrenergic mechanisms in the process. Recently, we found that daily pretreatment with an adrenergic \( \alpha \)-blocker, phentolamine, or a \( \beta \)-blocker, propranolol, completely blocked the development of tolerance to morphine during their combined treatment for 10 days (8). Thus, in addition to the opioid mechanism, the possible involvement of an adrenergic mechanism in the suppressive effect of SIAs on the development of morphine tolerance can not be excluded.

The blockade of the development of tolerance without affecting the analgesic effect of morphine by the combination of FS- or PSY-stress provides further evidence that the analgesic effect of morphine is separable from its tolerance liability as we have demonstrated previously by the pretreatment with naloxone or adrenergic blockers (8, 9).

References
1 Izumi, R., Takahashi, M. and Kaneto, H.: Involvement of different mechanisms, opioid and non-opioid forms, in the analgesia induced by footshock (FS) and immobilized-water immersion (IW) stress. Japan. J. Pharmacol. 33, 1104-1106 (1983)
2 Takahashi, M., Tokuyama, S. and Kaneto, H.: Implication of endogenous opioid mechanism in the production of antinociceptive effect induced by psychological stress in mice. Japan. J. Pharmacol. 44, 283-281 (1987)
3 Takagi, H., Takashima, T. and Kimura, K.: Antagonism of the analgesic effect of morphine in mice by tetrabenazine and reserpine. Arch. Int. Pharmacodyn. Ther. 149, 484-492 (1964)
4 Verri, R.A., Graeff, F.G. and Corrado, A.P.: Antagonism of morphine analgesia by reserpine and \( \alpha \)-methyltyrosine and the role played by catecholamines in morphine analgesic action. J. Pharm. Pharmacol. 19, 264-265 (1967)
5 Vedernikov, Yu P. and Afrikanov, I.I.: On the role of a central adrenergic mechanism in morphine analgesic action. J. Pharm. Pharmacol. 21, 845-847 (1969)
6 Takahashi, M., Izumi, R. and Kaneto, H.: The role of the catecholaminergic mechanism in foot shock (FS) stress- and immobilized-water immersion (IW) stress-induced analgesia in mice. Japan. J. Pharmacol. 35, 175-179 (1984)
7 Takagi, H., Inukai, T. and Nakama, M.: A modification of Haffner's method for testing analgesics. Japan. J. Pharmacol. 16, 287-294 (1966)
8 Kihara, T. and Kaneto, H.: Important role of adrenergic function in the development of analgesic tolerance to morphine in mice. Japan. J. Pharmacol. 42, 419-423 (1986)
9 Kaneto, H., Yamazaki, A. and Kihara, T.: Evidence for the dissociation of morphine analgesia, tolerance and dependence. J. Pharm. Pharmacol. 37, 507-508 (1985)