ABSTRACT — The site of action involved in the suppression by exposure to footshock (FS)- and psychological (PSY)-stress of the development of antinociceptive tolerance to morphine has been investigated. Daily treatment with 10 mg/kg, s.c.; 3 μg, i.t.; and 5 μg, i.c.v. of morphine, regardless of the administration route, resulted in the development of tolerance. Daily exposure to FS- or PSY-stress suppressed the development of tolerance to s.c. and i.t. administered morphine but not that to i.c.v. administered morphine. Pretreatment with 2 mg/kg, i.p. of nor-binaltorphimine (nor-BNI) abolished the suppressive effect of PSY-stress on the development of tolerance to morphine given s.c. The suppression by PSY-stress was also antagonized by 2 μg, i.t. of nor-BNI and not by 2 μg, i.c.v. of nor-BNI. Thus, the development of tolerance in the spinal cord due to interaction of morphine at μ-opioid receptors can be suppressed by exposure to these stresses, probably through the descending signals from the supraspinal area, and activation of δ-opioid receptors in the spinal cord could also participate in the suppression by PSY-stress.
purchased and housed in groups 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 weights ranging between 23–26 g, they were employed for the experiments.

Compounds and application procedure

Morphine (Takeda Pharm. Co.) and norbinaltorphimine (nor-BNI, gift from Dr. H. Nagase, Toray) were used. These drugs, concentrations expressed in terms of their salts, were freshly prepared with saline and were administered in a volume of 0.1 ml/10 g of body weight for subcutaneous (s.c.) injections and in a volume of 10 µl/mouse for intrathecal (i.t.) and intracerebroventricular (i.c.v.) injections. I.t.-injections were carried out according to the method described by Hylden and Wilcox (11). Briefly, a lumbar puncture was performed using a 30-gauge needle directly connected to a microsyringe. The needle was inserted between L5 and L6. I.c.v. injection was made according to the method of Haley and McCormick (12). Animals were exposed to an inescapable and unsignalled FS (2 mA, 0.2 Hz, 1 sec duration) for 15 min (FS stress) or psychological stress for 5 min using a communication box (PSY-stress). For exposure to PSY-stress, 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 exposure to each stress have been described elsewhere (13, 14). FS- and PSY-stress were applied from 5 and 15 min, respectively, after 10 mg/kg, s.c. or equipotent 3 µg/mouse of i.t.-administered or 5 µg/mouse of i.c.v.-administered morphine, to synchronize the induction of the antinociceptive effect of morphine with that of stress. Nor-BNI (2 mg/kg, i.p.; 2 µg/mouse, i.t.; or 2 µg/mouse, i.c.v.) was administered 10 min before morphine.

Measurement of antinociception

The antinociceptive effect was measured by the tail pinch method (15). Measurements were made at 20 min and 30 min after the administration of morphine and then performed at intervals of 15 min for the following 60 min. Antinociceptive effect was expressed as the area under the curve (AUC) that was obtained by plotting the increase in response time (sec) on the ordinate and the time interval (min) on the abscissa. The antinociceptive effect of morphine alone or in combination with exposure to stress and/or nor-BNI was determined daily for 5 days. A decrease in the analgesic response was considered to indicate the development of tolerance.

Statistical analysis

The results were expressed as the mean ± S.E. Following a 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; P < 0.05 was considered to indicate a significant difference.

RESULTS

Morphine produced antinociception dose-dependently upon s.c., i.t. and i.c.v. administration; and 10 mg/kg, s.c.; 3 µg, i.t.; and 5 µg, i.c.v. of morphine were nearly equipotent. Daily treatment with these doses of morphine, regardless of the administration route, resulted in the development of tolerance (Fig. 1). Combination of morphine administration with exposure to FS- or PSY-stress suppressed the development of tolerance to s.c. and i.t. administered morphine without affecting morphine antinociception. Exposure to these stresses did not influence the antinociceptive effect of i.c.v.-administered morphine or the development of tolerance to morphine by this route of administration.

The suppressive effect of PSY-stress expo-
Consistent with our earlier findings (10), concurrent FS- and PSY-stress exposure suppressed the development of antinociceptive tolerance to morphine given daily systemically. Repeated injections of morphine into the spinal and supraspinal areas resulted in tolerance to the analgesic effect. Interestingly, both FS- and PSY-stress exposure suppressed tolerance that developed in the spinal cord, but not that developed in the supraspinal area. These results indicate that the blockade by stresses of the development of tolerance to morphine administered systemically is mainly attributable to the suppression of the spinal, but not supraspinal tolerance to morphine. DeLander et al. (16) have demonstrated that rats that are chronically treated with morphine do not develop tolerance when μ-receptors in the
Fig. 2. Effect of i.p. administered nor-binaltorphimine on the blockade by footshock (FS) and psychological (PSY) stress of the development of antinociceptive tolerance to morphine. Two mg/kg, i.p. of nor-binaltorphimine was given daily 10 min prior to 10 mg/kg, s.c. of morphine in combination with FS-stress exposure (left) or with PSY-stress exposure (right) for 5 days (closed symbols). Control mice received saline instead of nor-binaltorphimine (open symbols). Morphine alone (○); morphine + FS-stress (△, left) or morphine + PSY-stress (△, right). Each point is the mean ± S.E. of 8–24 animals. *P < 0.05, **P < 0.01, compared with the corresponding value on the 1st day. #P < 0.05, ##P < 0.01, compared with the control group treated daily with morphine.

Fig. 3. Effect of i.t.- or i.c.v.-administered nor-binaltorphimine on the blockade by psychological (PSY)-stress of the development of antinociceptive tolerance to morphine. Nor-binaltorphimine (2 μg/mouse, i.t. or 2 μg/mouse, i.c.v.) was given daily 10 min prior to 10 mg/kg of morphine, s.c. in combination with PSY-stress exposure for 5 days. Control mice received saline instead of nor-binaltorphimine. Morphine alone (○), morphine + PSY-stress (△), nor-binaltorphimine + morphine (●), or nor-binaltorphimine + morphine + PSY-stress (▲). Each point is the mean ± S.E. of 8–24 animals. *P < 0.05, **P < 0.01, compared with the corresponding value on the 1st day. ###P < 0.01, compared with the control group treated daily with morphine.
spinal cord are alkylated by the irreversible μ-opioid receptor antagonist β-funaltrexamine (β-FNA), suggesting the relative importance of the spinal cord in the development of morphine tolerance. However, since it has been reported that pretreatment with reserpine abolished the analgesic effect of morphine given i.c.v. (17), and likewise, the analgesic effect induced by FS- and PSY-stress exposure is not recognized in reserpinized mice (10, 13), the effect of exposure to stress on the supraspinal level could not be excluded. It is likely that the exposure to stress indirectly alters or modulates the spinal function through the descending pathway and consequently suppresses the development of antinociceptive tolerance without affecting the supraspinal morphine antinociception and tolerance.

We have reported that the site of action of adrenergic blockers, phentolamine and propranolol, in suppressing the development of analgesic tolerance to morphine is located in the supraspinal areas rather than the spinal cord (9). Thus, it may be plausible that supraspinal and spinal areas play independent roles in the production of morphine antinociception and tolerance, and that compounds or procedures that block the development of tolerance act selectively on supraspinal or spinal sites.

Nor-BNI, a selective κ-opioid receptor antagonist (18, 19), nullified the suppressive effect of PSY-stress but not that of FS-stress on the development of tolerance to morphine. The results suggest that FS- and PSY-stress differentially act on the spinal cord, and that κ-opioid receptor mechanisms are mainly involved in the suppression by PSY-stress. This may be supported by our earlier findings (10, 20) that an application of PSY-stress produces analgesia through the mediation of κ-opioid receptors, whereas FS-stress produces analgesia by different opioid receptor mechanisms, i.e., through the mediation of μ-opioid receptors.

Thus, we conclude that these stresses suppress the development of morphine tolerance by acting on the spinal cord, probably through the descending signals from the supraspinal area, and also that the development of tolerance in the spinal cord due to interaction of morphine at μ-opioid receptors can be suppressed by activation of κ-opioid receptors in the spinal cord.

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